Volume 1 • Number 5 • October 2007

Journal of Clinical Lipidology

Official Journal of the National Lipid Association

FONDAZIONE GIOVANNI LORENZINI MILAN, ITALY
GIOVANNI LORENZINI MEDICAL FOUNDATION HOUSTON, USA

Weill Cornell Medical College

DALM 2007
XVI International Symposium on
DRUGS AFFECTING LIPID METABOLISM

October 4-7, 2007
Hilton New York Hotel
New York, New York (USA)

Abstracts Issue

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ISSN 1933-2874

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INSTRUCTIONS TO AUTHORS

Journal of Clinical Lipidology is the Official Journal of the National Lipid Association.

STATEMENT OF PURPOSE

The Journal of Clinical Lipidology is published to support the diverse array of medical professionals who work to reduce the incidence of morbidity and mortality from dyslipidemia and associated disorders of lipid metabolism.

Journal of Clinical Lipidology provides rapid online electronic and print publication of the most important current scientific developments in the field of lipids and lipidology. The Journal publishes both clinical and basic original, peer-reviewed articles devoted to diagnosis and treatment lipid disorders. Sections of the Journal address pioneering studies and the clinicians who conduct them, case studies, ethical standards and conduct, professional guidance such as ATP and NCEP, editorial commentary, letters from readers, National Lipid Association (NLA) news and upcoming event information, as well as abstracts from the NLA annual scientific sessions and the scientific forums held by its chapters, when appropriate.

In general, review articles are invited, and unsolicited submissions will be considered. All articles are sent for peer-review.

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Manuscripts submitted must be original, with no portion under simultaneous consideration for pub-

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lication elsewhere or previously published, except for an abstract of fewer than 400 words. Studies involving experimental animals and humans must conform to the guiding principles of the Declaration of Helsinki, and human subjects must have given informed consent of a study that has been approved by the Institutional Committee on Human Research at the authors' institution. Any financial or other relations must be disclosed. Cover letters must include affirmation of the above.

Title Page (save as a separate file): Please include a brief and descriptive title of the article, a short title of fewer than 50 characters, the authors' full names, academic degrees, hospital and academic affiliations, acknowledgment of ALL sources of financial support, potential conflicts of interests for all authors, and the name, address, phone and fax numbers, and E-mail of the individual responsible for editorial correspondence and/or reprint requests.

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For Reviews and Case Studies, please follow the format instructions below:

Reviews: (250 words) Background (justify relevance to readership), Sources of material, abstract of findings and conclusion.

Case Studies: Brief of overview of the problem and major management decisions required to resolve the case.

Glossary of abbreviations used in the manuscript: Avoid ALL abbreviations other than standard units of measurement and common abbreviations, such as RV, LV, etc.

Text: Begin the text on page 3 and organize into sections: Introduction, Methods, Results, Discussion, and Conclusion, with appropriate subheadings to make the sections easily understood. Explain abbreviations at first mention, followed by the abbreviation in parentheses. References, tables, and figures should be cited in numerical order. Avoid jargon, cliches, and laboratory slang. References to medical devices, equipment and drugs must adhere to code structures and usage conventions set forth by NLA code. Place acknowledgments at the end of the text, before references. The manuscript should not exceed 20 double-spaced typed pages, 8 figures, 3 tables, and 35 references. Authors whose native language is not English are STRONGLY advised to seek appropriate grammatical assistance. Poorly written manuscripts are at a disadvantage. Do not include any author contact information within the text.

References: Number references in the numerical order in the text. Include references to unpublished material or personal communications in the text in parentheses. Abbreviate titles of periodicals according to the style of Index Medicus, National Library of Medicine. List all authors in each reference following exactly the format and punctuation shown below.

Journal Article–Example
Huikuri HV, Tapanainen JM, Lindgren K, Raatikainen P, Makikallio TH, Juhani Airaksinen KE, Myerburg RJ. Prediction of sudden cardiac death after myocardial infarction in the beta-blocking era. *J Am Coll Cardiol.* 2003;42:652–658.

Chapter in Book with Different Author and Editor–Example
La Rovere MT, Schwartz PJ. Baroreflex sensitivity. In: Opie L, ed. *Drugs for the Heart, Sixth Edition.* Philadelphia: WB Saunders; 2006:67–93.

Authors are responsible for the accuracy and completeness of their references and for correct text citation.

Tables: Tables must be self-explanatory and supplement, not duplicate, the text. Number brief titles in Arabic numerals according to the order of mention in the text. Each table should be typed on a separate page and designed for economy of space and readability. Do not imbed tables within the text. Notes designated in the tables and all abbreviations should be defined in a footnote. Abbreviations should be identified in alphabetical order. Footnotes should be used in the following order: *,†,§.

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Original Contribution

Case Studies. Case studies are limited to 4 authors, 8 double-spaced pages total, including text, references (<20), figure legends, and 4 figures. No abstract included.

Reviews. Contemporary and Historical Reviews (Solicited and Unsolicited)

Review articles should contain a brief abstract and be 5000 total words, including references, tables, and figures. References are no more than 30 and must be under 5 years old. Use other articles to incorporate older references.

Letters to the Editor: Letters should be double-spaced, not exceeding 450 words. Letters will be reviewed and are subject to editing. They should not contain original data or figures. If accepted for publication, a copy of the letter will be sent to the author(s) of the original article, if applicable. The author(s) will have an opportunity to respond with new material that will be considered for publication with the letter.

Editorial Commentary. All articles by invitation only.

Commentaries should be confined to articles in the current issue or a very specific topic that is current and high interest to the readership.

News from the NLA.
Critique of Recent Publications: This should be a commentary on recent publications that are of very high interest to the readership such as large trials of lipid altering drugs, new laboratory methods, and new genetic linkage studies of lipid disorders. The author should be invited based on intimate knowledge of the area of study and an understanding of the implications of the clinical impact. The Critique should give: title, reference, study question, major methods, results, conclusion of the authors, and an opinion statement as to the impact this will have on our thought process and/or our actions in the clinical arena. This should be limited to approximately 5 publications within the previous 6 months. The 5 publications/articles to be critiqued should be construed as essential material that all clinical lipidologists should know in some detail. The abstracts for these areas should be organized like those for the Review Articles. Examples of topics to be addressed can be found at http://www.journals.elsevierhealth.com/periodicals/jacl/authorinfo.

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Foreword

From the Editor-in-Chief

This issue of the *Journal of Clinical Lipidology* contains the abstracts of presentations to be made at the Drugs Affecting Lipid Metabolism (DALM) meeting to be held from October 4 through 6 in New York City. This continues a tradition begun 42 years ago of a tri-annual gathering of academic investigators and experts within the pharmaceutical industry from around the world to share new information about disorders of lipid metabolism and new therapies to treat such disorders. This meeting has been sustained by the Lorenzini Foundation and its staff (please see the description of this Foundation on pages 303–304). The tradition has been to alternate the location between an American city and one located in Italy. The presentations and discussions during the fifteen previous meetings of DALM have stimulated the development of new drugs, refined the use of those already available and enticed young investigators to develop and continue successful careers in the study of lipid disorders and their treatment. This meeting reflects a tremendous growth of the science and thought in our field. From the content of the abstracts, it should live up to the tradition of the past and provide an intense learning experience for all those devoted to Clinical Lipidology.

The stated goals and objectives of the symposium are to enhance the ability of those attending to:

1. Assess the benefits of lipid modification in clinical trials on endpoints of cardiovascular events and surrogate endpoints, such as progression /regression of atherosclerosis and endothelial dysfunction;
2. Evaluate the importance of traditional and novel risk factors, including genetic polymorphisms, as they relate to atherosclerosis and cardiovascular disease and contribute to the response to drug therapy;
3. Differentiate the multiple effects of various classes of medication, alone and in combination, on lipid modification and measures of vascular function and disease;
4. Appraise new drugs, neutraceuticals, and dietary approaches and their application to the treatment of lipid disorders;
5. Evaluate the role of new technologies (such as CT, MRI, and IVUS) and new biochemical markers in identifying the atherosclerotic burden and the vulnerable plaque;
6. Review and evaluate new research regarding the underlying and environmental causes of the metabolic syndrome;
7. Assess the role of the cardiovascular and metabolic risk factors in the development of cardiovascular disease and diabetes in special groups of patients;
8. Describe recent clinical trials and assess their impact on the prevention and treatment of atherosclerosis and cardiovascular disease; and
9. Determine the practical basis for the appropriate use and the implementation of guidelines.

Some 800 presentations will be made at plenary sessions, in workshops, and with posters. It is the hope of the staff of the *Journal of Clinical Lipidology* and of the meeting organizers that the availability of the abstracts will provide the core of the information to those who were not able to attend. For those to be in attendance, we hope it will help guide your choices of the multiple simultaneous presentations.

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1933-2874/$ -see front matter
doi:10.1016/j.jacl.2007.08.016

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News from the National Lipid Association

Lipid Luminations update

So far, so good is the word on our NLA-sponsored radio program, *Lipid Luminations.* Our broadcasts are on XM Radio channel 233, ReachMD. To date, we've recorded 7 broadcasts on a range of topics, featuring our thought leaders and spokespersons. Podcasts of past broadcasts are available. To download podcasts, check the upcoming broadcast schedule, or listen in live, click on the ReachMD link on the NLA homepage at www.lipid.org. Our recent programs include:

Lipid Issues in Primary Care
Combination Lipid Therapy . . . What Works?
The Journal of Clinical Lipidology
OTC Supplement Discussion
Becoming a Full Time Lipidologist
Cardiovascular Effects of Estrogen
The ABCs of Preventing Heart Disease

New publications from the NLA

One of the great benefits of NLA membership is the educational tools we provide to our members. We are pleased to announce the pending release of two new NLA Complex Lipid Management Self-Assessment Programs (CLM-SAPs) and an additional Self-Study Mondule (SSM):

CLM-SAP 11: *Clinical Applications of Advanced Lipoprotein Testing, Inflammatory Markers and Non-invasive Assessments of Atherosclerosis* (Sept. 2007)
NLA-SSM: *Pharmacology and Safety of Lipid Altering Therapies* (Nov. 2007)
CLM SAP 12: *Management of Low HDL-C* (Dec. 2007)

CLM-SAPs provide problem-solving, case-based questions that involve common pharmacological issues faced by lipid specialists. The NLA CLM-SAP series, together with the NLA Self-Study Modules (SSMs), are the main preparatory mechanisms for the certifying examinations in Clinical Lipidology. Each module is CME-certified by the NLA for 5 credit hours and is offered as direct-mail enduring material.

All NLA active members, at the time of printing, receive the CLM-SAPs and NLA-SSMs free of charge as a benefit of membership. To order back copies of older editions (2004–2006) of the CLM-SAP, visit www.proevalinc.com. NLA members receive a significant discount on the fee.

NLA Meetings in 2008

Dates	Meeting	Location	Contact information	Room rate and cut-off date
Feb 22–24	NELA 4th Annual Scientific Forum	Philadelphia, PA	The Loews Hotel 215-627-1200	$185/night January 25, 2008
May 29–Jun 1	PLA/NLA 2008 Annual Scientific Sessions	Seattle, WA	The Westin 888-627-8513, ask for the PLA/NLA room rate	$199/night May 6, 2008
Jul 18–20	SWLA 3rd Annual Scientific Forum	Denver, CO	Marriott City Center 800-444-2206, ask for SWLA room rate	$179/night June 25, 2008
Aug 22–24	SELA 11th Annual Scientific Forum	Hilton Head, SC	Westin Hilton Head 800-937-8461, ask for the SELA room rate	$209/night $10/night resort fee July 21, 2008
Sep 26–28	MWLA 5th Annual Scientific Forum	Chicago, IL	Westin Michigan Avenue 888-627-8385, ask for the MWLA room rate	$229/night August 27, 2008

1933-2874/$ -see front matter
doi:10.1016/j.jacl.2007.08.001

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www.lorenzinifoundation.org

From the Lorenzini Foundation

The **Fondazione Giovanni Lorenzini Medical Science Foundation** consists of two not-for-profit scientific organizations, one based in **Milan, Italy** (established in 1969) and the second in **Houston, Texas, USA** (established in 1984): both are committed to international scientific exchange and education in basic and medical research.

The mission of the Foundation is the transfer of basic scientific research results to clinical and applied research and the collaboration with academia to ensure constant updating of physicians and basic scientists.

The Lorenzini Foundation has been actively involved and has long-standing experience in running **Educational Campaigns on Public Health** both at national and international levels, such as the Italian Cholesterol Campaign (national: 1986–1992), the Campaign on Triglycerides as a Risk Factor (international: 1989–1992), the Italian Campaign on Women's Health and Menopause (national: 1995–1999), the Italian Campaign for the Prevention of the Global Cardiovascular Risk (directed at clinicians, GPs, and the public: 2003—still in progress)

The activities of the Lorenzini Foundation include the organization of international **Congresses and Meetings** and the publication of proceedings, highlights, websites, CD-ROMs on specific topics; of particular note are the following series of international symposia: Women's Health and Menopause, Drugs Affecting Lipid Metabolism, Triglycerides and HDL, Prostaglandins and Eicosanoids (the next one will be organized in July in Beijing, China).

The Lorenzini Foundation also coordinates all the activities of the **International Society of Atherosclerosis** (a Federation of 54 national societies with more than 12,000 members).

The Lorenzini Foundation has extensive experience in **promoting and coordinating interdisciplinary international panels** and in publishing comprehensive documents, such as **Position Papers** or **Guidelines**. The most recent activities in this area are:

1) a joint monograph entitled "International Position Paper on **"Women's Health and Menopause: A Comprehensive Approach" published in July 2002 together with the National Heart, Lung, and Blood Institute/NIH and the NIH Office of Research on Women's Health**: this publication has been developed by an international panel of more than 80 experts *(NIH Publication No. 02-3284, July 2002, 1-297).*
2) international guidelines entitled **"Harmonized Clinical Guidelines on Prevention of Atherosclerotic Vascular Disease" developed in collaboration with the International Atherosclerosis Society and its 54 Constituent Member Societies.**

Because of the above-mentioned experience, the Lorenzini Foundation is able to easily **combine the scientific interests of regulatory agencies, health organizations, academia, clinicians, scientific societies, and industry** to solve complex problems.

At present the Lorenzini Foundation is developing a project on **the use of Integrated Biomarkers in Cardiovascular Diseases,** which include **Courses and Symposia** directed at clinicians, bioinformatics experts, regulatory officers, scientists, and industry researchers. Other courses are planned in the area of chemotherapy and neurosciences.

The Courses and Symposia are part a **more comprehensive project** that also includes the coordination of an **International**

1933-2874/$ -see front matter
doi:10.1016/j.jacl.2007.08.002

Panel whose purpose is to discuss the state of the art of research and use of biomarkers (alone or integrated) and to propose recommendations on future research and the better use of biomarkers (alone or integrated) with the objective of increasingly personalized medicine. With this project, the Lorenzini Foundation aims to **create a methodology model of collaboration among academia, regulatory agencies, scientists, and industry** that may also be translated to oncology and diseases of the central nervous system.

The Lorenzini Foundation also coordinates the **development of web sites**, as permanent sources of information, dedicated to metabolic diseases and atherosclerosis, in collaboration with the International Atherosclerosis Society (**www.athero.org**) and the Italian Heart Foundation (**www.fondazionecuore.it**). See also **www.cardiometabolica.org** on the metabolic syndrome.

Upcoming international symposia organized by the Lorenzini Foundation:

October 22–25, 2008
6th International Symposium on Multiple Risk Factors in Cardiovascular Diseases—Prevention and Intervention—Health Economics
Co-chairs: A.M. Gotto, Jr. (New York, NY, USA), S.M. Grundy (Dallas, TX, USA), and R. Paoletti (Milan, Italy)
Venice (Lido), Italy.
For more information, please contact: info@lorenzinifoundation.org

June 14–18, 2009
XV International Symposium on Atherosclerosis
Co-chairs: A.M. Gotto, Jr. (New York, NY, USA) and E.J. Schaefer (Boston, MA, USA)
Boston, MA, USA
For more information, please contact: info@athero.org

Journal of
Clinical Lipidology

VOLUME 1, NUMBER 5, OCTOBER 2007

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These abstracts were presented at the **XVI International Symposium on Drugs Affecting Lipid Metabolism** (October 4–7, 2007, Hilton New York Hotel, New York, NY, USA). The Final Program can be found at www.lorenzinifoundation.org.

For Keywords, refer to pages 546–548.

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Abstracts

Thursday
October 4, 2007

Opening Remarks
8:30 AM - 8:45 AM
Chairs: *R. Paoletti, Milan, Italy and A.M. Gotto, Jr., New York, NY, USA.*

1
WELCOME AND OPENING REMARKS
Antonio M. Gotto, Jr. *Weill Cornell Medical College, New York, NY, USA.*

The field of drugs affecting lipid metabolism has become increasingly sophisticated over the last few years. Statins remain at the forefront of pharmacological approaches to managing dyslipidemia, but the primacy of low-density lipoprotein (LDL) in discussions of lipid-based cardiovascular risk has given way to greater consideration of the other lipid fractions, such as high-density lipoprotein (HDL) and triglycerides. Explorations of the targeting of these fractions to change the course of atherosclerosis have yielded novel insights that will guide future investigations. Along the same lines, greater understanding of the biological bases of the atherosclerotic plaque has suggested new targets of therapy. In sum, these advances indicate an exciting and promising future for the field.

Funding: None

Plenary Session 1 "HDL"
8:45 AM - 10:45 AM
Chairs: *R. Paoletti, Milan, Italy and A.M. Gotto, Jr., New York, NY, USA.*

2
ANTIATHEROGENIC PROPERTIES OF HDL
Philip Barter*. The Heart Research Institute, Sydney, Australia.*

An inverse relationship between the concentration of cholesterol in high density lipoproteins (HDLs) and the development of premature coronary heart disease (CHD) has been observed in many large-scale prospective studies. In several of these, the level of HDL cholesterol (HDL-C) has been the single most powerful lipid predictor of future CHD events. In support of these human population studies, there are numerous intervention studies in animals showing that an increase in the concentration of HDLs inhibits the development of atherosclerosis. Evidence in humans that increasing the concentration of HDLs protects against cardiovascular disease is more limited but growing. There are several properties of HDLs that have the potential to protect against the development of atherosclerosis. The best documented is the ability of HDL to promote the efflux of cholesterol from cells in the artery wall. However, HDLs have a number of additional potentially anti-atherogenic properties that may be unrelated to their role in plasma lipid transport. For example, HDLs bind lipopolysaccharide, promote endothelial repair by enhancing the migration of cells from neighbouring undamaged tissue and by recruiting progenitor endothelial cells from plasma into damaged endothelium. HDLs inhibit the synthesis of platelet-activating factor by endothelial cells. HDLs are anti-thrombotic. They modulate endothelial function, probably by stimulating endothelial NO production. HDLs also possess antioxidant and anti-inflammatory activities. The degree to which any or all of these non-lipid transport functions of HDL contribute to a protection against atherosclerosis is still uncertain, although evidence is mounting that at least some of them may be of substantial importance.

Funding: National Health and Medical Research Council of Australia

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HDL, REVERSE CHOLESTEROL TRANSPORT, AND NUCLEAR RECEPTORS

***Jean-Charles Fruchart**, Bart Staels, Jean-Louis Junien. Pasteur Institute of Lille, Lille, France; Genfit, Lille, France.*

High density lipoprotein (HDL) cholesterol levels are a strong increase predictor of cardiovascular events. PPARα (peroxisome-proliferator activated receptor α) and LXRα (liver X receptor α) are transcription factors that regulate the expression of genes that control lipid and lipoprotein metabolism as well as the inflammatory response. PPARα and LXRα control the first steps of reverse cholesterol transport by acting on microphages in different ways. PPARα agonists enhance the expression of CLA-1 and the ABC transporter ABCA1 by an indirect mechanism involving the stimulation of LXRα expression. LXRα agonists promote cholesterol efflux from macrophages through the induction of ABCA1, ABCG1 and ABCG4. PPARα and LXR agonists increase the expression of NPC1 and NPC2 leading to an enrichment of cholesterol in the plasma membrane and to a stimulation of cholesterol mobilization by PPARα and LXR activation. On the basis of these findings, the development of new molecules targeting selectively these nuclear receptors provide exciting opportunities to reduce atherosclerosis and its complications. We have developed selective nuclear receptor modulators with characteristic cofactors binding patterns in order to activate desirable target genes and to repress the transcription of undesirable genes. At this stage, a growing body of evidence from in vitro and in vivo study in animals and humans, indicates that these drugs are safe and have beneficial effects in atherosclerosis.

Funding: None

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UPDATE ON HDL METABOLISM AND POTENTIAL OF HDL AS A THERAPEUTIC AGENT IN THE TREATMENT OF CARDIOVASCULAR DISEASE

***H. Bryan Brewer**. Medstar Research Institute, Washington, DC, USA.*

HDL has been proposed to protect against atherosclerosis by several mechanisms including reverse cholesterol transport, protection of LDL from oxidation, as an anti-inflammatory agent, and by modulation of endothelial function with increased NO production. A major advance in HDL metabolism has been the elucidation of a dual pathway for HDL mediated cholesterol efflux. These pathways include poorly lipidated apoA-I the preferred cholesterol acceptor for the ABCA1 transporter and αHDL which facilitates efflux by the SR-BI receptor and ABCG1 transporter. Current drug therapy to increase HDL includes statins, fibrates, and niacin. Future approaches to increase HDL include both acute HDL infusion therapy for acute coronary syndrome (ACS) patients and chronic oral HDL therapy for stable cardiovascular disease patients. Of clinical interest is the use of acute HDL infusion therapy to potentially decrease cardiac events in ACS patients. The initial clinical study with acute HDL therapy utilized apoA-I Milano/phospholipids complexes and was associated with regression in coronary artery atherosclerosis. Recently, infusions of autologous delipidated HDL have been initiated to further establish the efficacy of apoA-I infusions to reduce coronary atherosclerosis. ApoA-I mimetic peptides are also being developed as an approach for infusions in ACS patients. Further clinical studies will be required to definitively establish if acute HDL infusion therapy with apoA-I/phospholipid complexes and apoA-I mimetic peptides will result in regression of coronary atherosclerosis and decreased cardiac events.

Funding: Medstar Research Institute

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DRUGS AFFECTING HDL

***Cesare Sirtori**. University of Milano, Milano, Italy.*

Modifying high density lipoprotein (HDL) metabolism is a major objective in present day pharmacology. HDL, by their capacity to remove cell cholesterol, provide a crucial tool for prevention as well as for eliciting regression of established disease. Raising HDL can be attempted by the activation of, eg the PPAR system, by PPARα agonists (fibrates), and also by nicotinic acid, apparently acting on all three PPARs (α, δ, γ). Both fibrates and nicotinic acid act on the ABCA1 transporter; fibrates, to a variable extent, exert a powerful antiinflammatory activity. The failure of the cholesterol ester transfer protein (CETP) inhibitor torcetrapib, in spite of the significant HDL rise, was predictable, based on epidemiological data underlining a lack of protection of CETP gene mutations both in the coding and promoter regions. CETP inhibition leads to large, but dysfunctional HDL particles; this failure underlines the risk of antagonizing a transfer/exchange process typical of higher species. Potentially better results can be achieved by activators of the CETP system, eg probucol, a powerful stimulator, leading to lower HDL-C levels but to positive effects on the IMT, xanthomas, etc. The analog AGI-1067, less active on the CETP system, provided inadequate preventive activity in high risk patients in the ARISE study. Among different agents, statins have a variable activity on HDL-C, but there is as yet no clear link between the HDL raising action and cardiovascular benefit. Finally, among the A-I mimetics, apoA-I_{Milano} being the forerunner, a number of molecules hold hope. The recent possibility of monitoring lesions by an MRI technology even in very small arteries, will lead to the direct testing of these last agents by non-invasive approaches.

Funding: None

Plenary Supported Session 1
"CHOLESTEROL BALANCE, LIPOPROTEIN METABOLISM, AND CARDIOVASCULAR RISK"
11:15 AM - 12:45 PM
***Chairs:** A.L. Catapano, Milan, Italy and E.A. Fisher, New York, NY, USA.*

6

CHOLESTEROL BALANCE AND THE REGULATION OF PLASMA LIPID LEVELS

***David Cohen**. Brigham and Women's Hospital, Boston, MA, USA.*

Cholesterol is synthesized by most cells in the body, and is also obtained in the diet. Adult humans synthesize cholesterol at rates of up to 1.2 g/day. Absorption from the diet is incomplete, with percentages that range from 25 to 80. Dietary cholesterol contents average approximately 0.4 g daily. Because excess cholesterol is toxic to cells, each day an amount equal to endogenous synthesis plus absorbed cholesterol must be routed from peripheral tissues to the liver for elimination. This is achieved when HDL in plasma accept excess cholesterol from cells. HDL cholesterol is then esterified by the circulating enzyme lecithin: cholesterol acyl transferase to form cholesteryl esters. These molecules accumulate in the cores of HDL particles and are returned to the liver. A fraction of the cholesteryl esters in HDL take an alternate route to the liver. They are transferred by cholesteryl ester transfer protein to triglyceride-rich lipoproteins. The liver must maintain a steady state content of free cholesterol that is suitable for hepatocellular function. Hepatic cholesterol is derived from lipoproteins, from de novo synthesis that is rate-limited by HMG-CoA reductase and from hydrolysis of stored cholesteryl esters. Excess cholesterol may be exported into plasma by incorporation into HDL or VLDL particles. Cholesterol may also be eliminated from the body via bile. This is achieved by conversion to bile salts at rates averaging 0.4 g/day and by secretion of cholesterol unmodified into bile at rates of up to 2 g/day. Fecal losses of bile salts balance synthetic rates, accounting for 0.4 g/day of cholesterol losses. Approximately 50% of biliary cholesterol is reabsorbed in the intestine.

Funding: National Institutes of Health (R01 DK048873 and R01 DK056626); American Heart Assocation Established Investigator Award

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IMAGING AND BIOMARKERS TO ASSESS CARDIOVASCULAR RISK AND GUIDE TREATMENT

John J.P. Kastelein. *Academic Medical Center / University of Amsterdam, Amsterdam, Netherlands.*

Novel treatment modalities for cardiovascular prevention are emerging rapidly. Since it is virtually impossible to evaluate all these new compounds in long-term trials using clinical end points, there is an urgent need for validated surrogate markers of atherosclerosis to save both time and costs. Over the last decade, the use of imaging markers has been widely introduced into drug-development strategies. Here the most commonly used techniques will be discussed. Whereas both testing of endothelial function, assessed as flow-mediated dilation, and assessment of carotid intima-media thickness have been shown to predict future cardiovascular events, predominantly intima-media thickness has been used successfully as a surrogate marker in intervention studies. More recently, standardization of intravascular ultrasound has also enabled reproducible assessment of coronary atheroma volume. Multislices computed tomography and electron-beam computed tomography have proven useful in providing quantitative information on plaque burden and coronary calcium content, respectively. The imaging modalities reviewed here all provide specific information on either functionality or morphology of the vasculature. The value of carotid intima-media thickness for cardiovascular risk prediction has been studied most extensively. Whereas assessment of plaque burden using intravascular ultrasound appears to be the most direct way to quantify coronary changes, its predictive value for future cardiovascular events remains to be established. Awaiting further technical improvements, MRI is expected to provide the most valuable information for the evaluation of atherosclerosis in the near future.

Funding: None

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UPDATE ON NEW LIPID-ORIENTED CLINICAL TRIALS

Edward A. Fisher. *NYU School of Medicine, New York, NY, USA.*

Although it is well established that risk factor modification lowers coronary artery disease event rates, there are still a number of outstanding issues that ongoing clinical trials will try to resolve. Some examples include, what are the appropriate goals for even well-established risk factors like LDL and blood pressure in patients at different levels of risk? Are there additive effects of treating multiple risk factors? In metabolic syndrome and diabetic patients, what is the relative importance of raising HDL and lowering triglycerides to lowering LDL? Is CRP or SPLA2 an independent risk factor for CAD? Do non-statin drugs that lower LDL cholesterol give similar benefits to statins? How compelling are imaging studies to regulatory agencies and guideline panels, compared to end point studies? These and other questions will be discussed in the context of recent, completed studies, ongoing studies, and the new pharmacological approaches on the horizon.

Funding: None

Supported Session 2 "MANAGEMENT OF MIXED HYPERLIPIDEMIA: BEYOND LDL CHOLESTEROL"
2:00 PM - 4:00 PM
Chairs: *C.M. Ballantyne, Houston, TX, USA and E.J. Schaefer, Boston, MA, USA.*

9

APPROACH TO THE PATIENT WITH MIXED HYPERLIPIDEMIA: RATIONALE FOR NON-HDL-C OR ApoB AS A TARGET FOR THERAPY

Christie M. Ballantyne. *Baylor College of Medicine, Houston, TX, USA.*

Clinical trials of statin therapy have demonstrated significant relative risk reductions in cardiovascular events and mortality with increasingly aggressive reductions in LDL-C, yet patients still have events despite intensive statin therapy. Patients with mixed dyslipidemia present a unique challenge because they have increased triglyceride-enriched lipoproteins, small dense LDL particles, increased apo B levels, and frequently low HDL-C levels. Although lipid fractions such as triglycerides and HDL-C have been shown to be associated with cardiovascular risk in epidemiological studies, clinical event trials have not examined the effects of decreasing triglycerides or increasing HDL-C. Therefore, LDL-C remains the primary target of lipid-lowering therapy in the U.S. National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines, and the ATP III guidelines establish non-HDL-C as a secondary therapeutic target in patients with triglycerides ≥200 mg/dL. Non-HDL-C has the advantage of using information already available in a standard lipid profile to assess the levels of all atherogenic, apo B–containing lipoproteins. However, recommended non-HDL-C targets are infrequently achieved in high-risk patients. Measurement of apo B level may also be useful in assessing cardiovascular risk, and some authorities recommend an apo B target of <90 mg/dL in high-risk patients. However, even among patients who achieve LDL-C goals, achieving this apo B target is difficult and may require more aggressive therapy than that required to reduce LDL-C to goal. Achieving an apo B target of <90 mg/dL in patients with elevated triglycerides has been shown to require reducing non-HDL-C to <100 mg/dL and reducing LDL-C to <70 mg/dL. While all current lipid-modifying drugs affect multiple lipid fractions, greater benefit on multiple lipid fractions often requires combination therapy, and combining agents with complementary mechanisms may provide greater improvements for the entire lipid profile.

Funding: None

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LIPOPROTEIN METABOLISM IN THE PATIENT WITH ELEVATED TRIGLYCERIDES

Henry Ginsberg*. Columbia University College of Physicians and Surgeons, New York, NY, USA.*

Hypertriglyceridemia, together with reduced plasma levels of HDL cholesterol and a predominance of smaller, cholesterol ester depleted LDL, is the hallmark dyslipdemia of insulin resistance and type 2 diabetes mellitus. Increased fatty acid flux to the liver, increased hepatic lipogenesis, and increased remnant lipoprotein delivery of TG to the liver are all stimuli to increased assembly and secretion of VLDL. However, there appear to be qualitative and quantitative differnces in the effects each has on VLDL TG and apoB secretion. Hyperinsulinemia, another central feature of insulin resistance, can stimulate VLDL secretion independently through the stimulation of lipogenesis, but insulin can also inhibit VLDL secretion by targeting apoB for degradation in the hepatocyte. Insulin-mediated targeting of apoB for degradation may be defective in an insulin resistant liver, leading to more VLDL secretion. Once in the plasma, VLDL TG lipolysis is regulated by lipoprotein lipase and apoCIII. In insulin resistance, apoCIII secretion is increased, leading to less efficient lipolysis of VLDL TG. The products of lipolysis, VLDL remnants and LDL, are cleared from the plasma by a number of pathways, including heparin sulfate proteoglycan mediate trapping in the liver and LDL receptor mediated uptake. Both of these processes can be affected by insulin resistance and diabetes. Reduced HDL levels result in part from CETP mediated exchange of core lipids between HDL and both VLDL and chylomicrons: TG-enriched HDL are lipolyszed and apoA-I is lost from plasma. Small dense LDL also results from CETP mediated processes.

Funding: R01 HL55638 (H. Ginsberg, PI); R01 HL73030 (H. Ginsberg, PI)

11

OMEGA-3 FATTY ACIDS FOR TREATMENT OF HYPERLIPIDEMIA: MECHANISMS AND EVIDENCE

William S. Harris. *University of South Dakota, Sioux Falls, SD, USA.*

Omega-3 fatty acids (FAs) contain 18 to 22 carbons and a signature double bond at the third position from the methyl (or n, or omega) end of the molecule. These FAs include the plant-derived α-linolenic acid (ALA, 18:3n-3), and the fish-oil–derived eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). The latter two FAs, when consumed together at 3-4 g/d, have well-described triglyceride (TG) lowering effects. In a recent evidence-based review, typical TG reductions were 22-30%, HDL and LDL increased by 1.6% and 6%, respectively. In patients with severe hypertriglyceridemia (TG>500 mg/dL) treated with prescription omega-3 acid ethyl esters (4 g/d), a net (vs placebo) 50% reduction in TG was observed (from 816 to 408 mg/dL), along with a 10% decrease in non-HDL-C, and a 9% increase in HDL-C (from 22 to 24 mg/dL). This was accompanied by a 50% increase in LDL-C (from 89 to 133 mg/dL), an effect also seen with fenofibrate in this patient population. The mechanisms by which omega-3 FAs lower TG levels include reduced hepatic lipogenesis, increased hepatic beta-oxidation, and enhanced lipolysis of circulating TG. Although not yet established in humans, these effects appear to be mediated by omega-3 FA-induced increases in PPAR-alpha and suppression of SREBP-1c. In addition to effects on serum lipids, EPA and DHA have important effects on membranes, eicosanoid metabolism and gene transcription which together appear to lower heart rate and blood pressure, decrease platelet aggregation, alter ion channel function and ultimately reduce risk for cardiac arrhythmias.

Funding: None

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WEIGHING THE EVIDENCE FOR ADDING A SECOND DRUG TO STATIN IN THE PATIENT WITH MIXED HYPERLIPIDEMIA

Michael H. Davidson. *The University of Chicago, Chicago, IL, USA.*

After achievement of LDL cholesterol goals, non-HDL cholesterol can be managed more aggressively by lowering LDL-cholesterol or by using strategies that target a reduction in very-low-density lipoprotein cholesterol. As the prevalence of hypertriglyceridemia is increasing, enhanced efforts to improve non-HDL cholesterol goal achievement has the potential to produce a substantial effect on CHD. With the recognition that the residual risk is significantly elevated in patients at very high risk, the NCEP ATP III panel recommended the therapeutic optional goal of LDL <70 mg/dL and non-HDL <100 mg/dL. Cholesterol lowering therapy with statins has been established as an effective method of reducing death and myocardial infarction among patients with CHD. However, a significant amount of individuals who are receiving statin therapy continue to have high residual risk. An important clinical challenge exits in reducing residual CHD risk with optimal therapies without increasing adverse effects. Combination therapy appears most appropriate for patients with a high rate of events of residual risk despite optimal statin therapy. In addition to lifestyle modification, the use of combination therapy in CHD is an acknowledged strategy in optimal management to prevent or delay the morbidity and mortality associated with CHD and its risk factors. Current recommendations for CHD prevention and treatment advise the use of combination drug therapy for high-risk patients including those with combined hyperlipidemia and diabetic dyslipidemia. Hypertriglyceridemia is prevalent among several growing at-risk populations of individuals with obesity, type 2 diabetes, and metabolic syndrome. Many of these patients may be unable to achieve non-HDL-C targets with a statin alone. Thus, there is a clinical need for an effective and well-tolerated combination therapy to lower non-HDL-C. Potential combinations with a statin that have demonstrated additive efficacy include niacin and fibrates. In a recent study, the addition of 4 g/d of prescription omega-3 to ongoing simvastatin 40 mg in subjects with persistent hypertriglyceridemia was effective for providing additional non-HDL-C, VLDL-C, and

TG lowering. Combination therapy improved the overall atherogenic lipid profile, without attenuating statin efficacy.

Funding: None

Supported Session 3 "TREATING RESIDUAL RISK: REDUCING TRIGLYCERIDES"
4:30 PM - 6:30 PM
Chairs: *J.-C. Fruchart, Lille, France and A.M. Gotto, Jr., New York, NY, USA.*

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BURDEN OF CVD AND MICROVASCULAR COMPLICATIONS IN PATIENTS WITH DIABETES

Antonio M. Gotto, Jr.. *Weill Cornell Medical College, New York, NY, USA.*

Both Type I and Type II diabetes mellitus are independent risk factors for atherosclerosis. Coronary heart disease (CHD) occurs more frequently and at a younger age among diabetic individuals than in the general population and is the cause of death for more than half of the adult diabetic population. Diabetic patients, particularly those with Type II, often present with elevated levels of triglycerides (TG), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) particles and decreased levels of high-density lipoprotein (HDL)—the so-called Lipid Triad. This phenotype is also common in patients with the metabolic syndrome, which is characterized by dyslipidemia, hyperinsulinemia, hypertension, obesity, and increased risk for cardiovascular disease. Clinical trials of lipid modification provide a rationale for treating lipids in these patients to prevent macrovascular disease. Microvascular complications of diabetes are also common; prevention of these sequelae may require other approaches.

Funding: None

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ROLE OF ELEVATED TRIGLYCERIDES AND REDUCED HDL-C IN RESIDUAL CVD RISK REMAINING AFTER STATIN THERAPY

Jean-Charles Fruchart. *Pasteur Institute of Lille, Lille, France.*

Intervention trials using statins to lower LDL cholesterol have consistently shown substantial reduction in major cardiovascular events in the treated groups. However in all of the statin trials, it remains a substantial residual risk in the treated groups. Population studies have demonstrated that high density lipoprotein (HDL) cholesterol and triglyceride levels are strong, independent predictors of cardiovascular disease. The post-hoc analysis of the TNT trial evaluated the impact of HDL cholesterol in patients with CHD who were receiving statin therapy to reduce LDL cholesterol. Across the entire study cohort in multivariate analysis, HDL cholesterol was a significant predictor of subsequent major cardiovascular events. The PPARα activating Fenofibrate which can be used in association with statins improves the plasma lipid profile by lowering triglycerides and by increasing HDL and reverse cholesterol transport. These effects are achieved by a variety of mechanisms such as an increase in lipoprotein lipase expression, reduction of Apo-CIII expression, increased oxidation of fatty acids. Recent results from our laboratory demonstrate that Fenofibrate and PPARα agonists induce cholesterol trafficking in macrophages resulting in an enhanced availability of plasma membrane cholesterol for cholesterol efflux. This mechanism may contribute to the observed clinical effects of PPARα agonists on reverse cholesterol transport and HDL metabolism. Close review of the literature showed an increase risk of the use of statins in combination with Gemfibrozil but not with Fenofibrate. The recent FIELD study showed no adverse effects of the combined use of statins with Fenofibrate.

Funding: None

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CLINICAL TRIAL EVIDENCE SUPPORTING FIBRATE THERAPY TO REDUCE CVD AND MICROVASCULAR COMPLICATIONS IN PATIENTS WITH DIABETES

W. Virgil Brown. *Emory University School of Medicine, Atlanta, GA, USA.*

Fibrates have demonstrated significant reductions in major coronary events in men with no evidence of cardiovascular disease (Helsinki Heart Study) and in those with proven heart disease (VA HIT). In subgroup analyses, type 2 diabetics (DM2) were found to have similar if not greater reductions in CVD. Those with the metabolic syndrome were particularly benefited. Only two placebo controlled trials with vascular endpoints have been completed using fibrate therapy in DM2 patients exclusively. These were the DIAS study with an angiographic endpoint and the FIELD trial assessing multiple clinical events. Both of these trials used fenofibrate. The DIAS trial reported a significant reduction in the progression of stenotic lesions and a trend toward reducing the mean luminal diameter of the entire segments of coronary arteries that were assessed. The latter measure did not achieve statistical significance when the treated group was compared to placebo. The FIELD trial found a highly significant reduction of 25% in the major vascular events in some 7600 diabetics without vascular disease at baseline but when the 2400 patients with known vascular disease was included, the reduction in events (11%) with the active drug did not fulfill the statistical test. Myocardial infarction rates were significantly reduced in the total FIELD cohort. Both FIELD and DIAS demonstrated statistically significant reductions in progression of proteinuria and retinopathy with fenofibrate therapy.

Funding: None

Workshop 1 "HDL"
2:00 PM - 4:15 PM
Chairs: *G. Franceschini, Milan, Italy and A. Tall, New York, NY, USA.*

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LIPOPROTEIN ACCEPTOR FUNCTION IN CELL CHOLESTEROL EFFLUX: INSIGHTS FROM GENETIC HDL DISORDERS

Guido Franceschini*, Laura Calabresi, Elda Favari, Franco Bernini. University of Milano, Milano, Italy; University of Parma, Parma, Italy.*

The atheroprotective function of HDL has been related to their ability to act as an acceptor of cholesterol from macrophage foam cells in the arterial wall. Cell cholesterol efflux occurs through several distinct pathways. Passive diffusion, which may be facilitated by scavenger receptor, class B, type I (SR-BI), has broad acceptor specificity for a range of phospholipid-containing particles and does not result in net cholesterol efflux unless driven by a cholesterol concentration gradient. ATP-binding cassette (ABC) transporters promote net movement of cholesterol from cells to extracellular acceptors, either lipid free apolipoproteins and preβ-HDL (ABCA1), or mature α-HDL (ABCG1). Cell cholesterol efflux thus depends on the presence of specific transporters/receptors in the cell membrane, on the presence and avidity of extracellular cholesterol acceptors, and on extracellular proteins promoting HDL remodeling and generation of cholesterol gradients. The relative importance of these various factors in determining the efficiency of the whole process is largely unknown. Genetic HDL disorders due to mutations in a variety of HDL-related genes, which selectively affect the structure and/or levels of one or more of the players in this complex process may provide a clue to better understand the role of each factor in cell cholesterol efflux, reverse cholesterol transport and cardiovascular protection.

Funding: This work was supported in part by grants from Telethon-Italy (GGP02264), Fondazione Cariplo (2003-1753), and the Italian Ministry of University and Research (PRIN 2005)

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ABC TRANSPORTERS, CHOLESTEROL EFFLUX, AND CETP INHIBITION

***Alan Tall**. Columbia University Medical Center, New York, NY, USA.*

Cholesterol efflux from macrophage foam cells to HDL or its apolipoproteins is thought to have an important anti-atherogenic role. Two major ABC transporters are involved in this process: ABCA1 which promotes cholesterol efflux to lipid-poor apolipoproteins, and ABCG1 which promotes cholesterol efflux to HDL particles. While bone marrow transplantation studies have indicated an anti-atherogenic role of macrophage ABCA1, results with ABCG1 deficient bone marrow have been mixed. We reported a decrease in atherosclerosis in LDLR-/- mice transplanted with ABCG1-/- bone marrow. This unexpected result appeared related to compensatory up-regulation of ABCA1 and apoE secretion in ABCG1-/- macrophages. To assess this hypothesis we recently transplanted double KO ABCA1-/-ABCG1-/- bone marrow into LDLr deficient mice, resulting in a dramatic increase in atherosclerosis compared to WT or single KO BM recipients. This indicates overlapping roles and mutual compensation of ABCA1 and ABCG1 in macrophage cholesterol efflux and athero-protection. However, ABCG1 appears to have a specific role in protecting macrophages from apoptosis induced by oxidized LDL, related to the ability of ABCG1 (but not ABCA1) to promote efflux of specific oxysterols such as 7-ketocholesterol from macrophages to HDL. Large HDL that accumulates in CETP deficiency is enriched in apoE and LCAT and is highly efficient at promoting macrophage sterol efflux via the ABCG1 pathway. It is interesting to speculate that this could have specific ability to reverse oxysterol-mediated macrophage apoptosis and inflammatory gene expression in atherosclerotic lesions.

Funding: Pfizer; Merck; Astrazeneca; Amira; BMS; Boehringer Ingelheim

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THE ROLE OF PHOSPHOLIPID TRANSFER PROTEIN (PLTP) IN REVERSE CHOLESTEROL TRANSPORT

***Christian Ehnholm**, Matti Jauhiainen, Larbi Krimbou, Jacques Genest. National Public Health Institute, Helsinki, Finland; McGill University, Montreal, Canada.*

The anti-atherogenic role of HDL is well documented. Plasma HDL is heterogeneous due to continuous remodeling by plasma factors. A key protein in HDL remodeling is the phospholipid transfer protein (PLTP). PLTP has two functions: i) transfer of phospholipids between lipoproteins and ii) HDL conversion, a process generating large fused HDL particles and small preß-HDL particles. The role of PLTP in the reverse cholesterol transport (RCT) is not known. We have studied the role of two forms of PLTP, low-active (LA-PLTP) and high-active (HA-PLTP) in cholesterol efflux from THP-1 macrophages. Incubation of HDL with HA-PLTP increased preß-HDL and caused a 42 % increase in cholesterol efflux while LA-PLTP neither formed preß-HDL nor increased cholesterol efflux. Removal of preß-HDL by immunoprecipitation decreased cholesterol efflux by 47 %. The large fused HDL conversion particles generated by HA-PLTP also acted as efficient cholesterol acceptors. These observations demonstrate that PLTP activity is a pre-requisite for the PLTP –facilitated increase in cholesterol efflux and that only HA-PLTP plays a role in RCT by increasing cholesterol efflux via formation of efficient cholesterol acceptors, preß-HDL and large fused HDL. To investigate the role of PLTP in the metabolic pathway of nascent HDL particles, labeled LpA-I particles were incubated with plasma and purified PLTP. These incubations demonstrated that i) PTLP is required for phospholipid transfer between nascent HDL and apoß-containing lipoproteins, ii) PLTP is essential for the conversion of nascent LpA-I particles to pre-ß and α-LpA-I. In conclusion, PLTP plays an essential role in the metabolism of nascent HDL-particles and RCT.

Funding: None

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MODULATION AND ROLE OF PENTRAXIN 3 DURING ATHEROGENESIS

***Giuseppe D. Norata**, Cecilia Garlanda, Giulia Chiesa, Alberto Mantovani, Alberico L. Catapano. University of Milan, Milan, Italy; Ospedale Bassini, Cinisello Balsamo, Italy; Istituto Clinico Humanitas, Rozzano, Italy.*

Pentraxin 3 (PTX3) is a protein of the acute phase and the prototype member of the long pentraxin family. Although the expression was observed in atherosclerotic plaques, the role of PTX3 in cardiovascular disease is still ill defined. Aim of our study was to investigate the modulation and the role of PTX3 during atherogenesis. We first identified PTX3 as an HDL responsive gene, indeed incubation with HDL increased PTX3 expression in human umbilical vein endothelial cells (2,79 fold vs control cells) following PI3k/Akt and PKC activation. In vivo the expression of PTX3 was increased in mice overexpressing human Apo AI compared to Apo AI-/- mice and e.v. injection of HDL in C57Bl6 mice significantly increased PTX3 plasma levels. The expression of PTX3 is also significantly higher in carotid plaques from asymptomatic patients (5.63 fold), compared to plaques from symptomatic patients. To address the involvement of PTX3 in atherogenesis we developed double KO mice (ApoE -/-, PTX3 -/-). These mice showed increased aortic atherosclerotic lesion areas (+37%) compared to ApoE -/- mice and investigation of the gene expression patterns in the vascular wall revealed the activation of SMC. Of note collagen deposition in the plaque was lower in double KO mice despite similar levels of collagen 1A1, 1A2 and 3A1 mRNA. Our data suggest that PTX3, unlike other proteins of the acute phase, may play an important atheroprotective role affecting lesion progression and plaque stability.

Funding: This work was supported by grant from the Ministry of Research

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HIGHER APO A1, BUT NOT HIGHER HDL-C, MAY BE CARDIOPROTECTIVE: OBSERVATIONAL DATA FROM THE IDEAL TRIAL

***Anders Olsson**, Ingar Holme, Matti J. Tikkanen, John J.P. Kastelein, Nilo B. Cater, Christina Lindahl, Terje R. Pedersen. Linkoping University, Linkoping, Sweden; Ulleval University, Oslo, Norway; University of Helsinki, Helsinki, Finland; Academic Medical Center, Amsterdam, Netherlands; Pfizer, New York, USA.*

Background: Because the relationships of risk of major coronary events (MCE) and high HDL-C and/or high Apo A1 are unclear, a posthoc analysis of the database of the 5-year Incremental Decrease through Aggressive Lipid Lowering (IDEAL) trial was conducted to gain further insights into these relationships. **Methods:** Data from patients who had MCE (time to first occurence of myocardial infarction, CHD death, resuscitated cardiac arrest) during the first 6 months were excluded; data from 679 patients were included. Regression on-treatment analysis of lipid data (average of 3 and 6 months) and Cox fixed covariate analysis of MCE risk were performed with HDL-C or Apo A1 as the main exposure variables. Apo B and Apo A1 were adjusted in some models, HDL-C and Apo B in others. All models were adjusted for age, gender, and smoking but not for lifestyle factors or concomitant medications or conditions. **Results:** Apo A1 was associated with decreased MCE risk across the range >1.25 g/L (p for trend=0.03 unadjusted for HDL-C and Apo B, p for trend=0.05 adjusted). In contrast, HDL-C, after adjustment for apo B and Apo A1, had a non-linear relationship to MCE risk and was a significant risk factor for MCE (p=0.038). HDL-C >70 mg/dL was significantly associated with increased risk (RR for HDL-C 70-80mg/dL= 2.17, 95%CI 1.11, 4.23; RR for HDL-C>80mg/dL=2.46, 95%CI, 1.03, 5.88). **Conclusions:** Higher Apo A1 levels may be cardioprotective regardless of HDL-C, but very high HDL-C levels may not, unless Apo A1 are also higher.

Funding: Pfizer

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APOA-I MIMETIC PEPTIDE 5A-POPC HAS A DIFFERENTIAL EFFECT ON LIPOPROTEINS OF WILD TYPE, APOE K/O AND SR-BI K/O MICE

***Marcelo J.A. Amar**, Stephen Demosky, John Stonik, Alan Remaley. NIH, Bethesda, MD, USA.*

The 5A peptide is a bi-helical amphipathic peptide that mediates cholesterol efflux via the ABCA1 transporter and has reduced cytotoxicity compared to other apoA-I mimetic peptides (Curr Op Inv Drugs 2007,8(3):201). When associated with SM/DPPC, it decreased by ~50% atherosclerosis in ApoE K/O mice. To understand the metabolic effects of this peptide, as well as its mechanism of action, we infused 5A-POPC (30mg/kg, bolus) in wild type(WT), ApoE K/O and SR-BI K/O mice. Similar to 5A-SM/DPPC, 5A-POPC lead to an early rise of cholesterol levels and an effect that lasted for over 24h. Compared to baseline (mg/dL) (TC=103±6, TG=134±16, PL=220±11, FC=40±2), WT mice had significant changes at 1h (p<0.03) (TC=118±6, TG=462±44, PL=322±14, FC=64±2), and 24h after injection (TC=119±6, TG=117±9, PL=212±13, FC=37±2, CE=82±4). FPLC analysis showed an increase in HDL after injection, lasting over 24h. We next evaluated the effect of 5A-POPC in apoE K/O mice, a model of elevated apoB-Lp, in which over 80% of the cholesterol is on ApoB-Lp . No significant changes were observed in plasma lipids demonstrating the specific HDL related action of 5A. In more detailed analysis, FPLC revealed a reduction of 40% of LDL after 3h of infusion. Finally, after a bolus injection of 5A-POPC on SR-BI K/O mice, no significant changes were observed up to 72h supporting previously published observation that SR-BI is an important receptor for ApoA-I peptide mimetics. In summary, treatment with 5A-POPC led to an increase in HDL in WT mice, a decrease of LDL in apoE K/O mice and no changes in SR-BI K/O mice.

Funding: National Institutes of Health, MD

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ANTIOXIDANT EFFECTS OF AMPHIPATHIC K CLASS PEPTIDE MODELS OF APOLIPOPROTEIN A-I HELIX 6

*Polixeni T. Mantani, Maria P. Petraki, Konstantinos Harilogis, Andromaxi A. Dimitriou, Afroditi P. Tambaki, Maria Sakarellos-Daitsiotis, **Alexandros D. Tselepis**. University of Ioannina, Ioannina, Greece.*

Objective: Apolipoprotein A-I (apoA-I), the main protein component of high density lipoprotein (HDL), significantly contributes to the antiatherogenic activities of HDL. We synthesized amphipathic K class peptide models of helix 6, domain 147-159 of apoA-I and studied their capability to inhibit oxidation of low density lipoprotein (LDL). **Methods:** The following peptides were synthesized using the Fmoc-strategy: (1) Ac-R**M²**SDSAERAVDRL-NH_2 (2) Ac-R**A²**SDSAERAVDRL-NH_2. The effect of the above peptides on Cu^{2+}-induced LDL oxidation as well their possible influence on the antioxidant potency of the high-density lipoprotein (HDL) subfraction HDL3c were determined. **Results:** Both peptides significantly inhibit LDL oxidation in a dose-dependent manner, the peptide (2) being more potent inhibitor than peptide (1). HDL3c (50-100 μg protein/ml inhibits LDL oxidation since it induces a 30-60% prolongation of the lag time of LDL oxidation. Both peptides enhanced the antioxidant potency of HDL3c up to 2-fold at doses that do not influence LDL oxidation, the peptide (2) being more potent than peptide (1). **Conclusions:** Amphipathic K class peptide models of helix 6, domain 147-159 inhibit LDL oxidation and increase the antioxidant potency of HDL3c. Such peptide models may be useful for the development of potentially antiatherogenic agents based on apo A-I structure.

Funding: The research project is co-funded by the European Union-European Social Fund (ESF) and National Sources, in the framework of the program "Pythagoras II" of the Operational Program for Education and Initial Vocational Training" of the Community Support Framework of the Hellenic Ministry of Education

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A NOVEL DRUG RVX-208 RAISES PLASMA APOLIPOPROTEIN A-I AND HDL CHOLESTEROL

Ravi Jahagirdar, Chris D. Nicholls, Henrik C. Hansen, Sarah Attwell, Kevin G. McLure, Gregory S. Wagner, Jan Johansson, ***Norman C.W. Wong****. Resverlogix Corporation, Calgary, AB, Canada.*

Objective: To examine RVX-208, the lead compound from a new class of synthetic small molecules that raise ApoA-I production. **Methods:** RVX-208 treated HepG2 cells and human hepatocytes were assayed for ApoA-I promoter activity, mRNA abundance and protein secretion. Next, RVX-208 was given orally to human ApoA-I (hApoA-I) expressing transgenic (hTG) or C57/BL6 (WT) mice & analyzed for plasma ApoA-I protein, HDL abundance, 2D gel of αHDL, serum cholesterol efflux activity & toxicity. **Results:** In HepG2 cells, RVX-208 increased ApoA-I transcription, mRNA and protein by 5-, 5.2- & 2.3-fold, respectively. Similarly, RVX-208 increased human hepatocyte expression of ApoA-l mRNA and secreted protein by 2.2- & 1.7-fold, respectively. Next, RVX-208 (10-60 mg/kg b.i.d.) given orally yielded a dose-dependent increase of plasma ApoA-I in hTG and WT mice. At 30 mg/kg, ApoA-I increased by 120 and 70%, respectively above baseline. As expected, ApoA-I mRNA in hTG rose 80 and 70% above baseline in liver & small intestine, respectively and hepatic mRNA in WT mice rose by 50%. In sera of both mouse models, RVX-208 increased HDL, 2-D gels showed substantially higher αHDL & these sera elicited more cholesterol efflux from loaded macrophages vs control. Additional studies showed that RVX-208 induction of ApoA-l expression did not involve PPAR or LXR receptors. Toxicology studies in rats, mice & cynomolgus monkeys showed oral administration of RVX-208 to be safe at very high plasma exposures. **Conclusions:** RVX-208 increases ApoA-I production in HepG2 cells. In two mouse models, orally administered RVX-208 increases ApoA-I mRNA in liver & small intestine leading to higher plasma ApoA-I protein and HDL cholesterol.

Funding: Resverlogix Corp.

Workshop 2 "HDL AND REVERSE CHOLESTEROL TRANSPORT"
4:30 PM - 6:40 PM
Chairs: *G. Assmann, Muenster, Germany and D.J. Rader, Philadelphia, PA, USA.*

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UPDATE ON MOLECULAR REGULATION OF REVERSE CHOLESTEROL TRANSPORT

Daniel J. Rader*. University of Pennsylvania, Philadelpha, PA, USA.*

Reverse cholesterol transport (RCT) is believed to be a major mechanism by which HDL protects against atherosclerotic vascular disease. Proof of its direct relationship to atherosclerosis and insight into its molecular regulation have been hampered by the inability to directly quantitate the rate of RCT in vivo in animal models and humans. We developed an assay to directly assess the rate of RCT from macrophages to feces in vivo in mice. Using this assay, we demonstrated conclusively that apoA-I overexpression promotes macrophage RCT and apoA-I deficiency slows macrophage RCT. We also showed that hepatic SR-BI expression is a positive regulator of macrophage RCT in the opposite direction of its effects on plasma HDL-C levels, proving the concept that steady-state concentrations are not predictive of the rate of RCT. Introduction of CETP expression resulted in reduced HDL-C levels but increased macrophage RCT in wild-type mice but not LDL receptor knockout mice; CETP expression also restored to normal the rate of RCT in SR-BI knockout mice. An LXR agonist was shown to induce a marked increase in the rate of macrophage RCT in vivo. We adapted the method to mouse peritoneal and bone marrow macrophages and used this approach to show that macrophage ABCA1 and ABCG1, but not SR-BI, contribute quantitatively and additively to macrophage RCT in vivo. Additional data regarding genetic manipulations of genes such as LCAT and pharmacologic interventions such as PPARα agonists, niacin, and ezetimibe will be presented. In addition, the concept and potential of targeting macrophage RCT therapeutically in humans will be reviewed.

Funding: This work was supported by P01-HL22633 and P01-HL59407 from the National Heart, Lung, and Blood Institute, and an alternative

drug discovery initiative award to the University of Pennsylvania from GlaxoSmithKline

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REVERSE CHOLESTEROL TRANSPORT AS A PHARMACOLOGICAL TARGET

Franco Bernini. *University of Parma, Parma, Italy.*

Reverse cholesterol transport (RCT) is an antiatherosclerotic process that occurs through: 1) cholesterol efflux from cells, 2) cholesterol uptake by the liver, 3) cholesterol excretion into bile and 4) sterol elimination into the feces. Multiple genes/proteins are involved in this process, leading to the identification of several targets, whose pharmacological modulation may provide antiatherosclerotic, beneficial effects. Compounds that promote lipid efflux may result in substantial protective effect related to the reduction of cholesterol content of peripheral cells. This effect is gained by PPAR and LXR agonists that increase cellular lipid release through up-regulation of ABCA1 and contribute to the formation of nascent HDL. The LXR synthetic agonists T0901317 and GW3916 has shown to promote the in vivo RCT that specifically occurs from macrophages. We demonstrated that a possible mechanism related to this effect is the increased potential of sera to promote cholesterol efflux via passive diffusion and SR-BI-mediated mechanisms. Since HDL play an active role in several steps of RCT factors that increases their level could promote the whole process. Despite of torcetrapib failure inhibition of CETP is currently a promising approach. Modulation of hepatic lipid uptake may be reached by agents that stimulate SR-BI activity, as agonists of thyroid hormone receptors beta, whose administration in mice resulted in an improved fecal sterol excretion. The stimulation of the last step of RCT represents a novel approach, supported by the recent discovery that in vivo stimulation of LXR promotes the elimination of sterols into the feces through a biliary cholesterol independent pathway.

Funding: Grants from Istituto Nazionale di Ricerche Cardiovascolari and the Italian Ministry of University and Scientific Research

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ADVANCES IN THE MEASUREMENT OF CHOLESTEROL (C) EFFLUX AND REVERSE CHOLESTEROL TRANSPORT (RCT)

Marc K. Hellerstein. *UC, Berkeley, USA; UC, SF, CA, USA.*

The functional significance of RCT remains controversial, largely because fluxes have not been measurable in vivo. I will discuss results from a recently developed stable isotope method for measuring the 2 arms of RCT- efflux from tissues into blood and excretion from blood to fecal sterols. Schwartz' work provided the basis for a simple approach to measure efflux, by tracer dilution. Technically, the key validating observation was isotopic plateau in plasma free C after ~16 hr in humans, with a rapid turnover pool ~5-8 g. Efflux was 400-500 mg/hr in man, consistent with input from liver being largely removed (equilibrated) by the model, with variability among but not within (C-V 10%) subjects. Acceptor capacity was rate-limiting for efflux in rodents, as rHDL infusion caused an immediate and persistent increase. Efflux was altered by small molecules (e.g.,LXR agonists).The 2nd arm of RCT responded to several epatobiliary therapeutic targets in animals (C or bile acid re-absorption, LXR). Interestingly, fecal sterol balances were insensitive to measured changes in metabolic source (e.g., contribution from hepatic de novo synthesis vs plasma C). Brief infusions of rHDL increased efflux without altering excretion. In summary, 1) C efflux from tissues is routinely measurable *in vivo* in humans and is an order of magnitude greater than LDL delivery of C to tissues (suggesting a potent target); and 2) potential flux-generating steps in RCT are now testable (e.g, ABC-A1), as are drug effects (e.g. CETPi) and functional differences in HDLs (e.g, A1-Milano). The central questions concerning RCT can now be addressed: are RCT fluxes relevant to atherosclerosis (i.e., reflect macrophage events) and is efflux per se, without altering whole-body excretion, a therapeutic target?

Funding: CA BioStar; KineMed, Inc.

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EFFECT OF rHDL ON CHOLESTEROL EFFLUX *IN VIVO*

***S.M. Turner**, J.N. Voogt, S. Killion, J. Stark, C. Fessler, M.K. Hellerstein. KineMed, Emeryville, CA, USA.*

The first step in reverse cholesterol transport is efflux of free cholesterol from tissues, however plasma HDL cholesterol concentrations do not reflect efflux rate, so a direct, *in vivo* measurement is required. We have developed a method for quantifying the efflux rate of cholesterol from tissues into the rapidly exchanging cholesterol pool in blood and liver. Efflux of cholesterol is measured by the constant infusion isotope-dilution technique, through IV infusion of 13C-cholesterol. In rats, IV administration of human reconstituted HDL (rHDL) resulted in an immediate dilution in plasma free cholesterol (i.e., lower enrichment), representing an increase in efflux of unlabeled cholesterol into the bloodstream. A persistent, dose responsive increase in cholesterol efflux was observed over the 4 hours following a bolus of 22 or 45 mg apoA1. Subsequent studies demonstrated that prolonged continuous infusion of rHDL resulted in a persistent increase in cholesterol efflux. Pre-labeling by long infusions of 13C-cholesterol did not prevent the increase in efflux induced by rHDL administration. Results of these studies support the model of a rapid-turnover pool in plasma and liver that is in communication with a large store of extra-hepatic cholesterol. Moreover these findings demonstrate that cholesterol acceptor capacity (e.g., pre-beta HDL availability), not efflux transporter activity (e.g., ABCA1 content) is rate limiting for whole body cholesterol efflux in the rat. In humans, we have shown that cholesterol efflux is a highly dynamic process (~12-15 g/day/subject) relative to whole-body excretion (~1.0-1.5g/day). Application of this technique in both animal models and humans could provide new insights into the physiological and pharmacological factors regulating cholesterol efflux and RCT.

Funding: KineMed Inc.

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REVERSE CHOLESTEROL TRANSPORT (RCT) IN METABOLIC SYNDROME

***P. Nestel**, D. Sviridov, A. Hoang, E. Ooi, G. Watts. Baker Heart Research Institute, Melbourne, Australia; University Western Australia, Perth, Australia.*

Objective: Statins reduce progression of atherosclerosis possibly due to increased RCT from plaque macrophages facilitated by HDL. Effects of rosuvastatin on indices of RCT were studied. **Methods:** 25 men with metabolic syndrome in a randomised, placebo-controlled trial of 4 weeks' treatment with 40mg/d rosuvastatin. Measurements: plasma lipoproteins, HDL profile, plasma CETP, LCAT, and cholesterol efflux assayed using THP-1 macrophages. **Results:** Compared with placebo, rosuvastatin reduced levels of plasma cholesterol 44%, LDL cholesterol 60% and triglyceride 38%. HDL cholesterol (mean [SD]) rose (0.97 [0.17] to 1.05[0.17] mmol/L; $P<0.05$). HDL LpA-I increased ($P<0.05$) whereas LpA-I/A-II fell. LCAT activity fell (0.55[0.13] to 0.35[0.07] nmol/mL/h $P<0.05$); CETP activity fell from 89[13] to 80[11] nmol//L/h ($P<0.05$), indicating reduced cholesterol esterification and cholesteryl ester transfer. Cholesterol efflux *in vitro* fell from 7.1 [1.8]% (placebo) to 6.2 [1.7]% (rosuvastatin); $P<0.05$, but with plasmas depleted of apoB lipoproteins the difference in efflux was not significant. Efflux was paradoxically inversely correlated with HDL-C ($P=0.016$) and LpA-I ($P=0.035$) concentrations but was absent after rosuvastatin. **Conclusions:** Reduced capacity of plasmas following statin treatment to stimulate cholesterol efflux *in vitro* was partly due to reduction in apoB lipoproteins and reduced activities of CETP and LCAT. The paradoxical inverse correlation between HDL levels and cholesterol efflux *in vitro* in metabolic syndrome subjects suggests a novel example of HDL dysfunctionality in this syndrome. After rosuvastatin this abnormality was partially reversed possibly due to formation of larger HDL.

Funding: Partially funded by grant from AstraZeneca

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SUCCINOBUCOL (AGI-1067) PROMOTES REVERSE CHOLESTEROL TRANSPORT IN THE RAT THROUGH ENHANCED HEPATIC HDL-CHOLESTEROL ESTER UPTAKE

***Cynthia Sundell**, Scott Turner, Jason Voogt, Jayraz Luchoomun, Charles Kunsch, Mark Hellerstein, Robert Scott. AtheroGenics, Inc, Alpharetta, GA, USA; KineMed, Inc, Everyville, CA, USA.*

Succinobucol is an antioxidant, anti-inflammatory and lipid-modulating compound that has anti-atherosclerotic properties. To explore its mechanism of action we investigated its ability to promote reverse cholesterol transport (RCT) in normal rats using stable isotopes. Male Sprague Dawley rats were administered succinobucol (N=12) at a dose of 200 mkd or vehicle (N=13). After 14d, rats were given an IV infusion of ^{13}C cholesterol as well as an i.p. bolus of $^{2}H_{2}0$. Food intake and fecal output were monitored for the next 7d. Data from d14-18 were used to determine cholesterol efflux from peripheral tissues to the plasma as well as endogenous cholesterol excretion. After the final dose on d20, rats were fasted overnight and tissue samples collected on d21 for determination of de novo cholesterol synthesis (DNCS). Succinobucol decreased VLDL, LDL and HDL cholesterol by 75%, 48%, and 6%, respectively compared to controls. Cholesterol efflux was unchanged in the succinobucol group compared to controls. The excretion of endogenous cholesterol via neutral sterols and the bile acid pathway was significantly increased by 78% and 120%, respectively. Muscle DNCS, a complementary measurement of RCT, was also increased by 34%. In a separate study, co-treatment of human liver HepG2 cells with succinobucol (1–10 μM) and ^{3}[H]-cholesterol ester (CE) HDL (50 μg/ml) for 3.5 h, resulted in a dose-dependent increase in CE uptake. Taken together these data indicate that despite a reduction in HDL cholesterol levels, succinobucol promotes RCT in this model through a mechanism that likely involves enhanced hepatic HDL-CE uptake.

Funding: AtheroGenics, Inc.

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APOLIPOPROTEIN A-I BREAKDOWN IS INDUCED BY THROMBOLYSIS IN CORONARY PATIENTS

*Monica Gomaraschi, Ivano Eberini, Elisabetta Gianazza, Giuliana Mombelli, Silvio Klugman, Guido Franceschini, Cesare R. Sirtori, **Laura Calabresi**. University of Milano, Milano, Italy; Niguarda Hospital, Milano, Italy.*

Objective of the present work was to evaluate whether high density lipoprotein (HDL) proteins, quite sensitive to enzyme degradation, may undergo direct lysis following thrombolytic treatment. Sera from a total of 40 patients with acute myocardial infarction (AMI), unstable angina (UA) and dilative cardiomyopathy (controls) were investigated. AMI patients underwent either immediate PCI or were treated with prior tenecteplase thrombolysis. From AMI patients undergoing thrombolysis, blood was collected at three time intervals, ie before thrombolysis, and 4 and 12 h thereafter. Products of extensive proteolysis of apoA-I, the major protein component of HDL, were found in many acute coronary patients treated with tenecteplase. On the contrary, no apoA-I degradation was observed in controls and in UA patients, as well as in AMI patients undergoing immediate PCI. ApoA-I degradation by tenecteplase occurs at both the N- and C-termini, and was due to the selective degradation of the protein in small pre-beta-migrating HDL. ApoA-I proteolysis was observed *in vitro* after incubation of control serum with tenecteplase (0.01 mg/ml) at 37° for 2h. As observed *in vivo*, apoA-I proteolysis was associated with the disappearance of a specific HDL subpopulation, the small pre-beta-migrating particles. In conclusion, significant apoA-I fragmentation occurs in AMI patients after thrombolytic treatment. The degradation of apoA-I likely reduces HDL functionality, particularly the ability to promote cell cholesterol efflux, but can also affect the anti-inflammatory and antioxidant properties of HDL.

Funding: None

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EFFECT OF ROSUVASTATIN (RSV) ON REMODELLING OF HIGH DENSITY LIPOPROTEINS (HDL) IN ATHEROGENIC DYSLIPIDAEMIAS: ROLE OF CHOLESTERYL ESTER TRANSFER PROTEIN (CETP)

***John M. Chapman**, Muriel Caslake, Maryse Guerin, Paul Durrington, Mike K. Palmer, Fergus McTaggart, Chris J. Packard. INSERM, Paris, France; Royal Infirmary, Glasgow, United Kingdom; Royal Infirmary, Manchester, United Kingdom; Astra Zeneca, Cheshire, United Kingdom.*

Objective: To investigate the effect of RSV on HDL particle remodelling in hypercholesterolaemic patients with either normal or elevated triglyceride (TG) levels. **Methods**: Patients with either hypercholesterolaemia (HC, n=13) or mixed hyperlipidaemia (HL; TG≥2.0 mmol/l; n=15) were treated for 8 wk with R 40 mg. HDL subpopulations (LpAI, LpAIAII), particle size distributions and chemical compositions were determined. **Results**: RSV increased HDL-cholesterol (HDL-C) by about 10% in both groups. In HC, elevations occurred primarily in large LpAI particles (+29% from baseline; $p<0.05$) resembling HDL_2. In HL, by contrast, HDL-C elevation reflected a preferential increase in large LpAIAII particles (+22%; $p<0.05$). A marked increase in the ratio of CE to TG occurred in both HDL_2 and HDL_3 consistent with the RSV-induced fall in plasma CETP activity and mass. At 8 wks, VLDL-TG levels were lower by 28% ($p<0.05$) and 41% ($p<0.01$) compared to placebo in HC and HL groups respectively. **Conclusion**: RSV induced an increase of CE in HDL particles probably as a result of reductions in CETP activity and mass and in numbers of apo-B-containing particle acceptors, notably in TG-rich VLDL. These actions resulted in a preferential remodelling of HDL to larger LpAI particles in HC, but to LpAIAII particles in HL. VLDL-TG levels may be key determinants both of CETP action and of HDL particle remodelling induced by RSV in atherogenic dyslipidaemias.

Funding: AstraZeneca funded the study

Workshop 3 "LIPID METABOLISM"
2:00 PM - 3:55 PM
***Chairs:** R. Zechner, Graz, Austria and I. Goldberg, New York, NY, USA.*

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FATTY TISSUES: GETTING IT IN AND STORING IT UP

***Ira J. Goldberg**. Columbia, New York, NY, USA.*

The heart is the most energy-requiring organ in the body. Although the primary source of cardiac energy is fatty acids, hearts will increase their use of glucose during diabetes, with heart failure, or ischemia, and in the presence of abnormalities in fatty acid oxidation. Although in some of these situations, switching from fatty acid to glucose may be a compensation, it is also possible that abnormalities of cardiac lipid utilization are pathological. To test this, we created and studied mice with overexpression or tissue specific knockout of cardiomyocyte lipoprotein lipase (LPL). LPL deficient hearts compensated by increasing basal glucose uptake. Surprisingly, these mice developed cardiac dysfunction characterized by decreased fractional shortening, and interstitial and perivascular fibrosis. When stressed with angiotensin-induced hypertension, cardiac LPL knockout mice did more poorly than controls. Normal, but not LPL deficient, hearts had increased glucose uptake with hypertension. Excess lipid uptake and storage in the heart also leads to cardiac dysfunction. We have created two forms of cardiac dysfunction associated with excess lipid storage, lipotoxicity. Mice with transgenic expression of myocardial anchored LPL (LPL^{GPI}) develop dilated cardiomyopathy. This dysfunction is reduced, but not eliminated, by reduction of cardiac ceramide. Mice with transgenic overexpression of PPAR gamma in the heart also developed cardiac dysfunction with increased expression of fatty acid uptake and oxidation genes, and GLUT4. Thus high level of expression of PPAR gamma can lead to cardiomyopathy.

Funding: NIH

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LIPOLYSIS: EFFECTS ON LIPID- AND ENERGY-HOMEOSTASIS

***Rudolf Zechner**. University of Graz, Graz, Austria.*

Adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) are important triacylglycerol (TG) hydrolases that act coordinately with monoglyceride lipase (MGL) to catabolize stored lipids in adipose and non-adipose tissues. Orthologs of ATGL are known in essentially all genomes across eukarya including yeast, fruit flies, worms and vertebrates. HSL is less conserved and mostly found in vertebrates. Absence of ATGL or HSL often results in opposing phenotypes. For example, whereas ATGL deficiency in mice and humans causes the accumulation of excess fat in adipose and non-adipose tissues (particularly in cardiomyocytes), HSL deficiency is associated with decreased TG levels in adipose tissue, liver and muscle. This suggests that both enzymes, although active within the same biochemical pathway, differ in their specific physiological functions. We have analyzed cardiac- and skeletal muscle, adipose tissue, and liver tissues of induced mutant mouse lines that lacked ATGL or HSL to investigate downstream effects of lipase action on metabolic processes. Large-scale microarray analyses revealed altered expression profiles of numerous genes in response to ATGL/HSL deficiency. These genes are involved in metabolic pathways that affect glucose homeostasis, lipogenesis and fatty acid metabolism. Many of these adaptive metabolic processes to lipase deficiency can be reverted by the transgenic expression of the missing lipase. Our findings demonstrate that changes in the activity of metabolic lipases affects a plethora of cellular processes in muscle and adipose tissue with a variety of physiological consequences for tissue function.

Funding: None

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APOLIPOPROTEIN CIII INHIBITS INSULIN-DEPENDENT ENDOTHELIAL NITRIC OXIDE SYNTHASE FUNCTION IN ENDOTHELIAL CELLS THROUGH PROTEIN KINASE βII

***Akio Kawakami**, Frank M. Sacks, Shimokado Kentaro, Masayuki Yoshida. Tokyo Medical and Dental University, Tokyo, Japan; Harvard School of Public Health, Boston, MA, USA.*

Objective: Apolipoprotein (apo)CIII is elevated in patients with type2 diabetes and insulin resistance, and predicts cardiovascular disease. Endothelial dysfunction is associated with insulin resistance. The present study investigated the effect of apoCIII on insulin-dependent endothelial nitric oxide synthase (eNOS) function in vitro and in vivo. **Methods and Results:** ApoCIII (100μg/ml, 30min.) inhibited insulin-induced IRS-1/PI3-kinase/Akt activation in human umbilical vein endothelial cells (HUVECs). Furthermore, ApoCIII reduced insulin-stimulated eNOS activation and NO release into the media. ApoCIII activated PKCβII in HUVECs, resulting in IRS-1 phosphorylation at pSer616. Impaired insulin signaling was restored by PKCβ inhibitor. In addition, treatment of C57BL/6J mice with apoCIII resulted in impaired insulin-stimulated eNOS pathway in the aorta. PKCβ inhibitor attenuated inhibitory effects of apoCIII. Finally, injection of apoCIII-rich VLDL, but not apoCIII-deficient VLDL, impaired these processes. **Conclusion:** Our data suggest that apoCIII in VLDL impairs insulin-stimulated NO production by vascular endothelium. This adverse effect of apoCIII is mediated by of PKCβII which inhibits the IRS-1/PI3-kinase/Akt/eNOS pathway. These results indicate that apoCIII not only modulates lipoprotein metabolism, but also may directly contribute to the development of diabetic complications through endothelial dysfunction.

Funding: This study was supported by grants from Takeda Science Foundation and Ono Medical Research Foudation

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INFLUENCE OF SHORT-TERM ROSUVASTATIN THERAPY ON ENDOTHELIAL PROGENITOR CELLS AND ENDOTHELIAL FUNCTION

***Massimo R. Mannarino**, Matteo Pirro, Paolo F. Romagno, Francesco Bagaglia, Elmo Mannarino. Internal Medicine, Angiology and Arteriosclerosis Disease, Perugia, Italy.*

Endothelial progenitor cells (EPCs) are thought to maintain endothelium integrity by replacing injured mature endothelial cells. Cholesterol-lowering therapy may promote the mobilization of EPCs from bone marrow and improves endothelial function. Whether improvement of endothelial function may be attributed to an increased EPCs availability is unknown. The aim of our study was to investigate whether a relationship exists between EPCs and endothelial function variations after short-term intervention with rosuvastatin. Thirty hypercholesterolemic patients were assigned to 4-week rosuvastatin (10 mg daily) treatment. The number of EPCs, brachial artery flow-mediated vasodilatation (FMV), as an index of endothelial function, and lipid profile were measured before and after the 4-week intervention. At baseline we found a positive correlation between the number of EPCs and FMV ($r=0.48$, $p=0.027$). At the end of the 4-week intervention there was a 32% reduction in LDL cholesterol ($p<0.001$), that was paralleled by a 90% increase in the number of EPCs ($p=0.04$) and in brachial artery FMV (from 4.9±4% to 8.9±3%, $p=0.01$). In univariated analysis a positive correlation emerged between variation of circulating EPCs and change in FMV ($r=0.55$, $p=0.01$), this correlation being still present and significant after controlling for blood cholesterol reduction. No correlation was found between the reduction in colesterol and the increase in the number of EPCs.In conclusion, short-term rosuvastatin therapy improves endothelial function by increasing the number of circulating EPCs. This latter effects appears to be independent from reduction in cholesterol levels.

Funding: None

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THE UNDERLYING MECHANISM FOR DIACYGLYCEROL-MEDIATED AMELIORATION IN POSTPRANDIAL LIPIDS AND ENERGY HOMEOSTASIS

***Hidekatsu Yanai**, Hiroshi Yoshida, Yoshiharu Tomono, Yuji Hirowatari, Kumie Ito, Noriko Sato, Norio Tada. The Jikei University School of Medicine, Kashiwa, Japan; The Jikei University School of Medicine Kashiwa Hospital, Kashiwa, Japan; Tosoh Corporation, Ayase, Japan.*

Objective: Diacylglycerol (DAG) has been suggested to suppress postprandial hyperlipidemia and to prevent obesity by increasing postprandial energy expenditure due to different physicochemical dynamics in the small intestine from triacylglycerol (TAG). To understand how DAG affects postprandial lipid and energy metabolism, we measured postprandial serum lipids and plasma serotonin, which is mostly present in the small intestine and mediates peripheral sympathetic thermogenesis. **Methods:** The study was designed in a randomized crossover style. Seven male participants ingested DAG or TAG oil (30 g fat/m^2) with 40 gram of carbohydrate with a 2-week interval. Measurements of metabolic parameters were performed before and at 2, 4 and 6 h after fat ingestion. **Results:** The substitution of DAG for TAG decreased VLDL-cholesterol by 45.6% at 2 h, and decreased serum insulin by 41.3% at 4 h after ingestion. The area under the curve (AUC) for VLDL-cholesterol was positively correlated with the AUC for insulin. Concurrently, DAG elevated plasma serotonin levels by 47.3% at 2h, while TAG did not influence. **Conclusion:** The present study indicates that the substitution of DAG for TAG suppresses the postprandial increase in VLDL-cholesterol and insulin. This study also demonstrates that DAG ingestion increases plasma serotonin, proposing a possible mechanism for anti-obesity by DAG.

Funding: This study was supported by the Jikei University Research Fund

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FENOFIBRATE INCREASES HDL-CHOLESTEROL BY REDUCING CHOLESTERYL ESTER TRANSFER PROTEIN EXPRESSION

Willeke De Haan, Caroline C. Van der Hoogt, Marit Westerterp, Geesje M. Dallinga-Thie, Johannes A. Romijn, Hans M.G. Princen, Wouter Jukema, Louis M. Havekes, ***Patrick C.N. Rensen****. LUMC, Leiden, Netherlands; EUR, Rottterdam, Netherlands; TNO, Leiden, Netherlands.*

Objective: In addition to efficiently lowering VLDL-triglycerides (TG), fenofibrate increases HDL-cholesterol levels in humans. We investigated whether the fenofibrate-induced increase in HDL-cholesterol is dependent on the expression of the cholesteryl ester transfer protein (CETP). **Methods and Results:** *APOE*3-Leiden* (*E3L*) transgenic mice without and with the human CETP transgene, under control of its natural regulatory flanking regions, were fed a Western-type diet with or without fenofibrate. Fenofibrate (0.04% in the diet) decreased plasma TG in *E3L* and *E3L.CETP* mice (-59% and -60%; $P<0.001$), caused by a strong reduction in VLDL. Whereas fenofibrate did not affect HDL-cholesterol in *E3L* mice, fenofibrate dose-dependently increased HDL-cholesterol in *E3L.CETP* mice (up to +91%). Fenofibrate did not affect the turnover of HDL-CE, indicating that fenofibrate causes a higher steady-state HDL-cholesterol level without altering the HDL-cholesterol mass flux through plasma. Analysis of the hepatic gene expression profile showed that fenofibrate did not differentially affect the main players in HDL metabolism in *E3L.CETP* mice as compared to *E3L* mice. However, in *E3L.CETP* mice, fenofibrate reduced hepatic *CETP* mRNA (-72%; $P<0.01$) as well as the CE transfer activity in plasma (-73%; $P<0.01$). **Conclusions:** Fenofibrate increases HDL-cholesterol by reducing the CETP-dependent transfer of cholesterol from HDL to (V)LDL, as related to lower hepatic CETP expression and a reduced plasma (V)LDL pool.

Funding: Netherlands Heart Foundation (2003B136)

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LIPID-ALTERING EFFICACY AND FLUSHING PROFILE OF ER NIACIN/LAROPIPRANT IN PATIENTS WITH DYSLIPIDEMIA

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Niacin has proven lipid-modifying efficacy and cardiovascular benefit, but is underutilized due to flushing, primarily mediated by prostaglandin D2 (PGD2). Laropiprant (LRPT) is a PGD2 receptor antagonist that reduces extended release niacin (ERN)-induced flushing. We evaluated the lipid efficacy and flushing of ERN/LRPT (tablet containing 1g ERN and 20 mg LRPT) alone or added to ongoing statins. In this double-blind, 24-wk study, dyslipidemic patients (pts) (66% on statins), were randomized to ERN/LRPT 1g (n=800), ERN 1g (n=543), or placebo (P) (n=270). After 4 wks, active treatments were doubled to 2g (2 tablets) for 20 wks. Endpoints included effects of ERN/LRPT 2g vs. P on lipids/lipoproteins, and of ERN/LRPT vs. ERN on pt-reported flushing symptoms. Relative to P, ERN/LRPT produced significant LS mean changes in LDL-C (-18.4%), TG (-25.8%), non-HDL-C (-19.8%), Apo B (-18.8%), HDL-C (20.0%) and Apo A-I (6.9%). Similar results were observed regardless of treatment with statins. ERN/LRPT produced significantly less flushing than ERN during initiation (Wk 1) and maintenance therapy for all prespecified endpoints (incidence, intensity, bother, sleep difficulty, discontinuation). ERN pts reported more "moderate or greater" flushing than ERN/LRPT pts ($p<0.001$ across wks 2-24). ERN/LRPT was well tolerated. ERN/LRPT 2g, alone or with a statin, produced significant, durable improvements in multiple lipid/lipoprotein parameters. The improved tolerability of ERN/LRPT supports a simplified 1g→2g dosing regimen and should allow more pts to reach and maintain a 2g therapeutic dose of niacin, a therapy proven to reduce cardiovascular risk.

Funding: Merck

Workshop 4 "LIPID LOWERING THERAPY"
4:30 PM - 6:25 PM
Chairs: *E. Leitersdorf, Jerusalem, Israel and M.-R. Taskinen, Helsinki, Finland.*

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TREATMENT TO TARGET IN FAMILIAL HYPERCHOLESTEROLEMIA: IS IT A "MET NEED"?

Eran Leitersdorf*. Hadassah University Hospital, Jerusalem, Israel.*

Familial hypercholesterolemia (FH) has been a "human model" for the impact of lifelong hypercholesterolemia on cardiovascular health. Since the discovery of the low density lipoprotein (LDL) receptor, numerous mutations and the associated phenotypic characteristics were described. Inhibition of cholesterol synthesis and more recently cholesterol absorption resulted in marked favorable changes in the lipid profile with associated impact on morbidity. Regrettably, even under maximal therapy, a significant number of FH patients are not yet on target lipid levels as recommended by the NCEP ATP III guidelines. Several reasons may account for this observation including: 1. High baseline cholesterol levels associated with specific mutations, gene-gene interaction with other lipid modifying genes, and co-morbidities (Obesity, Metabolic Syndrome and Diabetes). 2. Reduced response to statins related to specific mutant alleles of the LDL receptor or other genes controlling cholesterol metabolism or drug metabolism. Recent studies revealed beneficial effects and even atheroma regression in high risk individuals especially in those with established CHD. These observations may soon lead to recommendation of even lower target LDL-C levels. For FH patients it would mean that the currently available pharmacotherapy will not be sufficient. It may therefore be concluded that FH is still an unmet need and there is a room for additional cholesterol lowering drugs including those which are currently in late development stages.

Funding: Sarah and Moshe Mayer Research Foundation

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PHARMACOKINETIC CONSIDERATIONS IN COMBINED LIPID LOWERING THERAPY

Alberto Corsini*. University of Milan, Milan, Italy.*

The strategy for designing a combined lipid-lowering therapy (LLT) should have a strong pharmacological rationale from a pharmacokinetic (PK) and a pharmacodynamic point of view. It is fundamental to underline that we are developing an *add on therapy* since we cannot avoid the use of statin. Drug transport, along with drug metabolism via cytochrome P450 (CYP) are considered as the main integral part of the overall PK profile of statins. Statins are substrates of efflux or uptake transporters, expressed in intestine, liver and kidney. All statins undergo extensive microsomal metabolism by CYP isoenzymes, with the exception of pravastatin, which is transformed enzymatically in the liver cytosol, and of rosuvastatin which is only partly metabolized by CYP2C9. The CYP3A4 isoenzyme is responsible for the metabolism of lovastatin, simvastatin, and atorvastatin. Fluvastatin is metabolized primarily by CYP2C9. UGT-mediated lactonization of open acid form of statins is a common enzymatic pathway leading to a rapid metabolism by cytochromes. This information on the PK profile of statins and the potential relevance of drug-drug interaction (DDI) must be considered for the development of new therapeutic entities. Since we do not have an experimental model validated to predict DDI, it is important to take advantage of the strategies utilized to study ezetimibe. Indeed, several clinical studies investigated ezetimibe together with drugs recognized by different metabolic pathways as well as with a variety of drugs utilized in CHD patients. Thus in vivo clinical study is, so far, the only predictable approach for investigating the potential DDI profile of new drugs. The use of combination therapy, however rationalized regarding efficacy, is not without counterbalancing risk (e.g. statins/gemfibrozil; atorvastatin/torcetrapib).

Funding: None

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LIPIDS, CREATININE, HOMOCYSTEINE AND CLINICAL OUTCOMES IN THE FENOFIBRATE INTERVENTION AND EVENT LOWERING IN DIABETES (FIELD) TRIAL

***James Best**, Anthony Keech. University of Melbourne, Melbourne, Australia; University of Sydney, Sydney, Australia.*

In the FIELD trial, fenofibrate reduced several macro- and micro-vascular outcomes in 9795 patients with type 2 diabetes, but not non-fatal MI/coronary death. Fenofibrate reduced LDL-C (12%), TG (29%) and raised HDL-C (5%). Increases of creatinine (12%) and homocysteine (35%) resolved within 8 weeks after fenofibrate cessation at study close, with no increase in renal adverse outcomes. We assessed baseline and on-study lipids, creatinine and homocysteine as independent risk factors for CVD events. Treatment effect was assessed after adjustment for 1-year on-study biomarkers in a landmark analysis, and by changes in biomarkers during a 6-week active run-in pre-randomization. Landmark analysis suggested that changes in lipids could explain most of the effects of fenofibrate. In contrast, active run-in analyses showed that lipid changes with short-term treatment did not significantly alter treatment benefit. After adjustment in landmark analysis for on-study creatinine and homocysteine levels, the estimated effect of fenofibrate was increased, suggesting that increases in these parameters attenuated the effect on CVD events. However, active run-in analyses showed that creatinine rise with short-term fenofibrate was a risk marker for CVD, but did not attenuate treatment. Further analyses of homocysteine after active run-in will explore the possibility that treatment-induced changes in homocysteine may predict both risk of CVD events and benefit from fenofibrate therapy. At present, there is no basis for excluding (or favouring) patients for fenofibrate therapy based on levels of biomarkers or changes in biomarkers with initial treatment.

Funding: Supported by Laboratoires Fournier SA, Dijon, France

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APOLIPOPROTEIN B AND NON–HIGH-DENSITY LIPOPROTEIN CHOLESTEROL ARE TIGHTLY CORRELATED DURING STATIN THERAPY

***Christie M. Ballantyne**, Joel S. Raichlen, Valerie A. Cain. Baylor College of Medicine, Houston, TX, USA; AstraZeneca LP, Wilmington, DE, USA.*

Objective: Hypercholesterolemic patients may reduce LDL-C to a predetermined goal yet have excess atherogenic lipoproteins. Apolipoprotein B (apo B) provides a measure of atherogenic lipoproteins and may be a superior predictor of CHD events. An apo B target <90 mg/dL has been proposed as an alternative to non–HDL-C <130 mg/dL, particularly for hypertriglyceridemic patients. Correlation of apo B to non–HDL-C during therapy with various statins was examined. **Methods:** MERCURY II enrolled patients with LDL-C 130 to 250 mg/dL, TG <400 mg/dL, and high risk for CHD. Apo B and lipids were measured at baseline and after therapy with rosuvastatin 10 mg (n=374) or 20 mg (n=742), atorvastatin 10 mg (n=185) or 20 mg (n=186), or simvastatin 20 mg (n=190) or 40 mg (n=191), given wk 8 to 16. The relation of apo B to non–HDL-C was analyzed by linear regression. **Results:** Baseline data showed good correlation of apo B and non–HDL-C (r=0.88 to 0.90 among the 6 arms); apo B <90 mg/dL correlated with non–HDL-C <130 mg/dL. Regression lines on statin therapy were steeper and correlations between apo B and non–HDL-C increased (r=0.94 to 0.96). Although lipid responses among statin groups varied widely (-29% to -49% for non–HDL-C), regression lines among groups were statistically indistinguishable. Data showed that reaching apo B <90 mg/dL required reducing non–HDL-C to <100 mg/dL on statin therapy. **Conclusions:** Non–HDL-C may be a reasonable surrogate for apo B across a wide range of lipid responses to statin therapy. To reach apo B <90 mg/dL requires reducing non–HDL-C to <100 mg/dL, the optional target for very high risk patients.

Funding: This study and analysis were funded by AstraZeneca LP

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ATORVASTATIN SAFETY IN PATIENTS 75 YEARS AND OLDER

***Judith Hey-Hadavi**, Rachel Laskey, Andrei Breazna. Pfizer Inc., New York, NY, USA.*

Objective: Although older patients benefit significantly from statin treatment, they may be less likely to receive treatment due to safety concerns. This pooled analysis evaluated atorvastatin safety in patients ≥75 years. **Methods:** Patients aged ≥75 receiving placebo or atorvastatin (10-80 mg dose range) were identified from 45 randomized completed atorvastatin trials (1994-2005) in the analysis of treatment-associated adverse events (AEs), serious AEs and enzyme elevations, and from 54 trials (1992-2005) in the analysis of albuminuria, hematuria, and myalgia. **Results:** Pooling identified 3145 patients ≥75 years (placebo=834, atorvastatin 10mg=531, 20mg=125, 40mg=216, 80mg=1439). The number of patients with AEs was similar in all groups. The most frequently reported treatment-associated AEs were related to the digestive system (4-15% in atorvastatin groups; 19%, placebo). Treatment-associated AEs seldom led to withdrawal in any group. Persistent elevation of liver function tests (>3xULN) was seen in 2 (0.2%), 2 (0.4%), 0, 0, and 14 patients (1.0%) in the placebo, atorvastatin 10mg, 20mg, 40mg, and 80mg groups, respectively. Persistent CPK elevations (>10xULN) were not seen in any group. Among 2128 patients available for analysis of muscle and liver-related AEs (placebo=419, atorvastatin 10mg=433, 20mg=119, 40mg=154, 80mg=1003), the incidence of treatment-associated myalgia was low (<2.5%), no cases of rhabdomyolysis were reported, and treatment-related cases of albuminuria and hematuria were rare. **Conclusions:** The incidence of AEs in atorvastatin-treated patients ≥75 did not increase with dose and was similar to that seen with placebo. These results support the favorable safety profile of atorvastatin across the dose range and should be considered when managing cardiovascular risk, including stroke, in patients ≥75 years.

Funding: Pfizer Inc.

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ONCE WEEKLY DOSE OF ROSUVASTATIN FOR PATIENTS WITH PRIOR STATIN INTOLERANCE

***Patrick M. Moriarty**, Cheryl A. Gibson, Jim Backes. University of Kansas Medical Center, Kansas City, KS, USA.*

Background: Adverse effects (AE) from statin use, though small (3%-4%), may prevent patients from adequately reducing their cholesterol levels and lowering their risk of cardiovascular disease (CVD). Rosuvastatin has the highest bioavailability of any statin and a plasma 1/2 life of 20 hours. It has also been shown that 1 milligram of rosuvastatin can reduce LDL-C by 34%. We examined the feasibility of prescribing statin therapy once a week to patients previously intolerant to once daily dosing. **Methods:** We conducted a prospective trial involving 12 patients (9 female, average age 62 +/- 11 years and 50% with CVD) previously resistant to statin therapy (myalgias 75%, GI upset 8%, elevated liver enzymes 17%). After a 4 week washout period patients initiated once weekly rosuvastatin 2.5mg, 5mg, 10mg, or 20mg (mean dose 10mg) depending on patient characteristics. During this time period no other lipid lowering medication was added to the patient's drug regimen. **Results:** Following 4.25 (+/- 1.5) months of therapy results indicated a 30% reduction of LDL-C, 155 mg/dL vs 108 mg/dL ($p<0.004$) and a 26% reduction of total cholesterol, 232 mg/dL vs 177 mg/dL ($p<0.018$) without any significant intolerance to therapy. Both triglycerides and HDL-C were not significantly altered from baseline. **Conclusion:** When patients require marked LDL-C reduction present with a prior statin intolerance, it often poses a major challenge for the practitioner. Once a week dosing of statin (rosuvastatin) therapy may be an effective alternative for patients intolerant to standard daily therapy.

Funding: None

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EFFICACY AND SAFETY OF THE CETP INHIBITOR MK-0859 IN DYSLIPIDEMIC PATIENTS

***D. Bloomfield**, G. Carlson, A. Sapre, D. Tribble, J.M. McKenney, T.W. Littlejohn, III, C.M. Sisk, Y.B. Mitchel. Merck, Rahway, NJ, USA; Isis Pharma, Research, Carlsbad, CA, USA; VCU & NCRI, Research, Richmond, VA, USA.*

We evaluated the lipid-altering efficacy and safety of the CETP inhibitor MK-0859 as monotherapy or coadministered with atorvastatin (ATV) 20 mg in 589patients (pts) with primary hypercholesterolemia or mixed hyperlipidemia (30% with low HDL-C: 44 mg/dL in men and 54 mg/dL in women). Pts were randomized equally to placebo (P), ATV 20 mg, or MK-0859 10 mg, 40 mg, 150 mg, or 300 mg doses alone or coadministered with ATV 20 mg daily for 8 wks. An equal proportion of pts had TG >150 mg/dL in each group. At each visit, replicate blood pressure (BP) measurements were obtained with an automated cuff. LS mean % changes from baseline in LDL-C, the primary endpoint, and other lipid/lipoprotein parameters were evaluated for monotherapy doses vs. P and coadministration doses vs. ATV 20 mg. For MK-0859 monotherapy (P, 10 mg, 40 mg, 150 mg and 300 mg), changes from baseline to Wk 8 were 2%, -16%, -27%, -40% and -39%, respectively, for LDL-C and 4%, 44%, 86%, 139% and 133%, respectively, for HDL-C ($p<0.001$ vs. P for all doses). Coadministration of MK-0859 with ATV produced significant incremental LDL-C reductions and similar HDL-C increases vs. monotherapy. Significant dose-dependent decreases in apo B and increases in apo A-I were observed with MK-0859. LDL-C reductions were similar in patients with baseline TG levels > and the median. The incidence of AEs was similar for P and all active treatment groups. Importantly, MK-0859 was not associated with a mean increase in BP in any treatment arm. MK-0859, alone or with ATV 20 mg, produced favorable, significant, dose-dependent changes in LDL-C and HDL-C. MK-0859 was well-tolerated with no effect on BP.

Funding: Merck

Workshop 5 "DIABETES AND CVD"
2:00 PM - 4:00 PM
***Chairs:** R. Lauro, Rome, Italy and A. Chait, Seattle, WA, USA.*

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WHY IS ATHEROSCLEROSIS AND DIABETES A FATAL COMBINATION?

***Marja-Riitta Taskinen**. University of Helsinki, Helsinki, Finland.*

Type 2 diabetes is the major threat to the public healthy in the 21st century as a risk factor for cardiovascular disease (CVD). Type 2 diabetes and the clustering of cardiometabolic risk factors are driven by epidemics of obesity worldwide. Once a person has developed vascular complications the prognosis is poor and it is therefore important to prevent the first myocardial infarction (MI) in people with diabetes. The atherosclerotic disease often remains asymptomatic until a high-risk plaque ruptures and stroke or myocardial infarction ensures. Proinflammatory/prothrombotic state is a seminal component of cardiometabolic risk factor cluster and highly frequent in Type 2 diabetes. Atherosclerotic plaques in diabetic subjects show characteristics associated with plaques instability and rupture. Advanced glycation endproducts (AGE) have adverse effects on diabetic vasculature when expression of receptors for AGEs (RAGE) are increased. In the setting of hyperglycemia and excess FFA the formation of reactive oxygen species (ROS) is enhanced. Vascular and coronary circulatory dysfunction are seen across the spectrum of insulin resistance with increasing severity in Type 2 diabetes. Atherosclerosis and diabetes is a particularly fatal combination due to the coexistence of coronary artery disease, heart failure (HF) and hypertension. This cluster in people with diabetes has been nominated as a cardiotoxic triad. Diabetes increases the risk of HF but about 80 % and adversely affects the prognosis of people with HF. Each 1% increase in HbA_{1c} is associated with an 8% increased risk of HF. Maladaptive fuel shifts of cardiac energy metabolism leads to cardiac dysfunction. Importantly PPARα is an essential component in cardiac substrate switching. A multifactorial treatment strategy should be used to achieve optimal risk reduction in people with T2DM.

Funding: None

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THE IMPACT OF OBESITY AND DIABETES ON MACROPHAGE APOPTOSIS IN ADVANCED ATHEROSCLEROSIS

***Ira Tabas**, Tracie Seimon, Jerry Arellano, Dongying Cui, Yankun Li, Wahseng Lim, Seongah Han, Chien-Ping Liang, Alan Tall, Domenico Accili. Columbia University, New York, NY, USA.*

Macrophage death in advanced atherosclerosis causes plaque necrosis, which promotes plaque disruption and acute atherothrombotic vascular events. Of interest, plaque necrosis and atherothrombotic disease are markedly increased in diabetes and metabolic syndrome. We discovered a "multi-hit" macrophage apoptosis pathway that appears to be highly relevant to advanced atherosclerosis. The hits include **(1)** a pro-apoptotic branch of the endoplasmic reticulum stress pathway known as the Unfolded Protein Response (UPR), which is mediated by the UPR effector CHOP; **(2)** a pathway involving the MAP kinase JNK, which is activated by combinatorial pattern recognition receptor signaling involving the type A scavenger receptor (SRA) and toll-like receptor 4; and **(3)** a pathway involving cytosolic calcium, calcium/calmodulin-dependent protein kinase II (CaMKII), and STAT1. Macrophages with defective insulin signaling show enhanced components of this pathway and increased susceptibility to apoptosis when exposed to UPR activators and SRA/TLR4 ligands. Moreover, the advanced lesions of atherosclerosis-prone mice reconstituted with insulin-resistant macrophages show increased macrophage apoptosis and plaque necrosis. Finally, a number of adipocytokines that are altered in obesity have profound effects on this multi-hit pathway of macrophage apoptosis. Based on these findings, we propose that one mechanism of increased plaque necrosis and atherothrombotic vascular disease in insulin resistant syndromes is amplification of a multi-hit signal transduction pathway involved in advanced lesional macrophage death.

Funding: NIH grants HL79801, HL75662, and HL57560

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DO MARKERS OF PRECLINICAL ATHEROSCLEROSIS INFLUENCE GLOBAL CARDIOVASCULAR RISK?

***Salvatore Novo**, Francesca Bonura, Egle Corrado, Ida Muratori, Salvatore Montalto, Giuseppina Novo. University Hospital P. Giaccone, Palermo, Italy.*

Several reports suggest the importance of preclinical ATS in increasing CV risk beyond that deriving from the charts. The most recognised markers of preclinical ATS are: endothelial dysfunction (ED), carotid intima-media thickening (IMT) and reduced ankle-brachial index (ABI). We can study ED by the evaluation of flow mediated vasodilation (FMD) after an ischemic stimulus. FMD progressively reduces increasing the number of risk factors (RF); it is a marker of coronary ATS and independent predictor of events. Patients with IMT have an increase of CV events in the follow up, more important of that deriving from the risk score evaluation, as shown in the ARIC, CHS, Rotterdam, Finnish Study and in own experience. IMT is increased in patients with a cluster of RF and in particular DM, IGT, cholesterol, hypertension and smoking. Moreover, IMT improve the ability of stress testing in predicting coronary ATS. A recent meta-analysis revealed that IMT is a strong predictor of vascular events with an adjusted risk of 1.26 for AMI and 1.32 for stroke. An ABI < 0.90 is an indicator of multifocal ATS and of CV events as demonstrated in the PARTNERS, Edinburgh, CAPRIE and other studies. So, today it is possible to detect precociously preclinical ATS in subjects with a cluster of traditional RF. These subjects need to be investigated for CAD with one non-invasive test (stress test, echo stress or SPECT scintigraphy) or with multidetector CT of coronary bed. Although guidelines don't recommend a target of LDL-C for those patients, it is reasonable to consider a target < 100 mg%. Moreover, it is very important to plan studies to evaluate the effects of statins and of different levels of LDL-C for these patients.

Funding: None

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CONFIGURATION OF A CELL BASED ASSAY FOR HUMAN GPR120, A RELEVANT TARGET FOR DIABETES, USING THE Ca^{2+} ACTIVATED PHOTOPROTEIN PHOTINA®

***Maria Grazia Giribaldi**, Anna Della Bella, Lia Scarabottolo. Axxam, Milano, Italy.*

Objective: GPR120 is a G-protein-coupled receptor, Gq-coupled, long-chain free fatty acids activated. GPR120 plays a critical role in physiological glucose homeostasis. In particular, GPR120 represents an important therapeutic target for diabetes as its stimulation promotes glucagon-like peptide-1 secretion with consequent circulating insulin increment. In this study, we developed a functional, high sensitive, cell based assay for the human GPR120 using the new and improved Ca^{2+} activated photoprotein, Photina®. This assay is particularly suitable for high-throughput screening (HTS) campaigns for the identification of GPR120 modulators. **Methods:** CHOmitoPhotina® cell line, previously generated and characterized, was stably transfected with the human GPR120 cDNA. The best functional clones were identified by measuring flash luminescence emitted by the photoprotein Photina® upon α-linolenic acid stimulation. The assay was developed in the 384 multiple well plate (384 MTP) format. **Results:** A GPR120 CHOmitoPhotina® pure clone was obtained after sequential limiting dilutions and it was pharmacological characterized by functional assay using α-linolenic acid as ligand. Receptor stimulation leads to very strong and dose dependent luminescence kinetics, showing α-linolenic EC_{50} in the micromolar range with a signal to background up to 150 fold. A fully automatic protocol was optimized for our screening station, in 384 MTPs. **Conclusions:** The GPR120 CHOmitoPhotina® clone obtained demonstrated high sensitivity, robustness and signal stability over time. This cell line represents a reliable functional cell based assay for studying intracellular Ca^{2+} mobilization mediated by GPR120 activation and for HTS of compound libraries.

Funding: None

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THE EFFECT OF A LOW GLYCEMIC INDEX DIET ON GLUCOSE CONTROL AND MARKERS OF CARDIOVASCULAR DISEASE RISK IN TYPE 2 DIABETES

***Cyril W.C. Kendall**, Andrea R. Josse, Monica Banach, Sophie Ares, Sandra Mitchell, David J.A. Jenkins. St. Michael's Hospital, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada.*

Objective: To determine the effect of a low glycemic index (GI) diet on markers of long-term glycemic control and coronary heart disease (CHD) risk in subjects with type 2 diabetes taking oral hypoglycemic agents. **Methods:** Approximately 200 non-obese subjects with type 2 diabetes and baseline HbA1c between 6.5-8%, will be randomized to a 6 month study of parallel design. Subjects will be instructed to follow either a high cereal fibre-normal GI diet (mean GI=80; control) or a low GI diet (mean GI<70; test) with a comparable fibre content to the control. Fasting blood samples will be taken monthly to measure HbA1c, glucose, insulin and blood lipids. Anthropometrics, blood pressure and 7-day food records will also be assessed at each visit. **Results:** The average dietary GIs for the first 18 subjects to complete this study were: 66 in the low GI group and 77 in the high cereal fibre group. Preliminary data also show significant reductions in HbA1c (0.004 %, P=0.04); systolic (10 mmHg, P=0.01) and diastolic blood pressure (2 mmHg, P=0.04); and a significant increase in HDL-cholesterol (1.05±0.06 mmol/L, P=0.03) from 0 to 24 weeks in the low GI group. There were no significant changes observed in the high cereal fibre group. **Conclusions:** In subjects with type 2 diabetes on oral hypoglycemic agents, preliminary data suggest that a low GI diet may be beneficial for long-term glycemic control and may also improve other CHD risk factors. Final data will be presented.

Funding: The Canadian Institutes of Health Research, Ottawa, Canada; The Barilla Group, Parma, Italy

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ADIPONECTIN MODULATES THE EFFECT OF ATORVASTATIN ON PLASMA HDL CHOLESTEROL IN PATIENTS WITH TYPE 2 DIABETES

*Mandy van Hoek, **Geesje M. Dallinga-Thie**, Hans Jansen, Eric J.G. Sijbrands, Arie van Tol. ErasmusMC, Rotterdam, Netherlands; AMC, Amsterdam, Netherlands.*

Adiponectin plays an important role in lipid homeostasis and affects insulin sensitivity. We studied the effects of atorvastatin on plasma lipoproteins and adiponectin levels in patients with type 2 diabetes. In the DALI study, a randomized placebo-controlled study on the effects of aggressive atorvastatin treatment in patients with type 2 diabetes, plasma adiponectin levels, lipoproteins, as well as lipoprotein lipase (LPL) and hepatic lipase (HL) activities were assessed at baseline and after 6 months of treatment with placebo, 10mg (A10) or 80 mg atorvastatin (A80). At baseline, positive relationships were found between adiponectin and LPL activity (r=0.19 p=0.012) and HDL cholesterol (r=0.46, P<0.001). Negative relationships were present between adiponectin and HL activity (r=-0.17 p=0.022) and triglycerides (r=-0.52, P<0.001). Atorvastatin treatment had no effect on adiponectin levels. However, adiponectin levels at baseline significantly interacted with the effect of atorvastatin treatment on HDL-cholesterol (p=0.007), i.e. patients with the highest baseline plasma adiponectin concentration (tertile 3) displayed the largest increase in plasma HDL cholesterol during treatment (10%), while the increase in the lowest tertile group was negligible (1%). Adjustment for HL and LPL did not change the results. We conclude that plasma adiponectin is related to LPL (positive) and HL activity (negative). Furthermore, high adiponectin levels increase the HDLcholesterol response to atorvastatin treatment.

Funding: None

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THE METABOLIC SYNDROME DOES NOT ADD TO CAROTID ATHEROSCLEROSIS BEYOND THAT EXPECTED BY RISK FACTOR COUNTING OR RISK SCORING

***José P. Werba**, Samuela Castelnuovo, Beatrice Frigerio, Mauro Amato, Alessio Ravani, Elena Tremoli, Damiano Baldassarre. IRCCS, Milan, Italy; Milan University, Milan, Italy.*

Objective: The aim of this study was to assess if the Metabolic Syndrome (MS) has any add-on effect on subclinical atherosclerosis beyond that expected by risk factors (RF) counting or risk scoring. **Methods**: Intima-media thickness (IMT) of carotid arteries was assessed by using B-mode ultrasound in 1805 patients (56+/-13 y; 52% women) attending a cardiovascular prevention program. Patients with (cases) or without (controls) MS according to NCEP ATP III criteria were 1:1 matched for sex, age and either the number of conventional RF (Analysis 1) or the Framingham risk score (Analysis 2). For Analysis 1 not more than 2 components of the MS were accepted as RF in the control group. **Results**: Case:control matches were 211 for Analysis 1 and 244 for Analysis 2. No significant differences in carotid IMTmean and carotid IMTmax were found between cases and controls in both analyses (Analysis 1: IMTmean 1.03+/-0.38 vs 1.07+/-0.37; IMTmax 1.90+/-0.96 vs 1.95+/-0.90. cases and controls, respectively; Analysis 2: IMTmean 1.03+/-0.36 vs 1.01+/-0.33; IMTmax 1.91+/-0.94 vs 1.83+/-0.81, cases and controls, respectively; all p>0.1). **Conclusions**: According to our results the metabolic syndrome does not add to the extent of carotid subclinical atherosclerosis beyond that expected by RF counting or risk scoring. These findings do not support any particular harmful synergism between components of the metabolic syndrome in determining carotid atherosclerosis.

Funding: None

Workshop 6 "GENETICS AND CVD"
4:30 PM - 6:40 PM
Chairs: *A.J. Lusis, Los Angeles, CA, USA and A. Tall, New York, NY, USA.*

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NUTRITION AND GENETICS: PARTNERS IN CARDIOVASCULAR HEALTH AND DISEASE

Jose M. Ordovas*. JM-USDA-HNRCA at Tufts University, Boston, MA, USA.*

The concept of personalized nutrition is appealing. However, despite its appearance in the medical literature 35 years ago, this notion remained in hibernation for the ensuing 25 years. It was not until the turn of the Century that genetics began to percolate into nutrition research triggering a deluge of publications that have provided both the proof-of-concept to support genetic basis for the individuality of nutritional needs, but also set high expectations among health professionals and the public. The current evidence shows that variants at candidate genes for lipid metabolism (i.e., LIPC, APOE, APOA5) and inflammation (i.e., IL1, CRP) are associated with plasma levels of classical and new biomarkers of cardiovascular disease risk. Moreover, the data support significant interactions between these genes and the responses of these biomarkers to both dietary interventions and habitual dietary practices. However, in order to make good use of the increasing amount of data being generated, this information has to be balanced with reliable and comprehensive phenotypic information gathered over time in very large numbers of subjects. Moreover, the evidence needs to be supported by properly designed intervention studies. Therefore, whereas it is accepted that our responses to the environment (i.e., diet) are largely determined by our genetic make up, our knowledge is not enough to successfully implement the use of genetics to personalize dietary advice for the prevention of cardiovascular diseases. However, personalized nutrition is a valid concept and it is important to keep in mind that "the future belongs to those who prepare for it today".

Funding: Supported by contract 58-1950-9-001 from the US Department of Agriculture Research Service and by NIH grants U 01 HL72524, HL 54776 and DK075030

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INTEGRATIVE GENETIC APPROACH TO UNDERSTANDING COMPLEX GENETIC AND ENVIRONMENTAL INTERACTIONS IN ATHEROSCLEROSIS

Aldons J. Lusis*. UCLA, Los Angeles, CA, USA.*

Human genome wide association studies will likely identify the most prominent genetic factors contributing to cardiovascular disease susceptibility over the next few years. But such studies will not have sufficient power to address complex gene-gene and gene-environment interactions. Traditional studies in mice, such as transgenic studies, emphasize single gene effects rather than complex interactions. We are using an "integrative genetics" approach, combining genetics and expression array analyses to help bridge the gap between the DNA variation and complex clinical traits. A key aspect of our approach is the use of multiple, common, naturally occurring genetic variations affecting gene expression between inbred strains of mice or cells isolated from different human donors. We have used these variations to model reference gene networks for tissues or cells under normal conditions. We feel that understanding molecular interactions in the complex states in which they operate, will provide a superior context in which to interpret associations between genes and diseases.

Funding: NIH HL30568, HL28481

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ABSTRACT WITHDRAWN

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A FREQUENT POLYMORPHISM IN THE LOW-DENSITY LIPOPROTEIN RECEPTOR GENE IS THE CAUSE OF FAMILIAL HYPERCHOLESTEROLEMIA (FH)

***Joep C. Defesche**, Ellen J. Schuurman, Lisette N. Klaaijsen, Kah-Lin Khoo, Albert Wiegman. Academic Medical Center at the University of Amsterdam, Amsterdam, Netherlands; Cardiology Clinic, Kuala Lumpur, Malaysia.*

Objective: To assess the effect of SNPs in the LDL-receptor (*LDLR)* and apolipoprotein B (*APOB)* genes on LDL-receptor function. **Methods:** *LDLR* and parts of *APOB* of 6705 patients with the clinical diagnosis of FH were sequenced. RNA was isolated and cDNA was prepared. Neutral DNA variants were analyzed for their effect on splice sites and branch points. **Results:** In total 136 different SNPs were identified. Two SNPs were found to interfere with normal RNA splicing: **G186G** (621C→T in exon 4; 88 heteroz., 2 homoz.) created an additional 3'-splice donor site and **R385R** (1216C→A in exon 9; 9 heteroz.) created an additional 5'-splice acceptor site. Probability: 88.9 vs 75.9% for G186G and 93.3 vs 85.3% for R385R (SpliceSiteFinder). G186G resulted in an in-frame deletion of 75 bp and a LDL-receptor protein lacking the last 25 amino acids 3' in exon 9. R385R caused a frame shift-deletion of 31 bp resulting in a premature stop codon after M391 and probably in an unstable mRNA. All patients with G186G were of Dutch origin. Of the families with R385R, one was of Chinese descent but living in The Netherlands, and one was from Malaysia. R385R has recently been published (Bourbon et al, Atherosclerosis 2007,doi 10.1016/j. atherosclerosis. 2007.01.034). **Conclusion:** The finding of G186G and R385R demonstrated that seemingly neutral DNA variants may result in severe pathogenic mutations affecting LDLR-function. All patients with G186G and R385R had a classical FH phenotype with severely elevated LDL-cholesterol levels and in many cases tendon xanthomas and premature cardiovascular disease.

Funding: None

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LPLS447X IS ASSOCIATED WITH ATTENUATED ALBUMINURIA RELATED CARDIOVASCULAR RISK IN MALES

***Melchior C. Nierman**, Mike Zuurman, Barbara A. Hutten, Hans L. Hillege, Paul E. de Jong, Gerjan Navis, Jan Albert Kuivenhoven, John J.P. Kastelein, Erik S.G. Stroes, Wiek van Gilst. Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; University Medical Center Groningen, University of Groningen, Groningen, Netherlands.*

Objectives: Higher albumin excretion rates (≥15mg/24hr) are associated with increased CVD risk and a pro-atherogenic lipid profile (i.e. high TG and low HDL-C). Since LPLS447X is associated with exactly opposite efffects, we evaluated its effects on albuminuria related CVD risk. **Methods:** We used data from the PREVEND (Prevention of REnal and Vascular ENdstage Disease) study (n=8592), a prosp. population-based cohort study. Subjects with baseline CVD or macroalbuminuria were excluded. Remaining subjects were categorized according to their albuminuric status (I: 0-15, II: 15-300 mg/24hr; n=5427 and n=2071, respectively). Primary endpoint was a major cardiovascular event. Multivariate Cox regression analysis was used to calculate hazard ratios (HR). **Results:** LPLS447X was associated with decreased TG and elevated HDL-C. After correction for age, smoking, blood pressure, lipids and the use of antihypertensive and lipid lowering drugs, LPLS447X was strongly associated with reduced CVD risk in males ($HR_{II\ vs.\ I}$: 0.44 [95% CI: 0.21- 0.92], p=0.029), but not in females ($HR_{II\ vs.\ I}$: 0.80 [95% CI: 0.24 - 2.73], p=0.72). **Conclusion:** This study confirms that LPLS447X is associated with an anti-athogenic lipid profile. In addition, LPLS447X was able to attenuate albuminuria associated CVD risk in males. Clearly, this frequent LPL variant can truly be classified as a 'gain of function' mutation.

Funding: None

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GENETIC VARIATION IN THE ANGIOPOIETIN-LIKE 3 GENE IN HYPERTRIGLYCERIDEMIC PATIENTS

***Sigrid W. Fouchier**, Augustinus H.M. Smelt, Joep C. Defesche, Ko Willems van Dijk, Rune R. Frants. Leiden University Medical Center, Leiden, Netherlands; Academic Medical Center, Amsterdam, Netherlands.*

Objectives: Hypertriglyceridemia (HTG) is a risk factor for cardiovascular disease. Angiopoietin-like 3 (ANGPTL3) influences triglyceride (TG) levels by modulating LPL activity, and the gene is regulated by LXR. Since the effect of genetic variation in the ANGPTL3 gene on TG levels in human is unknown, we investigated whether genetic variants in this gene influence TG levels in a human HTG cohort. **Methods:** We selected 200 HTG patients and sequenced the complete coding region, including promoter and intronic boundaries of ANGPTL3. Two hundred normolipidemic individuals were used as controls. **Results:** Six genetic variants were identified. These variants were located in the promoter region (1-914insA, 1-795delATTCA, and 1-153C>T), exon 1 (N76K), intron 4 (836-23delATG), and intron 5 (931+53C>T). All variants showed allele frequencies of ≤0.007. The variants 1-153C>T, N76K and 836-23delATG were exclusively found in the HTG population. The functional LXR binding site in which 1-153C>T was present, was disrupted by the C to T transition (Matinspector). The Asn at position 76 within the N76K variant is highly conserved between human, cow, pig, rat and mouse, and is located in the region of the protein which is thought to play a role in controlling TG levels. **Conclusion:** Our results indicate that genetic variation in the ANGPTL3 gene is rare. The ANGPTL3 variants found in our HTG patients may potentially influence levels of ANGPTL3 and explain the HTG phenotype. To further elucidate association between the ANGPTL3 variants and TG levels, family investigation is ongoing.

Funding: Nutrigenomics Consortium

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EVALUATION OF INSULIN RECEPTOR SUBSTRATE 2 AS A SUSCEPTIBILITY GENE FOR ATHEROSCLEROSIS AND CORONARY HEART DISEASE

***Daniel A. Hagg**, Margareta Jernas, Olle Wiklund, Dag S. Thelle, Bjorn Fagerberg, Per Eriksson, Anders Hamsten, Bob Olsson, Bjorn Carlsson, Lena M.S. Carlsson, Per-Arne Svensson. Institute of Medicine, Sahlgrenska Academy, Goteborg, Sweden; Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway; King Gustaf V Research Institute, Karolinska University Hospital, Stockholm, Sweden.*

Objective: Genetic susceptibility strongly affects the development of atherosclerosis. Our aim was to search for and evaluate susceptibility genes for atherosclerosis. **Methods:** DNA microarray expression profiles of lipid-loaded macrophages were used to identify genes with an altered expression in subjects with atherosclerosis (n = 15) compared to matched controls (n = 15). **Results:** Insulin receptor substrate 2 (IRS2) had increased expression in lipid-loaded macrophages from atherosclerotic subjects compared with controls. Individuals homozygous for C of the -765C→T SNP in the promoter region of IRS2 had an increased risk for coronary heart disease in the INTERGENE study (case = 512, control = 512), odds ratio 1.43, 95% CI 1.10-1.86, P = 0.010. This was not confirmed in the SCARF study (case = 315, control = 315). **Conclusion:** Increased macrophage expression of IRS2 may be a risk factor for atherosclerosis and coronary heart disease in certain populations.

Funding: Swedish Research Council (11285), the Swedish Heart Lung Foundation, AFA, Sahlgrenska University Hospital foundation, The National Board of Health and Welfare, Wilhelm och Martina Lundgren Foundation, The Swedish Diabetes Foundation, Goljes minne Foundation, Swegene, Fredrik and Ingrid Thuring Foundation and the Swedish federal government under the LUA/ALF agreement

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THE LEIPZIG HEART STUDY (LE-HEART STUDY) – PHENOTYPING OF CARDIOVASCULAR CHARACTERISTICS, ENVIRONMENTAL FACTORS AND RISK FACTORS IN ANGIOGRAPHICALLY ASSESSED CHD PATIENTS FOR GENOME WIDE ANALYSES

Daniel Teupser, Frank Beutner, Stephan Gielen, Annegret Schink, Alexander Leichtle, Ralph Burkhardt, Gerhard Schuler, ***Joachim Thiery****. Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Leipzig, Germany; Heart Center of Cardiology, Leipzig, Germany.*

Purpose: Coronary artery disease (CAD), myocardial infarction (MI) and stroke are the leading causes of morbidity and mortality. While traditional risk factors play an important role in the pathogenesis of CAD, heritability estimates indicate that genetic factors contribute to a large amount (50%) of disease variability. The aim of our study is to obtain an excellently phenotyped cohort for identification of genetic factors of CAD-susceptibility. **Method:** In the prospective LE-Heart Study, consecutive patients with suspected CAD (n= 2000) are included before undergoing diagnostic coronary angiography. A standardized questionnaire about disease status, family history of CAD, risk factors and physical activity is performed. Anthropometric measurements, extensive clinical chemistry, an exercise stress test, echocardiography and measurements of peripheral atherosclerosis (IMT, ABI) are also performed. Severity of CAD is assessed by coronary angiography. LE-Heart also provides DNA for genome-wide studies, a cell-bank of PBMC and RNA from whole blood for transcriptome analyses. We will report about the phenotypic characterization of the first 500 patients. **Conclusions:** The LE-Heart Study investigates the association between genetic and lifestyle factors modulating the severity of CAD. First results of cardiovascular characteristics and metabolic phenotypes will be presented.

Funding: Roland-Ernst-Foundation for Healthcare

Friday
October 5, 2007

Plenary Supported Session 4 "BEYOND STATIN MONOTHERAPY: RAISING HDL-C, LOWERING CVD RISK"
7:00 AM - 8:30 AM
Chairs: *P.J. Barter, Sydney, Australia and H.B. Brewer, Jr., Washington, DC, USA.*

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LOW HDL-C IS A SIGNIFICANT INDEPENDENT CVD RISK FACTOR AND THERAPEUTIC TARGET FOR REDUCING CVD RISK

Philip Barter*. The Heart Research Institute, Sydney, Australia.*

Intervention trials using statins to lower LDL cholesterol (LDL-C) have consistently shown substantial reductions in major cardiovascular events (MCEs) in both primary and secondary prevention, in those with and without diabetes or hypertension and in people with a wide range of baseline lipid levels. Furthermore, the magnitude of the reduction in events is a function of the extent of LDL lowering, with each 40 mg/dL decrease in LDL-C equating with a 24% reduction in MCEs. This benefit has been demonstrated with reductions of LDL-C down to levels as low as 70 mg/dL. However, in all of the statin trials, including those in which a proportion of subjects achieved low levels of LDL-C, there is a substantial residual risk of MCEs despite adequate treatment with statins. In part, this reflects the residual risk associated with a low level of HDL-C. The fact that a low HDL-C remains predictive of MCEs during statin therapy supports the view that HDL-C should be considered as a therapeutic target, independent of achieved levels of LDL-C. This view was further supported by the results of the Treating to New Targets (TNT) study of more than 10,000 subjects, of whom many achieved an on-treatment LDL-C of less than 70 mg/dL. There was an inverse relationship between The incidence of MCEs correlated inversely and significantly with the on-treatment (3 months) level of HDL-C in the overall study. This inverse relationship remained apparent in the group with HDL levels less than 70 mg/dL. There was no evidence of an interaction between HDL-C and LDL-C. Thus, a low level of HDL-C should be

considered as a potential therapeutic target for reducing the residual CVD risk that persists even in patients who achieve very low levels of LDL-C when treated with statins.

Funding: None

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NATIONAL GUIDELINES AND EVIDENCE SUPPORTING THE USE OF HDL-RAISING AGENTS TO REDUCE CVD RISK

H. Bryan Brewer. *Medstar Research Institute, Washington, DC, USA.*

Results from two decades of clinical trials have established that lowering LDL is associated with approximately a 25% decrease in clinical cardiac events. Based on this residual risk of cardiovascular disease in patients taking statin additional therapeutic approaches to reduce cardiac events are needed in the treatment of high risk patients. Several lines of evidence suggest HDL may be an effective potential therapeutic target. Epidemiological studies have established that low HDL is an independent cardiovascular disease (CVD) risk factor. Studies with animal models including apoA-I transgenic mice and rabbits, as well as infusions of HDL in hypercholesterolemic rabbits suggest that increased HDL may reduce atherosclerosis. HDL has also been shown to increase cholesterol efflux from cholesterol loaded cells, reduce adhesion molecules on endothelial cells, decrease vascular inflammation and increase nitrous oxide production. The combined preclinical results have suggested that increasing HDL may be a new therapeutic target to further decrease cardiac events in patients at risk for cardiovascular disease. Clinical trials focused on raising HDL are limited. Initial clinical trials including HATS, infusions of apoA-IMilano/phospholipid complexes, and Arbiter 2 and 3 have all been consistent with the additional clinical benefit of raising HDL in conjunction with reducing LDL with statin therapy. Drug therapy to increase plasma HDL includes statins, fibrates, and niacin. New oral drugs under development include CETP inhibitors and niacin preparations with decreased flushing. Additional clinical trials will be required to definitively establish that raising HDL is safe and associated with decrease clinical cardiovascular events and mortality.

Funding: Medstar

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USE OF COMBINATION THERAPY WITH STATINS TO PROVIDE OPTIMAL REDUCTION IN RESIDUAL CVD RISK

Karol E. Watson. *David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.*

Current guidelines for the prevention and treatment of coronary heart disease focus on LDL-C as the primary target of therapy. However, there has been growing interest in raising HDL-C as a secondary target of therapy, based on strong epidemiologic data suggesting that HDL-C levels are inversely related to the development of atherosclerosis. In the Framingham Heart Study, patients with the highest HDL-C levels had the lowest risk of developing coronary artery disease during the ensuing 35 years. Data from Framingham suggests that each 1% increase in HDL-C was linked to a 2% reduction in the development of coronary artery disease. Lifestyle modification remain the first line of therapy for patients with low HDL-C. Obesity, cigarette smoking, high saturated fat intakes, and sedentary lifestyle all reduce HDL-C levels, and altering these risk factors can increase HDL-C. For many patients however, lifestyle modification may not be enough to achieve optimal HDL-C levels. A number of medications also impact HDL-C levels. Statins, which are the most efficatious LDL-C lowering medications, have a modest HDL-C raising effect. Fibric acid derivatives (fibrates) are effective therapy for patients with high triglycerides and low HDL-C, as is nicotinic acid (niacin). Niacin is the most potent drug currently available for raising HDL-C and has been shown to reduce the risk of cardiovascular events. In clinical trials that have combined an LDL-C lowering strategy with an HDL-C raising strategy, clinical event reduction and atherosclerosis regression has been seen. The use of combination lipid lowering therapy will be discussed in this presentation, focusing on the efficacy of this approach as well as the safety issues and practical applications.

Funding: None

Plenary Session 2 "NUTRITION, DIABETES AND CARDIOVASCULAR DISEASE"
8:30 AM - 10:30 AM
Chairs: *W.V. Brown, Atlanta, GA, USA and E.J. Schaefer, Boston, MA, USA*

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METABOLIC SYNDROME: CLASSIFICATION, DIAGNOSIS, AND TREATMENT

Scott Grundy*. University of Texas Southwestern Medical Center, Dallas, TX, USA.*

The metabolic syndrome represents a clustering of cardiovascular risk factors. These include atherogenic dyslipidemia, elevated blood pressure, elevated plasma glucose, a prothrombotic state, and a proinflammatory state. Most persons with the metabolic syndrome are insulin resistant. Obesity, particularly abdominal obesity, appears to be the driving force behind the population increase in metabolic syndrome. The syndrome is associated with a doubling of risk for atherosclerotic cardiovascular disease (ASCVD) and a five-fold increase in risk for type 2 diabetes. In most countries of the world, about one fourth of the adult population has the metabolic syndrome. Several organizations have proposed clinical criteria for the metabolic syndrome. The most widely used is that proposed by the United States National Cholesterol Education Program. According to these criteria, a diagnosis of the metabolic syndrome can be made if a patient has three of five of the following abnormalities: increased waist circumference, elevated triglycerides, reduced HDL, higher blood pressure and impaired fasting glucose or type 2 diabetes. Lifestyle interevention (weight loss and increased physical activity) is the primary therapy for the syndrome. However, in patients in whom lifestyle therapy does not normal the major risk factors for ASCVD, it may be necessary to use specific drug therapy to treat individual risk factors.

Funding: None

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OBESITY, INFLAMMATION, DIABETES, AND CARDIOVASCULAR DISEASE

Alan Chait*, Savitha Subramanian, Chang Yeop Han. University of Washington, Seattle, WA, USA.*

Obesity is an inflammatory disorder associated with an increased predisposition to the development of diabetes and vascular disease. Obesity also is associated with adipocyte hypertrophy and macrophage accumulation in adipose tissue. We have performed *in vitro* studies with differentiated 3T3-L1 adipocytes and used a mouse model to understand the relationships amongst obesity, inflammation and vascular disease.

Differentiated 3T3-L1 cells were made hypertrophic by exposure to high levels of glucose. Cellular hypertrophy was associated with increased expression of MCP-1, serum amyloid A (SAA)3, and hyaluronan synthase (HAS)2, which led to increased production of hyaluronan (HA). SAA3 HA coexisted in a complex at the cell surface. This complex and MCP-1 each accounted for about a third of the chemotactic activity produced by adipocytes.

To study these events *in vivo*, we fed LDLR-/- mice a diabetogenic (high fat, high sucrose) diet +/- added cholesterol (0.15%). Both diabetogenic diets resulted in obesity and macrophage accumulation in intra-abdominal fat, but the addition of cholesterol strikingly increased macrophage accumulation, despite equivalent weight gain. of Macrophages accumulation in intra-abdominal fat was associated with several downstream consequences, including insulin resistance, systemic inflammation and increased atherosclerosis; all of which were worse in the presence off added dietary cholesterol. SAA3 mRNA and HA were increased in the hypertrophic adipose tissue in these animals, consistent with our *in vitro* findings.

These findings suggest that the extent of adipose tissue macrophage accumulation is a determinant of insulin resistance, systemic inflammation and atherosclerosis. Moreover, they suggest that the complex of SAA3 and HA produced by hypertrophic adipocytes plays a role in macrophage accumulation. They also are consistent with a "two-hit" hypothesis, in which obesity leads to changes in adipose tissue, which is then exacerbated by the addition of dietary cholesterol.

Funding: NIH HL30086, DK02346

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EMERGING THERAPEUTIC STRATEGY BASED ON ADIPOCENTRIC HYPOTHESIS

Yuji Matsuzawa. *Sumitomo Hospital, Osaka, Japan.*

Clustering of multiple risk factors such as impaired glucose metabolism, lipid disorders and hypertension has been shown to be major background of atherosclerotic diseases and disease entities such as metabolic syndrome become popular as highly atherogenic state. Multiple risk factors clustering in metabolic syndrome does not occur by accident. Visceral fat accumulation has been shown to cause impaired glucose metabolism, lipid disorders and hypertension, therefore visceral fat accumulation is considered to be a key player in metabolic syndrome. We studied on the molecular characteristic of adipose tissue and adipocytes by investigating expressed genes in visceral and subcutaneous adipocytes and revealed that adipocytes, especially visceral adipocytes are secreting a variety of bioactive substances, adipocytokines. We demonstrated that visceral fat accumulation causes abnormalities of adipocytokine secretion such as hypersecretion of PAI-1 which is related to thrombogenic vascular diseases. More importantly, we also discovered adiponectin which protects against the development of diabetes mellitus, hypertension, inflammation and atherosclerotic vascular diseases. Plasma levels of adiponectin decreased in the subjects with visceral fat accumulation and hypoadiponectinemia caused by visceral fat accumulation might be one of major causes of metabolic syndrome. In this lecture, I would like to show the molecular mechanism of metabolic syndrome with respect to visceral fat accumulation focusing on adiponectin and I would like to discuss about therapeutic strategy which may improve adipocytokine secretion especially about foods and drugs which may increase plasma levels of adiponectin.

Funding: Supported by the Ministry of Education, Culture, Sport, Science and Technology; Grant in Aid for Scientific Research on Priority Area

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DIET AND LIFESTYLE, AND ATHEROSCLEROSIS RISK REDUCTION

Alice H. Lichtenstein. *Tufts University, Boston, MA, USA.*

Habitual diet and lifestyle behaviors can alter cardiovascular disease (CVD) risk factors. Current guidance to minimize risk for developing CVD includes adhering to a dietary pattern rich in vegetables, fruits, whole grains, low/non-fat dairy products, legumes, fish and lean meats coupled with achieving and maintaining a healthy body weight, engaging in regular physical activity and eliminating exposure to tobacco products. Such guidance is flexible and can be adapted for individuals with a wide range of lifestyles and dietary preferences, and to meet the unique needs for growth, development, and aging. Additional advice includes maintaining a moderate fat intake (25% to 35% of energy) rich in unsaturated rather than saturated and trans fat; limiting sodium intake; favoring higher fiber food options; consuming alcohol in moderation and adhering to heart healthy dietary principles when eating food prepared both at home and outside the home. Hypercholesterolemic patients benefit from consuming 1.6 to 2.0 grams of plant sterols/stanols per day. Hypertrigylceridemic patients benefit from consuming a diet at the higher end of fat intake recommendations, and minimizing alcohol and simple carbohydrate intake. In both cases weight loss and increased levels of physical activity in overweight and obese patients has shown to favorably affect CVD risk factors. Although many claims are made with regard to the value of herbs and supplements and their relationship to CVD risk, to date few if any have been substantiated in the long term. Although diet and lifestyle modification may not eliminate the need for pharmacological intervention, it can delay initiation or stabilize a patient at a given dose. The critical challenge is to develop strategies to maximize adherence to these diet and lifestyle recommendations.

Funding: NHLBI

Plenary Supported Session 5 "TARGETING HDL IN THE MANAGEMENT OF MIXED DYSLIPIDEMIA: THE NEXT FRONTIER IN CV RISK REDUCTION"
11:00 AM - 12:30 PM
Chairs: *L. Ose, Oslo, Norway and D.J. Rader, Philadelphia, PA, USA.*

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REDUCING RESIDUAL CV RISK: THE NEED TO LOOK BEYOND LDL-C

Leiv Ose*. Lipid Clinic, Oslo, Norway.*

Reducing Residual CV Risk: The Need to Look Beyond LDL-C Leiv Ose, MD, PhD Lipid Clinic Rikshospitalet University Hospital Oslo, Norway Lowering low-density lipoprotein cholesterol (LDL-C) has been the primary goal of lipid management. Statins and other LDL-lowering medications have consistently demonstrated an ability to reduce levels of LDL-C with a corresponding reduction in cardiovascular risk. Meta-analyses of major statin trials demonstrate a strong correlation between LDL-C and cardiovascular event rates. Combining statins with other agents with different mechanisms of action may result in greater reductions in LDL-C than with statins alone, and numerous ongoing studies will assess the effects of these combinations on clinical end points such as major vascular events, death, and intima-media thickness. Despite its success in reducing cardiovascular risk, approximately a third of high-risk patients receiving statin therapy will still experience a major vascular event within 5 years. It has been suggested that a considerable degree of this residual risk may be attributable to low high-density lipoprotein (HDL), which remains an independent predictor of cardiac risk, even when LDL-C is low. Looking beyond LDL lowering and incorporating HDL-boosting therapies into lipid management will be an important step in the evolution of cardiovascular risk reduction.

Funding: None

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THE IMPACT OF INCREASING HDL ON CV RISK: EVIDENCE FROM CLINICAL TRIALS

B. Greg Brown*. University of Washington, School of Medicine, Seattle, WA, USA.*

A substantial and growing body of data exist on the lipid and clinical effects of niacin preparations, including once-daily extended-release niacin, given in combination with LDL-lowering drugs. The evidence points, in all cases, to a superiority of these combinations when compared with placebo or statin monotherapy in terms of LDL-C- and TG-lowering, increase in HDL-C, HDL2, and LDL size and buoyancy. Furthermore, these combinations, even at similar amounts of LDL-C-lowering, reverse (regress) atherosclerosis and markedly reduce the frequency of clinical cardiovascular events. These observed benefits, at the lipid, arterial, and clinical event level, substantially exceed the well-established expectations for statins alone (which include slowed stenosis progression - not regression; and 25-35% cardiovascular event rate reduction). As a whole, the clinical trial data on niacin in combination with LDL-C-lowering drugs (five different trials) suggest that they more than double the clinical benefits of statin monotherapy. While consistently favorable at arterial and clinical levels, and very compelling, these combination therapy trials must be considered strong pilot evidence because their total number just exceeds 700 patients.

Funding: 1983-1993 NHLBI P01 program project: Human lipoprotein pathophysiology. John J. Albers, PhD, PI. $4.6 million. Project 8: Colestipol and lovastatin or niacin for prevention of atherosclerosis progression. BG Brown, PI. $1,250,000, incl. $450,000 suppl. funding from Merck, Inc.; 1993-1998: NHLBI R01: Does atherosclerosis regress with therapy for low HDL-cholesterol? B.G. Brown, PI. $3.6 million, extension to 2000; suppl. funding from Merck ($450,000) and Upsher-Smith ($120,000); 2005-2011: NIH R01 081616 AIM-HIGH – Statin plus niacin to prevent vascular events. B Greg Brown, MD, PhD, PI; Ruth McBride Co-PI. $22 million. Principal funding for AIM-HIGH. Additional $21.3 million support to Ruth McBride from Kos Pharmaceuticals

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TARGETING HDL IN THE MANAGEMENT OF MIXED DYSLIPIDEMIA: CLINICAL STRATEGIES ON THE HORIZON

Daniel J. Rader. *University of Pennsylvania School of Medicine, Philadelphia, PA, USA.*

Low-density lipoprotein cholesterol (LDL-C)–lowering therapies have been shown to lower cardiovascular risk by approximately a third, but considerable residual cardiovascular risk remains. Interventions that increase high-density lipoprotein cholesterol (HDL-C) may further reduce cardiovascular risk. Niacin, the most effective currently available agent for elevating HDL-C, causes flushing in many patients, limiting its clinical utility. Recent advances in our understanding of HDL-C metabolism and its role in the reduction of atherosclerosis have illuminated a number of potential targets to modify HDL-C levels and improve cardiovascular risk. Laropiprant blocks cutaneous DP-1 receptors and thus diminishes the flushing and burning associated with niacin administration. Studies are ongoing to assess the effect of niacin in combination with this DP-1 antagonist on vascular outcomes in high-risk patients receiving medication to lower LDL-C. Other potentially promising routes to further reductions of cardiovascular risk include direct intravenous infusions of a recombinant/synthetic HDL-C made from a mutant apolipoprotein (apo) A1 called apo A1 Milano; orally effective synthetic peptides that mimic the biological effects of apo A1, such as the D4F peptide; nuclear hormone receptor agonists that stimulate various steps in the reverse cholesterol transport pathway; ex vivo delipidation of HDL-C to improve its functionality; and HDL-C–based gene transfer. Further study is needed to corroborate the clinical benefits of these approaches in larger, naturalistic samples. This presentation will review these emerging clinical strategies to improve the treatment and further reduce the cardiovascular risk of patients with mixed dyslipidemia.

Funding: None

Lecture in Memoriam of Prof. David Kritchevsky and XXXIII Lorenzini Lecture
12:30 PM - 1:30 PM
Chairs: *R. Paoletti, Milan, Italy and A.M. Gotto, Jr., New York, NY, USA.*

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BUILDING ON THE PAST, SECURING THE FUTURE: THE PROCAM STUDY REACHES 50,000 MEN AND WOMEN

Gerd Assmann. *Universitaet Muenster, Muenster, Germany.*

Building on the past, securing the future: PROCAM now includes 50.000 participants The **Pro**spective **Ca**rdiovascular **M**uenster (PROCAM) Study was initiated in 1979 and has been following an occupational cohort recruited in the north-west of Germany since then. The 50.000th participant has just been recruited and the study now has data on more than 1.000 clinical endpoints, mostly cardiovascular disease and cancer. In the past, emphasis has been given to conventional risk factors, leading to the development of risk scores and algorithms that have now entered general use throughout Europe and many other parts of the world. I will report our most recent endpoint data. In addition, I will outline preliminary results on novel genetic risk factors in the PROCAM cohort.

Funding: Governmental funding

Supported Session 6 "NOVEL APPROACHES TO REDUCING LDL OR ATHEROGENIC LIPOPROTEINS: BEYOND STATINS?"
2:00 PM - 4:00 PM
Chairs: *M.-R. Taskinen, Helsinki, Finland and E. Stein, Cincinnati, OH, USA.*

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BIOCHEMICAL MECHANISMS AND GENETIC VARIABLES CONTROLLING STATIN-INDUCED MUSCLE TOXICITY

Reijo Laaksonen. *Tampere University Hospital, Tampere, Finland.*

To study metabolic effects in muscle during high dose statin treatment, we performed whole genome expression profiling of muscle specimens and UPLC/MS based lipidomics analyses of plasma samples obtained in a randomized trial from patients either on high dose simvastatin (80 mg), atorvastatin (40 mg), or placebo. We found 111 differentially expressed genes (1.5-fold change and p-value < 0.05) in the high dose simvastatin group, while expression of only one and five genes was altered in the placebo and atorvastatin groups, respectively. The Gene Set Enrichment Analysis identified 23 affected pathways (FDR q-value < 0.1) in muscle following high dose simvastatin, including eicosanoid synthesis and phospholipase C pathways. These changes were accompanied with a significant decrease (-47%) in muscle mitochondrial DNA during simvastatin treatment measured as mtDNA / nuclear DNA ratio. This finding was in accordance with muscle mitochondrial respiratory chain enzyme activity assays suggesting decreased total mitochondrial volume per cell in the simvastatin group. Using lipidomic analysis we identified previously uncharacterized drug-specific changes in the plasma lipid profile despite similar statin-induced changes in plasma LDL-cholesterol. We also found that the plasma lipidomic changes following simvastatin treatment correlate with the muscle expression of the arachidonate 5-lipoxygenase-activating protein. We demonstrated that high dose simvastatin affects multiple metabolic and signalling pathways in skeletal muscle. The plasma lipidomic profile may serve as a sensitive biomarker of statin-induced metabolic alterations in muscle and may thus allow us to identify patients who should be treated with a lower dose to prevent a possible toxicity.

Funding: The Tampere University Hospital research fund, Academy of Finland and Tekes

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NOVEL THERAPIES IN LDL LOWERING

Anthony S. Wierzbicki. *Guy's & St Thomas' Hospitals, London, United Kingdom.*

Low density lipoprotein cholesterol is recognised as one of the major risk factors for cardiovascular disease. Many interventions ranging from diet through bile acid sequestrants, cholesterol absorption inhibitors and statins reduce LDL-C levels and most if these have been shown to reduce cardiovascular events. Current intestinally acting agents reduce LDL-C by 15-20% while statins reduce LDL-C by up to 50%. All these therapies show marked variations in individual response and management of drug 'resistant' patients forms an increasing challenge. In addition many patients cannot tolerate sufficient doses of statin, while intestinal agents have lesser efficacy and so cannot reach the LDL-C targets that are now desirable. Thus, the opportunity exists for additional agents synergistic with current drugs that could help reduce LDL-C to the eventual potential target of 1 mmol/L. Approaches to lower LDL beyond statins include gut-based absorption inhibition including NPC1L1 or other transporter blockade, bile acid sequestrants and ileal bile acid transport inhibitors. It is possible to modulate LDL-receptor function through PCSK-9 or via berberine and to increase cholesterol metabolism into bile acids through the farnesoid-X receptor. LDL reduction can also be achieved by further blockade of the cholesterol synthesis pathway e.g. squalene synthase inhibition or disruption of synthesis of VLDL particles either through inhibiton of microsomal transfer protein or apolipoprotein B-100 either by traditional compounds or through the use of inhibiting RNA species. This presentation summarises new developments in the field of novel agents to lower LDL-cholesterol.

Funding: None

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OVERVIEW OF SQUALENE BIOSYNTHESIS INHIBITORS

Evan Stein. *Metabolic & Atherosclerosis Research Center, Cincinnati, OH, USA.*

The benefit of reducing elevated LDLc is extremely well established due to a huge amount of data generated by large, clinical end-point, placebo controlled trials mostly with HMG CoA reductase inhibitors ('statins'),[1] but but also other modalities. Based on two decades of well controlled clinical end point trials guidelines for prevention of CVD have resulted in lower and lower targets for LDLc and its Apo B containing and related precursors VLDL and IDL. Despite the fact that statins are an extremely effective class of Apo B and LDLc reducing agents they often fail to achieve current targets in high risk patients. In addition there are large and growing patient populations who are unable to tolerate statins. Alternative LDLc lowering agents are less effective and some have yet to have been shown to reduce CVD risk. Inhibition of the cholesterol pathway at a point further down from where statins operate, such as Squalene Synthase (SSI), thus offer an attractive target for reducing LDLc for a number of reasons; they may have a 'statin sparing' effect both in terms efficacy by reducing the need for high dose statin and also safety by increasing some of the intermediary metabolites thought to be depleted by statins (see figure below). The advantage of this mechanism is that the ultimate manner by which plasma LDLc is reduced is the same as that for statins, namely LDL receptor upregulation and by extrapolation the CVD risk reduction can be assumed. While over the past decade a number of pharmaceutical companies have evaluated SSI only one compound TAK-475/lapaquistat) has remained in clinical development and is now in advanced phase III. 1. CTT Trialists. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005;366:1267-1278.

Funding: Takeda

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INHIBITION OF SQUALENE SYNTHASE IS EFFECTIVE IN STABILIZATION OF CORONARY PLAQUES

Masashi Shiomi. *Kobe University School of Medicine, Kobe, Japan; Takeda Pharmaceutical Company Limited, Osaka, Japan.*

Objective: To examine whether inhibition of squalene synthase suppress coronary atherosclerosis, we administered TAK-475, a novel squalene synthase inhibiter (SSI), to WHHLMI rabbits. **Methods:** WHHLMI rabbits were divided into 3 treatment groups: oral TAK-475 (100 or 200 mg/kg) or placebo administered for 32 weeks. Serum lipid levels were monitored every 4 weeks. Coenzyme Q10 levels in plasma, liver, and soleus muscle were measured. Coronary atherosclerosis was evaluated as cross-sectional vascular narrowing; coronary artery sections were stained immunohistochemically or histopathologically, and lesional components measured by computer-assisted image analysis. **Results:** TAK-475 decreased plasma cholesterol and triglyceride levels. In the coronary artery plaques of animals treated with TAK-475, macrophages and extracellular lipid deposits decreased and collagen fibers increased. There were no differences in smooth muscle cells among treatment groups. Areas positive for matrix metalloproteinase-1, plasminogen activator inhibitor-1, and oxidized LDL decreased in the coronary plaques of TAK-475-treated animals. Thus, TAK-475 treatment also appeared to suppress destabilization of coronary artery plaques. In addition, TAK-475 increased anti-oxidative coenzyme Q10 levels in plasma and peripheral tissues, but did not affect proliferation or apoptosis of arterial cells. **Conclusions:** Inhibition of squalene synthase was effective in delaying progression of atherosclerosis and suppressing destabilization of coronary plaques in this animal model.

Funding: This study was supported by Takeda Pharmaceutical Company Limited, Osaka, Japan.

Supported Session 7 "THE ENDOCANNABINOID SYSTEM AS A NEW APPROACH TO REDUCING RISK FOR CARDIOVASCULAR DISEASE"
4:30 PM - 6:30 PM
Chairs: *M.J. Chapman, Paris, France and R.H. Eckel, Denver, CO, USA.*

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INTRODUCTION TO THE ENDOCANNABINOID SYSTEM, AND ITS REGULATION IN THE FRAMEWORK OF ENERGY BALANCE AND METABOLISM

Vincenzo Di Marzo. *Consiglio Nazionale delle Ricerche, Pozzuoli, Napoli, Italy.*

The endocannabinoids (ECs) anandamide and 2-arachidonoylglycerol are endogenous agonists of the CB_1 and CB_2 cannabinoid receptors, the former of which is widespread in mammalian tissues, with the highest concentrations in the brain. ECs are biosynthesized from neurons following the Ca^{2+}-sensitive hydrolysis of lipid precursors and are released "on demand" to act as local mediators "when and where needed". ECs are produced in response to stressors to help re-establishing the homeostasis of other mediators. However, some pathological states lead to a longer-lasting and less specific activation of CB_1 receptors, which can contribute to the symptoms of these disorders (Di Marzo and De Petrocellis, Annu. Rev. Med., 2006). CB_1 receptors are present in all organs controlling energy balance, and are necessary to induce food intake after a short period of food deprivation, help accumulate fat into adipocytes, induce lipogenesis in the liver, prolong nutrient retention in the intestine and reduce energy expenditure (Matias and Di Marzo, Trends Endocrinol. Metab., 2007). CB_1 stimulation modulates the release and/or expression of hypothalamic anorexic and orexigenic mediators, of adipokines from adipocytes and of metabolic enzymes in the liver and skeletal muscle. CB_1 expression and activation by ECs increases during, and participates in, differentiation of preadipocytes into adipocytes, and a complete EC system is present in pancreatic islets and liver. Recent data suggest that a permanent elevation of EC levels in some peripheral organs occurs in rodents following prolonged high fat diets, and in humans with hyperglycemia or visceral obesity. The contribution of this phenomenon to dyslipidemia and insulin insensitivity will be discussed.

Funding: Sanofi-aventis

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THE ENDOCANNABINOID SYSTEM AND ITS ROLE IN OBESITY AND METABOLIC SYNDROME

Daniela Cota. *University of Cincinnati, Cincinnati, OH, USA.*

Recent evidence indicates that the endocannabinoid system (ECS) modulates several physiologic functions through central and peripheral mechanisms, and dysregulation of the ECS may be linked to abdominal obesity. In the central nervous system, cannabinoid type 1 (CB_1) receptors and their respective ligands, the endocannabinoids, modulate food intake and the motivation to consume palatable food. In peripheral tissues, such as liver, white adipose tissue, muscle, and pancreas, CB_1 receptors have also been found and the ECS has thus been involved in the regulation of metabolic homeostasis. Diet-induced obese animal models, as well as genetic models of obesity are characterized by increased endocannabinoids levels and CB_1 expression in several tissues. Obese humans also have an increase in plasma endocannabinoids (particularly 2-AG) levels which directly correlates with levels of visceral adiposity. Moreover, both subjects with eating disorders and patients with type 2 diabetes and uncorrected hyperglycemia have increased circulating levels of endocannabinoids. Several hypotheses have been formulated to explain the dysregulation of the ECS. Diet and stress might affect the normal activity of the ECS. Moreover, alteration in the function or in the circulating levels of hormones involved in the regulation of energy balance (ie, leptin, CCK, ghrelin) might be causally linked to dysregulation of the ECS. Furthermore, gene polymorphisms for CB1 or for enzymes regulating the degradation of endocannabinoids, might also contribute to the development of eating disorders and obesity. Although the exact cascade of events that leads to dysregulation of the ECS remains to be delineated, targeting the ECS activity in obesity appears to be an important approach for improving body weight and metabolic control.

Funding: None

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ENDOCANNABINOIDS AND WEIGHT LOSS

***Samuel Klein**. Washington University School of Medicine, St Louis, MO, USA.*

The endocannabinoid system has recently been recognized as an important regulator of energy balance. Endocannabinoids are lipid mediators that increase appetite by activating cannabinoid 1 (CB-1) receptors. CB1 receptors in the hypothalamus, limbic forebrain, and peripheral sensory nerve terminals have been implicated in appetite regulation. Administration of CB-1 receptor antagonists have been shown to decrease body weight in animal models and in human clinical trials. Data from multicenter clinical trials conducted in thousands of obese men and women demonstrated that treatment with the CB-1receptor antagonist, Rimonabant, causes about a 5% greater weight loss at 1 year of treatment than that achieved with placebo therapy. Moreover, continued therapy with Rimonabant for second year effectively in preventing weight recidivism, whereas discontinuation of treatment resulted in rapid weight regain.

Funding: None

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ENDOCANNABINOIDS CLINICAL APPLICATIONS

***Louis J. Aronne**. Weill Medical College of Cornell University, New York, NY, USA.*

The Rimonabant in Obesity (RIO) trials compared a selective cannabinoid-1 receptor blocker—rimonabant—with placebo. The agent down-regulates overactivity of the endocannabinoid system and directly impacts the physiology of diet-induced obesity. In the trial, patients taking rimonabant experienced significant reductions in body weight and waist circumference when measured at one year. The agent also appears to have direct peripheral effects on metabolic cardiovascular risk parameters beyond and apart from the central effects on food intake and energy homeostasis; rimonabant decreased insulin resistance and improved lipid and glucose profiles. A recent study designed with HbA1c as a primary endpoint demonstrated its efficacy as a treatment for type 2 diabetes and additionally showed improvements in weight, dyslipidemia, and inflammatory markers. The premature discontinuation rate in this trial was 15%, comparable to trials of statins and anti-diabetic agents. Adverse events included depressed mood, anxiety, nausea, dizziness, upper respiratory tract infection, nasopharyngitis, influenza, diarrhea, arthralgia, insomnia, viral gastroenteritis, and fatigue. The proportions of patients who had adverse events, serious adverse events, and discontinuation due to adverse events was higher in patients receiving rimonabant 5 mg and 20 mg than in those receiving placebo. The difference in adverse events between treated and placebo groups declined over time. Rimonabant has been approved in 37 countries, and more than 100,000 patients have been treated. Recent information on post-marketing surveillance and the benefit vs. risk of using rimonabant in an obese population will be discussed.

Funding: None

Workshop 7 "ANTIOXIDANTS"
2:00 PM - 4:00 PM
Chairs: *F. Violi, Rome, Italy and I. Jialal, Davis, CA, USA.*

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DIETARY FACTORS THAT MODULATE INFLAMMATION

Ishwarlal (Kenny) Jialal*. UC Davis Medical Center, Sacramento, CA, USA.*

Much evidence supports a pivotal role for inflammation in all phases of atherosclerosis from the initiation of the fatty streak to the culmination in acute coronary syndromes. The largest amount of published data from prospective studies supports a role for CRP as a risk marker for CVD. Various studies have reported weight loss to be the best non-pharmacological modality to reduce biomarkers of inflammation such as CRP. There is a strong correlation between CRP reduction with weight loss (r^2= 0.87, $p<0.05$). Among the n-3 fatty acids, alpha-linoleic acid and conjugated linoleic acid appears to be anti- and pro-inflammatory, respectively. Meal modulation of inflammation via combination of soy proteins, viscous fiber, plant sterols and almonds has been shown to decrease inflammation. Importantly, the Mediterranean diet has been reported to result in reduced inflammation. Furthermore, the existing body of data reveals RRR-AT and to some extent gamma-enriched mixed tocopherols, to be beneficial for reduction in CRP and other biomarkers of inflammation. Interestingly, a clinical trial with RRR-AT (1200 IU/day) or placebo for 2 years in patients with stable CAD reveal significant ($p<0.05$) reduction in CRP in RRR-AT group as compared to placebo. Much further research is needed before firm conclusions can be drawn for individual dietary factors, both macronutrients and micronutrients.

Funding: NIH

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SHORT- AND LONG-TERM EFFECTS OF ANTIOXIDANT VITAMIN SUPPLEMENTATION ON CARDIOVASCULAR EVENTS: A LARGE RANDOMISED CONTROLLED TRIAL

Louise Bowman, on behalf of the Heart Protection Study Collaborative Group*. CTSU, University of Oxford, Oxford, United Kingdom.*

Objectives: Observational data suggest that increased intake of antioxidant vitamins might reduce the incidence rates of vascular disease. Clinical trials have generally failed to confirm benefits. One possible explanation is their short duration. Continued follow-up of all participants in the MRC/BHF Heart Protection Study (HPS) may show whether any delayed effects of antioxidant vitamin supplementation on vascular events do eventually emerge. **Methods:** During the scheduled treatment period of HPS, 20 536 patients at increased vascular risk were allocated antioxidant vitamin supplementation (600 mg Vitamin E, 250 mg Vitamin C and 20 mg β-carotene daily) or matching placebo. No significant reductions in any type of vascular disease were seen during the scheduled 5-year treatment period. In the post trial period, all survivors are followed-up by annual postal questionnaire and/or via their GP, supplemented by information from central registries. **Results:** During 4 years' post-trial follow-up, there were no significant differences in vascular deaths (857 [9.8%] previously allocated vitamins vs 834 [9.5%] previously allocated placebo; RR 1.03 [95% CI 0.93-1.13]) or major vascular events (1363 [18.8%] vs 1364 [18.8%]; RR 0.99 [0.92-1.07]). The number of major coronary events (887 [10.6%] vs 855 [10.2%]; RR 1.03 [0.94-1.14]) and strokes (360 [4.3%] vs 350 [4.2%]; RR 1.02 [0.88-1.19]) were similar in both groups. **Conclusions:** 5-years of antioxidant vitamin supplementation is safe, but without evidence of any short- or long-term cardiovascular benefit.

Funding: HPS (1994-2001) was funded by the UK Medical Research Council, the British Heart Foundation (BHF), Merck and Co., Inc. and Roche Vitamins. The post-trial follow-up was funded by the BHF

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EARLY INCREASE OF OXIDATIVE STRESS AND SOLUBLE CD40L IN CHILDREN WITH HYPERCHOLESTEROLEMIA

***Francesco Violi**, Pasquale Pignatelli, Roberto Carnevale, Eliana Morrone, Francesco Morrone. University of Rome "La Sapienza", Rome, Italy.*

Background: Oxidative stress is suggested to play a major role in premature atherosclerosis. **Objective:** Aim of the study was to analyse the behaviour of oxidative stress and its interplay with CD40L, a proatherosclerotic protein, in hypercholesterolemic children. **Participants:** 41 hypercholesterolemic children (mean age 9.28±0.5) and 40 normocholesterolemic children (mean age 9.02±0.69) matched for sex and age. Within each group children were classified as having or not a family history of cardiovascular disease, (CAD). **Methods:** 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative stress, and soluble CD40L (sCD40L) were measured. In a subgroup of children with high (n= 8) or normal (n= 8) levels of serum cholesterol, platelet p38 MAP kinase phosphorylation, a protein involved in NADPH oxidase activation, was determined. **Results:** Hypercholesterolemic children had higher values of 8-OHdG and sCD40L compared to controls (0.55±0.06 vs 0.21±0.02 ng/ml, $p<0.001$ and 0.55±0.04 vs 0.19±0.03 ng/ml, $p<0.001$, respectively). A correlation between 8-OHdG and sCD40L was observed in either children with high ($r=0.676$, $p<0.001$) or normal ($r=0.878$,$p<0.001$) levels of cholesterol. Children with family history of CAD tended to have higher values of 8-OHdG and sCD40L but the difference was not significant. Platelet p38MAP kinase was phosphorylated more in children with hypercholesterolemia compared to controls (36.8±5.80 vs 8.0±4.5 AU, $p<0.001$ respectively). **Conclusion:** hypercholesterolemic children have an early increase of oxidative stress that may be responsible for up-regulation of CD40L and potentially predispose to premature atherosclerosis.

Funding: None

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ENHANCED LIPID PEROXIDATION AND PLATELET ACTIVATION AS POTENTIAL CONTRIBUTORS TO INCREASED CARDIOVASCULAR RISK IN THE "LOW-HDL" PHENOTYPE

***Giovanni Davì**, Francesca Santilli, Antonella Ganci, Carlo Maria Barbagallo, Valentina Davì, Angelo Baldassarre Cefalù, Stefano Lattanzio, Davide Noto, Giovanni Ciabattoni, Maurizio Averna. "G. d'Annunzio" University of Chieti, Chieti, Italy; University of Palermo, Palermo, Italy.*

Objective: Low levels of high-density lipoprotein (HDL) cholesterol are identified as a major independent risk factor for coronary heart disease (CHD). Interventions aimed at increasing HDL cholesterol levels prevent the progression of CHD. Anti-atherogenic properties have been recognized for HDL, including protection from low-density lipoproteins oxidation and its deleterious consequences. Our aim was to examine whether HDL levels are related to in vivo oxidative stress and platelet activation, as potential contributors to increased cardiovascular risk. **Methods:** Urinary 8-iso-prostaglandin (PG)$F_{2\alpha}$ and 11-dehydro-thromboxane (TX)B_2, in vivo markers of oxidative stress and platelet activation, respectively, were measured in 45 CHD patients with HDL < 35 mg/dL (36 M, aged 61±9 yrs), compared to 30 CHD patients with HDL > 35 mg/dL (20 M, aged 61±10 yrs). **Results:** Patients with HDL<35 mg/dL showed significantly higher levels of 8-iso-$PGF_{2\alpha}$ (301±163 pg/mg creatinine) and 11-dehydro-TXB_2 (620±416 pg/mg creatinine) as compared to patients with high HDL (207±114 and 351±192 pg/mg creatinine, respectively). A significant direct correlation was found between urinary 8-iso- $PGF_{2\alpha}$ and 11-dehydro-TXB_2 in both groups. **Conclusions:** We conclude that a low HDL phenotype is associated with increased lipid peroxidation and platelet activation, thus providing novel insight into the mechanisms linking low HDL and occurrence of cardiovascular disease.

Funding: This research was supported by EC FP6 funding (LSHM-CT-2004-0050333)

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CIRCULATING OXIDIZED LOW DENSITY LIPOPROTEIN BIOMARKERS IN SUBJECTS HETEROZYGOUS FOR LECITHIN: CHOLESTEROL ACYLTRANSFERASE GENE DEFECTS

***Adriaan G. Holleboom**, G. Kees Hovingh, John J. Kastelein, Jan A. Kuivenhoven, Sotirios Tsimikas. Academic Medical Center, Amsterdam, Netherlands; University of California, San Diego, CA, USA.*

Lecithin:cholesterol acyltransferase (LCAT) displays phospholipase activity and may have antioxidant properties. Previous studies suggested that IgG autoantibodies to oxidized low-density lipoprotein (OxLDL) are positively associated with increased cardioavascualr risk, whereas IgM autoantibodies are inversely associated. However, the relationship of LCAT function to oxidative stress in humans is not well studied. IgM and IgG autoantibodies to malondialdehyde (MDA) and copper modified LDL, IgG and IgM apoB-100-immune complexes (IC) (IC/apoB) and the oxidized phospholipid content on apolipoprotein B-100 lipoproteins (OxPL/apoB) were measured in 53 subjects heterozygous for LCAT gene defects and 62 unaffected family controls. The relationships of OxLDL biomarkers to carotid intima media thickness (IMT) was determined. IgM MDA-LDL levels [median relative light units (interquartile range)] [7822(4581-9802) vs. 8303(5767-10643, $p<0.001$)] and IgM IC/apoB [4585(2869-6496) vs. 5972(4263-8349), $p=0.0123$] were lower in subjects with LCAT gene defects compared to controls. In contrast, IgG IC/apoB [3989(2681-6028) vs. 3586(2747-4805), $P=0.025$] and total IgG apoB-IC [3755x103(2481-6209) vs. 3060x103(2493-4068), $P=0.012$] were higher. In general, IgM OxLDL biomarkers were inversely associated with carotid IMT and IgG OxLDL biomarkers were positively associated with IMT in univariate analysis, but not in multivariate analysis. These data suggest that reduced LCAT activity is associated with a systemic pro-oxidant state.

Funding: None

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STRAWBERRIES AND THEIR ANTIOXIDANT EFFECTS AS PART OF A CHOLESTEROL-LOWERING DIETARY PORTFOLIO

***Tri H. Nguyen**, David J.A. Jenkins, Cyril W.C. Kendall, Balachandran Bashyam, Andrea R. Josse, Howard D. Sesso, Britt Burton-Freeman, Robert G. Josse, Lawrence A. Leiter, William Singer. University of Toronto, Toronto, ON, Canada; St. Michael's Hospital, Toronto, ON, Canada; Brigham & Women's Hospital and Harvard Medical School, Boston, MA, USA; University of California, Davis, CA, USA.*

Objective: To assess the antioxidant effects of strawberries added to an effective cholesterol-lowering diet (dietary portfolio). **Methods:** Twenty-eight hyperlipidemic subjects who had followed a cholesterol-lowering dietary portfolio, consisting of soy, viscous fiber, plant sterol and nuts, for a mean of 2.5 years were randomized to take strawberries (454 g/d, 112 kcal) or additional oat bran bread (65 g/d, 112 kcal, ~2g β-glucan) (control) each for one month in a cross-over design separated by a two-week washout. **Results:** Both the strawberry and oat bran bread diet significantly increased serum protein thiols ($P<0.001$ and $P=0.001$, respectively), indicating protection from oxidative damage by both treatments. Protection from oxidative damage to LDL-C was seen with the strawberry diet only, as determined by the reduction in TBARS in LDL-C fraction ($P=0.004$) but not with the oat bran bread diet ($P=0.816$). The difference between treatments was significant ($P=0.014$). No effect of treatment was seen on conjugated dienes as an additional indicator of LDL-C oxidative damage. **Conclusion:** Both strawberries and oat bran bread reduced oxidative damage to serum proteins. However, only strawberries provided evidence of increased protection of LDL-C from oxidation.

Funding: Canada Research Chair Endowment of the Federal Government of Canada; California Strawberry Commission

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FLAVONOID-RICH CHOKEBERRY FRUIT EXTRACT INHIBITS ENDOTHELIAL PROGENITOR CELLS SENESCENCE INDUCED BY OXIDIZED LDL

***Marek Naruszewicz**, Andrzej Parzonko, Anita Kosmider, Iwona Laniewska, Miroslaw Dluzniewski. Medical University of Warsaw, Warsaw, Poland.*

Many studies have shown the important role of endothelial progenitor cells (EPCs) in the process of neovascularisation of the vascular wall damaged by atherosclerosis risk factors. The purpose of this study was to assess the effect of chokeberry flavonoids on EPC senescence in vitro. **Methods.** EPCs were isolated from healthy volunteers and plated on fibronectin-coated plates. After 2 days of culture, non-adherent cells were removed and EPCs were incubated with ox-LDL (10 μg/ml) in the presence or absence of increasing concentrations (20 to 50 μg) of the extract containing flavonoids (anthocyans 25%, polymeric procyanidines 50%, phenolic acid 9%). The experiment was conducted in 3 cycles of 72 h. EPC senescence was determined by measurement of telomerase activity with the use of the TeloTAGGGPCR ELISA kit. **Results.** In three independent experiments we showed that flavonoids lowered the effect of ox-LDL on telomerase activity in EPCs in a dose-dependent manner and this effect was significant with $p<0.000$ at the presence of 50 μg of chokeberry extract. The preliminary investigations in MI patients (n=10) in whom we used chokeberry extract (3x100mg/day) for 30 days demonstrated that their EPCs had a higher telomerase activity as compared to placebo. **Conclusions.** These results confirm the potential usefulness of natural antioxidants in the treatment of atherosclerosis, also through their effect on EPC senescence inhibition.

Funding: This work was supported in part by non-restricted grants from Polish Society for Atherosclerosis Research and from Agropharm (Aronia extract producer)

Workshop 8 "NUTRITION AND CVD"
4:30 PM - 6:45 PM
***Chairs:** S.M. Grundy, Dallas, TX, USA and D. Jenkins, Toronto, ON, Canada.*

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DIETARY EFFECTS ON ATHEROGENIC DYSLIPIDEMIA OF METABOLIC SYNDROME

***Ronald M. Krauss**. Children's Hospital Oakland Research Institute, Oakland, CA, USA.*

The atherogenic dyslipidemia of the metabolic syndrome consists of elevated plasma levels of triglyceride-rich lipoproteins and small, dense LDL particles, and reduced HDL-cholesterol (C). We sought to determine the effects on this lipoprotein profile of dietary carbohydrate reduction (54% vs. 39% vs. 26%) both with and without weight loss in 178 healthy men with BMI 29.2 ± 0.2 kg/m2. In addition, effects of lower (9%) vs. higher (15%) saturated fat intake were tested on the 26% carbohydrate diet. The 26% carbohydrate low-saturated fat diet reduced triglyceride, apoB, small, dense LDL mass and total/HDL-C ratio and increased LDL peak diameter compared with 54% carbohydrate. Following subsequent diet-induced weight loss and stabilization (-5.1 ± 1.8 kg), all these variables showed significantly greater changes, and LDL-C showed significantly greater reductions in the group that remained on the 54% carbohydrate vs. the group on the 26% carbohydrate diet. Lipoprotein changes with higher vs. lower saturated fat in the 26% carbohydrate diet were similar except for greater LDL-C reduction with low saturated fat. There were no differences, however, in reductions of small, dense LDL; the lesser reduction in total LDL-C with high saturated fat was due to greater offsetting increases in larger, more cholesterol-enriched LDL particles. Thus, similar improvements in atherogenic dyslipidemia can be achieved by both reduced carbohydrate intake in the absence of weight loss and by weight loss on a higher carbohydrate diet. Hence, for overweight individuals unable to maintain weight loss, moderate carbohydrate restriction offers a means of improving lipid-related cardiovascular disease risk. Moreover, on such a diet, reduction in small, dense LDL is independent of saturated fat intake.

Funding: National Dairy Council

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A DIETARY PORTFOLIO TO REDUCE BOTH LIPID AND OTHER RISK FACTORS FOR CHD

***David Jenkins**, Andrea R. Josse, Tri H. Ngyuen, Nishant J. Fozdar, Cyril W.C. Kendall. St. Michael's Hospital, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada.*

Objective: To design effective cholesterol lowering diets using combinations of recognized cholesterol lowering foods or food components. **Method:** Studies are now being undertaken using combinations of foods or food components recognized by the Food and Drug Administration (FDA) as reducing serum cholesterol. These include plant sterols, soy proteins, viscous fibers and nuts (almonds). Studies have tended to be of one month duration, especially those that are metabolically controlled. However, longer term *ad libitum* studies have also been undertaken up to 3 years duration. **Results:** Studies of combinations of 2 components given as supplements or incorporated into a dietary strategy have resulted in 4.0-17.5% reductions in low density lipoprotein cholesterol (LDL-C). Where 4 components have been combined in metabolic diets (Dietary Portfolio), 28.6-35.0% reductions in LDL-C have been achieved. The same dietary approach in an *ad libitum* context has resulted in LDL-C reductions in a group of 30 subjects, who completed 3 years of the Dietary Portfolio, of 17.1% at 1 year, 11.9% at 2 years and 11.6% at 3 years. In the Dietary Portfolio studies, reductions were also seen in C-reactive protein (CRP) neutrophil: lymphocyte ratio and blood pressure. **Conclusion:** As recommended by the NCEP ATP III, diets combining specific cholesterol lowering foods or food components may increase the effectiveness of dietary strategies for cholesterol reduction.

Funding: NSERC, Loblaws, Unilever, Almond Board of California, Canada Research Chair Endowment

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MACRO AND MICRO LIPID NUTRIENTS AND CARDIOVASCULAR DISEASES

***Claudio Galli**. University of Milan, Milano, Italy.*

Lipid macronutrients (fatty acids, FA) represent a major and qualitatively / quantitatively variable component of the diet. The amounts / proportions of the main FA in the diet and biological systems, i.e. saturates, monounsaturates and Omega 6 PUFA, affect various parameters with an impact on the CV system mainly through modulation of metabolic processes. In contrast, various nutrients, ingested in amounts of few tens to few hundred mg / day, e.g. lipid associated micronutrients and long chain (LC) Omega 3 FA, modulate CV parameters, directly affecting cell functions. Various steps are involved in the overall process. a. Micronutrients (lipophilic, e.g. Vitamin E, carotenoids, or hydrophilic compounds dispersed in lipid, e.g. phenolics in oils). The following steps play a major role: bioavailability (surface / volume of microdispersions), interactions with enterocytes (transporters, transcription factors, competition, inflammation interacting with the CV system). b. Selected and minor (less than 200 mg/day) FA, but relevant to CV health, i.e. the LC Omega 3 EPA and DHA. Still unresolved issues/inadequate data on: available sources; bioavailability from different sources, conversion of the short chain to the LC products; Omega 3 FA status in population groups, in relation to physiological and life style parameters; lipid pools and cellular targets of incorporation of the two main LC Omega 3 FA, EPA and DHA; differential mechanisms of action of these FA on CV processes; correlations between body levels and pathophysiological processes. More information is requested for proper recommendations and treatments. The above points will be discussed and pertinent data will be presented.

Funding: None

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LEPTIN RESISTANCE IN THE FRUCTOSE-FED RAT MODEL OF METABOLIC SYNDROME. INVOLVEMENT OF THE MAPK/ERK PATHWAY

Laia Vilà, Núria Roglans, María V. Perna, Marta Alegret, ***Juan C. Laguna****. University of Barcelona, School of Pharmacy, Barcelona, Spain.*

Fructose (F) ingestion in liquid solution induces hepatic leptin resistance in male Sprague-Dawley (SD) rats, with hyperleptinemia, a deficit in STAT3 phosphorylation at Ser 727 (PSer-STAT3) and a lack of AMPK activation (Roglans, *Hepatology* 2007;45:778-788). To ascertain the point in the leptin transduction cascade affected by F, two groups of 10 male SD rats each had free access to water (control group C) or to a 10 % F solution (F group). After 14 days, plasma and liver samples were obtained for determining plasma analytes and liver triglycerides, fatty acid B-oxidation activity (BOX), c-fos mRNA and protein levels of total and phosphorylated forms of leptin receptor (LR), ERK, MEK, STAT3, LKB1 and AMPK. Statistical analysis was done by the unpaired *t*-test, $P<0.05$. F *versus* C rats had hyperleptinemia (1.9 Fold Induction), hypertriglyceridemia (1.3 FI), increased liver triglycerides (1.6 FI) and STAT3 phosphorylation at Tyr 705 (1.9 FI), and decreased BOX (0.8 FI) and no change in PSer-STAT3 and P-AMPK. Protein levels of total and phosphorylated forms of ERK 1/2, MEK 1/2, and LKB1, kinases involved in the transduction of the leptin signal, were unmodified in F rats. c-fos mRNA level, increased by an active MAPK pathway thorough ERK 1/2, was also unchanged. F rats showed a 1.9 FI in the amount of LR phosphorylated at Tyr 1138, responsible for STAT3 activation (phosphorylation at Tyr 705), while the amount of LR phosphorylated at Tyr 985, related to the MAPK/ERK pathway activation, was unchanged. Thus, hepatic leptin resistance is related to a deficit in the MAPK/ERK pathway signal due to a lack of LR phosphorylation at Tyr 985.

Funding: Supported by FPCNL, IBUB, REDIMET and grant FIS PI 060247

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ANTHOCYANIN PREVENTS CD40-ACTIVATED PROINFLAMMATORY SIGNALING IN ENDOTHELIAL CELLS BY REGULATING CHOLESTEROL DISTRIBUTION

Wenhua Ling*. Sun Yat-Sen University, Guangzhou, China.*

Objective: Rafts are considered to function as platforms for the dynamic association of TNF receptor family members including CD40, which is critical for the activation of immune responses. However, whether signals induced by CD40 depend on this microdomains in endothelial cells, which were relevant to atherogenesis, are unknown. This study was to investigate the influence of anthocyanin on composition of lipid rafts and CD40-mediated proinflammatory events in human endothelial cells. **Methods and Results:** In this study, we reported that upon CD40 ligand (CD40L) stimulation of HUVECs with anthocyanin prevented from CD40-induced inflammation, measured by production of IL-6, IL-8, and MCP-1 through inhibiting CD40-induced NF-kappa B activation. TRAF-2 played pivotal role in CD40-NF-kappa B pathway as TRAF-2 small interference RNA (siRNA) diminished CD40-induced NF-kappa B activation and inflammation. TRAF-2 overexpression increased CD40-mediated NF-kappa B activation. Raft disorganization after methyl-beta-cyclodextrin diminished TRAF-2 localization into rafts and rendered it dominant negative for the activation of NF-kappa B by CD40. Exposure to anthocyanin not only interrupted TRAF-2 recruitment to lipid rafts but also decreased cholesterol content in insoluble lipid rafts. **Conclusion:** These findings identify a physiological role for lipid rafts as a critical regulator of CD40-mediated signaling and raise the possibility that certain pathologic conditions may be treated by altering CD40 signaling with drugs affecting its raft localization.

Funding: Supported by National Natural Science Foundation of China Research grants and China Medical Board of New York Inc (grant CMB 98-677)

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LUPIN PROTEINS REDUCE PROGRESSION OF LIPID-RICH CAROTID PLAQUES IN RABBITS FED A CHOLESTEROL-RICH DIET

Marta Marchesi, Cinzia Parolini, Stuart K. Johnson, Erika Diani, Elena Rigamonti, Lorena Cornelli, Cesare R. Sirtori, ***Giulia Chiesa****. University of Milan, Milan, Italy.*

Objective: In spite of the health claim, approved by the FDA, on the role of soy proteins in reducing the risk of coronary heart disease, soybean based foods are used to a modest extent in Western Europe. Lupin is a valuable alternative to soy, being a protein-rich legume, poor in anti-nutritional factors. The aim of the study was to evaluate the effect of lupin protein based diets on the progression of atheromatous lesions in New Zealand White rabbits. **Methods:** Focal lesions were induced, by electric current, on common carotid arteries of 18 rabbits. After surgery, animals were fed for 90 days a diet containing 1% cholesterol, 15% saturated fatty acids and 20% protein. The protein source was 20% casein (C), 10% casein and 10% lupin protein isolate (C+L), or 20% lupin protein isolate (L). Total cholesterol (TC) and triglyceride (TG) levels were measured at 0, 30, 60, 90 days of dietary treatment. At 90 days after surgery, rabbits were sacrificed and histological analysis of carotids was performed. **Results:** L fed rabbits displayed lower TC levels, compared to C fed animals, at 60 and 90 days after surgery ($p<0.05$). No differences in TG plasma concentrations were observed among the three groups at each time point analyzed. Histological analysis of carotids showed a significant reduction of focal lesion progression in L vs C fed rabbits (1.04x109±0.43 x109 vs 1.66x109±0.30 x109mm3; $p<0.05$). **Conclusions:** The results indicate, in this animal model, a cholesterol lowering activity and a reduced atherosclerosis progression associated with lupin protein based diets.

Funding: EEC project, Healthy-Profood, QLRT 2001-2235

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SOY PROTEINS: MECHANISM FOR CHOLESTEROL REDUCTION

Maria R. Lovati*, Cristina Manzoni, Anna Pizzagalli, Marcello Duranti. University of Milano, Milano, Italy.*

7S soy globulin and its alfa prime subunit have been shown to positively modulate LDL receptor (LDL-R) activity both in vitro and in vivo. Aim of the study was to evaluate the effect of a short chain of alfa prime subunit (S-alfa prime) from 7S soy globulin and of a synthetic peptide corresponding to a sequence which differs between the alfa and alfa prime subunits, on cell cholesterol homeostasis. Hep G2 cells were incubated in the presence/absence of alfa prime subunit ($3.5x10^{-6}$M) or the S-alfa prime or the synthetic peptide at concentration of 10^{-5} M and 10^{-6} M. Dose-dependent LDL-R up-regulation has been detected in cells exposed to S-alfa prime versus that found in controls. Moreover, LDL uptake (+ 192%) and degradation (+ 143%) of cells tested to the highest dose of S-alfa prime ($8x10^{-6}$ M) was similar to that found in cells incubated with 10^{-6}M simvastatin. Hep G2 cells exposed to the synthetic peptide showed a dose-dependent increase in the ^{125}I-LDL uptake (+ 38% and + 42%, respectively) and degradation (+46% and +53%, respectively) vs values recorded in non exposed cells; these results were confirmed by an increase in SREBP2 mRNA and LDL-R mRNA, by RT-PCR. These findings open new prospects in the studies aimed at identifying the regulatory peptide/s from soybean proteins and the mechanism/s involved in this biological response, in view of their potential utilization in the management of human lipid disorders.

Funding: Supported in part by a grant of MIUR of Italy

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ASSESSMENT OF THE EFFECTS OF A DIETARY PORTFOLIO OF CHOLESTEROL LOWERING FOODS IN HYPERCHOLESTEROLEMIA AFTER 3 YEARS

Nishant Fozdar, Tri H. Nguyen, David J.A. Jenkins, Cyril W.C. Kendall, Julia M.W. Wong, ***Andrea R. Josse****, Dorothea A. Faulkner, Kristi K. Srichaikul, Augustine Marchie, Edward Vidgen, Candice Holmes, Robert G. Josse, Lawrence A. Leiter, William Singer. St. Michael's Hospital, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada.*

Objective: To determine how sustainable the low density lipoprotein-cholesterol (LDL-C) and total cholesterol:high density lipoprotein cholesterol ratio (TC:HDL-C) reductions were over 3 years on a combination diet of cholesterol-lowering foods (dietary portfolio). **Methods:** Sixty-seven hyperlipidemic subjects were prescribed diets high in plant sterols (1.0 g/1000 kcal), soy protein (22.5 g/1000 kcal), viscous fibers (10 g/1000 kcal) and almonds (23 g/1000 kcal). LDL-C and TC:HDL-C data on the 31/66 subjects who completed the first 3 years are presented. **Results:** At 1, 2 and 3 years, the LDL-C and TC:HDL-C reductions were: year 1, -17.7% and -15.7%; year 2, -12.4% and -13.2%; and year 3, -12.4% and -13.6% (all results significant, $P<0.010$). **Conclusions:** These data indicate a reduction in dietary effectiveness after the first year on the diet. However, in this group of subjects, who represented the just under 50% who completed the three years, a sustained long term benefit was seen, especially in the TC:HDL-C ratio.

Funding: Canada Research Chair Endowment of the Federal Government of Canada

Workshop 9 "INFLAMMATION AND CVD"
2:00 PM - 3:40 PM
Chairs: *F. Crea, Rome, Italy and P.M. Ridker, Boston, MA, USA.*

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INFLAMMATION AND ACUTE VASCULAR EVENTS

Filippo Crea*. Catholic University of the Sacred Heart, Rome, Italy.*

It is well recognised that atherogenic stimuli like hypertension, hypercholesterolemia, smoking and diabetes cause endothelial dysfunction followed by chemo-attraction of inflammatory cells, which then migrate in the subendothelium and originate the atherosclerotic plaque. The latter can remain clinically silent for years or even for ever or become suddenly unstable. In a sizeable proportion of patients the sudden transition from the asymptomatic or stable phase of coronary artery disease to acute coronary syndromes is associated to an inflammatory outburst. The sudden activation of inflammatory cells in the unstable plaque results in the release of cytokines which have the potential to cause endothelial activation, plaque fissuring and vasoconstriction followed by thrombus formation. Recent observations suggest the intriguing possibility that inflammation is not limited to the culprit stenosis, but it is widespread in the whole coronary circulation. The triggers of inflammation associated to acute coronary syndromes are probably multiple and still largely unknown. Several studies have shown that dysregulation of T cell repertoire may play a key pathogenetic role. The intensity of the inflammatory outburst associated to coronary instability, as assessed by measuring serum levels of markers of inflammation, is a powerful independent predictor of short-medium term outcome, even in the presence of optimal currently available treatment. A better knowledge of the triggers and mechanisms of inflammation is warranted to further improve the outcome in this setting.

Funding: None

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INFLAMMATION AND THE CHRONIC DISEASES OF OLDER AGE

Russell P. Tracy. *University of Vermont, Colchester, VT, USA.*

"Inflammation" is a set of interconnected processes including innate and adaptive immunity, coagulation and fibrinolysis, and metabolic or endocrine pathways such as glucose and cholesterol metabolism. Contrary to previous belief, these systems are chronically activated at very low levels. Inflammation biomarkers reflect these systems, yield similar pathophysiological and clinical information, have different contributions to aging across the lifespan and different meanings at different ages with respect to disease prediction. Some of the evidence that contributes to this thinking has been obtained through the study of results obtained from clinical trials of drug-based interventions; e.g., drugs that affect cholesterol metabolism. Some interventions may have different effects at different disease stages and/or ages; e.g., anti-TNF therapy in rheumatoid arthritis compared to congestive heart failure. We pose the hypothesis that aging is the accumulation of damage which results from the chronic activity of these systems. Through the constant utilization of these interfacial response mechanisms, the organism trades short-term benefit for long-term damage, a position that is at least moderately supported by evolutionary biology concepts. In support, we and others have determined that inflammation biomarkers are associated with most (all) chronic diseases of aging. While this makes these biomarkers less specific, it does point out the interconnectedness of this aging-related pathophysiology. In support, genetic polymorphisms in genes related to inflammation are emerging as risk factors for these chronic diseases. Understanding these systems in enough detail to interrupt inflammation in a targeted manner – leaving important responses systems intact – may be a critical endeavor in the near term.

Funding: Supported by NIH RO1 HL077499

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PPARα ACTIVATION OF MACROPHAGES INHIBITS THE SECRETION OF INFLAMMATORY CYTOKINES IN CULTURED ADIPOCYTES

Yasushi Saito, *Kentaro Murakami, Hiroyuki Unoki, Hideaki Bujo. Chiba University Graduate School of Medicine, Chiba, Japan.*

The relationship between adipocytes and infiltrated macrophages in fat tissue is important for the pathogenesis of insulin resistance. PPARs play a role in the regulation of cytokine secretion. The aim is to study the effect of the activation of macrophages by PPARα agonist, K-111 (2,2-dichloro-12-(4-chlorophenyl) dodecanoic acid) on the modulation of TNFα expression in adipocytes using a cell culture system. A conditioned medium of LPS-stimulated RAW264.7 cells, a macrophage cell line, induced the level of TNFα mRNA in 3T3-L1 adipocytes. This effect was inhibited by the addition of neutralizing antibody against IL-6 in the conditioned medium or the preincubation of RAW264.7 cells with K-111. K-111 reduced both the IL-6 production and mRNA expression in RAW264.7 cells, and its effect was stronger than that of Rosiglitazone. The activation of the stress-activated protein kinase/c-Jun NH2-terminal kinase (SAPK/JNK) pathway and NF-κB subunits of p65 was significantly inhibited by K-111. The blocking of IL-6 production through the SAPK/JNK pathway or by transfection with siRNA specific for IL-6 abolished the inhibitory effect of K-111 on the TNFα expression in the 3T3-L1 adipocytes. As a result, the IL-6 produced by RAW264.7 cells is an inducer of TNFα expression in 3T3-L1 adipocytes, and the IL-6 secretion is inhibited by the activation of PPARα. K-111 may suppress the pathogenetical secretion of TNFα in adipocytes through the functional modulation of infiltrated macrophages.

Funding: Granti-in-aid for Scientific Research 2006

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ANTI-INFLAMMATORY AND ANTI-ATHEROGENIC EFFECTS OF ACETYL-11-KETO-b-BOSWELLIC ACID IN LPS-CHALLENGED APOLIPOPROTEIN E-DEFICIENT MICE

***Mustapha Rouis**, Clarisse Cuaz-Perolin, Eric Bauge, Daniel Scott-Algara, Thomas Simmet. INSERM, Lille, France; Pasteur Institute of Paris, Paris, France; University of Ulm, Ulm, Germany.*

Objectives- Micro-organisms might play a role in chronic inflammatory disease such as atherosclerosis. We studied the effect of acetyl-11-keto-b-boswellic acid (AKbBA), a molecule isolated from the gum resins of various *Boswellia* species, on atherosclerotic lesion development in apolipoprotéin E deficient (apoE$^{-/-}$) mice. **Methods-**Atherosclerotic lesions were induced by weekly lipopolysaccharide (LPS) injection in apoE$^{-/-}$ mice to mimic chronic inflammation. **Results-** Our results showed that LPS alone increases atherosclerotic lesion size and that the treatment with AKbBA-cyclodextrin significantly reduced it (~50 % reduction vs. control (100%), n=8, $p<0.001$). Moreover, the activity of the nuclear transcription factor NF-kB was also reduced in LPS-injected apoE$^{-/-}$ mice and treated with AKbBA-cyclodextrin as judged by the increase of the IkBa in the cytosol and the decrease of nuclear staining of the p65 subunit. As a consequence, a significant down-regulation in the expression of several genes, among which MCP-1, MCP-3, IL-1a, MIP-2, VEGF and TF was observed. By contrast, neither g-cyclodextrin nor AKbBA-cyclodextrin complex were able to affect the plasma concentration of triglycerides, total cholesterol, anti-oxidized LDL antibodies and various subset of lymphocyte-derived cytokines. **Conclusion-** The inhibition of NF-kB activity by plant resins from species of the *Boswellia* family might represent an alternative for classical medicine treatments for chronic inflammatory diseases such as atherosclerosis.

Funding: Grants from the Académie Nationale de Médecine, INSERM and CHRU de Lille

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THE MIAMI STUDY (MARKERS OF INFLAMMATION AND ATORVASTATIN EFFECT IN PREVIOUS MYOCARDIAL INFARCTION): RESULTS OF A PROSPECTIVE, OPEN-LABEL, MULTICENTER STUDY

***M. Amato**, D. Baldassarre, B. Porta, M. Camera, M. Arquati, F. Veglia, E. Tremoli, M. Cortellaro, on behalf of the MIAMI Study Group. Cardiologico Monzino Center, IRCCS, Milan, Italy; University of Milan, Milan, Italy; L. Sacco Hospital, Milan, Italy.*

Objective: To investigate the relationship between changes induced by atorvastatin in carotid IMT (C-IMT) and changes in soluble markers of inflammation, thrombosis and endothelial function. **Methods**: Patients with stable ischemic heart disease (n=85) were treated with 20 mg/day of atorvastatin for 20±4 months. C-IMT, soluble markers (sVCAM-1, sICAM-1, sE-selectin, IL-6, IL-8, IL-18, TNFα, hsCRP, vWF, CD40L, MMP9, fibrinogen) and lipids were measured at times 0, 12 and 24 months. **Results:** Atorvastatin induced C-IMT regression ($p=0.004$ for IMT_{mean}) and significantly reduced plasma levels of triglycerides, total-C, LDL-C, vWF, sICAM-1, sE-selectin, fibrinogen (all $p<0.0001$), IL-8 ($p=0.004$), MMP9 and TNFα (both $p<0.05$). HDL-C, IL-6 and CD40L increased in response to therapy ($p<0.05$), whereas hsCRP, IL-18, and sVCAM-1 did not change. Changes in lipids and in soluble markers were poorly correlated with C-IMTs changes when analyzed singly. In contrast, the combination of changes in soluble markers (soluble marker-score), soluble markers and lipids (total-score) or biologically-related variables (inflammatory-score, interleukin-score and adhesion molecule-score) strongly correlated with the effects of atorvastatin on carotid IMT ($p=$ 0.007, 0.002, 0.04, 0.003 and 0.17, respectively). **Conclusion:** The anti-atherosclerotic effect of atorvastatin could be explained, at least in part, by pleiotropic effects on markers of inflammation, thrombosis and endothelial dysfunction.

Funding: None

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EFFECTS OF PITAVASTATIN ON LIPID PROFILES AND HIGH-SENSITIVITY CRP IN JAPANESE SUBJECTS WITH HYPERCHOLESTEROLEMIA

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Effects of pitavastatin (Ptv, 1-2mg, for 12 months) on lipoproteins and high-sensitivity C-reactive protein (hs-CRP) were examined in 181 Japanese hypercholesterolemic subjects, including those with type 2 diabetes (DM; n=102). Serum low-density lipoprotein cholesterol and remnant-like particle cholesterol levels were significantly decreased by 28.3 and 25.2%, respectively. In subjects previously not treated with other statins, Ptv (2mg) lowered LDL-C by 36.3%. Serum triglycerides were decreased by 15.4% in subjects with basal levels above 150mg/dL. High-density lipoprotein cholesterol was increased by 6.8% in total and by 17.9% in subjects with basal levels below 40mg/dL. Hs-CRP was significantly decreased (median: 0.69 to 0.45mg/L, -34.8%, $p<0.01$). Furthermore, hs-CRP was more dramatically decreased in subjects with baseline values in the highest quartile (median: 2.8 to 1.31mg/L, -53.2%, $p<0.01$). Ptv similarly reduced hs-CRP levels in subjects with DM. No serious adverse events were observed, including glycosylated hemoglobin levels. In conclusion, Ptv improves lipid profiles and reduces inflammation in hypercholesterolemic subjects with safety.

Funding: Funded by Kowa

Workshop 10 "ATHEROSCLEROTIC PLAQUE"
4:30 PM - 6:25 PM
Chairs: *A. Newby, Bristol, UK and G. Steiner, Toronto, ON, Canada.*

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LIPIDS, EXTRACELLULAR PROTEASES, AND PLAQUE INSTABILITY

Andrew C. Newby*. University of Bristol, Bristol, United Kingdom.*

Oxidised LDL is taken up in to endothelial cells, vascular smooth muscle cells and macrophages at all stages of atherosclerosis. One consequence of Ox-LDL uptake is increased expression of extracellular proteinases including metalloproteinase, plasminogen activators and cathepsins. At early stages of atherogenesis extracellular proteinases synergise with growth factors and cytokines to promote migration and proliferation of vascular cells, which probably results in increasing plaque burden and fibrous cap formation. In late stages extracellular proteinases promote matrix destruction, neoangiogenesis and apoptosis of vascular cells all of which are associated with plaque rupture leading to myocardial infarction. This presentation will briefly summarise available clinical and experimental data that supports the dual role of proteases in plaque progression and instability. Successful drug therapy to stabilise vulnerable plaques depends on identifying those features of extracellular proteolysis that are specifically associated with instability. The talk will describe new experimental approaches to study foam cell macrophages and consider 2 hypotheses: instability is caused simply by higher expression levels of the same proteases, certain proteases or combinations of proteases are expressed selectively in vulnerable plaques. Candidate drug therapies based on each hypothesis will be discussed. In particular the efficacy of direct protease inhibitor treatment will be compared and contrasted with therapies based on reducing protease production from vascular cells.

Funding: British Heart Foundation, European Genomics Network

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DIABETES AND CORONARY RISK

George Steiner. *Toronto General Hosp & University of Toronto, Toronto, ON, Canada.*

The prevalence of diabetes, particularly type 2 diabetes, is increasing worldwide at an epidemic rate. Two-thirds or those with diabetes will die of cardiovascular disease. There is general agreement that the coronary risk of those with diabetes is 2 to 4 times greater than that in those without diabetes. However, risk calculators such as that of the UKPDS demonstrate that the actual risk in those with type 2 diabetes is also dependent on the coexistence of other risk factors. Several studies suggest that the coronary risk in those with diabetes is equivalent to that in people who have had a myocardial infarct but do not have diabetes. On the other hand several studies, while demonstrating an increased coronary risk do not find it to be as great as that in people with coronary disease alone. The differences in conclusions probably relate to study design, gender studied, endpoint chosen and duration of follow-up. All agree that the greatest risk is in those with diabetes who have had a prior infarct. Furthermore, the short and long term outcome after a heart attack for those with diabetes is much poorer than in those without diabetes. The current LDL-C targets for those with diabetes are based on these considerations. There is less evidence with respect to HDL and triglyceride. However, if they are still abnormal when the LDL-C is at target, current reasoning suggests that combining the LDL-C lowering therapy with treatment designed to correct abnormalities in HDL-C and triglyceride should be beneficial.

Funding: None

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MACROPHAGE EXPRESSION OF STEROIDOGENIC ACUTE REGULATORY PROTEIN (STAR;STARD1) ENHANCES APOA1 DEPENDENT CHOLESTEROL EFFLUX

J.M. Taylor, *C. Bartholomew, A. Graham. Glasgow Caledonian University, Glasgow, United Kingdom.*

This study utilises StarD1 protein to increase cholesterol transfer to mitochondrial CYP27A1, increasing macrophage cholesterol efflux to apoAI. Murine RAW264.7 macrophages were stably transfected with pCMV.5, pCMV.5_*StarD1* or pCMV.5_*StAR(R181L)*. Macrophage [^{3}H]cholesterol efflux (24h) was measured in the presence or absence of apoA1 (20μg ml^{-1}), dibutyryl cAMP [$(Bu)_2$cAMP; 0.3mM], and/or CYP27A1 inhibitor (GW2739297x, 1μM), LXR agonist (T01317, 10μM), and LXR antagonist (geranyl geranyl pyrophosphate, GGPP, 10μM). Mature StarD1 protein (30kDa) was detected in macrophages transfected with pCMV.5_*StarD1*, but not in empty vector (EV) or wild type (WT) controls. Expression of StarD1 enhanced (1.8-1.9 fold; $p<0.001$) apoAI-dependent cholesterol efflux in the presence of $(Bu)_2$cAMP, compared to EV, or WT cells. Macrophages stably expressing mutated StarD1(R181L) did not show enhanced cholesterol efflux compared with EV controls. The positive impact of StarD1 on cholesterol efflux was reduced ($p<0.001$) to that in EV controls, by inhibition of GW2738287x, or GGPP. By contrast, addition of T01317 induced a greater increase in cholesterol efflux in EV controls, (62.6%; $p<0.001$) compared to macrophages expressing StarD1 (24.5%, $p<0.05$). Midway (12h) during the efflux period, where maximal cholesterol efflux was induced, levels of ABCA1 protein were substantially higher in macrophages expressing StarD1 protein relative to GADPH. Macrophage expression of StarD1 increases apoAI-dependent cholesterol efflux, via a pathway involving CYP27A1 activity, LXR agonism and induction of ABCA1 protein expression, suggesting a novel strategy for atheroma regression.

Funding: Our work was funded by the British Heart Foundation (PG/04063/17186)

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EXPRESSION OF STARD1 AND STARD4 SUB-FAMILIES OF LIPID BINDING PROTEINS DURING MACROPHAGE DIFFERENTIATION AND METHYL-β-CYCLODEXTRIN CHOLESTEROL DEPLETION

***F. Borthwick**, J.M. Taylor, A. Graham, C. Bartholomew. Glasgow Caledonian University, Glasgow, United Kingdom.*

Steroidogenic acute regulatory protein (StAR) related-lipid transfer proteins may play a role in cholesterol homeostasis and atherogenesis, and their conserved lipid binding 'START' domains represent potential drug targets. We investigated expression of these proteins during phorbol ester-induced differentiation of human THP-1 macrophages, and in response to cholesterol depletion/lipid raft disruption by methyl b-cyclodextrin (MCD, 10mM; 1h), using qualitative/quantitative PCR and Western blotting techniques. Twelve of 15 START family genes are expressed in THP-1 macrophages, including StAR (STARD1), previously assumed steroid-specific. Differentiation increases (3.3-5.8-fold; $p<0.05$) macrophage cholesterol, phospholipid and triacylglycerol mass, and up-regulates mRNA for STARD1 and STARD4 subfamilies. STARD1 protein levels increases (5.2-fold; $p<0.05$), but STARD3 protein levels decrease (2.6-fold; $p<0.05$) after 4 days differentiation. Cholesterol depletion (30%; $p<0.05$) by MCD down-regulates STARD1, STARD3 and STARD5 mRNA 4h post-treatment. Cholesterol (1.4mM)-MCD complex increases macrophage cholesterol mass (3-fold; $p<0.05$), and up-regulates STARD1, STARD3 and STARD5 mRNA levels, 4h post-treatment. By contrast, MCD up-regulates STARD4 mRNA levels, while cholesterol-MCD has the opposite effect, 4h post-treatment; this effect is maintained 24h post-treatment. Regulation of STARD1 and STARD4 subfamilies of 'START' lipid transport proteins may reflect changes in macrophage intracellular cholesterol trafficking, suggesting these proteins may influence macrophage lipid phenotype and atherogenesis.

Funding: The British Heart Foundation support JMT (PG/04/063/17186)

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CHOLESTEROL EFFLUX MECHANISMS IN ENDOTHELIAL CELLS OF THE HUMAN PLACENTA

***Ute Panzenboeck**, Jasminka Stefulj, Cornelia Schweinzer, Gernot Desoye, Christian Wadsack. Medical University, Graz, Austria.*

Cholesterol is essential for fetal development. A large proportion of the fetal cholesterol demand is synthesized in fetal organs. However, fetuses with defects in endogenous cholesterol synthesis exhibit no birth defects, demonstrating the existence of external cholesterol sources. For maternal-fetal transfer, maternal cholesterol must be taken up on the maternal, apical side of the syncytiotrophoblast layer, released at the basolateral side, and subsequently traverse the fetal endothelial cell layer of the placenta, whose apical surface directly connects to the fetal circulation. During the present *in vitro* study we analysed the final step in this proposed pathway of transplacental transfer of (lipoprotein) cholesterol, efflux of cholesterol from fetal placenta endothelial cells (PECs). Primary arterial and venous PECs from human placenta were separately isolated. The capacity of PECs to efflux cholesterol to either HDL_3 or lipid-free apoA-I was tested using [^{3}H]-cholesterol labeled PECs. Significant time- and dose-dependent cholesterol efflux to both acceptors was observed. Induction of cholesterol efflux pathways upon oxysterol treatment suggested the involvement of LXR-regulated lipid transporters, ABCA1, ABCG1 and/or ABCG4. In line, oxysterol-inducible cholesterol efflux was reflected by up-regulated mRNA and protein expression levels of ABCA1 and ABCG1 in PECs pre-treated with LXR agonists. Our data suggest that the apoA-I/ABCA1 and the HDL_3/ABCG1 cholesterol efflux pathways are operative in PECs and can be modulated by LXR activation.

Funding: Supported by the Medical University Graz – Research Focus Reproduction & Pregnancy, and by the Austrian Science Fund P1474-B09

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DENSE HDL3 POTENTLY ATTENUATE APOPTOSIS INDUCED BY OXIDISED LDL (oxLDL) IN ENDOTHELIAL CELLS: IMPLICATION OF APOLIPOPROTEIN A-I AND SPHINGOSINE-1-PHOSPHATE

J.A. de Souza, C. Vindis, A. Negre-Salvayre, P. Therond, S. Chantepie, R. Salvayre, ***M.J. Chapman****, A. Kontush. Hopital Pitie, Paris, France; CHU Rangueil, Toulouse, France.*

Plasma HDL exert antiapoptotic activity. The relationship of HDL particle heterogeneity to antiapoptotic activity is indeterminate. Human microvascular endothelial cells (HMEC-1) were incubated with mildly oxLDL in the presence or absence of physicochemically-defined HDL subfractions isolated from normolipidemic human plasma (n=7) by density gradient ultracentrifugation. All HDL subfractions protected HMEC-1 against oxLDL-induced apoptosis as revealed by nucleic acid staining, annexin V binding, quantitative DNA fragmentation, inhibition of caspase-3 activity and reduction of cytoplasmic release of cytochrome c. Dense HDL3c displayed twofold superior intrinsic cytoprotective activity (as determined by cellular mitochondrial dehydrogenase activity) relative to HDL2b and 3a ($p<0.05$). Equally, all HDL subfractions attenuated oxLDL-induced generation of intracellular reactive oxygen species; such antioxidative activity diminished progressively from HDL3c to HDL2b ($p<0.01$). Apolipoprotein components accounted for 70% of HDL antiapoptotic action. HDL particle content of sphingosine-1-phosphate, a bioactive lipid, was specifically enriched in HDL3 vs. HDL2 (3-fold; $p<0.01$) and positively correlated with both HDL antiapoptotic and antioxidative activities. Finally, HDL antiapoptotic activity was attenuated (-68%) by antibody ligation of scavenger receptor BI (SRBI). In conclusion, dense HDL3c potently protect endothelial cells from oxLDL-induced apoptosis and from intracellular ROS generation via an SRBI-dependent pathway; apolipoprotein A-I and sphingosine-1-phosphate are implicated in such vasculoprotective activity.

Funding: INSERM, ARLA, ANR

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DAMAGED ENDOTHELIAL GLYCOCALYX IN FAMILIAL HYPERCHOLESTEROLEMIA PARTIALLY RECOVERS DURING STATIN THERAPY

Marijn C. Meuwese*, Max Nieuwdorp, Bart van Lith, Roos Marck, Hans Vink, John J. Kastelein, Erik S. Stroes. Academic Medical Center, Amsterdam, Netherlands; Maastricht University, Maastricht, Netherlands.*

Objectives: The endothelial glycocalyx, covering the inner surface of the vessel, constitutes a protective barrier between the flowing blood and the endothelium. Experimental models have indicated that loss of glycocalyx increased atherogenic vulnerability. We evaluated whether hypercholesterolemia is associated with glycocalyx perturbation in humans and whether this could be reversed by intensive statin treatment. **Methods:** We measured systemic glycocalyx volumes in 13 patients with familial hypercholesterolemia (FH) after >4 weeks cessation of therapy and after 8 weeks of intensive statin therapy (rosuvastatin 40 mg QD), as well as in 13 normocholesterolemic controls. Glycocalyx volume was estimated by subtracting the intravascular distribution volume of a glycocalyx impermeable tracer (erythrocytes) from that of a glycocalyx permeable tracer (dextran 40). **Results:** Glycocalyx volume in untreated FH patients (LDL 225±57 mg/dL (mean±SD)) was substantially reduced compared to controls (LDL 93±24 mg/dL); 0.8±0.3 L vs. 1.7±0.6 L ($p<0.001$). LDL was negatively correlated to glycocalyx volume ($\rho=-0.73$, $p<0.001$). In spite of normalization of LDL upon therapy (95±33 mg/dL) glycocalyx volume increased without reaching control levels (1.1±0.4 L; $p=0.04$). **Conclusions:** Glycocalyx volume is strongly reduced in FH patients and partly restores upon intensive statin therapy. Awaiting trials validating the protective role of an intact glycocalyx, the present findings suggest a need for novel interventions aimed at additional restoration of the glycocalyx.

Funding: AstraZeneca funded Rosuvastatin

Workshop 11 "SPECIAL POPULATIONS"
2:00 PM - 4:05 PM
Chairs: *E. Barrett-Connor, La Jolla, CA, USA and K.E. Watson, Los Angeles, CA, USA.*

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MANAGEMENT OF DYSLIPIDEMIA IN WOMEN

JoAnne Foody*. Yale University School of Medicine, New Haven, CT, USA.*

Cardiovascular disease (CVD) ranks first among all disease categories in hospital discharges for women and nearly 39 percent of all female deaths in America occur from CVD, which includes coronary heart disease (CHD), stroke and other cardiovascular diseases. CVD is a particularly important problem among minority women. Given these staggering statistics, it is imperative to aggressively modify risk factors in an effort to prevent CVD events. The lowering of LDL cholesterol is a potentially important strategy to reduce risk in a wide range of women at risk or with CVD. Epidemiology of CVD in women, unique aspects of dyslidemia in women and strategies for the modification of LDL cholesterol will be discusssed.

Funding: None

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MANAGING VASCULAR RISK IN THE ELDERLY

James Shepherd*. Royal Infirmary, Greater Glasgow & Clyde NHS, Glasgow, United Kingdom; Glasgow Royal Infirmary, Glasgow, United Kingdom.*

Appropriateness of giving statins to seniors in our population has fomented lively debate ever since these drugs were introduced into clinical practice in the late 1980s. The antagonists say that since serum cholesterol no longer predicts vascular risk in the aging, there seems little point in squandering limited valuable resources on statin therapy for them. On the other hand, acceptance of the value of statin therapy for preventing vascular disease in the elderly has recently gained momentum from analysis of the benefits seen in limited numbers of older subjects recruited into the early statin endpoint trials. The Scandinavian Simvastatin Survival Study, the CARE (Cholesterol and Recurrent Events) trial, LIPID (Long-term Intervention with Pravastatin in Ischaemic Disease) and, most recently, the Heart Protection Study all contained subgroups of participants aged >70 years. They all showed consistent benefit in terms of reduced vascular risk. These subgroup analyses were strongly buttressed by the results of the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER), a trial seeking to determine whether pravastatin could specifically reduce the impact of vascular disease on an older cohort (70-82 years) of 5,804 men and women with a history of existing vascular disease or who were at high risk for this condition. Statin treatment for 3.2 years produced a 15% reduction in the combined risk of fatal and nonfatal heart attack and strokes and a 24% fall in coronary heart disease death. Translation of the results of PROSPER into clinical practice would encourage physicians to intervene actively with statins in older adults, many of whom are living with a higher risk of vascular disease.

Funding: None

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LIPIDS, ETHNICITY, AND RISK: HOW LIPID LEVELS MEAN DIFFERENT THINGS IN DIFFERENT RACES

***Karol E. Watson**, Preethi Srikanthan. Geffen School of Medicine at UCLA, Los Angeles, CA, USA.*

There is a complex interplay between genetic and cultural factors that influences the expression of lipid levels in various individuals and between groups of people. It is therefore not surprising that differences in lipids have been reported between ethnic groups. The epidemiologic relationship between serum cholesterol and the risk of coronary heart disease (CHD) is well documented, and despite differences in the prevalence of CHD between various populations, the relative increase in CHD mortality rates for a given cholesterol increase is remarkably consistent. Differences in plasma lipoprotein levels, however, have been reported between ethnic groups and because of the multi-cultural nature of today's society; this has important implications for clinical practice. In recent years information on the relationship between lipid levels and insulin resistence has also been studied. African Americans as a group, have higher rates of insulin resistance than non Hispanic Whites, yet paradoxically, have lower rates of the characteristic dyslipidemia of insulin resistance (low HDL-C, high triglycerides and small, dense, triglyceride enriched LDL-C). In clinical practice elevated triglycerides are often used as evidence that insulin resistance is present while normal triglycerides are used as evidence that insulin resistance is absent. The relationship between insulin resistance and triglyceride levels, however, differs by race-ethnicity and may confuse or confound the diagnosis of insulin resistance. Understanding of differences between lipid levels in various ethnic-minority groups will help to identify individuals at risk for development of cardiometabolic disease and will likely improve outcomes.

Funding: NIH/NHLBI N01-HC-95160

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RISK FACTORS FOR CARDIOVASCULAR DISEASE, WAIST CIRCUMFERENCE AND BODY MASS INDEX IN CHILDREN

***P. Schwandt**, G.M. Haas, K.G. Parhofer, E. Liepold. Arteriosklerose-Praeventions-Institut, Muenchen, Germany; LMU, Muenchen, Germany.*

Objectives: International references for BMI and blood pressure (BP) are available for children.We developped age- and gender specific percentile curves on waist circumference (WC) and lipids for German children aged 3-11 years. **Subjects and methods**: In 1571 children (780 boys) from 92% of all elementary schools in Nuremberg percentile (pc) curves for WC and fasting lipids were constructed by LMS Chart Maker. For statistics SPSS 14.0. **Results:** Girls had higher lipids ($p<0.01$) and lower WC than boys, while BP was not different. The prevalence of risk factors in girls/boys was for: LDL-C >130 mg/dl 18/13%, HDL-C <40 mg/dl 11/6%, TG >110 mg/dl 8/4%, hypertension >95th pc 6/3%, overweight (BMI >90th pc) 5/5%, WC >90th pc 4/9%. In both sexes hypertension correlated ($p<0.001$) with overweight (BMI as well as WC) and low HDL-C with hypertriglyceridemia. 31% of the girls had one and 8% two risk factors Vs 22% and 5% of the boys. The area under the ROC curves for 2 risk factors was >0.7 for BMI and WC, >0.6 for lipids in boys and in girls >0.7 for non-HDL-C, >0.6 for BMI, WC and for all the other risk factors except HDL-C. Age- and gender specific LMS curves show at the 50th p.c. a continuos decrease for LDL-C and increase for HDL-C in both sexes. In boys triglycerides decreased continuously by 13 mg/dl from age 3 to age 11, and increased by 5 mg/dl in girls. WC continuously increased in boys by 15 cm (29%), in girls by 12 cm (24%), as did BMI by 2.5 (17%) in boys and by 3.1 kg/m² (22%) in girls. BP curves did not differ. **Conclusions:** Age- and gender-specific percentile curves in children <12y show considerable differences for different age.

Funding: Foundation for the Prevention of Arteriosclerosis, Bavarian Ministry of Health

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A SYSTEMATIC REVIEW AND META-ANALYSIS OF STATIN THERAPY IN CHILDREN WITH FAMILIAL HYPERCHOLESTEROLEMIA

***Hans J. Avis**, Maud N. Vissers, Evan A. Stein, Frits A. Wijburg, Mieke D. Trip, John J.P. Kastelein, Barbara A. Hutten. AMC, Amsterdam, Netherlands; Metabolic and Atherosclerosis Research Centre, Cincinnati, OH, USA.*

Objectives: Functional and morphological changes of the arterial wall already present in young children with heterozygous familial hypercholesterolemia (HeFH) suggest that treatment should be initiated early in life to prevent premature atherosclerotic cardiovascular disease. This study assesses the efficacy and particularly safety of statin therapy in children with HeFH. **Methods:** We performed a meta-analysis of randomized, double-blind, placebo-controlled trials evaluating statin therapy in children aged 8-18 years with HeFH. **Results:** Six studies (n=798) with 12 to 104 weeks of treatment were included. Total cholesterol, LDL-cholesterol and apolipoprotein B were significantly reduced whereas HDL-cholesterol and apolipoprotein A1 were significantly increased by statin therapy. No statistically significant differences were found between statin and placebo treated children with respect to the occurrence of adverse events (RR 0.99; 95%CI: 0.79 to 1.25), sexual development (RR of advancing ≥ 1 stage Tanner classification 0.96; 95%CI: 0.79 to 1.17), muscle toxicity (RR of CK ≥ 10 times the upper limit of normal (ULN) 1.38; 95%CI: 0.18 to 10.82) or liver toxicity (RR of ≥ 3 times the ULN for ASAT 0.98; 95%CI: 0.23 to 4.26 and for ALAT 2.03; 95%CI: 0.24 to 16.95). We found a minimal difference in growth in favour of the statin group (0.33 cm; 95%CI: 0.03 cm to 0.63 cm). **Conclusion:** In addition to the fact that statin treatment is efficacious, our results support the notion that statin treatment in children with HeFH is safe.

Funding: None

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AGE- AND GENDER-SPECIFIC WAIST CIRCUMFERENCE PERCENTILES IN GERMAN AND TURKISH CHILDREN PARTICIPATING IN THE PEP FAMILY HEART STUDY

***G.M. Haas**, E. Liepold, P. Schwandt. Arteriosklerose-Praeventions-Institut, Muenchen, Germany.*

Objective: Because no international reference values exist and > 25% in the multiethnic PEP Study are not German, waist circumference percentiles were assessed in German and Turkish children. **Subjects and methods:** 4784 children (3-11 y) participated in this study (74.6% German and 6.5% Turkish), WC was measured according to WHO recommendations by trained research assistants. For statistics SPSS 14.0 and for constructing percentile curves LMS Chart Maker Pro were used. **Results:** Smoothed age- and gender- specific percentile curves demonstrate higher WC values among Turkish as compared to German children at all percentiles in all age groups. Turkish children had higher mean WC values (by 2.9 cm) than the German at the 50th percentile. At the 97th percentile the mean WC values were higher by 4.2 cm in boys and 9.5 cm in girls. This difference between Turkish and German boys continuously decreased from age 3 to 11y from 8.1 cm to 1.3 cm at the 97th percentile. In contrast, among girls the mean WC difference between both ethnic groups increased from –0.3 to 20.1 cm at the age of 3 to the age 11 at the 97th percentile. The age- and gender-specific percentile LMS curves for BMI confirm that overweight and obesity in Turkish children is more prevalent than in German children: Their mean BMI at the 50th percentile is by 1.0 kg/m² higher in boys and by 1.4 kg/m² in girls and at the 97th percentile by 3.3 kg/m² respectively 3.7 kg/m². **Conclusions:** Turkish children aged 3-11 years living in Nuremberg have by 2.9 cm higher WC values than German children at the 50th percentile and even by 9.5 cm higher for girls at the 97th percentile. Abdominal overweight is more prevalent in children of Turkish migrants.

Funding: Foundation for the Prevention of Arteriosclerosis

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THE HORMONES PREVENT HEART DISEASE IN YOUNGER WOMEN HYPOTHESIS

***Elizabeth Barrett-Connor**. University of California San Diego, La Jolla, CA, USA.*

The news from the Women's Health Initiative was unexpected. Despite many observational studies showing postmenopausal hormone therapy (HT) was cardioprotective, and diverse in vivo and in vitro studies supporting biologic plausibility, none of the controlled clinical trials of HT showed a reduced risk of heart attack, including many smaller trials using different hormone regimens. Among many putative explanations, the one currently in favor is that the women were too old (they already had atherosclerosis), and that estrogen could not reverse plaque burden, but could prevent or delay it. Another hypothesis is that estrogen could both retard atherosclerosis and increase events in women who already had atherosclerosis. An ancillary study from the Women's Health Initiative could have tested this hypothesis by measuring coronary artery plaque burden in the cohort after the absent cardiac protection was observed. Ultrafast computed tomography makes it possible to examine coronary artery calcium score, shown to parallel the amount of pathology and predict future coronary events. The trial was large, so plaque burden imbalance at baseline would have been unlikely; the cost would have been modest compared to the cost of the Women's Health Initiative. Unfortunately the ancillary study of the Women's Health Initiative that recently measured coronary artery calcium in 1000 trial participants aged less than 60 included no older women. Thus there has been no possibility to answer the younger vs. older women question. The results of this study are not yet known, but the study design deserves discussion and debate. This and future studies and what to do meanwhile will be addressed, as will implications for women's health of this younger-women-only design.

Funding: None

Workshop 12 "NUCLEAR RECEPTOR AND LIPID METABOLISM"

4:30 PM - 6:40 PM

***Chairs:** J.-C. Fruchart, Lille, France and D.P. Hajjar, New York, NY, USA.*

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NUCLEAR RECEPTORS AND COREGULATORS IN THE CONTROL OF CELLULAR METABOLISM

*Nico Mitro, Emma De Fabiani, Federica Gilardi, Cristina Godio, Elena Scotti, Elise Gers, Andrea Galmozzi, Donatella Caruso, **Maurizio Crestani**. University of Milano, Milano, Italy.*

Nuclear receptors (NRs) are ligand-dependent transcription factors that regulate gene expression in response to a variety of environmental cues. Here, we discuss the role of some NRs and the associated coregulators in the regulation of bile acid metabolism and cholesterol homeostasis. By dissecting the transcription mechanisms of the gene (*CYP7A1*) encoding cholesterol 7α-hydroxylase, the rate-limiting enzyme of bile acid synthesis, we found that bile acids increase the nuclear concentration of histone deacetylase (HDAC) 7 and promote the assembly of a repressive complex containing also HDAC1 and 3, which associates to the nuclear receptor HNF-4 and leads to the repression of *CYP7A1*. Gene silencing with siRNA confirms that HDAC7 is the key factor required for the repression of *CYP7A1* transcription, whereas knock-down of the inhibitor SHP does not prevent the downregulation of *CYP7A1*. Administration of the HDAC inhibitors, valproic acid (VPA) or trichostatin A (TSA), to hypercholesterolemic *Lld-r*$^{-/-}$ mice increases Cyp7a1 mRNA, bile acid synthesis and consequently reduces total plasma and LDL cholesterol (190±20 mg/dl in control mice vs. 69±24 mg/dl in VPA treated mice and 40±7 mg/dl in TSA treated mice, $P<0.01$). VPA and TSA also display effects in extrahepatic tissues contributing to the beneficial action of these molecules in preventing the consequences of hyperlipidemia. Altogether, our study highlights the importance of HDACs in the feedback regulation of *CYP7A1* transcription and identifies them as potential targets to modulate bile acid and cholesterol metabolism.

Funding: Supported by grants from Telethon (GGP04252), the European Commission (NORTh) and the Italian Ministry of University and Research

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THE NUCLEAR RECEPTOR FXR: A PROMISING TARGET FOR THE METABOLIC SYNDROME?

Bart Staels. *U545 INSERM; Institut Pasteur de Lille; University of Lille II, Lille, France.*

The metabolic syndrome is an insulin-resistant state characterized by a cluster of cardiovascular risk-factors, including abdominal obesity, hyperglycemia, elevated blood pressure and combined dyslipidemia. In this presentation, we will discuss the role of the bile acid-activated receptor FXR in the modulation of the metabolic syndrome. Due to its regulatory actions on lipid and glucose homeostasis, FXR appears to be a potential pharmacological target. Moreover, the observation that FXR also influences endothelial function and atherosclerosis suggests a regulating role also in the cardiovascular complications associated with the metabolic syndrome. The pharmacological activation of FXR leads to a complex response integrating beneficial actions and potentially undesirable side effects. Thus, the identification of selective FXR modulators (SBARMs) will be required to develop compounds useful in the treatment of the metabolic syndrome.

Funding: None

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A SYNTHETIC FARNESOID X RECEPTOR (FXR) AGONIST PROTECTS AGAINST DIET-INDUCED DYSLIPIDEMIA

Douglas C. Harnish, Helen B. Hartman, KehDih Lai, Paige E. Mahaney, ***Mark J. Evans****. Wyeth, Collegeville, PA, USA.*

Farnesoid X receptor (FXR) plays a critical role in the regulation of triglyceride (TG) and cholesterol homeostasis. The synthetic ligand FXR-450 is a potent FXR agonist. Here we demonstrate that FXR-450 has lipid-lowering activity in several animal models. FXR-450 reduced serum LDL cholesterol levels in hypothyroid wildtype mice but not in hypothyroid FXRKO mice. FXR-450 also decreased serum TG and VLDL, LDL and HDL cholesterol levels in male LDLRKO mice. The Western diet elevations of hepatic cholesterol or triglyceride content and the repressions of cholesterol-regulated genes (HMG-CoA synthase and PCSK9) were all attenuated by FXR-450. This activity of FXR-450 was dependent upon SHP since FXR-450 activity was significantly reduced in LDLRKO/SHPKO mice. Surprisingly though, FXR-450 still lowered VLDL, LDL and HDL cholesterol levels in LDLRKO/SHPKO mice, suggesting a contribution by other FXR regulated pathways to this effect. In male hamsters, which have a bile acid physiology more closely resembling humans, FXR-450 treatment also reduced plasma TG levels and VLDL, LDL and HDL cholesterol levels. In rats, FXR-450 decreased triglyceride and VLDL cholesterol levels. However, in contrast to mice and hamsters, FXR-450 significantly increased HDL cholesterol levels in the rat. Although FXR has been suggested to modulate apoAI expression, FXR-450 did not repress apoAI gene expression in LDLRKO mice, human apoAI transgenic mice, or rats. The basis for the species-specific effects of FXR-450 on HDL cholesterol levels remains to be determined. These results suggest that synthetic FXR agonists may have use in the treatment of dyslipidemia.

Funding: This work was funded by Wyeth

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PHOSPHORYLATION OF LXR ALPHA SELECTIVELY AFFECTS TARGET GENE EXPRESSION IN MACROPHAGES

***Ines Pineda-Torra**, Jonathan E. Feig, Edward A. Fisher, Michael J. Garabedian. NYU School of Medicine, New York, NY, USA.*

Modulation of LXRalpha actions in macrophages is of great relevance to the development of pharmacological agents against atherosclerosis and other cardiovascular diseases. Here, we show that LXRalpha is phosphorylated in serine 198 (S198) in cholesterol-loaded RAW 264.7 macrophages as well as in primary bone marrow-derived macrophages and macrophage foam cells of atherosclerotic lesions. In RAW 264.7 cells cultured under basal conditions, LXRalpha is phosphorylated by Casein Kinase 2 (CK2); phosphorylation is enhanced by LXR ligands and is reduced both by CK2 inhibitors and by activation of the heterodimeric partner RXR with 9-cis-retinoic acid (9cRA). LXRalpha target gene selectivity is achieved by modulating the phosphorylation status of the receptor. Expression of some (AIM and LPL), but not other (ABCA1 or SREBP) established LXR target genes is increased in RAW 264.7 cells expressing the LXRalpha S198A phosphorylation-deficient mutant, compared to those expressing the wild-type receptor. Remarkably, a gene normally not regulated by LXRalpha in macrophages, the chemokine ligand CCL24, is activated in cells expressing LXRalpha S198A. Accordingly, inhibition of LXRalpha phosphorylation by 9cRA or by a CK2 inhibitor promotes CCL24 expression, thereby phenocopying the LXRalpha S198A mutation. Thus, our results reveal that LXRalpha phosphorylation is dynamic and selectively affects the expression of LXRalpha target genes, and underscore a previously unrecognized role for phosphorylation in restricting the repertoire of LXRalpha-responsive genes in macrophages.

Funding: AHA Postdoctoral fellowship

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IMPACT OF COMMON VARIANT OF BILE ACID RECEPTOR FXR GENE ON CARDIOVASCULAR RISK FACTORS

***Atsushi Nohara**, Toru Noguchi, Mika Mori, Hayato Tada, Masayuki Tsuchida, Mutsuko Takata, Masaaki Kawashiri, Akihiro Inazu, Junji Kobayashi, Masakazu Yamagishi, Hiroshi Mabuchi. Kanazawa University Graduate School of Medical Science, Kanazawa, Japan.*

Objective: Many nuclear receptors play important roles in energy homeostasis, inflammation and atherosclerosis. Bile acid receptor FXR is shown to have crucial roles in cholesterol conversion into bile acids, and also in glucose metabolism. We identified common polymorphism of FXR gene in Japanese population, and hypothesized that this polymorphism could affect energy homeostasis. **Methods:** All the coding regions of FXR gene were screened in 180 hyperlipidemic patients by PCR-DGGE analysis. Clinical characteristics were evaluated in 298 general males and in another 176 (50 diabetes and 126 non-diabetes) coronary artery disease suspected patients (CAD group) including 105 coronary angiography performed patients. **Results:** We identified FXR -1g->t variants, and its allele frequency was 0.32. FXR -1t carriers had significantly higher BMI both in general males (23.2 ± 2.4 vs. 23.9 ± 2.7 kg/m2, p=0.03) and in non-diabetes CAD group (22.2 ± 4.2 vs. 24.2 ± 4.1 kg/m2, p=0.01). In lipid analysis of non-diabetes CAD group, FXR -1t carriers showed lower lipid levels both in HDL-C (57 ± 22 vs. 49 ± 14 mg/dl, p=0.03), and in LDL-C (159 ± 59 vs. 136 ± 57 mg/dl, p=0.03). With ultrasonography, FXR -1t carriers showed resistance to development of fatty liver (59% vs. 32%, p=0.02). **Conclusion:** Common genetic variant of FXR seems to have deep impact in energy homeostasis associated with cardiovascular risk factors.

Funding: None

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HEPATIC NUCLEAR RECEPTOR EXPRESSION AND METABOLISM OF CHOLESTEROL AND BILE ACIDS IN HUMANS

***Marco Bertolotti**, Chiara Gabbi, Claudia Anzivino, Maurizio Crestani, Emma De Fabiani, Enrico Tagliafico, Lucia Carulli, Matteo Ricchi, Paola Loria, Nicola Carulli. University of Modena and Reggio Emilia, Modena, Italy; University of Milano, Milano, Italy.*

Aim of the present studies was to analyze the hepatic expression of different genes involved in the molecular regulation of cholesterol homeostasis in human liver. **Methods:** Liver biopsies were obtained in 32 untreated patients undergoing abdominal surgery, a subgroup of which with cholesterol gallstone disease (GS). mRNA levels of CYP7A1, related nuclear receptors and biliary transporters were assayed by real-time RT-PCR. **Results:** CYP7A1 mRNA best correlated with HNF-4 ($r = 0.55$, $p < 0.05$). Aging was inversely correlated with CYP7A1 mRNA levels and with serum IGF-1 which, in turn, was correlated with HNF-4 and CYP7A1 expression. In GS, PPAR-gamma coactivator 1 (PGC-1), a coactivator of CYP7A1, and bile salt export pump (BSEP) were less expressed and correlated with each other, whereas cholesterol transporters ABCG5 and ABCG8 were more expressed. **Conclusions:** Age-related reduction of bile acid synthesis might be related with reduced expression of hepatic HNF-4 and of CYP7A1; modifications of the GH/IGF axis likely play a role. In GS reciprocal alterations of genes involved in biliary transport are consistent with increased biliary saturation. PGC-1 appears to play a protective role; this might take place via interaction with FXR and target genes such as BSEP. The changes observed may help focus on novel molecular targets for the management of cholesterol accumulation conditions.

Funding: Supported by COFIN-PRIN grant 2004 067491 and 6th Framework Program grant LSHM-CT-2006-037498

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MBX-213, A NOVEL DUAL PPAR α/γ AGONIST WITH BENEFICIAL EFFECTS ON LIPID PROFILES AND INSULIN RESISTANCE

***Francine Gregoire**, Holly Clarke, Xin Chen, Edward Clemens, Brian Lavan. Metabolex, Hayward, CA, USA.*

Discovery of alternative PPAR modulators that retain the anti-diabetic and lipid-lowering properties of currently marketed drugs while avoiding side effects would be greatly beneficial. MBX-213 is a novel non-TZD dual α/γ agonist that displays unique *in vitro* characteristics and possesses impressive glucose and lipid lowering *in vivo*. *In vitro*, MBX-213 is a partial agonist that activates PPARγ (EC50 ~10 μM) and PPARα (EC50 ~5μM). In human mutant PPARγ (Tyr473Ala) reporter assay, MBX-213 interacts with PPARγ in a manner distinct from the full agonists. MBX-213 also displays reduced ability to recruit co-activators in a PPARγ co-activator assay. In functional cell-based assays, MBX-213 minimally induces adipogenesis while modulating expression of known PPARγ target genes and enhancing adipocyte insulin sensitivity. *In vivo* studies aimed at evaluating efficacy and side effects demonstrate that MBX-213 is an efficacious anti-diabetic and hypo-lipidemic agent that lacks the negative side effects observed with full PPARγ agonists. Significant glucose, insulin, triglyceride and FFA lowering are seen upon short-term treatment of ZDF rats while long term treatment of ZF rats results in increased insulin sensitivity and reduced triglyceride levels in the absence of body weight gain. Similarly, robust lipid lowering and anti-diabetic responses are also observed in db/db mice without body weight and fat mass gain. Moreover, increases in HDL cholesterol, HDL particle size and human apoA1 plasma levels occur in humanized apoA1 transgenic mice after a week of MBX-213 treatment. Taken together, these results position MBX-213 as a very promising agent for the treatment of dyslipidemia associated with type 2 diabetes.

Funding: None

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PROOF OF PRINCIPLE FOR A NEW TARGET FOR THE TREATMENT OF DYSLIPIDEMIA? EFFICACY AND SAFETY DATA FROM THE CARDIAC SPARING AND LIVER SELECTIVE THYROID RECEPTOR AGONIST KB2115 FOLLOWING 12 WEEKS TREATMENT IN 100 PATIENTS

Jens D. Kristensen, Olof Breuer, Karin Mellstrom, Jorma Strand, Mats Eriksson, ***Bo Angelin****. Karo Bio, Huddinge, Sweden; Karolinska Institute, Stockholm, Sweden; University of Oulu, Oulu, Finland.*

The thyroid receptor (TR) is a key regulator of metabolic pathways and holds the promise of a new target for the treatment of dyslipidemia. Thyroid hormones are known to reduce cholesterol levels through regulation of a number of key enzymes involved in synthesis, degradation and lipid transport in the liver. To take advantage of thyroid hormone effects on lipid metabolism for the treatment of dyslipidemia, the TR agonist KB2115 has been developed as a liver selective thyroid receptor agonist that can induce hyperthyroidism in the liver, while a euthyroid state is preserved in the extra-hepatic tissue. Preclinical data has shown pronounced LDL-cholesterol lowering effect without unfavorable extra-hepatic effects. We now report results from a proof of concept study of the liver selective TR agonist KB2115 in patients with dyslipidemia. A 12 week, placebo controlled, double blind, randomised, clinical phase II study was designed to explore whether clinical relevant LDL-lowering effect can be achieved without disturbing the extra-hepatic thyroid homeostasis. KB2115 or placebo was administered for 3 months to 100 patients with dyslipidemia. Efficacy of KB2115 on the lipid profile and biomarkers for cholesterol transport will be reported and related to safety data, including potential effects on extra-hepatic thyroid receptors. Mechanisms of action with focus on reverse cholesterol transport will be discussed.

Funding: The study was sponsored by Karo Bio AB, Sweden

Workshop 13 "HYPOLIPIDEMIC DRUGS (1st part)"
2:00 PM - 3:45 PM
Chairs: *B. Angelin, Huddinge, Sweden and S. Bellosta, Milan, Italy.*

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THE EFFECT OF SHORTENING LOCKED NUCLEIC ACID ANTISENSE OLIGONUCLEOTIDES, FROM 16 TO 12 NUCLEOTIDES, ON THE EXPRESSION OF apoB mRNA AND SERUM CHOLESTEROL LEVELS

Christoph Rosenbohm*, Jens B. Hansen, Niels Fisker, Maj Hedtjärn, Jacob Ravn, Michael Meldgaard, Henrik F. Hansen, Ellen M. Straarup. Santaris Pharma, Hoersholm, Denmark.*

Objectives: Oligonucleotides (ODN) are used to target and cause degradation of specific mRNA coding for apolipoprotien B. Locked Nucleic Acid (LNA) is a nucleic acid modification that increases affinity and improves stability of ODN. These characteristics are of major importance in the development of highly potent therapeutic ODN consisting of less than 20 nucleotides. We have previously published *in vivo* results obtained with 16-mer LNA ODN. Using apoB as target we have designed 12- to 15-mer LNA ODN than retain the specificity and show an increased *in vivo* potency compared to the 16-mer. **Methods:** C57BL/6 mice were dosed saline solutions of the different LNA ODN at 5 mg/kg/day on 3 consecutive days by *i.v.* injections. 24 hours after the last dosing liver was sampled for analysis of apoB mRNA expression by RT-qPCR and serum for cholesterol levels. **Results:** The apoB mRNA expression was down regulated in a length dependent manner. The 12-mer and 13-mer resulting in 90% down regulation, the 14-mer in 80%, 15-mer in 40%, and 16-mer in 30% down regulation compared to saline. Similar for the cholesterol levels in serum with 20% reduction in total cholesterol by dosing the 16-mer and 80% after dosing the 12-mer. The ALT and AST levels were similar for the saline, 16-, and 15-mer groups. The 14-mer showed a non significant increase in ALT and AST levels whereas these were doubled in the 12- and 13-mer group. **Conclusion:** The reduction in length of a 16-mer ODN targeting apoB resulted in a length dependent effect on both mRNA expression and serum cholesterol with the

12-mer oligonucleotide being the most potent LNA ODN *in vivo*.

Funding: Santaris Pharma

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LXR AGONIST STIMULATION OF GLOBAL REVERSE CHOLESTEROL TRANSPORT (RCT) RATES IN RATS MEASURED WITH STABLE ISOTOPES

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Activation of LXR target genes pharmacologically may offer a potent means of stimulating the flux through the RCT pathway for treatment of cardiovascular disease. We have developed an *in vivo* stable isotope method for quantifying cholesterol (C) efflux rate and flux through the global RCT pathway, and report here the effects of LXR treatment in rats. Efflux of free C from tissues into plasma is measured by the isotope dilution principle, through a constant IV infusion of $^{13}C_2$-cholesterol. Administration of an LXR agonist for 5 days (TO901317, 20 mg/day) increased efflux rates by 47% compared to controls. This was consistent with the observed increases in C transport-related LXR target genes in the liver and the periphery. Measurement of *de novo* C synthesis rates in peripheral tissues revealed a 110% increase with LXR treatment, consistent with negative C balance in tissues (ie RCT). By multiplying efflux rates by the fractional recovery of label in the stool over 4 days, global RCT (flux from tissue cholesterol to fecal sterols) was determined. LXR treatment in rats increased global RCT flux two-fold. In summary, efflux rates of C from tissues are quantifiable, and track with the measured effect of LXR agonists on transporter target genes and synthesis of C in the periphery. Combining efflux with label recovery in stool provides an integrated whole-body metric of the flux through the RCT pathway which is increased by anti-atherogenenic LXR agonist treatment. This technique can also be used in humans and could provide new insights into the physiological and pharmacological control of C efflux and RCT.

Funding: KineMed, Inc.

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TORCETRAPIB MONOTHERAPY INCREASES HDL AND REDUCES ATHEROSCLEROSIS IN E3L.CETP MICE, BUT DOES NOT ENHANCE THE ANTI-ATHEROSCLEROTIC POTENCY OF ATORVASTATIN

***Willeke de Haan**, Jitske de Vries-van der Weij, Jose W.A. Van der Hoorn, Johannes A. Romijn, J. Wouter Jukema, Louis M. Havekes, Hans M.G. Princen, Patrick C.N. Rensen. LUMC, Leiden, Netherlands; Gaubius Laboratory, Leiden, Netherlands.*

CETP inhibition is regarded as a promising strategy to reduce atherosclerosis by increasing HDL-cholesterol, but recent human trials showed that the CETP inhibitor torcetrapib did not add to the anti-atherosclerotic effect of atorvastatin. Therefore, we evaluated in the present study the effect of torcetrapib alone and in combination with atorvastatin on atherosclerosis in APOE*3-Leiden. CETP (E3L.CETP) transgenic mice with a human-like lipoprotein profile. E3L.CETP mice were fed a diet containing 0.25% cholesterol without or with torcetrapib (0.01%), atorvastatin (0.0023%) or both for 14 weeks, and atherosclerosis development was assessed in the aortic root. A single oral gavage of torcetrapib reduced plasma CETP activity (-59%, $P<0.01$), which was sustained by chronic treatment, while torcetrapib increased CETP mass (+33%, $P<0.0015$). Torcetrapib, atorvastatin, and the combination reduced plasma cholesterol (-20%, -42%, and -40%, all $P<0.01$). Besides lowering (V)LDL-C, torcetrapib increased HDL-C in absence and presence of atorvastatin (+25% and +41%) without increasing apoAI. Atherosclerotic lesion area (and severity) in the aortic root was decreased by torcetrapib (-42%, $P<0.05$), atorvastatin (-46%, $P<0.05$), and the combination (-60%, $P<0.05$). However, combination therapy did not significantly add to both single therapies. We conclude that torcetrapib monotherapy reduces atherosclerosis as related to an increase in HDL but does not enhance the anti-atherosclerotic potency of atorvastatin.

Funding: NHS 2003B136

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IDENTIFICATION AND CHARACTERIZATION OF MK-0859, A NOVEL CHOLESTERYL ESTER TRANSFER PROTEIN INHIBITOR

***Edward O'Neill**, C.P. Sparrow, Y. Chen, S. Eveland, B. Frantz-Wattley, D. Milot, P.J. Sinclair, A. Ali, Z. Lu, C.J. Smith, G. Taylor, C.F. Thompson, M.S. Anderson, A. Cumiskey, R. Rosa, J. Strain, L.B. Peterson. Merck & Co. Inc., Rahway, NJ, USA.*

Objectives: Identify a potent inhibitor of cholesteryl ester transfer protein (CETP) capable of increasing HDL-cholesterol and lowering LDL-cholesterol. **Methods:** An in vitro fluorescent transfer CETP assay suitable for kinetic measurements, with signal-to-noise >6, was used for high throughput screening and optimization of leads. Compounds were characterized by testing both cholesteryl ester and triglyceride transfer. Inhibitors were assayed in 95% human serum and tested in mice transgenic for CETP. Recombinant human CETP was expressed in Drosophila S2 cells for use in an inhibitor binding assay. CETP-HDL interaction assays were used to assess the mechanism by which inhibitors block CETP activity. **Results:** We identified a CETP inhibitor, MK-0859, having an IC50 of 15 nM in the screening assay and 57 nM in an assay containing 95% human serum. The compound yields an average 24 mg/dL increase in HDL-cholesterol when dosed at 10 mg/kg BID into C57BL/6 mice transgenic for CETP. Under an alternative dosing regimen in the same mice the compound increases HDL-cholesterol and particle size, and lowers LDL-cholesterol. This compound does not alter blood pressure in mice under conditions where torcetrapib shows significant increases in blood pressure. The compound competes for the binding of either torcetrapib or JTT-705 to purified CETP. Like torcetrapib the compound induces tight reversible binding of CETP to HDL, but unlike JTT-705 it does not form a covalent bond with CETP. **Conclusions:** We identified a novel CETP inhibitor, biochemically similar to existing CETP inhibitors.

Funding: Authors are employees of Merck & Co., Inc.

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MATRIX METALLOPROTEIN 13 EXPRESSION IS REDUCED BY ATORVASTATIN IN HUMAN ABDOMINAL AORTIC ANEURYSMS

***Morris Schweitzer**, Benjamin Mitmaker, Daniel Obrand, Nathan Sheiner, Cherrie Abraham, Melissa Meilleur, Lorraine Chalifour. SMBD-Jewish Gen. Hosp., Montreal, QC, Canada.*

Objectives: Extracellular matrix (ECM) destruction by proteinases characterizes expanding abdominal aortic aneuryms (AAA). We asked whether Atorvastatin therapy would decrease matrix metalloproteinase (MMP) expression or activity in the central region of human AAA tissue. **Methods:** Paired protein homogenates were prepared from the centre vs. distal (at least 2cm) region of AAA tissue collected at elective surgical repair from non-statin treated (NST, n=20) and Atorvastatin-treated (AT, n=20) patients. Western blots measured MMP1, MMP2, MMP3, MMP9, MMP13, TIMP1, TIMP3 and TIMP4, TGFß, SMAD2 and phosphoSMAD2 expression in comparison to GAPDH expression. Zymography measured TIMP, MMP2 and MMP9 activity. Data were analysed by student t-test. **Results:** The two groups did not differ in age, gender or AAA size. No patient had a first degree relative with AAA and no AAA had ruptured. Expression and activity of MMP1, MMP2, MMP3, MMP9, TIMP1, TIMP3 and TIMP4 was increased in the centre vs. distal region in all AAAs, but did not vary with treatment. However, MMP-13 protein expression was significantly reduced in the centre vs. distal region of AT vs. NST samples. TGFß, released from its latent protein by MMP activity, activates its receptor kinase so SMAD2 is activated. A non-significant trend towards reduced TGFß, SMAD2 and pSMAD2 in AT-treated samples suggests reduced endogenous MMP proteolysis. **Conclusions:** AT-treated AAA patients have reduced centre vs. distal MMP-13 protein at surgical repair. AT treatment may stabilize AAA progression by retention of ECM.

Funding: Pfizer Canada, Inc.

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COMPARISON OF EFFECTS OF PIOGLITAZONE AND ROSIGLITAZONE ON LIPID & LIPOPROTEIN METABOLISM

***T. Chahil**, C. Ngai, A. Bensadoun, I. Goldberg, M. Jahnes, S. Holleran, M. Rosenbaum, R. Ramakrishnan, H. Ginsberg. Columbia Univ Med Ctr, New York, NY, USA; Cornell Univ, Ithaca, NY, USA.*

High TG and low HDL-C are common in type 2 DM (T2DM). Pioglitazone (PIO) and rosiglitazone (ROSI) similarly improve insulin sensitivity, but PIO lowers TG and raises HDL-C more than ROSI. We examined effects of PIO and ROSI on lipid and lipoprotein metabolism in T2DM and dyslipidemia. 8 subjects were enrolled in a randomized, double-blind crossover study and given PIO (45 mg/d) or ROSI (4 mg bid) for 13 wks, followed by a 2wk washout period and then 13 wks of the opposite drug. At the end of each period we measured plasma lipids and lipoproteins, post-heparin plasma (PHP) LPL and HL mass and activity, LPL and PPARγ mRNA from abdominal sc adipose tissue, and plasma apo C-III. We also measured turnovers of VLDL TG and apo B. Age: 56 ± 12 yrs. Baseline lipids (mg/dL): TC 165, TG 186, HDL-C 40, LDL-C 92. Fasting TG fell 27% during PIO compared to ROSI (118 ± 32 vs 162 ± 70 mg/dL,p = 0.005). PIO also raised fasting HDL-C levels vs ROSI (43 ± 8 vs 40 ± 8 mg/dL,p = 0.03). LPL and PPARγ mRNA levels did not differ between treatment periods. Also, both PHP LPL mass and LPL activity were similar on PIO vs ROSI. PHP HL mass was higher in 7 of 8 subjects on PIO (114.4 ± 51.1 vs 69.2 ± 43.1 ng/ml,p = 0.11), while PHP HL activity was unchanged. Serum apo C-III levels were lower on PIO vs ROSI (15.4 ± 4.1 vs 18.5 ± 6.8 mg/dL,p = 0.047). PIO lowered TG more than ROSI; this was associated with decreased apo C-III, which could result in improved in vivo lipolysis of VLDL TG. Analysis of the turnover study data (pending) will either confirm better lipolysis of VLDL TG with PIO or increased VLDL TG secretion with ROSI.

Funding: Unrestricted, investigator-initiated grant (Takeda Pharmaceuticals)

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EFFECTS OF MAXIMAL DOSES OF ATORVASTATIN VERSUS ROSUVASTATIN ON SMALL DENSE LOW DENSITY LIPOPROTEIN CHOLESTEROL LEVELS

***Masumi Ai**, Seiko Otokozawa, Bela F. Asztalos, Katsuyuki Nakajima, Peter H. Jones, Evan A. Stein, Ernst J. Schaefer. Tufts University, Boston, MA, USA; Baylor College of Medicine, Houston, TX, USA; Metabolic and Atherosclerosis Research Center, Cincinnati, OH, USA.*

Objective: Atorvastatin and rosuvastatin are both highly effective in reducing low density lipoprotein (LDL) cholesterol (C), but rosuvastatin has been shown to be more effective than atorvastatin in lowering LDL-C. Small dense LDL (sdLDL) has been recognized as more atherogenic than larger LDL. It is important for preventing CHD to lower sdLDL-C. Our purpose in this study was compare the effects of Rosuvastatin 40 mg/day with atorvastatin 80mg/day on sdLDL-C. **Methods:** Hypercholesterolemic patients (n=271) were studied at baseline and 6 weeks after therapy with either atorvastatin 80 mg/day (n=136) or rosuvastatin 40 mg/day (n=135). A novel automated assay was used for measuring serum sdLDL-C level with measuring other variables. **Results:** Both atorvastatin and rosuvastatin caused significant and similar decreases in total-C (-39.0%, -40.9%), TG (-24.4%, -25.9%), and large LDL-C (-49.9%, -46.8%), but rosuvastatin was significantly ($p<0.01$) more effective than atorvastatin in reducing the total C/HDL-C ratio (-45.6% versus -39.4%), non HDL-C (-51.3% versus -48.2%), direct LDL-C (-52.3% versus -50.1%), and sdLDL-C (-52.8% versus -45.8%). **Conclusions:** Both statins, given at their maximal doses, favorably alter the entire spectrum of lipoprotein particles, but rosuvastatin is significantly more effective than atorvastatin in lowering the total C/HDL-C ratio, non-HDL-C, LDL-C, and small dense LDL-C levels.

Funding: Denka-Seiken Co. (Drs. Ai and Nakajima)

Workshop 14 "HYPOLIPIDEMIC DRUGS (2nd part)"
4:30 PM - 6:20 PM
Chairs: *L. Calabresi, Milan, Italy and J. Davignon, Montreal, QC, Canada.*

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PPARα IS A KEY REGULATOR OF HEPATIC FGF21

Thomas Lundåsen, Mary C. Hunt, Sabysashi Sanyal, ***Bo Angelin****, Stefan H.E. Alexson, Mats Rudling. Karolinska Institutet, Stockholm, Sweden.*

The metabolic regulator fibroblast growth factor 21 (FGF21) has antidiabetic properties in animal models of diabetes and obesity. Using quantitative RT-PCR, we here show that the hepatic gene expression of FGF21 is regulated by the peroxisome proliferator-activated receptor alpha (PPARα). Fasting or treatment of mice with the PPARα agonist Wy-14,643 induced FGF21 mRNA by 10-fold and 8-fold respectively. In contrast, FGF21 mRNA was low in PPARα deficient mice, and fasting or treatment with Wy-14,643 did not induce FGF21. Obese ob/ob mice known to have increased PPARα levels, displayed 12-fold increased hepatic FGF21 mRNA levels. The potential importance of PPARα for FGF21 expression also in human liver was shown by Wy-14,643 induction of FGF21 mRNA in human primary hepatocytes and PPARα response elements have been identified in both the human and mouse FGF21 promoters. Further studies on the mechanisms of regulation of FGF21 by PPARα in humans is of great interest.

Funding: Swedish Research Council, Karolinska Institutet, Stockholm City Council (ALF)

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THE mTOR INHIBITOR EVEROLIMUS PREVENTS ATHEROSCLEROSIS IN LDLR-/- MICE DESPITE SEVERE HYPERCHOLESTEROLEMIA

Frank Beutner*, Marc Mueller, Daniel Teupser, Desiree Brendel, Uta Ceglarek, Joachim Thiery. University Hospital Leipzig, Leipzig, Germany.*

Inflammatory processes play a critical role in atherogenesis. The immunosuppressant everolimus, a mammalian target of rapamycin (mTOR) inhibitor has been shown to modulate cell cycle, cell proliferation and to reduce transplant vasculopathy in humans and different animal models. The aim of this study was to investigate the effect of everolimus on immune response, lipid metabolism and atherosclerosis in hypercholesterolemic LDL-receptor-deficient mice. Solvent or Everolimus (0.05 mg/kg [A] or 1.5 mg/kg [B] per day) was administered subcutaneously by osmotic minipumps for 12 weeks. Atherosclerosis was quantified in aortic root and brachiocephalic artery and 23 circulating cytokines were measured by a multiplex immunoassay. The treatment resulted in dose-dependent plasma levels of everolimus with 0,31 and 531 µg/l, respectively. Atherosclerotic lesions in both treated groups showed significantly less lesion complexity. Moreover, everolimus reduced lesion size at the aortic root by 60% and in the brachiocephalic artery by 85% in group B($p<0.01$ and $p<0.001$). The reduction of atherosclerosis was observed in spite of a significant increase in plasma cholesterol and LDL cholesterol levels in the treatment groups (cholesterol 16.1±2.0, 23.8±2.6, 23.1±3.3 mmol/l in control, group A and B, respectively). Only 5 of 23 measured cytokines (IL-1α, IL-5, IL12p40, GM-CSF, KC) were effected by everolimus. These results demonstrate that everolimus inhibits atherosclerosis in LDL-receptor deficient mice, despite the presence of severe hypercholesterolemia. It opens new strategies for the treatment of atherosclerosis in hypercholesterolemic subjects.

Funding: Novartis Pharma AG Basel

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RECONSTITUTED HIGH-DENSITY LIPOPROTEIN CHOLESTEROL THERAPY FOR CARDIOVASCULAR DISEASE

***Shin-ichiro Miura**, Satoshi Imaizumi, Yoshihiro Kiya, Yoshinari Uehara, Masataka Sata, Kerry-Anne Rye, Keijiro Saku. Fukuoka University School of Medicine, Fukuoka, Japan; Heart Research Institute, Sydney, Australia; University of Tokyo Graduate School of Medicine, Tokyo, Japan.*

Reconstituted (r)HDL has been shown to produce the rapid regression of atherosclerosis in animal models and humans. We hypothesized that rHDL also contributed to the prevention of and recovery from cardiovascular disease (CVD) through its pleiotropic effects in addition to the efflux of cholesterol, and analyzed these effects using rat and mouse models. We found that treatment with the rHDL[POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine)/ ApoA-I], which we reconstituted, prevents reperfusion-induced ventricular fibrillation and tachycardia. The rHDL-induced nitric oxide (NO) production mediated by ABC (ATP-binding cassette transporter) A1 or ABCG1 through an Akt/ERK(extracellular-signal-regulated kinases)/ NO pathway in ECs suppresses reperfusion-induced arrhythmias. In Myocardial infarction (MI) rat model, rHDL-induced anti-apoptosis has beneficial morphological effects that result in the prevention of left ventricular remodeling after MI through a ERK pathway. In addition, rHDL directly stimulates the differentiation of endothelial progenitor cells via the PI3K/Akt pathway and enhances ischemia-induced angiogenesis in the ischemic hind limb mouse model. These findings represent an exciting new area in coronary atherosclerosis intervention. HDL-based therapy combined with the reduction of low-density lipoproteins may be able to dramatically reduce the incidence of CVD as well as aid in the recovery from CVD.

Funding: This work was supported by funds from the Central Research Institute of Fukuoka University, Japan

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CLINICAL DEVELOPMENT OF A NOVEL HDL THERAPEUTIC

***Daniel L. Sparks**, Teik Chye Ooi, William Dickie, Kenneth Sokoll. The University of Ottawa Heart Institute, Ottawa, ON, Canada; The Ottawa Hospital, Riverside Campus, Ottawa, ON, Canada; Liponex Inc., Ottawa, ON, Canada.*

CRD5 is a uniquely formulated soy phosphatidylinositol that is being developed as a therapeutic for the treatment and prevention of heart disease. CRD5 directly impacts plasma HDL levels through a MAPK, PPARα induced stimulation in apoA-I synthesis and secretion by the liver. Three Phase I human trials have been performed and have shown that a 5.6 gram daily dose of CRD5 taken with food is safe and can significantly increase plasma HDL-C levels by ~20% and reduce triglyceride levels by ~40%. A 12-week dose-ranging Phase I/II clinical trial in 50 patients with low HDL and high LDL and triglyceride levels was completed in Q1 2007. CRD5 was shown to be safe at all dosages. CRD5 was generally well tolerated at a dose of 1 gram per day. Modest increases in G.I. related adverse events were found with a 3 gram dose. CRD5 caused a modest but non-significant elevation in HDL-C of 5%, at a 1 gram dose (n=50). Subgroup analysis showed that the best responders (11% increase in HDL-C) were patients not on concomitant lipid altering medications and with baseline HDL-C of <0.9 mM (n=17). Patients taking drugs which neutralize stomach pH (e.g. proton pump inhibitors) showed the greatest increase in HDL-C of 16% (n=4 at a 1 gram dose). The data supports the view that CRD5 is sensitive to gastric degradation and additional Phase I/II testing will next evaluate the effect of enteric coating on improving the bioavailability and HDL elevating efficacy of the drug. CRD5 shows strong promise as a safe and effective therapeutic to raise plasma HDL levels.

Funding: CRD5 development is funded by Liponex Inc., a Canadian public company

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EFFECTS OF STATIN TREATMENT FOR REGRESSION OF CAROTID ATHEROSCLEROSIS: A LITERATURE REVIEW OF CLINICAL TRIALS ANALYZING CAROTID PLAQUES BY HISTOPATHOLOGY

***William Insull**. Baylor College of Medicine, Houston, TX, USA.*

Objective: To evaluate the statin-induced changes in human carotid artery plaque composition reported since 2001 by trials for plaque regression. **Methods:** Four controlled clinical trials met the review's eligibility criteria. The trials treated 201 patients by mono-therapy with pravastatin, simvastatin or atorvastatin for an average of 3.9 months, range 3 to 4.4 months, at doses 20 to 40 mg/d. Serum LDL-C on treatment averaged 90 mg/dl, range 74 -124 mg/dl. Carotid plaque tissues, from carotid endarterectomy, were analyzed by immuno-histological methods. **Results:** Significant reductions in plaque contents occurred for macrophages and lymphocytes, resp., means by – 57% and – 67%, with resp., p 0.07 to <0.0001, and p <0.05 to <0.0001. Smooth muscle cell content was reduced in one trial -21%, p 0.03, and in a second trial -9%, not statistically significant. Concurrently plaque total lipid content was reduced a mean of -72%, p's <0.05 to < 0.0001. Only the plaque content of collagen increased, a mean of +160%, range +88 to +233%, p's <0.003. Content of MMP-2 decreased -68% with p's <0.05 to <0.0001, and MMP-9 decreased -73%, p's <0.001. Contents of COX-2, mPGES-1 and oxidized LDL were reduced resp, -69%, -81% and -60%, p's usually <0.0001. **Conclusions:** Statins dosed over 3-4 months to LDL-C ~ 90 mg/dl promptly caused substantial favorable changes in plaques' cells, lipids, collagen and inflammatory oxidative processes, and probably caused true regression. To better understand and treat plaque components pivotal for atherogenesis, we need plaque regression trials with comprehensive analysis of plaque tissues after statin-induced LDL-C ≤70 mg/dl.

Funding: None

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EXTENDED-RELEASE NIACIN HAS MINIMAL FREE FATTY ACID REBOUND IN HEALTHY VOLUNTEERS

***Richard L. Dunbar**, Ramprasad Gadi, Grace A. Nathanson, Megan L. Wolfe, Rajesh Movva, Scott Lilly, Daniel J. Rader. University of Pennsylvania, Philadelphia, PA, USA.*

Objective: To test the hypothesis that extended-release (ER) niacin has a lower free fatty acid (FFA) rebound than immediate-release (IR) niacin. **Background:** Niacin acutely suppresses free fatty acid (FFA) levels, benefiting triglycerides, small, dense LDL, and indirectly, HDL. However, suppressed FFA is followed by a FFA rebound that drives insulin resistance from IR niacin. ER niacin has similar efficacy but less insulin resistance, perhaps because prolonged release blunts the FFA rebound. **Methods:** We are conducting a double-blinded, placebo-controlled, crossover trial of niacin in healthy adults, who took 1g of IR or ER niacin, or matching placebo while fasting on different weeks. We sampled blood over 12 hours. We report changes in FFA as mean (SD) of the absolute increment from baseline: incremental nadir (iNadir) and peak (iPeak) in mEq/L. **Results:** Among 7 subjects, niacin initially lowered FFA to the same degree: IR to an iNadir of -0.37 (0.25) mEq/L vs. ER's iNadir of -0.29 (0.12) (p=0.3). On IR niacin FFA rebounded with an iPeak of +1 (0.5), while ER niacin had an iPeak of +0.6 (0.3) similar to placebo's iPeak of +0.5 (0.2) (ER vs placebo p=0.43, but IR vs ER p=0.003). FFA area under the curve showed a similar pattern. **Conclusions:** Niacin formulations have different effects on fatty acid metabolism. Importantly, the increase in FFA on ER niacin was similar to that of placebo, though we confirm a marked rebound for IR niacin. As FFA rebound contributes to insulin resistance, this could explain why ER niacin reverses dyslipidemia with little impact on glucose metabolism.

Funding: Supported by NIH K12 RR017625-03

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ATORVASTATIN'S EFFECT ON INFLAMMATORY MARKERS (hsCRP, IL-6 AND IL-18) IN THE COMPARATIVE ATORVASTATIN PLEIOTROPIC EFFECTS (CAP) STUDY

***J. Davignon**, R. McPherson, J. Bonnet, A. Tedgui, P. Martineau, D. Simoneau, A. Nozza. CRIM, Montreal, QC, Canada; UOHI, Ottawa, ON, Canada; Inserm, Pessac, France; Inserm, Paris, France; Pfizer, Kirkland, QC, Canada; Pfizer, Paris, France.*

Background: CAP aimed to compare the effect of low and high dose atorvastatin on hsCRP in patients with CAD. To further explore the anti-inflammatory effects of atorvastatin, IL-6 and IL-18 concentrations were also measured. **Methods:** CAP was a 26-week, prospective, multicenter, double-blind, double-dummy study which enrolled 339 subjects with stable CAD. Subjects with LDL-C > 1.3 mmol/L but ≤ 3.9 mmol/L, hsCRP ≥ 1.5 mg/L but < 15 mg/L, and TG ≤ 4.6 mmol/L were randomized to atorvastatin 10 or 80 mg. **Results:** After 5 weeks, LDL-C decreased by 35.9% with 10 mg and by 52.7% with 80 mg ($p<0.001$) and remained stable thereafter. In the 10 mg group, mean hsCRP decreased by 25% at 5 weeks and remained stable thereafter. In the 80 mg group, hsCRP decreased by 36.4% at 5 weeks, but in contrast to the 10 mg group, hsCRP continued to decrease progressively over the 26-week study period (-57.1% at 26 weeks; $p<0.0001$ vs. week 5). Compared to 10mg, the 80 mg dose showed superior hsCRP reduction in all tertiles of baseline hsCRP. Concentrations of IL-6 and IL-18 did not change over time, but concentrations were significantly lowered in patients with baseline values in the highest baseline tertile. Atorvastatin was well tolerated in both study groups. **Conclusion:** Atorvastatin reduces hsCRP in a dose-dependent manner. High-dose atorvastatin induced a marked and progressive decline in hsCRP independently of changes in LDL-C. Moreover, atorvastatin 10/80mg exhibits anti-inflammatory action, as indicated by reduction of IL-6 and IL-18 in subjects with the highest baseline concentrations.

Funding: Pfizer

Saturday
October 6, 2007

Plenary Session 3 "THE ATHEROSCLEROTIC PLAQUE: FROM BIOLOGY TO CLINIC"
8:30 AM - 10:30 AM
***Chairs:** F. Mach, Geneva, Switzerland and C.M. Ballantyne, Houston, TX, USA.*

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INFLAMMATION AND IMMUNE SYSTEM IN ATHEROTHROMBOSIS

***Francois Mach**. Geneva University Hospital, Geneva, Switzerland.*

It is now generally recognized that atherosclerosis is a chronic inflammatory disease, characterized by over-recruitment of leukocytes to the site of inflammation. Vascular injury in response to cardiovascular risk factors promotes endothelial dysfunction, resulting in enhanced adhesion molecule expression and secretion of pro-inflammatory cytokines and chemokines. This, in turn, leads to adherence, migration and accumulation of leukocytes within atherosclerotic lesions. The recent findings on inflammatory processes involve in atherosclerosis development provide important links between risk factors and the mechanisms of atherogenesis. Thus, research interest has increasingly focused on inflammatory biomarkers as means of predicting the risk of future clinical events. Indeed, elevated plasma levels of molecules such as soluble intercellular adhesion molecule 1, interleukin-6 or C-reactive protein (CRP) have been shown to represent inflammatory markers of future cardiovascular risk. Among these, CRP has emerged as the most powerful and accessible for clinical use. Besides its predictive role in determining cardiovascular risk, C-reactive protein (CRP) may exert direct pro-atherogenic effects through pro-inflammatory properties. CRP is mainly produced by hepatocytes in response to interleukin-6 and is then released into the systemic circulation. Statins, significantly reduce cardiovascular events and mortality in patients with or without coronary artery disease and reduce plasma CRP levels in humans. We reported that statins limit both protein and RNA levels of IL-6-induced CRP in human hepatocytes.

These results demonstrate that statins reduce CRP production directly in hepatocytes. These findings furnish new evidence for direct anti-inflammatory properties of statins and provide new mechanistic insight into their clinical benefits.

Funding: None

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CURRENT METHODOLOGIES FOR ASSESSING CARDIOVASCULAR RISK

***Christie M. Ballantyne**. Baylor College of Medicine, Houston, TX, USA.*

Despite improvements in cardiovascular disease risk reduction by targeting major risk factors, many individuals who have cardiovascular disease events may not be identified on the basis of the major risk factors. Because inflammation plays an important role in the initiation and progression of atherosclerotic disease, including the occurrence of atherothrombotic events, increased understanding of the molecular basis of inflammation has led to the identification of biomarkers that are related to inflammation and may also be related atherothrombotic disease. Observational epidemiological studies and a few interventional trials have examined the association of biomarkers with atherosclerotic cardiovascular disease. In addition, surrogate endpoints such as carotid ultrasound have been widely used in epidemiological studies and clinical trials. Ongoing clinical studies are investigating the potential role of biomarkers in conjunction with noninvasive imaging to improve risk stratification, as surrogate measures of the efficacy of therapy, and as tools to select optimal medical treatment or to "personalize" therapy.

Funding: None

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TREATMENT OF CARDIOVASCULAR DISEASE: PRESENT AND FUTURE

***Antonio M. Gotto, Jr.**. Weill Cornell Medical College, New York, USA.*

Physicians must develop treatment plans that address the multiple cardiovascular risk factors that may be present in an individual patient, and one of these is often dyslipidemia. For many physicians, the 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase inhibitors, or statins, are first-line drug therapy for lipid disorders, based on several large clinical trials that have demonstrated substantial cardiovascular risk reductions with these agents. The re-examination of old therapeutic targets, identification of new targets, and development of new lipid-modifying agents will expand treatment options. Adjunctive drugs, such as cholesterol absorption inhibitors, may complement statin therapy for the optimal management of lipid disorders. In the area of new treatments, results of studies with inhibitors of cholesteryl ester transfer protein (CETP) have proved discouraging. Nevertheless, High-density lipoprotein (HDL) remains a valid target of investigation; data have suggested an anti-atherosclerotic benefit of transfusions using the mutant HDL protein, apo AI Milano, and other HDL-based therapies are in the pipeline. Despite the intriguing data on the horizon and the wealth of data in support of lowering low-density lipoprotein cholesterol, lipid disorders and other cardiovascular risk factors are not as aggressively managed as they should be, even in patients who may benefit the most. The issues that have facilitated such undertreatment will need to be resolved in order to maximize the utility of lipid-modifying strategies.

Funding: None

Plenary Supported Session 8 "BENEFITS OF STATIN THERAPY IN PATIENTS: NEW DIRECTIONS IN THE TREATMENT OF ATHEROSCLEROSIS"
11:00 AM - 12:30 PM
Chairs: *J. Shepherd, Glasgow, UK and J. LaRosa, Brooklyn, NY, USA.*

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EVOLUTION OF STATIN THERAPY: RISK REDUCTION AND THE ROLE OF LIPID THERAPY IN PRIMARY AND SECONDARY PREVENTION

John C. LaRosa*. SUNY Downstate Medical Center, Brooklyn, NY, USA.*

The introduction of HMG-CoA Reductase inhibiting drugs (statins) have revolutionized the care of patients with atherosclerosis. First and foremost, they have allowed adequate testing of the cholesterol "hypothesis." It is now firmly established that lowering low density lipoprotein-cholesterol (LDL-c) is proportionately and causally associated with a decreased risk of both morbid and mortal coronary and cerebravascular ischemic event. This benefit is more apparent in those at higher global risk of such an event and is evident across both age and gender boundaries. Even before wide-spread availability of generic statins, therapy was demonstrated to be cost effective, a finding that can only become more apparent as more powerful statins become available as generics. Questions regarding statin therapy remain, however. These include: (1) the relative value of statins in "primary" prevention, i.e. in individuals earlier in the course of atherosclerosis (2) the long-term safety of statins, particularly when taken over decades and with other medications and (3) the contribution of non-LDL lowering or "pleiotropic" effects of statins to their overall vascular benefits. Whatever the remaining uncertainties, it is clear that these drugs have dramatically improved the care of patients with symptomatic atherosclerosis.

Funding: None

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STATINS FOR HEART FAILURE: EVIDENCE AND MECHANISMS

David Waters*, San Francisco General Hospital, San Francisco, CA, USA.*

Patients with advanced heart failure were excluded from most of the randomized clinical trials that have shown statins to be effective in reducing cardiovascular events. Yet several mechanisms have been proposed whereby statins might benefit heart failure patients. By preventing recurrent ischemic events, statins prevent left ventricular damage and preserve LV function. Heart failure is characterized by endothelial dysfunction and statins reverse this abnormality. Statins also reduce the levels of proinflammatory cytokines that are associated with heart failure. Statins exert a favorable effect on LV remodeling in animal experiments, and in short studies of patients with non-ischemmic cardiomyopathy, statins have been shown to improve ejection fraction.

In the Treating to New Targets Trial (NEJM 2005;352:1425), 10,001 patients with coronary disease were randomized to 10 mg or 80 mg per day of atorvastatin and followed for a median of 4.9 years. The primary composite endpoint (cardiac death, MI resuscitated cardiac arrest and stroke) was reduced by 22% in the 80 mg compared to the 10 mg group (p=0/0002). Hospitalization for heart failure, a predefined secondary endpoint, was reduced by 26% in the 80 mg group (p=0.012). Among the 781 patients with a history of heart failure at baseline, the reduction in the incidence of heart failure hospitalizations during follow-up was 41% (p=0.008, Circulation 2007;115:576). Similar results were reported from the PROVE-IT Trial, where 80 mg per day of atorvastatin was compared to 40 mg of pravastatin among 4,162 patients followed for 24 months after an acute coronary syndrome episode. In two large ongoing trials among patients with heart failure, the effect of rosuvastatin is being compared to placebo on hard cardiovascular endpoints.

These data indicate that statins are likely to play an important role in the treatment of patients with heart failure.

Funding: None

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STATINS AND CV EVENT REDUCTION IN PATIENTS WITH RENAL DISEASE

***James Shepherd**, Royal Infirmary, Greater Glasgow & Clyde NHS, Glasgow, United Kingdom; University of Glasgow, Glasgow, United Kingdom.*

HMG CoA reductase inhibitors (statins) reduce the risk of cardiovascular events in a broad range of patients with and without pre-existing cardiovascular disease. Mild to moderate CKD patients have been underrepresented as a result of exclusion, or inadequate reporting of renal function. Limited data suggest, however, that such therapy is effective in preventing cardiovascular events. The post-hoc analysis of the TNT study outlined here was undertaken to investigate the effect of intensive lipid lowering with atorvastatin 80 mg on the risk of future cardiovascular events in CHD patients with and without CKD (stage 3 or greater). Atorvastatin 80 mg reduced major cardiovascular events compared with atorvastatin 10 mg in both patients with CKD and patients with normal or near normal eGFR levels at baseline. Indeed, the increased risk associated with CKD was reduced although not completely eliminated in these patients who were treated with 80 mg of atorvastatin. However, cardiovascular risk fell to a level near to that observed in patients with normal eGFR who received lower-dose atorvastatin. This study has implications for future CHD and CKD guidelines, indicating the value of serum creatinine screening in patients with CHD and promoting the concept that intensive LDL-cholesterol lowering should be recommended for patients with CHD and CKD who are at very high-risk of cardiovascular events.

Funding: Scientific Session supported by Pfizer

Debate 1 "WHERE DOES INSULIN RESISTANCE START?"

12:30 PM - 2:00 PM

***Moderators:** J.-C. Fruchart, Lille, France and F. Sacks, Boston, MA, USA.*

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WHERE DOES INSULIN RESISTANCE START?

***Guenther H. Boden**. Temple University School of Medicine, Philadelphia, PA, USA.*

Overabundance of fat (obesity) is associated with elevated plasma free fatty acid (FFA) levels. FFA, in turn, cause insulin resistance in all insulin targets including skeletal muscle, liver and endothelial cells and thereby contribute to the development of type 2 diabetes (T2DM), hypertension, dyslipidemia and non-alcoholic fatty liver disease. The mechanism through which FFA induce insulin resistance involves intramyocellular and intrahepatocellular accumulation of triglycerides and diacylglycerol, activation of several serine/threonine kinases, reduction in tyrosine phosphorylation of the insulin receptor substrate (IRS)-1/2 and impairment of the IRS phosphatidyl inositol 3 kinase pathway of insulin signaling. FFA also produce low grade inflammation in skeletal muscle and liver via activation of nuclear factor-kB resulting in release of several proinflammatory cytokines including TNF-a, IL-1b and IL-6 which can further impair insulin action.

Funding: This work was supported by National Institutes of Health grants R01-DK-58895, R01-HL-733267 and R01-DK-066003 and a mentor-based training grant from the American Diabetes Association

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WHERE DOES INSULIN RESISTANCE START? LIVER

Edward Horton. *Joslin Diabetes Center, Boston, MA, USA.*

Insulin resistance is characterized by decreased effectiveness of insulin to increase glucose uptake in skeletal muscle and adipose tissue and to decrease hepatic glucose output. These defects can be demonstrated by using the hyperinsulinemic-glucose clamp technique combined with infusion of glucose tracers. In the fasting state, blood glucose levels are maintained by a balance between peripheral glucose uptake (Rd) and hepatic glucose production (Ra). Following glucose ingestion, Ra is suppressed by a rise in insulin and a fall in glucagon levels. With hepatic insulin resistance there is both an increase in Ra during fasting and a failure to suppress Ra normally in response to meals, resulting in an elevation of both fasting and post-prandial blood glucose levels. These abnormalities in hepatic glucose regulation occur very early in insulin resistant states and can be demonstrated before any changes in blood glucose are observed. Underlying causes of hepatic insulin resistance are not fully understood. Genetic factors play a role in some cases, but the most compelling evidence supports nutrient overload, particularly increased liver fat content, as a major cause. This may or may not be associated with obesity. However, intra-abdominal obesity, increased free fatty acid influx and increased liver fat content are strongly associated with hepatic insulin resistance. At the cellular level, endoplasmic reticulum (ER) stress due to nutrient overload may be a critical factor. Reduction in liver fat content by caloric restriction, weight loss and increased physical activity improves hepatic insulin sensitivity as does treatment with medications such as metformin, thiazolidinediones or insulin.

Funding: None

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WHERE DOES INSULIN RESISTANCE START? THE ADIPOSE TISSUE

Stefano Del Prato. *University of Pisa, Pisa, Italy.*

Insulin resistance is a common condition in many of the metabolic disorders that afflict affluent societies, in particular type 2 diabetes and obesity. However, it is the latter that more frequently precedes development of type 2 diabetes, suggesting impaired insulin action may be sustained by adipose tissue excess, particularly when it accumulates in the visceral area. Epidemiologic observation has linked visceral obesity, insulin resistance and related metabolic abnormalities. Surgical removal of visceral fat improves insulin action and restore metabolic control. Visceral obesity is associated with increased FFA levels. Based on seminal studies by Randle and Newsholme, FFA have been shown to affect insulin-mediated glucose uptake in muscle by substrate competition. Later on, a direct effect of FFA on insulin-signalling, GLUT-4 and glycogen synthase was demonstrated. Excess of circulating FFA also may affect the β-cell thus favouring development of type 2 diabetes in insulin-resistant individuals. The metabolic mechanism of adipocyte-mediated insulin resistance is only one aspect of a complex picture. Adipose tissue expansion is indeed associated with altered production of adipokines. Increased TNFα results in serine-phosphorylation of IRS-1 and concomitant reduction of tyrosine phosphorylation, thus affecting insulin signalling. Interleukin-6 also is produced as it is, at least in rodents, resistin. Both adipokines hamper insulin action favouring development of insulin resistance. On the contrary, visceral obesity is associated with reduced adiponectin, an insulin "sensitizing" protein with interesting vascular protective effect. In summary, the adipose tissue, through modulation of energy depot and adipokines secretion, plays a pivotal role in the pathogenesis of insulin resistance.

Funding: None

Plenary Supported Session 9
"ATHEROSCLEROSIS, LDL-C, hs-CRP, AND HDL-C: MULTIPLE EFFECTS OF INTENSIVE STATIN THERAPY"
2:00 PM - 3:30 PM
Chairs: *R. Paoletti, Milan, Italy and S.M. Grundy, Dallas, TX, USA.*

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LDL-C AND BEYOND

Scott Grundy*. University of Texas Southwestern Medical Center, Dallas, TX, USA.*

Elevated plasma LDL is a major risk factor for atherosclerotic cardiovascular disease (ASCVD). In fact, it must be considered the primary risk factor because some elevation of LDL is required for the initiation and development of atherosclerosis. When LDL levels are very low, ASCVD rarely develops, even when other risk factors are present. Although LDL is the primary lipid risk factor, there is growing evidence that all lipoproteins containing apolipoprotein B-100 are atherogenic. This includes VLDL as well as LDL. In patients with the metabolic syndrome and type 2 diabetes, a significant portion of all apo B-containing lipoproteins are contained in VLDL. Thus total apo B, or LDL+VLDL cholesterol (non-HDL-cholesterol) carry a greater predictive power for ASCVD than does LDL cholesterol alone. It is possible that a low HDL promotes atherosclerosis as well. However, a low HDL is often a marker for a high VLDL level and other risk factors of the metabolic syndrome. For this reason, the atherogenic potential of a low HDL level is uncertain.

Funding: None

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THE ROLE OF hs-CRP IN DYSLIPIDEMIA

Paul M. Ridker*. Brigham and Women's Hospital, Boston, MA, USA.*

Inflammation plays a fundamental role in atherogenesis and the inflammatory biomarker hsCRP has proven to be an important risk factor for hard atherothrombotic events with a magnitude of risk equal to that of hyperlipidemia. In primary prevention, increased hsCRP indicates increased risk for MI, stroke, and vascular death (and incident type 2 diabetes). In the Reynolds Risk Score project which prospectively evaluated 24558 women over a 10-year period, the addition of hsCRP to traditional risk factors reclassified 40 to 50 percent of those at "intermediate risk" into clinically relevant higher- or lower-risk groups, and did so with markedly improved accuracy. Thus, for individuals at 5 to 20 percent 10-year risk, use of hsCRP allows more accurate targeting of statins to those with the most appropriate level of risk, minimizing toxicity and maximizing benefit. In ACS patients, results from PROVE IT-TIMI 22 and A to Z demonstrate that best clinical outcomes with statins are obtained when LDL-C levels are reduced below 70 mg/dL and when hsCRP levels are reduced below 2 mg/L. Thus, the concept of "dual goals" for statin therapy in terms of reducing both LDL and hsCRP has clinical relevance. The fully enrolled JUPITER trial of 17,802 primary prevention patients is testing whether statin therapy (rosuvastatin 20mg/d) should be given to apparently healthy individuals with low LDL but elevated hsCRP, a group at risk who do not currently qualify for treatment. Close to 40 percent of the JUPITER study population have metabolic syndrome, and thus this trial will also provide information on the efficacy of statins in this controversial group. Further, as the median LDL at entry is <110 mg/dl, JUPITER will provide important safety data on longterm agressive LDL reduction.

Funding: The JUPITER trial is an investigator initiated project funded by Astra-Zeneca

Workshop 15 "NEW HYPOLIPIDEMIC DRUGS (1st part)"
3:45 PM - 5:20 PM
Chairs: *H. Greten, Hamburg, Germany and H.B. Brewer, Jr., Washington, DC, USA.*

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ANTISENSE ApoB mRNA INHIBITION: FROM PROMISE TO PRACTICE

John Kastelein*. University of Amsterdam, Amsterdam, Netherlands.*

A novel promising treatment for hypercholesterolemia is antisense apolipoprotein B. ApoB is the main structural protein of all atherogenic particles, including VLDL, IDL, and LDL. Until recently direct inhibition of apolipoprotein could not be achieved by small molecule therapeutics. The antisense technology makes it possible to directly inhibit apolipoprotein B formation by blocking the translation of apoB mRNA. Antisense apoB is a 20-mer oligonucleotide, complementary to a part of the coding region of human apoB mRNA. A recent phase I trial demonstrated that treatment with short-term antisense apoB resulted in 50% apoB reduction with a concomitant 35% LDL-c reduction. Also in a dyslipidemic patient population treatment with this drug resulted in apoB and LDL-c reduction of up to 45%. Currently several phase II trials are ongoing addressing the safety and efficacy of treatment with antisense apoB in several patient populations.

Funding: None

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RNAi THERAPEUTICS FOR THE LOWERING OF LDLc CHOLESTEROL

Kevin Fitzgerald*, Maria Frank-Kamenetsky, Tracy S. Zimmermann, Amy C. Lee, Jay Horton, Akin Akinc, Birgit Bramlage, David Bumcrot, Matthew N. Fedoruk, Jens Harborth, James Hayes, Loyd B. Jeffs, Matthias John, Adam Judge, Victor Kotelianski, Kiew Lam, Muthiah Manoharan, Kevin McClintock, Lubomir Nechev, Martin Maier, Lorne R. Palmer, Timothy Racie, Ingo Rohl, Stephan Seiffert, Sumi Shanmugam, Vanda Sood, Jurgen Soutschek, Iva Toudjarska, Hans-Peter Vornlocher, Amanda Wheat, Ed Yaworksi, William Zeldis, Ian MacLachlan. Alnylam Pharmaceutical, Cambridge, MA, USA; Protiva, Burnaby, BC, Canada; Alnylam Pharmaceutical Europe, Kulmbach, Germany.*

Successful delivery of small interfering RNAs (siRNAs) *in vivo* is critical for the advancement of RNA interference (RNAi) therapeutics. In this work we demonstrate systemic delivery of siRNAs, and potent *in vivo* down-modulation of two non-druggable disease targets, apolipoprotein B (apoB) and proprotein convertase subtilisin kexin 9 (PCSK9). A single injection of formulated siRNA resulted in >90% silencing of apoB mRNA expression in the liver 48 h after administration. Reductions in apoB protein, and low-density lipoprotein (LDLc) levels were observed in 48 hours that lasted for at least 23 days, thus demonstrating an immediate, potent and durable biological effect. In addition to apoB we have shown down-modulation of PCSK9. PCSK9 has been closely implicated in LDLc regulation. We have demonstrated PCSK9 down-modulation in mouse, humanized mouse, rat, and primate models. Down-modulation of PCSK9 levels resulted in significant lowering of cholesterol (20-60%) in all animal models tested. These findings strongly support the potential of RNAi therapeutics as a new class of drug for metabolic and cardiovascular diseases. Our next steps include selecting the most potent lead molecule and moving it into GLP safety studies.

Funding: Alnylam Pharmaceuticals

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ANTISENSE INHIBITION OF MURINE APOLIPOPROTEIN B REDUCES HEPATIC APOLIPOPROTEIN B SECRETION WITHOUT INCREASING HEPATIC TRIGLYCERIDE OR PLASMA AMINOTRANSFERASE LEVELS CHARACTERISTIC OF MTP INHIBITORS

***Richard Lee**, Charles Whipple, Mark Graham, Rosanne Crooke. Isis Pharmaceuticals, Carlsbad, CA, USA.*

Objectives: There is a well established link between plasma apoB levels and atherosclerosis. Previous studies revealed that administration of an MTP small molecule inhibitor or an apoB antisense oligonucleotide (ASO) led to ~50% decreases in plasma apoB. However, mice treated with the MTP inhibitor also had elevations in hepatic TG, while mice treated with the apoB ASO had decreased hepatic TG. Our primary goal was to identify differences between hepatic apoB and MTP inhibition. **Methods:** HF-fed mice treated 6 weeks with either a control ASO, an MTP ASO, or an apoB ASO were injected with a ^{35}S-methionine/Triton WR-1339 and time points were collected. **Results:** TG secretion rates decreased in mice treated with apoB ASO (15.83 ± 0.5 mg/ml/min.) or MTP ASO (20.3 ± 6.0 mg/ml/min.) vs. controls (56.4 ± 5.1 mg/ml/min.). ApoB secretion decreased in apoB ASO treated mice at 60 min. (79.0 ± 13.5 % decrease) and 120 min. (77.2 ± 9.4%). MTP ASO treatment led to similar decreases in apoB at 60 min. and 120 min. However, mice treated with the apoB ASO had no change in plasma ALT or hepatic TG values (111.7 ± 32.9 mg/dl and 45.4 ± 1.1 mg/g tissue, respectively) vs. controls (50.3 ± 12.4 mg/dl and 41.6 ± 11.9 mg/g tissue, respectively), while MTP ASO treatment led to increases in both parameters (333.7 ± 85.5 mg/dl and 126.9 ± 18.1 mg/g tissue, respectively). **Conclusions:** ASO inhibition of hepatic apoB in HF-fed mice led to significant decreases in hepatic apoB and TG secretion without producing increased liver TG or elevations in ALT in contrast to ASO reduction of hepatic MTP.

Funding: This work was fully supported by Isis Pharmaceuticals, Inc.

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DISCOVERY AND CHARACTERIZATION OF A PARTIAL AGONIST OF THE NICOTINIC ACID RECEPTOR GPR109A

***E. Carballo-Jane**, J.G. Richman, K. Cheng, T.Q. Cai, A.K.P. Taggart, I. Gaidarov, J.S. Cameron, P.D. Boatman, R. Chen, G.L. Semple, D.T. Connolly, D.P. Behan, M. Hammond, S.D. Wright, S.L. Colleti, J.R. Tata, M.J. Forrest, M.G. Waters. Merck Research Laboratories (MRL), Rahway, NJ, USA; Arena Pharmaceuticals, Inc., San Diego, CA, USA; MRL, Rahway, NJ, USA.*

Nicotinic acid (NA) inhibits adipocyte triglyceride lipolysis, thereby lowering plasma free fatty acids (FFA). Decrease in FFA flux to the liver has been postulated to entrain the beneficial effects of NA: reduction of triglycerides, LDL cholesterol and Lp(a), and elevation of HDL cholesterol. However, NA also induces cutaneous flushing, limiting patient compliance. A G_i-coupled 7-transmembrane domain receptor expressed in adipocytes and macrophages, GPR109A, has been shown to mediate both NA-induced anti-lipolysis and cutaneous flushing in mouse models. Our goal was to identify a GPR109A ligand that maintains anti-lipolytic activity while avoiding flushing. MK-0354, a pyrazole tetrazole, acted as a partial agonist in cell-based assays in GPR109A-expressing CHO cells (reduction of intracellular cAMP). Whereas MK-0354 inhibited lipolysis in primary adipocytes to the same extent as NA, it failed to stimulate MAPK phosphorylation in transfected cells or primary macrophages. Consistent with these *in vitro* findings, MK-0354 had a marked therapeutic window between plasma FFA reduction and cutaneous flushing in mouse and dog. The pharmacological profile of MK-0354 in rodents and dogs suggested potential advantages over the currently available "low-flush" NA formulations. MK-0354 was therefore nominated for further examination in clinical trials.

Funding: Merck Research Laboratories

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EZETIMIBE IMPROVES HIGH FAT AND CHOLESTEROL DIET-INDUCED NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN MICE

Shuqin Zheng, Lizbeth Hoos, John Cook, Glen Tetzloff, Harry Davis, Margaret van Heek, ***Joyce J. Hwa****. Schering-Plough Research Institute, Kenilworth, NJ, USA.*

Objectives: Ezetimibe (EZE) is a novel cholesterol absorption inhibitor that reduces plasma LDL-C by selectively binding to the intestinal cholesterol transporter, Niemann-Pick C1-Like 1 (NPC1L1). Mice deficient in NPC1L1 are protected from high fat/cholesterol diet-induced fatty liver and hypercholesterolemia. The object of the present study was to determine if EZE treatment could reduce hepatic steatosis in diet-induced obese (DIO) mice. **Methods:** Mice were fed a diet containing high fat and cholesterol [HFC; 45% (Kcal) fat and 0.12% (w/w) cholesterol] from six weeks of age. After seven months of exposure to the HFC diet, mice (n=12/group) were treated with EZE (0, 0.5, 1.6, or 5 mg/kg/day) admix in the HFC diet for four weeks. **Results:** Compared to age matched chow fed mice, C57BL/6J mice chronically fed a HFC diet had significantly higher body weights (+60%), and enlarged livers (+180%) with elevated liver to body weight ratio (+75%). The DIO mice had 35, 24, and 3.8 fold higher levels of hepatic triglyceride (TG), cholesteryl ester (CE) and free cholesterol (FC), respectively. These livers of mice fed the HFC diet developed hepatic steatosis, with varying degree of fibrosis and steatohepatitis. 87% of the mice on the HFC diet for seven months had elevated plasma ALT activity, a biomarker for NAFLD. Four weeks of EZE treatment (5 mg/kg/day in diet) was able to reduce 40% of the TG, 80% of the CE and 50% of the FC that had accumulated in the liver after seven months of HFC feeding. Chronic EZE treatment also significantly decreased plasma ALT activity. **Conclusions:** These data suggest that reducing hepatic FC, CE and TG levels with EZE may be a novel treatment for NAFLD.

Funding: None

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IMPACT OF THE MTP-INHIBITOR, AEGR-733, ON THE SINGLE-DOSE PHARMACOKINETICS OF EZETIMIBE

Richard L. Dunbar, LeAnne T. Bloedon, Danielle Duffy, Ram Gadi, Rajesh Movva, William J. Sasiela, Daniel J. Rader, ***Marina Cuchel****. University of Pennsylvania, Philadelphia, PA, USA; Aegerion Pharmaceuticals, Bridgewater, NJ, USA.*

Background: Ezetimibe is a lipid-lowering drug that is typically combined with other agents to lower LDL-C to goal. Its use with non-statins is especially helpful in statin-intolerant patients. AEGR-733 is a microsomal triglyceride transfer protein (MTP) inhibitor that reduces LDL-C and triglycerides by a novel mechanism. Prior to clinical trials testing safety and efficacy of the combination, we assessed the potential for a drug-drug interaction between ezetimibe and AEGR-733. **Methods:** We enrolled 10 healthy volunteers, who took a single dose of ezetimibe 10 mg on day 1 followed by 7 days of monotherapy with AEGR-733 10 mg/day. On day 8, subjects combined the last dose of AEGR-733 with a repeat 10 mg dose of ezetimibe. We sampled blood over 24 hours on days 1 and 8 to determine the pharmacokinetic (PK) profile of the total, conjugated and unconjugated ezetimibe, and collected adverse event (AEs) and efficacy data. **Results:** AEGR-733 had no impact on the PK of ezetimibe. Comparing total ezetimibe from day 8 to day 1, the change in area under the curve (AUC) (0-inf) was -6% (90% CI, -26 to +20%, p=1), AUC (0-24) was +6% (90% CI -7 to +21%, p=0.2), and Cmax was +3% (90% CI -26 to +42%, p=0.4). Results for conjugated and unconjugated ezetimibe were similarly unaffected. Treatment with AEGR-733 10 mg lowered LDL by 28%, with few mild GI AEs and no clinically meaningful changes in liver enzymes. **Conclusion:** Our results demonstrate that AEGR-733 10 mg can be combined with 10 mg of ezetimibe to lower LDL-C without meaningful changes in the pharmacokinetics of ezetimibe.

Funding: Aegerion Pharmaceuticals

Workshop 16 "NEW HYPOLIPIDEMIC DRUGS (2nd part)"
5:30 PM - 6:35 PM
Chairs: *A. Corsini, Milan, Italy and M. Davidson, Chicago, IL, USA.*

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A BI-HELICAL ApoA-I MIMETIC PEPTIDE: EFFECT ON CHOLESTEROL EFFLUX AND ON ATHEROSCLEROSIS IN MICE

Alan T. Remaley*, Marcelo Amar, John Stonik. National Inst. of Health, Bethesda, MD, USA.*

ApoA-I peptide mimics that mediate cholesterol efflux by the ABCA1 transporter are currently being investigated as therapeutic agents. A potentially limiting property is that these peptides can also remove cholesterol by a cytotoxic microsolubilization process. The 37pA peptide (DWLKAFYDKVAEKLKEAF-P-DWLKAFYDKVAEKLKEAF) was modified by substituting up to 5 hydrophobic residues (L3, F6, V10, L14, F18) for A in either the C-terminal helix (5A) or both helices. 37pA (80 ug/mL) lysed in 1 h 30% of red blood cells, but substituting at least 4 residues with A in the second helix resulted in a peptide that was no longer hemolytic. Unlike 37pA, which largely effluxed cholesterol independent of ABCA1, the 5A peptide like apoA-I was almost completely dependent upon ABCA1 (HeLa cells: Km 10 ug/mL, Vmax 1.5%/18h; ABCA1-transfected HeLa cells: Km 8 ug/mL, Vmax 10%/18h). 5A was complexed with DPPC (1:7 molar ratio) and administered (IP 30mg/kg three times a week) to 8-week old apoE K/O on a normal chow diet for 9 weeks. A single injection of 5A resulted in a 45% increase of HDL-C at 6 h and a 165% increase in the ability of mouse serum to efflux cholesterol by ABCA1. At the end of the study, there were no apparent signs of toxicity from the peptide treatment, as assessed by liver and renal function tests, and the treated mice gained weight the same as controls. By en face analysis of the aorta, the 5A treated mice had a 53+15% ($P<0.002$) reduction in atherosclerosis. In conclusion, the 5A peptide containing a high and low lipid affinity helix showed the greatest ABCA1 specificity and the lowest cytotoxicity, and treatment with 5A can in increase HDL-C levels and reduce the progression of atherosclerosis in apoE K/O mice.

Funding: Intramural NHLBI funding

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IN VIVO AND IN VITRO STUDIES ON THE ANTIATHEROSCLEROTIC PROPERTIES OF EVEROLIMUS

Stefano Bellosta*, Roberta Baetta, Monica Canavesi, Nicola Ferri, Lorenzo Arnaboldi, Pascal Pfister, Agnese Granata, Richard Dorent, Alberto Corsini. University of Milan, Milan, Italy; Novartis Pharma AG, Basel, Switzerland.*

Everolimus (E) is an orally active immunosuppressive and antiproliferative compound derived from rapamycin. We investigated the potential antiatherosclerotic activity of E in cholesterol-fed rabbits subjected to perivascular carotid collar manipulation and in different cell culture models. In New Zealand White rabbits fed a 1% cholesterol diet for 4 weeks and randomized to everolimus (1.5 mg/kg given 1 day before collaring followed by 1 mg/kg per day for 14 days, administered by oral gavage) or vehicle control (N=14 per group), E reduced, at a plasma therapeutic concentration, the I/M ratio by 32%. Moreover, it decreased macrophage content in the plaque by 65% ($p<0.05$ vs vehicle), without affecting lipid plasma levels. Accordingly, in cultured cells, E (10^{-8} to 10^{-6} M) inhibited in a concentration-dependent manner monocyte chemotaxis up to 55% ($p<0.01$ vs control), and smooth muscle cell (SMC) proliferation by 60% ($p<0.01$ vs control) by blocking cell cycle progression in the G1 phase. Altogether the present findings highlight the ability of E to interfere, both *in vivo* and *in vitro*, with several processes involved in atherogenesis, such as macrophage accumulation and SMC proliferation within the intima, thus highlighting the potential antiatherosclerotic properties of everolimus.

Funding: The study was supported by Novartis AG, Basel, Switzerland

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DOSE-RELATED EFFECTS OF REPEATED RECOMBINANT APOA-IMilano ADMINISTRATIONS ON REGRESSION OF PLAQUES GENERATED IN RABBIT CAROTID ARTERIES

***Cinzia Parolini**, Marta Marchesi, Paolo Lorenzon, Mauro Castano, Elena Balconi, Luigi Miragoli, Linda Chaabane, Alberto Morisetti, Vito Lorusso, Bradley J. Martin, Charles L. Bisgaier, Brian Krause, Roger Newton, Cesare R. Sirtori, Giulia Chiesa. University of Milan, Milan, Italy; Bracco Imaging, Turin, Italy; Pfizer Inc., Ann Arbor, MI, USA.*

Objective: A recent clinical study has shown regression of atherosclerosis in coronary patients by administration of recombinant A-IM phospholipids complex (A-IM/POPC), a mutant form of apoA-I. Aim of the study was to evaluate in vivo, the minimal dose of A-IM/POPC able to induce atherosclerosis regression in a rabbit model of lipid-rich plaques. **Methods:** 36 rabbits underwent a perivascular injury at both carotids, followed by a 1.5% cholesterol diet. At 90 days after surgery, rabbits were randomly divided into 6 groups and treated, five times, with vehicle or A-IM/POPC at 5, 10, 20, 40, 150 mg/kg dose, by infusions, administered every four days. Plaque changes were evaluated in vivo by IVUS and MRI, performed before and at the end of the treatment period. **Results:** Atheroma volume in vehicle group strongly increased between the first and the second IVUS analysis (26.53±4.89% change). On the contrary, in A-IM/POPC-treated animals a reduced progression (15.28±0.87% and 8.60±0.54% changes, with 5 and 10 mg/Kg) or a regression (-1.56±1.17%, -3.40±2.07% and -6.83±2.86% changes, with 20, 40 and 150 mg/Kg) was detected. The results obtained by MRI analysis were in line with those at IVUS, and were significantly correlated (r=0.70599). **Conclusions:** A-IM/POPC repeated infusions proved effective in atherosclerosis treatment by determining from a lower progression at low doses to a clear-cut regression at the highest tested doses.

Funding: The study was funded by Pfizer Inc., USA

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EFFECT OF ACAT INHIBITION ON CAROTID ATHEROSCLEROSIS IN FAMILIAL HYPERCHOLESTEROLEMIA

***Marijn C. Meuwese**, Raphael Duivenvoorden, Aeilko H. Zwinderman, Lee R. Schwocho, Evan A. Stein, John J. Kastelein, Eric de Groot. Academic Medical Centre, Amsterdam, Netherlands; Daiichi Sankyo, Edison, NJ, USA; Cincinnati, OH, USA.*

Objective: The CAPTIVATE study investigated the effect of the acyl coenzyme A:cholesterol acyltransferase (ACAT) inhibitor pactimibe on carotid atherosclerosis in patients heterozygous for familial hypercholesterolemia (FH). **Methods:** CAPTIVATE was designed as a 2-year double-blind study with subjects randomized to either 100 mg pactimibe OD (n=371) or placebo (n=348), on top of standard lipid lowering therapy. Carotid atherosclerosis was assessed by ultrasound intima-media thickness (CIMT) measurements. **Results:** Due to unfavorable results in a parallel pactimibe study, ACTIVATE, using intra-coronary ultrasound, CAPTIVATE was prematurely terminated after 21 months. In-trial reproducibility in CAPTIVATE indicated that available B-mode ultrasound scans met the à priori set requirements to detect a relative ΔCIMT of at least 0.04 mm. Therefore, we assessed all images of patients with 2 or more scans at least 40 weeks apart. Covariance analysis showed that mean CIMT in the pactimibe group relatively increased if compared to placebo (Δ0.014 (SE 0.007) mm: p=0.04).**Conclusions:** The CAPTIVATE CIMT study demonstrated that pactimibe was associated with enhanced progression of atherosclerosis compared to placebo in FH. This is the third vascular imaging study to report that ACAT inhibition does not decrease atherosclerosis and may even promote atherogenesis.

Funding: Sankyo

Workshop 17 "IMAGING"
3:45 PM - 5:30 PM
Chairs: *E. Tremoli, Milan, Italy and Z.A. Fayad, New York, NY, USA.*

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MULTIMODALITY (PET/CT, MR, AND CT) IMAGING OF THE ATHEROSCLEROTIC PLAQUE: IMPLICATIONS FOR NEW REGRESSION CLINICAL TRIALS

Zahi A. Fayad*. Mount Sinai School of Medicine, New York, NY, USA.*

Atherosclerosis is an inflammatory disease, where the degree of inflammation, not the plaque size, determines risk of rupture and therefore likelihood of a clinical event. Magnetic Resonance Imaging (MRI) can image atherosclerotic plaque with high resolution, and several MRI parameters of disease extent in the carotid arteries and aorta have been shown to correlate with atherosclerotic risk factors. Dynamic-contrast-enhanced MRI (DCE-MRI) is a new technique for the study of plaque composition. In this study, the extent of plaque inflammation determined by FDG uptake was correlated with DCE-MRI. By providing a metabolic image of macrophage activity, F18-Fluorodeoxuglucose (FDG) positron emission tomography (PET) can image atherosclerotic plaque inflammation in patients and in animal models of disease, with a strong correlation between FDG uptake and plaque macrophage content. In addition, autoradiography has confirmed that the FDG signal originates from activated macrophages within the lipid core and fibrous cap of the plaque. This has led to the suggestion that FDG-PET might have a role in identifying 'high risk' plaques and monitoring their response to therapy. Computed tomography (CT) can be used in conjunction with PET to help co-register the PET images and for attenuation corrections. Moreover, CT with its exquisite coronary imaging has the potential to address atherosclerosis in the vessel wall of the coronary arteries. We review in this talk to use of multimodality imaging (MR, PET, and CT) for the study of inflammation of vessel wall may be useful in assessment of plaque vulnerability.

Funding: NIH NHLBI

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CAROTID INTIMA MEDIA THICKNESS AS MARKER OF ATHEROSCLEROSIS: RESULTS OF THE IMPROVE STUDY

Elena Tremoli*, Damiano Baldassarre. University of Milan, Milan, Italy; Monzino Cardiologic Center, Milan, Italy.*

The intima-media thickness (IMT) of extracranial carotid arteries, measured by high-resolution B-mode ultrasound has been proposed as an useful surrogate marker of atherosclerosis in carotid arteries and in other vascular regions. IMT is a good predictor of new myocardial infarction and it has been shown to be influenced by drugs known to reduce cardiovascular events, which supports the concept that IMT represents a biomarker of atherosclerosis. Carotid IMT alone has the same predictive capacity of VRFs. In a longitudinal - observational study, we have shown that the integrated use of VRFs included into the Framingham risk score and ultrasonic measurements of carotid IMT significantly increase their capacity to predict cardiovascular events in patients at low/intermediate risk. The integration of carotid IMT with non conventional VRFs may further optimize the stratification of patient risk. Another important carotid ultrasonic variable that may have predictive capacity, alone or when integrated with conventional or non conventional risk factors, is the progression of carotid IMT. A prospective, multicenter, longitudinal, long-term, observational study (The IMPROVE study) is currently ongoing. It aims to investigate the capacity of both cross sectional carotid IMT and overall IMT-progression to predict alone, or after integration with both conventional and non conventional VRF, the rate of new vascular events in an European population classified at high risk of cardiovascular disease for the presence of at least 3 VRFs. The patients' enrolment ended in April 2005 and a total of 3711 patients were recruited in 6 European countries.

Funding: Italian Ministry of Health, European Commission, IMPROVE project

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CORRELATION OF 3T-MRI CAROTID ARTERY WALL VOLUME AND B-MODE ULTRASOUND INTIMA-MEDIA THICKNESS MEASUREMENTS

***Raphael Duivenvoorden**, Aart Nederveen, Bart M. Elsen, Mieke D. Trip, Eik S. Stroes, John J. Kastelein, Eric De Groot. Academic Medical Center, Amsterdam, NH, Netherlands.*

Introduction: Cardiovascular MRI permits non-invasive, serial in vivo measurement of carotid arterial wall volume (AWV). We compared 3T-MRI AWV measurements with carotid ultrasound Intima-Media Thickness (CIMT) by descibing the correlation between both measures. **Methods:** 3T-MRI and B-mode ultrasound scans were done in the common carotid arteries of 10 young healthy volunteers (aged 28, SD 1.5). Axial T1-weighted TSE image stocks were acquired at end-diastole using a 5cm single-element microcoil (Philips, Hamburg, Germany). Sequence parameters were: slice thickness 3 mm, imaging matrix size 240, FOV of 60 x 60 mm, non-interpolated pixel size 0.25 x 0.25 mm, reconstruction matrix 240, TE 11 ms, TR according to the subjects heart rate. Active fat suppression (SPAIR) was applied together with a double inversion black blood prepulse. Carotid AWV was calculated by manual delineation of the carotid lumen volume and the outer carotid vessel volume. IMT measurements were performed using a standardized imaging protocol and a Sequoia 512 scanner equipped with an 8L5 transducers (Acuson-Siemens, Erlangen, Germany). IMT and AWV measurements were aggregated to a per subject scan CIMT and AWV. **Results:** 3T-MRI AWV was 245.5 (SD32.8)mm3 and ultrasound CIMT 0.619 (SD0.063)mm. The Pearson correlation coefficient for AWV and CIMT was 0.71 ($p=0.03$). **Conclusion:** 3T-MRI AWV measurements of the carotid artery are significantly correlated with B-mode ultrasound CIMT in youngh healthy volunteers. Further studies in 30 healthy younger and older human subjects, and in a porcine animal model are in progress.

Funding: None

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COMMON CAROTID ARTERY DIAMETER AS A PROMISING NEW CANDIDATE MARKER OF CARDIOVASCULAR RISK IN THE IMPROVE STUDY COHORT

***Damiano Baldassarre**, Elena Tremoli, on behalf of the IMPROVE Study Group. University of Milan, Milan, Italy; Monzino Cardiology Center, IRCCS, Milan, Italy.*

Aim: To explore whether inter-adventitial common carotid artery diameter (CCAD) detected by B-mode ultrasound may be considered a candidate marker of cardiovascular risk. **Methods:** The baseline data of the IMPROVE study cohort, including 1776 men and 1935 women representative of an European population at high risk of cardiovascular diseases, were analysed to explore whether CCAD is associated with the extent of carotid atherosclerosis, independently of traditional vascular risk factors and anthropometric variables. **Results:** After adjustment for possible confounders (height, centre, reader and sonographer), CCAD was positively associated with male gender, age, weight, body mass index, waist, hip, waist/hip ratio, systolic and diastolic blood pressure, pulse pressure, triglycerides, blood glucose, uric acid, cigarette packyears and family history of peripheral vascular disease (all $p<0.0001$). In contrast, years elapsed since smoking cessation, total-, HDL- and LDL-cholesterol were negatively related to CCAD (all $p<0.0001$). In the full model adjusted for all these confounders, CCAD was positively associated with carotid wall thickness ($p<0.0001$ for both IMT_{mean} and IMT_{max}) and with the prevalence of carotid segments with plaques ($p<0.04$). **Conclusion:** Inter-adventitial CCAD is positively associated with carotid atherosclerosis and with most vascular risk factors. These results suggest that CCAD is a promising candidate marker of cardiovascular risk. Completion of the IMPROVE study will allow to assess prospectively the value of CCAD to predict new vascular events, independently to VRFs and carotid IMT.

Funding: European Project, IMPROVE QLG1-CT-2002-00896

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EFFECT OF INTENSIVE LIPID ALTERING THERAPY ON THE SIZE OF ATHEROSCLEROTIC LESIONS IN THE SUPERFICIAL FEMORAL ARTERIES

***Joel Morrisett**, Christof Karmonik, Douglas Brown, Shawna Johnson, Vijay Nambi, Paul Sovelius, Kay Kimball, Christie Ballantyne, Alan Lumsden. Baylor College of Medicine, Houston, TX, USA; Methodist DeBakey Heart Center, Houston, TX, USA; The Methodist Hospital Research Institute, Houston, TX, USA.*

Objective: To determine if intensive lipid altering therapy reduces the size of atherosclerotic plaques in superficial femoral arteries (SFA) of patients with PAD. **Methods**: This is a double blind study of 120 patients with PAD requiring intervention on one leg, leaving the non-intervened contralateral leg for evaluating the effect of drug therapy. Subjects had untreated LDL-C >160 mg/dl; 60 received simvastatin 40mg, and 60 received simvastatin 40mg, ezetimibe 10mg, and niacin 1500mg. Volume of total artery (TA), total wall (TW), normal wall (NW), plaque (P), and lumen (L) of specific non-intervened SFA segments were measured by 3.0T MRI. Up to 25 slices 2mm thick were acquired, usually centered 8cm above the patella. Subjects were scanned at 0, 6, 12, and 24 months. Images were analyzed using a semi-automated edge detection algorithm. **Results**: The CV for TA, TW, NW, P, and L from SFA were comparable to those observed previously for carotid arteries. Thus far 45 patients have been randomized and >140 MRI exams completed. When changes in volumes of TA, TW, NW, P, and L are plotted vs slice position, profiles are generated that reflect the condition of the artery segment. **Conlusions:** Atherosclerotic plaques in SFA can be visualized non-invasively by 3T MRI and occur as both short intermittent and long extended lesions. Completion of this trial will allow comparison of the two lipid altering therapies.

Funding: HL078524, HL63090, T32 HL07812, Abbott (niaspan), Merck Schering Plough (ezetimibe)

Workshop 18 "STEM CELLS IN CVD"
5:30 PM - 6:40 PM
***Chairs:** Q. Xu, London, UK and S. Rafii, New York, NY, USA.*

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VASCULAR REPAIR BY STEM CELLS

***Qingbo Xu**. King's College London, London, United Kingdom.*

Although mature endothelial cells can proliferate and replace damaged cells in the vessel wall, recent findings indicate an impact of stem and progenitor cells in repair process. This presentation aims to briefly summarize the recent findings in stem cell research relating to the role of the cells in vascular repair. It has been demonstrated that endothelial stem cells present in the blood may be derived from a variety of sources, including bone marrow, spleen, liver, fat tissues and the adventitia of the arterial wall. In response to cytokine released from damaged vessel wall and adhered platelets, circulating stem cells home to the damaged areas. Recently, we demonstrated that laminar flow enhanced stem cell proliferation and differentiation into endothelial cells, as identified by endothelial marker gene expression at mRNA and proteins, increased endothelial numbers, and the enhanced capacity of in vitro tube formation and in vivo neovascularization. Over-expression of p53 and p21 enhanced endothelial marker reporter gene expression. Laminar flow up-regulated HDAC3 proteins level with concomitant increase of HDAC activity and p53 deacetylation. These data suggest that shear stress is a key regulator for stem cell differentiation into endothelial cells. To explore therapeutic possility, Sca1+ cells were were applied locally. While the injured vessel was completely occluded by neointimal lesions 2 weeks postoperatively, local transfer of shear stress-induced Sca1+ cells significantly reduced neointima lesions. In conclusion, stem cells contribute to vascular repair, during which shear stress promotes stem cell differentiation towards endothelial phenotypes. These findings indicate a potential of stem cell therapy for vascular disease in which shear stress should be considered as a crucial factor.

Funding: Supported by BHF and the Oak Foundation

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THE RELATIONSHIP BETWEEN ENDOTHELIAL PROGENITOR CELLS, THEIR APOPTOTIC MICROPARTICLES AND THE FRAMINGHAM RISK

***Matteo Pirro**, Francesco Bagaglia, Massimo R. Mannarino, Giuseppe Schillaci, Elmo Mannarino. Internal Medicine, Angiology and Arteriosclerosis Diseases, Perugia, Italy.*

Exposure to cardiovascular risk factors causes the release of pro-atherogenic microparticles (MPs) from vascular cells and reduces the number of atheroprotective endothelial progenitors (EPCs). We investigated whether MPs shedding from EPCs are detectable in cultures of EPCs and in the circulation of subjects with various degrees of cardiovascular risk. We investigated the relationship of EPCs-derived MPs to cardiovascular risk factors and aortic stiffness, a marker of cardiovascular risk and impaired vascular repair by EPCs. We estimated Framingham risk score in 105 individuals with various degrees of cardiovascular risk and measured aortic stiffness, as well as the number of circulating EPCs and EPCs-derived MPs (CD34+/KDR+) by FACS analysis. Release of apoptotic CD34+/KDR+ MPs was tested in cultures of EPCs exposed to incremental concentrations of the pro-apoptotic hydrogen-peroxide. The number of annexin-V positive EPCs-derived MPs increased from 1473/mL after vehicle exposure to 5719/mL after 1.5 mM hydrogen-peroxide exposure. The Framingham risk was associated with EPCs ($r=-0.47$, $p<0.001$) and CD34+/KDR+ MPs ($r=0.56$, $p<0.001$). Framingham risk ($b=0.40$; $p<0.001$) and EPCs ($b=-0.32$; $p=0.001$) were independently associated with EPCs-derived MPs. Low EPCs ($r=-0.59$, $p<0.001$) and high CD34+/KDR+ microparticle levels ($r=0.57$, $p<0.001$) were predictors of aortic stiffness, independent of the Framingham risk. In conclusions, EPCs undergo fragmentation into microparticles when exposed to a pro-apoptotic milieu. Increased microparticle shedding from EPCs reduce circulating EPCs levels and may thus contribute to increase aortic stiffness beside traditional risk factors.

Funding: None

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THE AMOUNT OF CD34+, CD 34-CD 133+ AND GPA+ IN PERIPHERAL BLOOD LIVING NUCLEAR CELLS IN PATIENTS WITH HEART FAILURE CAUSED BY CARDIO- AND HEPATOTROPIC VIRUSES

***Mamanti Rogava**, Zviad Gurtskaia, Tamar Bochorishvili. Acad.Nodar Kipshidze National Center of Therapy, Tbilisi, Georgia.*

Intensification of the regeneration of the damaged myocardium and other organs via the increase in number of stem cells in peripheral blood is of great interest. The aim of our research was to study amount of stem cells, progenitors and living nuclear cells in peripheral blood. In our study were involved 72 patients (57 men and 15 women),mean age 39±5.8). Patients were divided into two groups: I group -patients with chronic heart failure I-IV f.c. (NYHA) (dilated cardiomyopathy, CHD, myocarditis). Patients in the II group had no complaints due to cardiovascular system. Our study revealed that in patients with chronic heart failure amount of living cells in peripheral blood is diminished when compared to controls and healthy people, while the number of stem cells and progenitors (such as CD34+, CD34-CD133+) in peripheral blood increase parallel to progression of heart failure; GP-A cell number drops in this condition and starts to increase only when degree of heart failure severity due to successfully treatment is associated with the increase in living cell number. **Conclusion:** Changes in the number of these cells that go in parallel with aggravation of heart failure indicate to a possible reserve ability of the organism. In future it would be possible to implement these parameters in clinical practice to evaluate prognosis and therapeutic strategies for the condition.

Funding: Georgian International Society of Cardiomyopathy

Workshop 19 "METABOLIC SYNDROME"
3:45 PM - 5:25 PM
Chairs: *R. Carmena, Valencia, Spain and R.H. Eckel, Denver, CO, USA.*

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MANAGEMENT OF DYSLIPIDEMIA IN THE METABOLIC SYNDROME

Rafael Carmena*. University of Valencia, Valencia, Spain.*

Objective: Dyslipidemia is a central manifestation of the metabolic syndrome (MetS) and is present in at least 50% of subjects, and dyslipidemia is a consistent component of insulin-resistance. The MetS has been associated with an increased risk of cardiovascular disease (CVR) and death, and dyslipidemia is a major contributory factor for this risk. Although lifestyle changes are the cornerstone of therapy and will improve all components of the syndrome, they are rarely achieved in practice. Pharmacotherapy is usually required to reduce cardiovascular risk. Intensive treatment with statins or fibrates becomes an important aspect of the therapeutic programme. **Results:** In the VA-HIT, patients with MetS benefited more from gemfibrozil treatment. Similar results have been observed in *post-hoc* analysis of the BIP study The results of the FIELD study (using fenofibrate), however, could not confirm these findings. Statins are currently the mainstay therapy for dyslipidaemia, and have been shown to be effective in reducing the risk of CHD and stroke in patients with MetS. In the TNT trial, patients with MetS and stable CHD were randomized to either atorvastatin 10 mg/day or 80 mg/day. After mean follow up of 4.9 years, a primary event occurred in 13.0% of patients receiving atorvastatin 10 mg, compared with 9.5% of those receiving atorvastatin 80 mg (P <0.0001). **Conclusion:** Concerning CVR reduction, treatment of dyslipidemia with fibrates in MetS subjects has produced inconsistent results. Different intervention trials with statins have shown a consistent and significant reduction in the rate of major CV events including stroke.

Funding: Grant from Instituto de Salud Carlos III RCMN C03/08, Madrid Spain

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CUTTING THROUGH THE CONTROVERSIES OF THE METABOLIC SYNDROME

Robert H. Eckel*. University of Colorado at Denver and HSC, Aurora, CO, USA.*

The Metabolic Syndrome (MS) is a clustering of components (waist circumference, fasting triglycerides, HDL cholesterol, BP, and fasting glucose) that relates to the risk for CVD (adjusted risk ~1.5 fold) and type 2 diabetes (~3-6 fold). The NCEP and IDF definitions are most frequently used. The major difference is that the IDF defines abdominal obesity as a waist circumference ≥ 94 cm for Europid men and ≥ 80 cm for Europid women whereas the NCEP uses ≥ 102 and ≥ 88 cm, respectively. Adjustments for cerrtain populations, i.e. in Southern Asia, China, and ethnic Central and South America is recommended. In 2005, a joint ADA/EASD statement raised concerns about the MS including issues related to the definition, mechanisms, and therapeutics. Because this is a syndrome not a disease, the definition will likely change to best reflect mechanisms and disease risk. Hypertension excepted, insulin resistance explains most of the MS. Inflammation likely contributes to the insulin resistance. The MS identifies risk that is modifiable by lifestyle, extends beyond LDL cholesterol, is easily assessed and applied, economical and in the US is reimbursable (ICD-9 code, 277.7). With weight reduction, substantial modification of most if not all of the components of the syndrome are likely. Beyond lifestyle, weight loss drugs, bariatric surgery, high dose statins, niacin, ACE inhibitors, ARBs, and TZDs can all favorably modify more than 1 component of the syndrome. In summary, with additional data the definition of the MS will be refined. The identification of MS patients should promote a greater emphasis on lifestyle and risk factor modification to reduce the risk for CVD and diabetes.

Funding: None

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THE HYDROPHOBIC IMINOSUGAR AMP-DNM INCREASES INSULIN SENSITIVITY AND ENHANCES REVERSE CHOLESTEROL TRANSPORT IN MICE

Nora Bijl, Roelof Ottenhoff, Cindy P. van Roomen, Saskia Scheij, Johannes M. Aerts, ***Albert K. Groen****. Academic Medical Center, Amsterdam, Netherlands.*

Glycosphingolipids have been implicated to play a role in regulation of insulin sensitivity. It was the **aim** of this study to investigate the effect of a specific inhibitor of glycosphingolipid synthesis on parameters of the Metabolic Syndrome and cholesterol homeostasis. **Results:** Treatment of ob/ob mice with the hydrophobic iminosugar AMP-DNM (N- (5'-adamantane-1'-yl-methoxy)-pentyl-1-deoxynojirimycin) at a dose of 100mg/kg for 4 weeks ameliorated many symptoms of the Metabolic Syndrome; plasma glucose level and oral glucose tolerance tests normalized, HbA1c decreased 50% and hepatic triglyceride decreased by 50%. AMP-DNM decreased glycosphingolipid lipid content in liver by about 35%. The compound had no effect on liver cholesterol or phospholipid. Plasma triglyceride, FFA and phospholipid were unaltered but plasma cholesterol increased 10% and cholesterol synthesis (fecal neutral sterol) increased 50% indicating enhanced reverse cholesterol transport. This was probably due to increased biliary lipid secretion caused by upregulation of bile acid synthesis. **Conclusion:** Four weeks treatment with the iminosugar AMP-DNM normalizes glucose homeostasis in ob/ob mice and stimulates reverse cholesterol transport indicating that modulation of glycosphingolipid metabolism may develop into a novel treatment modality for Metabolic Syndrome.

Funding: Academic Medical Center, Amsterdam; MacroZyme, Amsterdam

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ROLE OF PHYSICAL ACTIVITY IN THE REDUCTION OF CARDIOVASCULAR RISK

Eduardo Farinaro*, Elisabetta Della Valle. University Federico II, Naples, Italy.*

The benefit of physical activity and fitness on plasma lipid levels, blood pressure, body weight glucose metabolism, endothelial function and insulin sensitivity has been demonstrated. Increasing physical activity can lead to a decreased clustering of cardiovascular risk factors associated with the metabolic syndrome. In our observations, people engaged in physical activity (2.5 hours a week) had lower plasma insulin levels, and the benefit also is extended to overweight and obese people. In hypertensive men in treatment with betablocking drugs after two months of physical exercise training all metabolic parameters improved, the most interesting finding was the increase of HDL cholesterol levels from 20 to 40 mg/dl.When the scientific community prescribes moderate physical exercise, the baseline individual's physical fitness should be taken into account; in fact, older or less fit people should consider the subjective rating of exertion. Before 1995, guidelines recommended vigorous intensity exercise for at least 20 minutes, three times a week. Subsequently, 30 minutes of moderate intensity, carried out almost daily was suggested. Physical activity should be performed regularly, and people of all ages should include a minimum of thirty minutes of moderate physical activity almost daily, for a total energy expenditure of 1000 kcal per week. Physical activity has numerous beneficial physiologic effects; therefore, programmes to improve lifestyle should have high priority in national policies to implement prevention of cardiovascular diseases.

Funding: The research has been carried out with the contribution of University Federico II Naples and National Coucil of Researches (CNR)

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KEY ROLE OF POSTPRANDIAL HYPERGLYCEMIA FOR THE PRESENCE AND EXTENT OF CORONARY ATHEROSCLEROSIS

***Christoph H. Saely**, Heinz Drexel, Harald Sourij, Stefan Aczel, Heidrun Jahnel, Robert Zweiker, Thomas Marte, Werner Benzer, Thomas C. Wascher. VIVIT Institute, Feldkirch, Austria; Medical University of Graz, Graz, Austria.*

Objective: To investigate the associations between abnormal glucose tolerance and angiographically characterized coronary artery disease (CAD). **Methods:** We enrolled 1040 consecutive Caucasian patients undergoing coronary angiography for the evaluation of CAD. An oral 75g glucose tolerance test was performed in patients without previously diagnosed diabetes. **Results:** From our patients, 394 had normal glucose tolerance (NGT), 190 impaired glucose tolearnce (IGT), 90 isolated postchallenge diabetes (postchallenge glucose ≥200 mg/dl), and 366 type 2 diabetes previously established or newly diagnosed on the basis of fasting glucose (conventional diabetes). Angiographically detectable CAD was more frequent in patients with impaired glucose tolerance (IGT), isolated postchallenge diabetes, or conventional diabetes when compared to NGT subjects (87.9%, 95.6%, 89.1% vs. 80.7%; p = 0.030, 0.001, 0.043, respectively). The prevalence of significant coronary stenoses ≥50%, compared to NGT subjects (57.4%), was similar in IGT patients (59.5%; p = 0.628), but significantly higher in patients with isolated postchallenge diabetes (77.8%; p = 0.001) or conventional diabetes (68.0%; p = 0.002). Also the number of significant stenoses was similar in IGT as in NGT subjects, but significantly higher in those with isolated postchallenge or conventional diabetes. Multivariate adjustment confirmed these results. **Conclusions:** In IGT, non-significant CAD is more frequent than in NGT; the prevalence and number of significant stenoses increases when postchallenge diabetes evolves.

Funding: There are no funding sources of commercial nature

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NIACIN RAISES GLUCOSE MORE IN METABOLIC SYNDROME PATIENTS WITH NORMAL FASTING GLUCOSE VERSUS IMPAIRED FASTING GLUCOSE

***Richard L. Dunbar**, Ramprasad Gadi, LeAnne Bloedon, Danielle Duffy, Amanda L. Baer, Daniel J. Rader, Frederick F. Samaha. University of Pennsylvania, Philadelphia, PA, USA.*

Objective: To determine whether niacin raises glucose more in metabolic syndrome (MetSyn) patients with impaired fasting glucose (IFG) than those with normal fasting glucose (NFG). **Background:** Niacin improves the dyslipidemia of MetSyn, but may provoke insulin resistance (IR) and rarely drug-induced diabetes. A subset of MetSyn has IFG (glucose >= 100 mg/dL), which may make them prone to greater increases in glucose from niacin. **Methods:** In this clinical trial, non-diabetics with MetSyn started extended-release (ER) niacin, titrating to 2g/day over 4 weeks. We compared baseline to 4-week fasting glucose, and report the mean change (95% CI, p vs baseline). **Results:** We studied 83 adults. Among 10 with IFG, glucose fell 5 mg/dl on niacin (-16.8 to +6.4, p=0.3), but rose 8 mg/dL in those with NFG (+4.5 to +12.3, p<0.00005) (IFG vs NFG, p=0.015). Change in glucose was inversely correlated with baseline glucose (Spearman's rho -0.31, p=0.005), and persisted adjusting for age, sex, the other 4 MetSyn criteria, and insulin. **Conclusion:** Surprisingly, those with IFG had no significant change in glucose on niacin, but those with NFG had significant increases. Both IFG and niacin are associated with peripheral IR. Perhaps the IR of IFG overshadows that of niacin, so that the incremental effect of ER niacin on IR is inconsequential. The small changes in both groups accord with milder effects of ER niacin on glucose than older formulations. In summary, our data challenge the notion that patients with IFG are more vulnerable to increasing glucose when starting niacin.

Funding: Abbott Laboratories

Session 1 "DOES CARDIOMETABOLIC RISK INTEGRATE METABOLIC SYNDROME?"
5:30 PM - 6:30 PM
Chairs: *E. Horton, Boston, MA, USA and M. Stern, San Antonio, TX, USA.*

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METABOLIC SYNDROME: AN UPDATE ON THE CURRENT DEBATE

Scott Grundy*. University of Texas Southwestern Medical Center, Dallas, TX, USA.*

The metabolic syndrome is associated with increased risk for both cardiovascular disease and type 2 diabetes. In addition, the frequency of the metabolic syndrome is increased with other conditions such as fatty liver, cholesterol gallstones, obstructive sleep apnea, and polycystic ovarian disease. The syndrome further is made up of several well established risk factors, e.g. dyslipidemia, dysglycemia, hypertension, and prothrombotic and proinflammatory states. Although there is a "common metabolic soil" to the syndrome, it is multifactorial in origin. For these many reasons, it is not surprising that considerable disagreement among scientists exists on several aspects of the syndrome including criteria for its clinical diagnosis. This metabolic complexity presents a challange for the presentation of the syndrome to the clinical world. There is little doubt that the syndrome is highly important as a multiplex cardiovascular risk factor, but efforts to simplify it for the medical world have not been entirely successful. Of course, the same is true for other complex conditions characteristic of clinical practice. But since the metabolic syndrome is a relatively new medical concept with unresolved issues, it will take some time before it will be widely acceptable as a practical concept that can modify clinical practice. Even so, the value of the concept is so great that efforts to better understand and articulate the meaning of the syndrome are worthwhile.

Funding: None

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METABOLIC SYNDROME: QUESTIONING THE CONCEPT

Michael Stern*. Univ. of TX Health Science Ctr at San Antonio, San Antonio, TX, USA.*

We evaluated whether the metabolic syndrome contributes additional information to identifying individuals at high risk of type 2 diabetes or CVD beyond that provided by a validated Diabetes Risk Model and the Framingham Risk Score. The incidence of diabetes and CVD were determined in Mexican Americans and non-Hispanic whites residing in San Antonio, TX. Metabolic Syndrome was defined according to the NCEP ATP III criteria. The metabolic syndrome and the risk scores were compared using the areas under Receiver Operating Characteristic curves (aROCs). The two risk scores had a significantly higher aROCs than the metabolic syndrome for predicting either endpoint. We explored several combined strategies in which high risk individuals were identified using different thresholds on the Framingham Risk Score to which were added individuals who fell below the Framingham threshold, but who met criteria for the metabolic syndrome. In every case superior predicting could be achieved by simply relaxing the Framingham threshold. The sensitivity and specificity were always higher using the Framingham Risk Score with the relaxed threshold than by using using the combined Framingham Score with the more stringent threshold plus the metabolic syndrome. We were unable to define a predicting strategy that incorporated the metabolic syndrome that was superior to using the Diabetes Risk Model or the Framingham Risk Score alone. Using these two risk scores, we created a user friendly Excel Spread Sheet which can be used to calculate the 10-year risk of either type 2 diabetes or CVD. We believe that the predicting properties of this tool are superior to those of the metabolic syndrome. The use of this tool will be demonstrated.

Funding: This work was supported by grants from the National Heart, Lung, and Blood Institute (R01 HL24799 and R01 HL36820)

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THE GLOBAL CARDIOMETABOLIC RISK INTEGRATES THE METABOLIC SYNDROME

H. Bryan Brewer. *Medstar Research Institute, Washington, DC, USA.*

During the last two decades, clinical trials have conclusively established that LDL are an independent risk factor for cardiovascular disease (CVD) and that reduction of LDL is associated with decreased cardiac events and mortality. However, clinical trials with statin treatment were associated with only a 25 % reduction in clinical events. The residual risk of CVD events has resulted in an unmet medical need to develop additional therapeutic approaches to decrease clinical events in high risk patients. In addition to the classical risks and high LDL a substantial number of patients with CVD have visceral obesity, insulin resistance, hypertension, and an atherogenic dyslipoproteinemia characterized by hypertriglyceridemia, dense LDL and decreased HDL. This cluster of risk factors is characteristic of patients with the metabolic syndrome and diabetes. The cluster of risk factors codified as the metabolic syndrome has been controversial due to disagreement regarding the pathophysiology and clinical usefulness of the classification. A major challenge for the cardiovascular field has been the search for the most effective treatment for the patient with the metabolic syndrome. Recently, visceral obesity and hypertriglyceridemia have provided a useful clinical approach to identify patients at risk for the development of CVD and diabetes. The residual risk of CVD in statin treated patients has led to the need of a more comprehensive clinical assessment of potential risk factors and the determination of a global cardiometabolic risk which includes the risk associated with the metabolic syndrome and diabetes. The future treatment of these high risk patients will require treatment with statins as well as the other identified risk factors including obesity, low HDL and insulin resistance.

Funding: Medstar

Workshop 20 "FATTY ACIDS, OMEGA-3 AND CVD"
3:45 PM - 5:25 PM
Chairs: *W.S. Harris, Sioux Falls, SD, USA and F. Sacks, Boston, MA, USA.*

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THE OMEGA-3 INDEX: A NEW RISK FACTOR FOR SUDDEN CARDIAC DEATH?

William S. Harris. *Univ of South Dakota, Sioux Falls, SD, USA.*

The cardioprotective effects of the long-chain omega-3 fatty acids (FA) eicosapentaenoic and docosahexaenoic acids (EPA and DHA) are widely recognized. Their greatest impact appears to be on risk for death, with weaker evidence for effects on myocardial infarctions per se. The doses at which these effects have been observed (<1 g EPA+DHA/day) have little to no effect on classic or emerging CHD risk factors. The risks for such events cannot currently be estimated using currently-available circulating biomarkers. The benefits of omega-3 FA all appear to derive from "membrane effects," which result in reduced susceptibility to cardiac arrhythmias or to plaque rupture and thrombosis. Since it is their presence in membranes that appears to be responsible for their ultimate metabolic effects, there is a compelling rationale for measuring membrane levels of these FA as biomarkers. For this reason, we have proposed that the red blood cell (RBC) content of EPA+DHA (expressed as the percent of total RBC FA; the omega-3 index) be considered as a potential risk marker, particularly for sudden cardiac death. The omega-3 index has much to recommend it - easily accessed and analyzed, virtually a pure phospholipid, responsive to changes in omega-3 FA intakes, relatively reflective of long term exposure, etc. Extrapolations from the literature suggest that an omega-3 index of 8% or more is associated with reduced risk. Future studies are needed to establish a standardized analytical method, to determine the extent to which the omega-3 index has incremental prognostic value beyond that provided by classic risk factors, and to establish target values for the omega-3 index for CHD risk assessment.

Funding: None

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HOW MUCH AND WHAT TYPE OF FAT IS OPTIMAL TO PREVENT CORONARY HEART DISEASE AND DIABETES

***Frank M. Sacks**. Harvard School of Public Health, Boston, MA, USA.*

Dietary approaches that use a low-fat, high-carbohydrate diet can be improved by replacing some of the carbohydrate with unsaturated fat and protein. Polyunsaturated fatty acids, especially omega-6, lower LDL cholesterol, and compared to carbohydrate raise HDL cholesterol. Monounsaturated fatty acids also raise HDL cholesterol. Several studies demonstrated that dietary approaches that emphasize unsaturated fat reduce blood pressure, and lower diurnal blood glucose and insulin responses. The DASH and OmniHeart trials demonstrate the substantial benefits to cardiovascular risk factors of healthy dietary approaches. The DASH diet, high in vegetables, nuts, fruits, and low-fat dairy products, and low in meats and sugar-containing beverages and desserts, reduces blood pressure and LDL cholesterol. Blood pressure responses are superior to drug regimens. However, the DASH diet has a low fat content, 27% and high carbohydrate content, 58%; and it lowers HDL cholesterol and does not reduce triglycerides. The OmniHeart study found that a lower carbohydrate version of the DASH diet that emphasized protein or unsaturated fat further reduced blood pressure and LDL cholesterol, and lowered triglycerides. Replacing carbohydrate with protein had a superior effect on triglycerides compared to unsaturated fat or carbohydrate, but protein reduced HDL cholesterol. Estimated risk reduction was 20% with the carbohydrate rich diet and 30% with the protein or unsaturated fat diet. Finally, in addition to benefits on risk factors for cardiovascular disease, several classic trials show that higher polyunsaturated fat diets, compared to traditional western diets, prevented cardiovascular events.

Funding: National Heart, Lung, and Blood Institute

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CIGARETTE SMOKE AFFECTS PLASMA FATTY ACID LEVELS: AN IN VIVO STUDY

***P. Risè**, S. Ghezzi, S. Mattavelli, C. Manzoni, D. Baldassarre, D. Caruso, C. Galli. University of Milan, Milan, Italy.*

Cigarette smoke (CS) contains thousand of compounds, mainly free radicals, that affect polyunsaturated fatty acids (PUFA) levels. In vitro, in different cell lines, CS inhibits the conversion of n-3 and n-6 fatty acids (FA) to their longer and more unsaturated derivatives. In vivo studies on the effects of CS on PUFA metabolism are scarce. **Objective:** to compare the FA profile in smoking (S) vs non smoking persons (NS). **Methods**: 12 S and 12 NS hyperlipidemic subjects were selected. FA in total lipids (TL), phospholipids (PL), triglycerides (TG) and cholesterol esters (CE) were analyzed by GC. **Results:** TG, LDL, HDL, glycemia, BMI and age are similar in the two groups. Although S show lower total cholesterol (TC) levels, their plasma oxysterols are higher (+53%) vs NS. In TL, S present higher levels of saturated (SFA) and monounsaturated FA (MUFA) vs NS. On the contrary PUFA are decreased: linoleic acid (LA, 18:2 n-3), arachidonic acid (AA, 20:4 n-6) and docosahexaenoic acid (DHA) are reduced in S than in NS. In addition, S present lower levels of PUFA in PL, TG and CE and higher levels of MUFA and SFA in PL and CE. The ANOVA linear regression shows, in TL, positive correlation between LA levels and TC, LDL only in NS. The correlation found in S is positive between 18:0 and TC, LDL, HDL; between SFA and TG; negative correlation between DHA and TC, EPA, DHA and TG. The relationship between FA levels and cigarette number or years of smoking were also investigated. **Conclusions:** Cigarette smoke significantly affects FA composition in TL, PL, TG and CE; S show decreased levels of PUFA and increased levels of SFA in relation to the number of years of smoking, while no correlation was seen between FA levels and number of cigarettes per day.

Funding: None

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PRESCRIPTION OMEGA-3 "ADDED ON" TO STABLE STATIN THERAPY: CHANGES IN LIPID PARAMETERS

***Harold Bays**, Michael Davidson, Robert Shalwitz, Ralph Doyle, Christie Ballantyne. Louisville Metabolic and Atherosclerosis Research Center (L-MARC), Louisville, KY, USA; Radiant Research, Chicago, IL, USA; Reliant Pharmaceuticals, Liberty Corner, NJ, USA; Methodist DeBakey Heart Center, Houston, TX, USA.*

Objective: Small clinical trials have previously evaluated the co-administration of prescription omega-3-acid ethyl esters (P-OM3) with statins in subjects with dyslipidemia, and have demonstrated greater lipid improvements than statin monotherapy. This larger "add-on" study investigated the change in lipid parameters when P-OM3 was added to stable statin therapy. **Methods:** This multicenter, randomized, double-blind, placebo-controlled trial assessed the effects of 4 g/d P-OM3 (Omacor®) on lipid parameters in 254 subjects with persistent hypertriglyceridemia stabilized (8 weeks) on simvastatin 40 mg/d. **Results:** Combination therapy (P-OM3 added to simvastatin) compared with statin monotherapy (placebo added to simvastatin) resulted in greater reductions in TGs (29.5% vs 6.3%; $P<0.0001$) and non-HDL-C (9.0% vs 2.2%; $P<0.0001$), and a greater increase in LDL particle size (1.0% vs 0.5%, $P=0.0066$). Furthermore, 20% of subjects in the combination-therapy group converted from LDL subclass pattern B to pattern A vs 4% in the simvastatin monotherapy group ($P=0.0002$). Lp-PLA_2, preferentially found on small, dense TG-enriched LDL particles, was also reduced (10.7% vs 1.4%, $P=0.002$), consistent with the presence of fewer small, dense LDL particles. **Conclusions:** Many patients with mixed dyslipidemia have persistent hypertriglyceridemia after statin monotherapy. When added to ongoing stable statin therapy, P-OM3 may result in further improvements in lipid parameters, which may provide complementary lipid benefits.

Funding: Reliant Pharmaceuticals, Inc.

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HIGHLY-PURIFIED EICOSAPENTAENOIC ACID TREATMENT IMPROVES NONALCOHOLIC STEATOHEPATITIS: A PILOT STUDY

***Naoki Tanaka**, Wataru Okiyama, Takero Nakajima, Toshifumi Aoyama. Shinshu University Graduate School of Medicine, Matsumoto, Japan; Shinshu University School of Medicine, Matsumoto, Japan.*

Background/Aims: Recent study using animal model has demonstrated that n-3 polyunsaturated fatty acids (n-3 PUFA) ameliorated activity of nonalcoholic steatohepatitis (NASH) through reducing hepatic tumor necrosis factor (TNF)-a expression and improving insulin resistance. Highly-purified eicosapentaenoic acid (EPA), one of the major components of n-3 PUFA, has been used as a lipid-lowering drug; however, the efficacy and safety of EPA for NASH has not been investigated. Thus, we conducted a pilot study among patients with biopsy-proven NASH. **Methods:** Twenty-five Japanese patients having both NASH and hyperlipidemia were enrolled. EPA (2700 mg/day) was administered for 12 months, and its efficacy was assessed by clinical and biochemical parameters. Adverse effects were also monitored. **Results:** All patients completed this study, and no adverse events occurred. Aspartate and alanine aminotransferase levels were significantly lower at 12 months compared to baseline (from 50 ± 17 to 38 ± 14 IU/L, P=0.003, and from 79 ± 36 to 46 ± 17 IU/L, P=0.007, respectively). Soluble TNF receptor 1 and 2 levels were also significantly decreased (from 1093 ± 273 to 984 ± 258 pg/mL, P<0.001, and from 2298 ± 605 to 1843 ± 621 pg/mL, P<0.001, respectively). However, body mass index, HOMA index, and serum adiponectin level did not change during the intervention. **Conclusions:** EPA treatment improved serum aminotransferase levels in patients with NASH. This beneficial effect seemed to result from its anti-inflammatory potential, not but improvement of insulin resistance. EPA might be one of the promising agents for the treatment of NASH.

Funding: None

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EFFECTS OF DIACYLGLYCEROL OIL VS. TRIACYLGLYCEROL OIL ON POSTPRANDIAL LIPEMIA IN A INSULIN RESISTANT POPULATION

***Gissette Reyes**, Koichi Yasunaga, Eileen Rothenstein, Wahida Karmally, Steve Holleran, Rajasekhar Ramakrishnan, Henry Ginsberg. Columbia University Medical Center, New York, NY, USA.*

Studies in rodents suggest that fatty acids (FA) in dietary1, 3 diacylglycerol oil (DAG) are not efficiently re-incorporated into chylomicrons after absorption from the intestinal lumen, resulting in greater FA oxidation, lower postprandial (PP) triglyceride (TG) levels and body weight. Diets enriched in DAG reduced PP TG levels in studies with normal subjects. IR is associated with elevated fasting and PP TG. We compared the acute and chronic effects of a diet enriched with foods containing DAG vs. a diet containing equal quantities of triacylglycerol (TAG) on fasting plasma lipids and PP TG in subjects with insulin resistance (IR). Seven men and 18 women with HOMA-R index >2.5, received DAG or TAG-containing foods for 5 weeks each, in a double blind, randomized crossover study. Fasting lipids (all mg/dl) were the same on DAG (183 TC, 171 TG, 41 HDLC, 108 LDLC) and TAG (183 TC, 169 TG, 40 HDLC, 110 LDLC) diets. At the end of each diet period, subjects had DAG and TAG PP TG studies. The areas under the curve above baseline (AUC) for plasma TG over 8 hrs (mean ± SD h.mg/dl) were similar for all four comparisons: DAG PP TG on DAG-enriched diet (DD) 505±380; DAG PP TG on TAG diet (DT) 503±439; TAG PP TG on DAG diet (TD) 517±638; and TAG PP TG on TAG diet (TT) 565±362. In conclusion, five weeks of a DAG enriched diet had no significant effect on fasting lipids, PP TG, or PP RLP C in IR subjects. It remains to be determined whether longer periods of consumption of DAG will have beneficial on PP TG in people with IR.

Funding: Educational grant

Session 2 "ALIGNING PHARMACEUTICAL INNOVATION WITH MEDICAL NEED: THE POTENTIAL IMPACT ON THE DEVELOPING WORLD"
5:30 PM - 6:30 PM
***Moderator:** R. Crystal, New York, NY, USA.*

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A PERSPECTIVE ON STUDENT ACTIVISM: GETTING TREATMENT TO AT-RISK POPULATIONS IN THE DEVELOPING WORLD

***Sandeep P. Kishore**. Weill Cornell Medical College / The Rockefeller University/ Memorial Sloan-Kettering Cancer Center, New York, NY, USA.*

Since 2001, the international student group Universities Allied for Essential Medicines (UAEM) has advocated that universities leverage their copious resources, intellectual property, and knowledge to make basic medicines available to treat diseases of the global poor. This campaign is highly relevant to cardiovascular disease (CVD), which has become the leading cause of death globally, with nearly 80% of deaths occurring the developing world. Currently, CVD claims twice as many lives as HIV/AIDS, tuberculosis and malaria in low-income countries. To date, there has been little effort to make medicines available to curb CVD and related chronic diseases for at-risk populations in the developing world. To address this issue, our UAEM chapter initiated a campaign with faculty and international experts to enhance access to life-saving medicines for heart disease. As one strategy, we successfully petitioned the World Health Organization (WHO) to include a statin drug on its Essential Medicines List (EML). This list is a guideline for developing countries to choose which high-priority drugs should be supplied to their citizens inexpensively. We chose generic simvastatin based on its worldwide availability and cost-effectiveness. According to the International Drug Price Indicator Guide and information from global pharmaceutical firms, generic statins will now cost US $40/year --10 cents/day -- down from the nearly $1,200/year of brand versions a couple of years ago. The addition of a statin to the EML makes it available for donation by United

Nations aid groups to 150+ national pharmaceutical inventories.

Funding: SPK is supported by NIH medical scientist training program grant GM 07739

Sunday
October 7, 2007

Plenary Session 4 "HYPOLIPIDEMIC THERAPY AND ORGAN DISEASE: BEYOND THE HEART"
8:30 AM - 10:30 AM
Chairs: *H.N. Ginsberg, New York, NY, USA and J. LaRosa, Brooklyn, NY, USA.*

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NEW INSIGHTS INTO PERIPHERAL ARTERIAL DISEASE AND ITS MANAGEMENT

John P. Cooke*. Stanford University, Stanford, CA, USA.*

There are few RCT that assessed the effects of risk factor modification in PAD. However, 4S showed that statin therapy reduced total mortality by 30%, with a comparable benefit in the PAD subgroup. There was also a 38% reduction in the risk of new or worsening intermittent claudication. Recent small trials showed that simvastatin, as well as atorvastatin, improve walking distance in patients with PAD. The ideal blood pressure goals in patients with PAD are not different from those with CAD. Notably, beta blockers do not reduce treadmill exercise time. However, for PAD the best data is with ACE inhibitors, in particular the HOPE study. In this trial, ramipril reduced MACE by about 20% in the PAD subset. Ramipril improved walking distance in a small clinical trial. The CAPRIE trial showed that in patients with PAD, clopidogrel was more effective than aspirin, with a 24% greater reduction in MACE. Clopidogrel might be the preferred antiplatelet agent in PAD. Supervised exercise programs improve symptoms and walking ability by about 100%. Cilostazol improves walking distance about 40%. Vascular regenerative therapies, including angiogenic and stem cell approaches, will be discussed.

Funding: Research supported by grants from the National Institutes of Health (R01 HL-63685; RO1 HL-75774; P01 AG18784; T32 HL07708; PO1AI50153), the Tobacco Related Disease Research Program of California (7RT-0128), and the California Institute for Regenerative Medicine. Stanford University owns patents on therapeutic modulation of angiogenesis related to the endothelial nicotinic acetylcholine receptor. Dr. Cooke receives royalties from these licenses. Dr. Cooke is a founder and holds equity in CoMentis, and is a consultant and/or speaker for, or has research collaborations with Ciphergen, Bristol Myers Squibb, Genzyme, and Quest

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STROKE

Pierre Amarenco*. Denis Diderot University and Medical School, Paris, France.*

The meta-analysis of all statin trials which have now included over 90,000 patients, shows relative risk reduction for stroke was 21% (OR 0.79 [0.73–0.85]) with no heterogeneity between trials. Fatal strokes were reduced, but not significantly, by 9% (OR 0.91 [0.76–1.10]). LDL reduction explained 34 to 80% of the observed benefit, leaving also room for other, pleotropic, effects. This effect is mainly driven by the extent of between-group LDL-C reduction.
Recent studies have confirmed that statins reduced the primary stroke in patients with coronary artery disease (HPS, TNT trial) and in other high risk populations – mainly diabetics (HPS, CARDS and hypertensives (ASCOT)– even with a normal baseline blood cholesterol level, which argues for a global cardiovascular risk-based treatment strategy. Statins have a good overall safety profile with no increased incidence of hemorrhagic stroke and cancer.
The Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study is a unique large randomized, placebo-controlled trials which brings definite answer to the question of using cholesterol lowering with atorvastatin 80 mg/day in 4,731 patients with recent (1 to 6 months) stroke or TIA and no past-history of cerebrovascular disease. Mean LDL cholesterol was 73 mg per deciliter on atorvastatin, and 129 mg per deciliter on placebo over the course of the trial. After 4.9 years median follow-up, a fatal or non fatal

stroke (primary endpoint) was reduced by 16% (P=0.03). Major cardiac events were reduced by 35% (P=0.002), any CHD event by 42%. Overall mortality was unchanged (216 deaths, atorvastatin versus 211 deaths, placebo, P=0.98). The rates of serious adverse events were similar except a small, significant excess of hemorrhagic stroke in the atorvastatin arm.

Based on 55,045 LDL-C measurements (with an average 11.6 measurement per patient performed during the follow-up), percent change in LDL-C from baseline was classified – post hoc – as no change from baseline, <50% reduction or ≥50% reduction. Compared to the group with no change or an increase in LDL-C, the group with the deepest LDL-C lowering (>50% from baseline) had a 31% relative risk reduction in stroke and no increase in brain hemorrhage.

What is next ? Next would be to show that patients with stroke/TIA that achieve LDL-C levels <70 mg/dL do better than those achieving the currently recommended LDL-C target after a stroke (<100 mg/dL). Drugs to be evaluated in stroke prevention include new statins, or therapy combining statin low dose and ezetimide, fibrates and PPARs.

Funding: None

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NONALCOHOLIC FATTY LIVER DISEASE: NEW INSIGHTS AND POTENTIAL NEW TREATMENTS

Henry Ginsberg. *Columbia University College of Physicians and Surgeons, New York, NY, USA.*

Individuals with insulin resistance and either the metablolic syndrome or type 2 diabetes mellitus commonly have both overproduction of very low density lipoprotein (VLDL) triglyceride (TG) and apolipoprotein B (apoB) from the liver and hepatic steatosis. There are several reasons for increased VLDL assembly and secretion in these people: increased fatty acid (FA) flux to the liver due to adipose insulin resistance with increased lipolysis, increased delivery of remnant lipoprotein TG to the liver, and increased hepatic de novo lipogenesis (glucose conversion to FA). In prior studies, we demonstrated that 1) increases in plasma FA levels stimulated apoB secretion even when TG secretion was unchanged and 2) PPARgamma2 gene expression was increased in fatty livers and was linked to increased lipogenesis. An additional stimulus to increased apoB secretion is hepatic insulin resistance: insulin normally targets apoB for intracellular degradation and this signal can be diminished if the liver is insulin resistant. In studies with mice specifically lacking hepatic insulin receptors, VLDL apoB secretion was increased despite reduced VLDL TG secretion. Thus, hepatic insulin resistance might protect against hepatic steatosis at the expense of increased VLDL (enriched in cholesterol) secretion. On the other hand, we now show that FA-induced endoplasmic reticulum (ER) stress, leading to the unfolded protein response (UPR), can inhibit the secretion of apoB100 in vitro and in vivo. The FA-induced inhibition of apoB100 secretion is complex, as mild ER stress leads to some UPR but not enough to interfere with FA-induced apoB secretion; more severe ER stress with greater UPR inhibits apoB secretion despite increasing steatosis.

Funding: R01 HL55638 (H. Ginsberg, P.I.); R01 HL73030 (H. Ginsberg, P.I.)

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DOES HYPOLIPEMIC THERAPY BENEFIT THE KIDNEY AS WELL AS THE HEART?

Marcello Tonelli. *University of Alberta, Edmonton, AB, Canada.*

The tremendous burden of cardiovascular (CV) morbidity and mortality in dialysis patients has been well documented. Dyslipidemia contributes significantly to cardiovascular death in patients with normal renal function, and cholesterol lowering with statins is effective for primary or secondary prevention of CV disease. If pharmacological treatment even modestly lowers the risk of CV events in patients with end stage renal disease (ESRD), the overall benefit in this high-risk group would be substantial. However, the relation between dyslipidemia and CV risk in patients with renal disease is less clear than in those with normal renal function, as is the efficacy of statins and other agents such as fibrates for preventing CV risk. This session will review the physiology of lipoprotein metabolism, the effect of lipoproteins on atherosclerosis and progressive loss of kidney function, and how lipid modifying therapy may interact with these processes. Special emphasis will be placed on issues relating to the management of patients with kidney disease.

Funding: None

Debate 2 "PHARMACOLOGICAL THERAPY: INCREASING HDL vs. DECREASING LDL"

11:00 AM - 12:00 PM

Moderator: *A.M. Gotto, Jr., New York, NY, USA.*

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PHARMACOLOGIC THERAPY: INCREASING HDL VERSUS DECREASING LDL

Ernst J. Schaefer. *Tufts University, Boston, MA, USA.*

Low HDL-C (< 40 mg/dl in men and < 50 mg/dl in women) is an independent CHD risk factor, and is observed in over 50% of patients with premature CHD in the following familial patterns: dyslipidemia (15%), combined hyperlipidemia (14%), and hypoalphalipoproteinemia (4%). Low HDL-C is also often associated with insulin resistance and male pattern obesity. Analysis of HDL by 2 dimensional gel electrophoresis indicates that CHD patients have decreased large alpha 1 and 2 HDL, and increased small pre-beta 1 and alpha 3 HDL. Weight loss, exercise, and alcohol increase HDL C, and increased dietary polyunsaturated fatty acids slightly decrease HDL C and enhance SR-B1 mediated reverse cholesterol transport. HDL also have anti-inflammatory effects. Statins have been shown to reduce CHD risk, associated with LDL C lowering, but there is often residual risk, which has been related to low HDL. Raising HDL with fibrates, statins, and niacin have all been associated with CHD risk reduction. Fibrates mainly exert their beneficial effects by lowering liver fat and inflammatory markers, and are especially beneficial in those with elevated insulin levels. Both statins (especially rosuvastatin) and atorvastatin) and niacin have very favorable effects on HDL particles. CETP inhibitors have not been shown to reduce CHD risk, but torcetrapib binds to CETP on HDL, while other CETP inhibitors do not do this. Raising HDL and infusions of ApoA-I Milano/phospholipid and reconstituted HDL have been shown to promote CHD regression. The future of lipid therapy is combining statins with HDL raising agents such as better niacin preparations and other agents in development.

Funding: Funding from the National Heart Lung, and Blood Institute, Unilever Research, the US Department of Agriculture Research Service, AstraZeneca, Abbott, and Pfizer

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INCREASING HDL VS. DECREASING LDL – CASE FOR LDLc

Evan Stein. *Metabolic & Atherosclerosis Research Center, Cincinnati, OH, USA.*

The debate over when, whom and how to treat adults with elevated LDL cholesterol (LDLc) is over due to a huge amount of data generated by large, clinical end-point, placebo controlled trials with HMG CoA reductase inhibitors ('statins')[1]and other modalities for reducing LDLc. The evidence for LDLc, and its Apo B containing and related precursors VLDL and IDL, has met all of the strictest criteria for being not just a risk factor but a cause or determinant of CVD. This includes evidence from biological, epidemiological, human genetic, animal experiments and most of all large placebo controlled clinical end point trials of LDLc lowering. The data has proven remarkably consistent from trial to trial, dose related and independent of mechanism by which LDLc is lowered. Any residual debate regarding LDL centers only on whether LDL represents the best representative of the Apo B containing 'atherogenic' particles or if nonHDLc or Apo B provide slight, but additional, targets for reduction. This debate is essentially only a refinement of the initial objectives surrounding LDL as reduction in these two latter parameters results, de facto, in reductions in LDL. Not only is LDL reduction now beyond question a proven mechanism for reducing morbidity and mortality from CVD and stroke, but it is infact the single most effective and simplest way to achieve such an objective. Furthermore lowering LDL more aggressively is currently the most effective way to reduce residual CVD risk from other risk factors such as elevated Lp(a), diabetes, smoking and even low HDLc. 1. Baigent C, Keech A, Kearney PM et al., for Cholesterol Treatment Trialists (CTT).Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005;366:1267-1278.

Funding: None

Thursday
October 4, 2007 —
Sunday
October 7, 2007

Poster Session 1 "ATHEROSCLEROTIC PLAQUE" - GROUP A

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IDENTIFICATION OF INTERLEUKIN-6 GENE EXPRESSION IN HUMAN CAROTID PLAQUES

Maja Zivkovic, *Djordje Radak, Aleksandra Stankovic, Tamara Djuric, Olja Stancic, Sandra Radak, Dragan Alavantic. VINCA Institute of Nuclear Sciences, Belgrade, Yugoslavia; Dedinje Cardiovascular Institute, Belgrade, Yugoslavia.*

Objective: Atherosclerosis is now recognized as a disease of arterial inflammation. Interleukin 6 (IL-6) is an inflammatory cytokine, which source is generally assumed to be macrophages activated by infection, or undergoing inflammatory activation in the vessel wall. It was reported that IL-6 is marker of coronary artery disease and unstable angina. The aim of our study was to investigate gene expression of IL-6 in human carotid plaques and to compare it between two groups of clinically defined plaque phenotypes: stable (fibrolipid, moderately or highly echogen) and unstable (complicated with low echogenicity and ulcerations). **Methods**: Atherosclerotic plaques from 27 patients (13 stable and 14 unstable) undergoing carotid endaterectomy were investigated. Real-time PCR was performed using ABI Prism 7500 and 18S rRNA as endogenous control for relative quantification of IL-6 gene expression. The $2^{-\Delta\Delta Ct}$ method was used to analyze the results. **Results**: In 36 percent of investigated plaque samples expression of IL-6 was not detected. In the rest of the samples (55% stable, 45% ustable), we did not detect significantly different expression of IL-6 between stable and unstable plaques. **Conclusion**: Our results show that the expression of IL-6 is not a useful predictor of plaque phenotype in advanced atherosclerotic lesions. These findings suggest that the role of IL-6 in pathophysiology of atherosclerosis might be important in the early phases of tissue remodeling and plaque formation, but not in advanced one.

Funding: This work was funded by Serbian Government Research Grant (Ministry of Science)

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THE ANGIOTENSIN-CONVERTING ENZYME 2 EXPRESSION IN HUMAN CAROTID PLAQUE

***Aleksandra Stankovic**, Maja Zivkovic, Djordje Radak, Tamara Djuric, Sandra Radak, Dragan Alavantic. VINCA Institute of Nuclear Sciences, Belgrade, Yugoslavia; Dedinje Cardiovascular Institute, Belgrade, Yugoslavia.*

Objective: Atherosclerotic plaques are conglomerates of dysfunctional endothelial cells, smooth muscle cells, lipid-laden macrophages and T lymphocytes. The major effector peptide of RAS, Ang II, is converted from Ang I by ACE. ACE/Ang II are thought to play an important role in atherosclerosis. Ang II also has important modulatory effects on vascular lipid metabolism in the vessel wall by enchansing LDL oxidation and infiltration in macrophages and endothelial cells. ACE2 is a newly described enzyme that can break down Ang I into Ang-(1-9) and Ang II into Ang-(1-7), thus removing Ang II from the atherogenic milieu might be atheroprotective. The aim of the study was to identify and quantify the ACE2 expression in atherosclerotic plaque and control artery. **Methods**: Atherosclerotic plaques from 14 patients undergoing carotid endaterectomy were investigated. Real-time PCR was performed using ABI Prism 7500 and 18S rRNA as endogenous control for relative quantification of ACE2 gene expression. The $2^{-\Delta\Delta Ct}$ method was used to analyze the results. **Results:** In human control artery without signs of atherosclerosis the expression of ACE2 was detected, but in human carotid plaque samples ACE2 expression was undetectable. **Conclusions:** ACE2 expression is diminished in advanced human carotid plaques. Our results support the hypothesis about atheroprotective role of ACE2 in vascular wall and implicate possible therapeutic approach of ACE2 upregulation as beneficial. Further investigations should include ACE2 analysis in different stages of atherosclerotic lesion formation.

Funding: Funded by Serbian Ministry of Science Reasearch Grant

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PERIVASCULAR CAROTID COLLAR PLACEMENT INDUCES NEOINTIMA FORMATION AND OUTWARD ARTERIAL REMODELING IN MICE INDEPENDENT OF APOLIPOPROTEIN E DEFICIENCY OR WESTERN-TYPE DIET FEEDING

*Roberta Baetta, **Stefano Bellosta**, Marta Uzzo, Ivano Eberini, Elena Donetti, Alberto Corsini. University of Milan, Milan, Italy.*

The perivascular collar model is an established method of arterial injury characterized by preservation of the anatomical integrity of the endothelium and absence of medial injury, interruption of blood flow or thrombus formation. We investigated the effect of apolipoprotein E deficiency ($apoE^{-/-}$) and Western-type diet on the development and composition of collar-induced carotid lesion in mice. $ApoE^{-/-}$ and wild-type mice with C57BL/6 background were fed a Western-type diet or chow diet for 4 weeks before collar surgery. Diets were continued after collar placement for 6 or 12 weeks. Compared to sham-operated arteries, collared carotids showed significant neointima formation, lumen loss, and outward remodeling in both $apoE^{-/-}$ and wild-type mice. These changes were not affected by either the genotype or the diet. Conversely, significant differences in lesion composition were detected between the 2 genotypes, with $apoE^{-/-}$ mice showing greater lipid deposition and lower SMC accumulation compared to wild-type mice, independent of the dietetic regimen. Altogether, the results of the present study indicate that although lesion composition may be influenced by genotype, neointimal formation and arterial remodeling in the perivascular carotid collar model occur independent of the exposure to atherogenic diet or the presence of a sensitized genotype such as $apoE^{-/-}$. This model would thus be suitable for investigating atherosclerotic lesion formation and its potential pharmacological modulation in the setting of different genetic and dietary conditions.

Funding: None

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SUSTAINED STATIC PRESSURE ACCELERATES CHOLESTEROL ACCUMULATION IN VASCULAR SMOOTH MUSCLE CELLS INDUCED BY OXIDIZED LOW DENSITY LIPOPROTEIN

Cheng Lai Xia, Kai Li, Xiao Yong Lei, ***Duan Fang Liao****. School of Life Science and Technology University of South China, Hengyang, Hunan, China.*

Objective: To investigate the accelerative effect of sustained static pressure on cholesterol accumulation in vascular smooth muscle cells (VSMCs) induced by oxidized low density lipoprotein (Ox-LDL). **Methods:** VSMCs line (A10) from mouse aorta were incubated with OX-LDL(50ug/ml) in a special incubator contenting 95% CO_2 and 5% air, in which the pressure was regulated from 0 to 180 mmHg. Cholesterol accumulation of VSMCs was detected by Oil Red O and HPLC. The protein levels and mRNA levels of caveolin-1, SREBP-1 were analyzed by western blot and RT-PCR, respectively. **Results:** Static pressure treatment (0, 60, 90, 120, 150, 180 mmHg for 24 hours) accelerated cellular cholesterol accumulation in VSMCs in present of Ox-LDL (50ug/ml) with a pressure-dependent manner. Oil Red O staining showed that there were more lipid-drops in VSMCs with static pressure than those without static pressure. The cellular contents of TC and FC were increased . Western blot analysis showed that ox-LDL down-regulated the protein expressions of caveolin-1 and SREBP-1. Interestingly, static pressure treatment further decreased the expression of these proteins. The mRNA of apo E was also significantly down-regulated by static pressure. **Consults:** Sustained static pressure could accelerate cholesterol accumulation in vascular smooth muscle cells. Down-regulation of caveolin-1, SREBP-1 and Apo E may play important role in mediating the effects of sustained static pressure. **Key Words:** sustained static pressure, vascular smooth muscle cell, cholesterol, caveolin-1, SREBP-1, Apo E.

Funding: NNSFC(30470719) and NMBRPC(973) (2006CB503808)

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CYTOSOLIC PHOSPHOLIPASE $A_2\alpha$ PROMOTES ATHEROSCLEROTIC LESIONS IN MICE

Hiromi Ii*, Mayuko Oka, Takashi Sato, Satoshi Akiba. Kyoto Pharmaceutical University, Kyoto, Japan.*

Objectives: We have studied the contribution of cytosolic phospholipase $A_2\alpha$ (cPLA$_2$) to the synthesis of cholesteryl ester (CE) in macrophages and formation of foam cells. In this study, we observed a decrease in the aortic root of atherosclerotic lesions in cPLA$_2$-knockout (KO) mice fed high-fat diets, and suggest possible reasons for the amelioration. **Methods:** Female wild-type and KO mice (C57BL/6, 6 weeks of age) were fed a normal chow diet or either of two kinds of high-fat diets, composed of 15% fat, 1.25% cholesterol, and 0.5% cholic acid (HF-1), or 20% fat and 1.25% cholesterol (HF-2), for 16 weeks, and fasted for 18 h. **Results:** In wild-type mice, HF-1 caused early atherosclerotic lesions in the aortic root with an increase in serum total cholesterol (T-C) levels and with a decrease in HDL-cholesterol (H-C) levels. However, no such lesion was observed with HF-2, which rather increased levels of T-C and H-C. Interestingly, in KO mice fed HF-1, atherosclerotic lesions were apparently reduced, and H-C levels were not decreased. We further examined the effects of high-fat diet on the liver, which is involved in the formation of HDL. Serum ALT and AST were increased in wild-type mice fed HF-1. Under the conditions, lipid vacuolation in hepatocytes and infiltration of inflammatory cells in the liver were observed. However, these alterations were reduced in KO mice fed HF-1. Meanwhile, in peritoneal macrophages from KO mice, oxidized LDL-induced synthesis of CE was not affected. **Conclusion:** We suggest that cPLA$_2$ is involved in the progression of atherosclerosis, and further that the decrease in atherosclerotic lesions in KO mice results from keeping HDL functions. The maintained H-C levels in KO mice may be due, in part, to the suppression of high-fat diet-induced liver damage.

Funding: None

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CONFIGURATION OF A HTS ASSAY AND IDENTIFICATION OF A STRUCTURAL MOTIF RESPONSIBLE FOR THE OLIGOMERIZATION AND CATALYTIC MODULATION OF ACAT2

L. Redaelli, ***L. Iuzzolino****, D. Carettoni. Axxam, Milan, Italy.*

Objective: Acyl coenzyme A:Cholesterol Acyltransferase 2 (ACAT2) is an oligomeric transmembrane enzyme that synthesizes cholesterol esters in liver and intestine. Although ACAT2 is a recognized potential target for the treatment of atherosclerosis, the high-throughput screening (HTS) for the identification of specific inhibitors has been hampered to date by its complex biochemistry and by the incompatibility of the available assay formats with the requisites of HTS. Hence, our study aimed to characterize the biochemical properties of ACAT2 and to configure a functional HTS assay. **Methods:** Recombinant versions of human ACAT2 were expressed in insect cells. The structure of the purified enzyme was analyzed by gel filtration. A library was screened to identify a HTS-compatible probe for the detection of the ACAT2 activity. **Results:** A structural motif of ACAT2 predicted to affect its quaternary structure and catalytic activity was identified by bioinformatic analysis. Deletion of this motif generated a chimeric form of ACAT2, named delta-ACAT2, with remarkable biochemical features: delta-ACAT2 displayed a monomeric structure and a 4-fold higher specific activity with respect to the native protein. These properties allowed the development of a HTS-compatible assay in 384 well/plate format, using a fluorescent probe to detect the ACAT2 activity. The reaction was optimized by screening a matrix of 250 conditions and its compliance with HTS was proven by using reference inhibitors. **Conclusions:** We have identified a motif of ACAT2 responsible for its structural and functional modulation. Moreover, we have configured a fluorescence-based HTS assay to measure its catalytic activity, which may facilitate the investigation of this target in the drug discovery.

Funding: None

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ENDOTHELIAL LIPASE ACTIVITY ON NOVEL HTS-COMPATIBLE SUBSTRATES: ENZYMATIC CHARACTERIZATION AND CHEMICAL REQUIREMENTS

L. Iuzzolino*, C. Sidoli, B. Bellanti, P. Arioli, D. Carettoni. Axxam, Milan, Italy.*

Objective: Endothelial lipase (EL) plays a primary role in remodelling of high density lipoproteins (HDL) associated with coronary atherosclerosis and metabolic syndrome. We investigated the enzymatic reaction of EL on surrogate substrates under different reaction conditions to shed light on the kinetic mechanisms and specific requirements for substrate recognition. **Methods:** We expressed functional recombinant human EL in insect cells and isolated the secreted protein by affinity purification. We optimized the enzymatic reaction in 384-format by testing a matrix of 250 conditions and screened a library composed of 100 ester compounds to profile the substrate specificity of EL. **Results:** The screening of the ester library identified 5 fluorogenic substrates hydrolyzed by EL. The kinetic characterization identified the most effective substrate among the primary hits, and this fluorogenic substrate was used to establish a HTS-compatible assay for EL. We further characterized the activity of EL on several classes of lipid-like compounds, such as natural phospholipids and triglycerides, HDL and LDL particles, and non-ionic and zwitterionic detergents. The same enzymatic characterization was performed with the homologous enzyme Hepatic Lipase (HL) revealing clearly distinct biochemical properties between these two homologous enzymes. **Conclusions:** The characterization of the enzymatic reaction of EL allowed the identification of a highly effective substrate amenable to setting up a fluorescence-based assay compatible with high-throughput screening. Moreover, the determination of the structural and chemical requirements for EL activity may contribute to designing specific inhibitors for the treatment of atherosclerosis and coronary heart disease.

Funding: None

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GENERATION OF A CELL BASED ASSAY SUITABLE FOR THE HIGH THROUGHPUT SCREENING OF ENDOTHELIAL NITRIC OXIDE STIMULATORS

*Giuliana Piazza, **Maria Grazia Giribaldi**, Chiara Liberati, Lia Scarabottolo. Axxam, Milan, Italy.*

Objective: Nitric oxide (NO) is an important intracellular and intercellular mediator involved in the modulation of many physiological processes in different tissues, including blood flow regulation, platelet aggregation, smooth muscle relaxation, central and peripheral neurotransmission. NO is synthesized by a family of three distinctive isoforms: nNOS, eNOS and iNOS. eNOS catalyzes the formation of NO and L-citrulline from L-arginine. In endothelial cells eNOS plays a crucial role in vascular tone, structure regulation. It also exerts an anti-inflammatory influence, inhibitions platelets adhesion and aggregation, and prevents VSMC proliferation and migration. Several lines of evidence link endothelial dysfunction, characterized by decreased bioavailability of NO, with the development of many pathological conditions such as heart failure, hypertension, diabetes and atherosclerosis and then represents an important pharmacological target. Goal of our work was the generation of a cell based assay suitable for the screening and identifications of eNOS stimulators. **Methods:** We have created a CHO cell line, which stably expresses: the reporter gene luciferase under the control of CREB response elements, a mutated soluble Guanylate Cyclase (sGCmut), which upon NO stimulation is able to convert ATP into cAMP, and the eNOS gene. **Results:** We have demonstrated that activation of eNOS can be detected by measuring luciferase increase, consequent to the increment of intracellular cAMP produced by sGCmut stimulation. The system, validated with known eNOS activators, shows strong signal to background responses. **Conclusion:** This assay represents a very sensitive and reliable functional cell-based assay, suitable for the HTS of human eNOS stimulators.

Funding: None

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SIMVASTATIN HALTS THE PROGRESSION OF ATHEROSCLEROSIS IN END-STAGE RENAL DISEASE PATIENTS

***Wahib J. Haykal**, Olexandr V. Kuryata. Batroun Regional Hospital, Batroun, Lebanon; Dnepropetrovsk State Medical Academy, Dnepropetrovsk, Ukraine.*

We aimed to evaluate the effects of simvastatin 10 mg/day on left ventricular hypertrophy (LVH) and endothelial vascular function in end-stage renal disease (ESRD) patients on haemodialysis (HD). 76 patients with ESRD on HD (45M, 31 F; mean age 42,2±3,6 years, mean time on HD 4,1±1,5 years) were enrolled. 33 (43,42%) patients with total cholesterol (TC) >=5,2 mmol/1 or LDL-C >= 3,5 mmol/1 received simvastatin 10 mg/day (study group), while 43 (56,57%) - without lipid damage (control group) received no statin treatment for 24 weeks. The lipid profile was measured in the blood. On echocardiography and doppler we determined the left ventricular mass index (LVMI) and the flow-mediated dilation (FMD) in the brachial artery during reactive hyperemia. 25 (75,75%) patients achieved the LDL-C goal <=2, 6 mmol/l and 30 (91%) - the TC goal <=4,5 mmol/l. Mean TC, LDL-C, TG and VLDL-C decreased by 22,3%, 43,2%, 36,2% and 28,3% respectively, while mean HDL-C increased by 17,5%. All patients had LVH at baseline. At week 24 in the study group LVMI decreased by 2,89%, since it was evidently increased in the control group by 5,17%. FMD was impaired in 61 (80,26%) patients. Decreased FMD was correlated with the LVMI ($r = -0,48$, $p < 0,01$). After treatment the FMD had been improved by 78,3% and normalized in 22 (66,6%) patients, while it impaired in the control group. Simvastatin may provide beneficial effects for the reduction of cardiovascular risk factors, morbidity and mortality in this population.

Funding: None

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THE PREVENTIVE AND THERAPEUTIC EFFECT OF T0901317 ON ATHEROSCLEROTIC LESIONS IN apoE$^{-/-}$ MICE MAY BE RELATED TO UPREGULATING ATP-BINDING CASSETTE TRANSPORTER A1 AND NIEMANN-PICK TYPE C1

***Chaoke Tang**, Xiaoyan Dai, Xiang Ou, Xinrui Hao, Dongli Cao, Yanwei Hu, Xiaoxu Li. Nanhua University, Hengyang, Hunan, China.*

Objective: We investigated the effect of LXR agonist T0901317 on the atherosclerotic lesions and ATP-binding cassette transporter A1 (ABCA1) and Niemann-Pick type C1 (NPC1) in apolipoprotein E knockout (apoE-/-) mice. **Methods:** Male apoE-/- mice were randomly divided into four groups, baseline group, vehicle group, prevention group and treatment group. All of the mice were fed a high-fat/high-cholesterol diet with or without LXR agonist T0901317 for 8 or 14 weeks. Oil red O staining was used to examine the aortic atherosclerotic lesions. TG, total TC, and HDL-C were determined by commercially enzymatic methods. Gene and protein expression was analyzed by gene microarray, real-time quantitative PCR, Western blot and immunohistochemistry, respectively. **Results:** T0901317 treatment resulted in a significant reduction of lesion area in prevention group and treatment group. Plasma TG, TC, HDL-C and apoA-I concentrations were markedly increased. Gene array analysis showed that LXR activation resulted in increased mRNA expression of LXRα and ABCA1, and decreased mRNA expression of some inflammatory genes including interleukin (IL)-1α,IL-6, and IL-7 in the liver. T0901317 significantly increased expression levels of ABCA1, NPC1, and NPC2 in the aorta and liver and small intestine. **Conclusion:** The synthetic LXR agonist T0901317 has a strong preventive and therapeutic effect on the atherosclerotic lesions in apoE-/- mice and upregulated ABCA1 and NPC1.

Funding: This work was supported by the grants from The National Natural Sciences Foundation of China (30470720) and Hunan Provincial Natrual Sciences Foundation of China (06jj5058)

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HIGH SENSITIVITY C-REACTIVE PROTEIN AND FLOW MEDIATED VASODILATATION IN HEALTHY SUBJECTS

***L.H. Kobbelgaard**, T. Madsen, E.B. Schmidt. Aalborg Hospital, Aarhus University Hospitals, Aalborg, Denmark.*

Objective: C-reactive protein is a predictor of cardiovascular disease. The earliest stages of atherosclerosis are characterized by low-grade inflammation and endothelial dysfunction. Flow mediated vasodilatation (FMD) is a non-invasive method to assess endothelial function. The aim of the present study was to investigate a possible relation between C-reactive protein and FMD in subjects with no overt cardiovascular disease. **Methods:** Twenty healthy men and 20 healthy women with a mean age of 48 ±12 years were included. Blood pressure, serum lipids and mean BMI were normal. After an overnight fast, C-reactive protein was measured with a high sensitivity assay (hs-CRP). FMD was measured in the right brachial artery. Vessel dimensions were recorded at baseline. Then an arterial occlusion cuff was inflated to 300 mmHg for five min, and 60 sec after deflation the peak diameter of the artery was recorded. The percent change in diameter from baseline was recorded as FMD. **Results:** The median hs-CRP in the population was 0.78 mg/L (interquartile range 0.48 – 1.37 mg/L), and the mean FMD (percent change) was 9.6 ± 4.1 %. Nine subjects (23 %) were smokers. No correlation existed between hs-CRP and FMD in the whole population (Spearman's rho -0.14; P = 0.39) or when looking at men (Spearman's rho -0.10; P = 0.69) and women (Spearman's rho -0.24; P = 0.33) separately. **Conclusion:** We found no relation between hsCRP and FMD in younger subjects. However, our participants were healthy and had low hs-CRP levels. A negative correlation may be present in subjects with higher levels of hs-CRP, lower values of FMD and with more cardiovascular risk factors.

Funding: None

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NEGATIVE CORRELATION BETWEEN FLOW-MEDIATED DILATION (FMD) AND ARTERIAL SIZE: TRUE ASSOCIATION OR MATHEMATICAL ARTEFACT?

***Fabrizio Veglia**, Mauro Amato, Alessio Ravani, Elena Tremoli, Damiano Baldassarre. Centro Cardiologico, Monzino, IRCCS, Milan, Italy; University of Milan, Milan, Italy.*

Objective: FMD is a marker of endothelial function often used as a surrogate endpoint in lipid lowering clinical trials. It is computed as the ratio of the brachial artery absolute diameter change (ADC), over the baseline diameter (BD). The rationale for this formula is the assumption of a proportionality between ADC and BD. A negative correlation between FMD and BD has been systematically reported in many contexts and explained with a variety of biological reasons. Besides this, the explanation may be purely mathematical, because BD is the denominator of the FMD formula. Aim of the present study was to assess the influence of mathematical artefacts on this correlation. **Methods:** we measured FMD on 119 dyslipidemic patients and we computed the correlation coefficient (R) between FMD and BD and between ADC and BD. We also run 1000 computer-simulations of the same correlations in virtual patients having, in place of BD and ADC, two variables totally random and uncorrelated, produced by a random number generator. The correlations obtained in the real and simulated contexts were then compared. **Results:** Similarly to what reported in the literature, in the real data the R between FMD and BD was -0.28 (95% C.I. -0.44 to -0.11) but the assumed proportionality between ADC and BD was not confirmed (r=-0.13, 95% C.I. -0.31 to 0.05). Using simulated data the R between FMD and BD was -0.27 (95%C.I. -0.39 to -0.12). **Conclusions:** The lack of proportionality between ADC and BD as well as the almost identical correlation coefficients obtained using the real and the simulated variables suggest that the association between FMD and BD is mostly attributable to mathematical artefacts.

Funding: None

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PITAVASTATIN INDUCES ATHEROPROTECTIVE GENE EXPRESSIONS IN THROMBOMODULIN LACZ-TARGETED MOUSE

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Objective: We have already reported that pitavastatin increased the levels of expression of atheroprotective factors, such as thrombomodulin (TM) and eNOS in HUVEC. Here we also reveal the in vivo induction of the factors by pitavastatin in TM lacZ-targeted mice. **Methods:** Male mice were administered pitavastatin 30, 100 mg/kg or the vehicle alone once at 7 a.m. by oral gavage. Six hours after the administration, the aortas were collected between aortic arch and thoracic aorta under pentobarbital anesthesia. For detection of lacZ gene expression, the samples were incubated with the chromogenic beta-galactosidase substrate X-Gal. Total RNA was extracted with a commercially available RNA extraction kit. To determine the levels of expression of TM, eNOS, PAI-1, tissue factor (TF), KLF2 and beta-actin, the real-time quantitative PCR was performed. **Results:** LacZ expressions based on TM message were localized at aortic arch and arterial branch points in the mouse. So we evaluated the gene expressions in the aortic arch. Pitavastatin increased the levels of expression of TM, eNOS and also KLF2, while those of PAI-1 and TF were not affected. Pitavastatin did not affect the plasma lipid levels in this study. **Conclusions:** Pitavastatin indeed induced the atheroprotective gene expressions in the mouse. It was possibly considered that these effects were attained by pitavastatin independently via its cholesterol lowering effect.

Funding: None

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PITAVASTATIN INDUCED KLF2 VIA A MEK5-ERK5-MEF2 SIGNALING PATHWAY IN HUVEC

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It has been considered that the pleiotropic effects of statins could be involved for the prevention of cardiovascular events besides cholesterol lowering. As to the endothelial function, it was reported that statins caused an induction of KLF2 (KRUPPEL LIKE FACTOR 2, known as a key regulator of anti-inflammatory genes) in HUVEC. However its molecular mechanism has not been fully elucidated. We thereby report the regulatory mechanism of KLF2 induction by pitavastatin. Pitavastatin (1 μmol/L) induced KLF2, thrombomodulin (TM) and eNOS mRNA in time- and concentration-dependent manner (the induction of KLF2 started at 2 hr after treatment, and that of thrombomodulin and eNOS did at 4 and 8 hr, respectively) in HUVEC. Interestingly, pitavastatin also induced ERK5 (EXTRACELLULAR SIGNAL-REGULATED KINASE 5) phosphorylation (generally thought to activate MEF2 protein) from 2hr after treatment. In the MEF2 (MADS BOX TRANSCRIPTION ENHANCER FACTOR 2) and ERK5 siRNA knock-down experiments, the induction of KLF2 by pitavastatin was disappeared. Furthermore, adenovirus expressing MEK5DN suppressed the induction of KLF2 by pitavastatin. These results indicate that pitavastatin induces KLF2 via a MEK5-ERK5-MEF2 signaling pathway. It suggests that the regulation of KLF2 induction by pitavastatin would contribute as a pleiotropic effect on anti-atherosclerotic action in addition to cholesterol lowering.

Funding: None

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DIFFERENCE IN EFFECT ON HUMAN ENDOTHELIAL CELLS AND ON ANGIOGENESIS AMONG HMG-COA REDUCTASE INHIBITORS, PRAVASTATIN, ATORVASTATIN, AND PITAVASTATIN

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Objective: It is certain that HMG-CoA reductase inhibitors (statins) possess biological actions in most of tissues beyond cholesterol lowering effect. The present study is designed to test whether the action on ECs and angiogenesis is common or specific among statins. **Methods and Results:** We used human epidermal microvessel ECs (HMVECs) and added statins from 0.005 to 1 μmol/L. More than 0.1 μmol/L of pitavastatin inhibited FGF-2-induced migration and proliferation as examined by scratch wound assay, chemotaxis assay and uptake of bromodeoxyuridine, and increased cell death assessed by trypan blue dye exclusion method and by DNA staining. In contrast, at 0.01 μmol/L of pitavastatin increased proliferation and migration of HMVECs, and reduced cell death significantly. Atorvastatin tended to increase migration and proliferation of HMVECs, and significantly reduced cell death at higher concentration (0.1 μmol/L) compared to at that of pitavastatin. Pravastatin increased migration and proliferation of HMVECs at 0.1 and 1 μmol/L, but did not affect cell death. The effect of the statins on angiogenesis was examined by quail chorioallantoic membrane (CAM) assay in response to 1 μg/embryo of FGF-2. High dose (0.5 μmol/embryo) of atorvastatin and pitavastatin significantly reduced angiogenesis (- 25% versus FGF-2 only), whereas the low dose (0.15 μmol/embryo) tended to increase FGF-2-induced angiogenesis (+12 % versus FGF-2 only). Pravastatin did not affected the angiogenesis at any concentrations. **Conclusions:** These results suggest that the responses of ECs and angiogenesis to statins are attributed to both common mechanism and to statin specific effect.

Funding: None

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EXPRESSIONAL CHANGES OF COPPER/ZINC SUPEROXIDE DISMUTASE AND ENDOTHELIAL NITRIC OXIDE IN ATHEROSCLEROTIC CORONARY ARTERIES

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Objectives: Oxidative stress plays a crucial role in atherogenesis. The redox state in the vasculature is determined by the balance between pro-oxidants and antioxidants. The aim of present study was to examine the role of copper/zinc superoxide dismutase (Cu/ZnSOD) and endothelial nitric oxide (eNOS) in the development of hypertension and atherosclerosis. **Methods:** We investigated by immunohistochemistry (Santa Cruz Biotechnology and DAKO reactive) the expressional changes of Cu/ZnSOD and eNOS in coronary arteries. We used human arteries, 7 nonatherosclerotic and 9 atherosclerotic segments from a. coronaria sinistra (r. descendens). These arteries were colected at autopsy from 16 cases within 5 hours of death. **Results:** In nonatherosclerotic coronary arteries, Cu/ZnSOD was expressed mainly in medial smooth muscle cells (SMC) and has only weak immunoreactivity in the endothelium, whereas eNOS was expressed mainly in endothelium. The expression of Cu/ZnSOD was significantly increased ($p<0.05$) in atherosclerotic coronary arteries in comparison with nonatherosclerotic. eNOS staining was more enhanced in atherosclerotic coronary arteries than on the group with nonatherosclerotic segments. **Conclusions:** In response to oxidative stress, the Cu/ZnSOD and eNOS were upregulated in atherosclerotic lesions. The imbalance between vascular antioxidant and oxidant systems might play an important role in coronary atherogenesis.

Funding: MU Pleven

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CIRCULATING MATRIX METALLOPROTEINASES (MMPs) AND THEIR INHIBITORS (TIMPs) BEFORE AND AFTER CORONARY ROTATIONAL ATHERECTOMY (RA)

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Objective MMPs and TIMPs had the potential role in coronary calcification as well as plaque development. To examine this possibility in the clinical settings, we measured circulating levels of MMPs and TIMPs before and after RA, to treat calcified coronary lesions, and compared their levels with calcification–related molecules (OPN and OPG). **Methods and results** We enrolled consecutive 47 patients (mean age: 70 years, M/F=29/18) treated successfully with RA. MMP2 levels (mean±SD, ng/ml) significantly ($p<0.001$) increased three hours after RA (from 696±127 to 754±153), and maintained the levels (752±131) of 24 hours after procedure, while MMP9 levels remained unchanged during the time course after RA. TIMP1 and TIMP2 levels (ng/ml) increased immediately and three hours after RA, and reached maximal levels (from 114±46 to 175±49, $p<0.001$ and from 52±13 to 59±17, $p<0.05$, respectively) at 24 hours after procedure. Preprocedural levels of MMP2 showed positive association with those of OPN and OPG ($r=0.335$, $p=0.0213$ and $r=0.511$, $p=0.0002$, respectively). By contrast, MMP9 levels showed no association with those of OPN and OPG. TIMP1 and TIMP2 levels were highly associated with those of OPN ($r=0.612$, $p=0.0001$ and $r=0.512$, $p=0.0002$, respectively) and OPG ($r=0.646$, $p=0.0001$ and $r=0.347$, $p=0.0167$, respectively). **Conclusions** Both MMP2 and TIMPs, not MMP9, may play important roles in coronary calcification, possibly in association with calcification–related molecules.

Funding: None

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EFFECT OF CIGARETTE SMOKING ON CAROTID IMT AND FRS

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Objective: To investigate 1) whether smokers and no smokers with identical Framingham risk score (FRS) have a different subclinical vascular damage and 2) whether moderate cigarette smoking throughout the life (in terms of packyears, a measure of total exposure to cigarette smoking not considered in the FRS) may be considered harmless as compared to the effects of heavy cigarette smoking on the extent of sub-clinical atherosclerosis. **Methods:** Carotid IMTs of never-, moderate- (packyears<30) and heavy-smokers (packyears≥30), matched for age, gender and FRS, were compared (n=72 per group). **Results:** As a result of the FRS-matching, moderate- and heavy-smokers were fully comparable for all the vascular risk factors (VRFs) included into the algorithm. In contrast, never-smokers had, consequently to the matching, higher levels of total and LDL-cholesterol, blood glucose, systolic and diastolic blood pressure (all $p<0.05$). The IMT_{mean} of both heavy- (1.05±0.31 mm) and moderate-smokers (1.02±0.27 mm) was significantly higher than in FRS-matched never-smokers (0.92±0.28 mm), ($p=0.01$ and $p=0.003$, respectively). Thus, regardless of a moderate or heavy smoking history, smokers show a worse carotid atherosclerotic profile than FRS-matched never smokers. **Conclusions:** Cigarette smoking, whatever moderate or heavy, induces a vascular damage that exceeds that observed in never smokers matched for FRS, which reveals the particularly strong atherogenicity of this risk factor. Moderate cigarette consumption, as defined in this study, may not be considered a harmless alternative to smoking cessation.

Funding: Research was supported in part by Philip Morris USA Inc. and Philip Morris International

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LIGHT OR HEAVY CIGARETTES CONSUMPTION AND CAROTID ATHEROSCLEROSIS

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Objective: To evaluate whether the pro-atherogenic effect of cigarettes is related to the content of tar, nicotine or carbon-monoxide (CO) reported on the pack**. Methods:** Carotid IMT of "light" cigarettes consumers was compared to that of "heavy" cigarettes consumers, matched for age, gender and packyears (n=71 per group). 71 never smokers, matched for age and gender, were included into the study as negative controls. Cigarettes were defined as "light" or "heavy" on the basis of the concomitant presence of the three components (tar, nicotine and CO) above or below 7, 0.7 and 7 mg, respectively. **Results:** In the unadjusted analysis, as well as in the full model adjusted for all possible confounders (body mass index, total cholesterol, blood glucose, uric acid, wine and beer consumption), all carotid IMT variables were greater in light cigarettes consumers than in never smokers ($p=0.011$, $p=0.019$, $p=0.037$, $p=0.01$, $p=0.039$ for $CC\text{-}IMT_{mean}$, $Bif\text{-}IMT_{mean}$, $ICA\text{-}IMT_{mean}$, IMT_{mean} and IMT_{max}, respectively), whereas no significant differences were observed between light and heavy cigarettes consumers (all $p > 0.05$). **Conclusions:** This assessment, based on measures of carotid IMT, supports that light cigarettes consumption is not associated with a significantly lower atherosclerotic risk than heavy cigarettes consumption, indicating that light cigarettes should not be considered a valid alternative to smoking cessation.

Funding: Research was supported in part by Philip Morris USA Inc. and Philip Morris International

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CLINICAL IMPLICATIONS OF CORONARY PLAQUES WITHIN THE PROXIMAL CORONARY SEGMENTS IN SUBCLINICAL CORONARY ATHEROSCLEROSIS

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Background: Plaques within the proximal third segments of major coronary arteries are considered "anatomically" vulnerable. However, the risk of coronary heart disease (CHD) and atherosclerotic burden with different plaques in subclincial people are unclear. **Methods:** 341 subjects without history of CHD receiving contrast-enhanced electron-beam computed tomography (EBCT) were enrolled. They were divided into four groups (without plaque, noncalcified, mixed and calcified) accroding to plaques within the proximal third segment of three major coronary arteries. Vascular inflammation (high-sensitive C-reactive protein, hs-CRP), Framingham 10-years absolute CHD risk, arterial atherosclerosis (maximal intima-media thickness of common carotid artery, CIMT) and total coronary artery calcification (CAC) were all recorded. **Results:** Subjects with mixed plaques had higher hs-CRP than the other three groups but did not reach statistical difference. The CHD risk was similar among subjects with plaques but was significantly lowest for those without plaque. The CIMT was the highest with mixed plaques, intermediate with non-calcified/calcified plaques, and the lowest with absence plaques ($p<0.001$). When comparing the total CAC, subjects with mixed plaques had the highest CAS followed by calcified/ noncalcified plaques and those without plaques ($p<0.001$). **Conclusion:** The risk of CHD and arterial atherosclerotic burden were highest with mixed coronary plaques, followed by the noncalcified/calcified plaques and lowest with absence of plaques. Vascular inflammation failed to differentiate the vulnerability of different coronary plaques.

Funding: None

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REPRODUCIBILITY OF CAROTID ARTERY WALL VOLUME MEASUREMENTS IN YOUNG HEALTHY VOLUNTEERS BY MEANS OF 3 TESLA MAGNETIC RESONANCE IMAGING

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Introduction: We developed 3T-MRI sequence protocols to perform carotid artery wall volumetric measurements. In this study we assessed reproducibility of our methods. **Methods:** Carotid arteries of 10 volunteers (aged 28, SD1.5) were imaged twice on different days. Axial T1-weighted end diastolic TSE images were acquired with a 5 cm single-element microcoil (Philips, Hamburg, Germany). Sequence parameters: slice thickness 3mm, imaging matrix size 240, FOV 60x60mm, non-interpolated pixel size 0.25 x 0.25mm, reconstruction matrix 240, TE 11ms and a heart rate triggered TR, active fat suppression (SPAIR) and a double inversion black blood prepulse. Carotid Artery Wall Volume (AWV), Wall-Outer Wall (WOW) ratio, mean Wall Area (MWA) and the mean Wall Thickness (MWT) were calculated by manual delineation of the carotid lumen volume and the outer carotid vessel volume. **Results:** Mean values of the initial and repeat scans were: AWV 245.5 (SD32.8)mm3 and 247.7 (SD30.6)mm3; WOW 0.22 (SD0.03) and 0.23 (SD0.03), MWA 9,09 (SD1,21)mm2 and 9,17 (1,13)mm2; MWT 0.427 (0.041)mm and 0.431 (SD0.039)mm. The average per subject paired mean differences were: AWV -2.2 (SD15.2)mm3, WOW 0.00 (SD0.01), MWA 0.01 (SD0.47) mm2, MWT 0.00 (SD0.01)mm. The intraclass correlation coefficients were: AWV 0.89 (95%CI 0.65-0.97), WOW 0.93 (95%CI 0.77-0.98), MWA 0.89 (95%CI 0.65-0.97), MWT 0.91 (95%CI 0.70-0.98) . **Conclusion:** 3T-MRI enables reproducible carotid artery wall volumetric measurements at high resolution non-invasively. Further reproducibility studies in 30 healthy younger and older human subjects are in progress.

Funding: None

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EVALUATION OF THE MRA FLOW QUANTIFICATION FOR THE ASSESSMENT OF THE AORTIC WALL DAMAGE IN ATHEROSCLEROSIS

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Purpose: The purpose is to study the blood flow features in the aortic arch and reveal the initial factors of atherogenesis and goals for arterial stenosis. **Methods:** 15 normal men (age from 27 to 35years) have been investigated by Magnetic Resonance Angiography (MRA). The phase images were carried out using Siemens-Avanto device. 1.5T. fl2D, TR-24.6ms, TE-1.5ms, FoV-348-360, SL6; TR-47ms, TE- 2.7ms. TA 19.30, FoV.292-360. SL6). The research of hemodynamic parameters was carried out in different sites and the opposite walls of the aortic arch. **Results:** Circular blood flow in the aortic arch during protodiastole is separated into the opposite flow streams, which are temporarily arrested at the certain sites creating the flat flow. At these specified places, the systolic velocity was found to be low. 5.3±1.2cm/s at the aortic arch, 0±0.2cm/s - isthmus area. The protodiastolic antegrade peak acceleration at the external wall of the isthmus area 6-fold surpasses the systolic one - place with a high accidence of the fatty lines/aortic dissection/calcification. High gradient pressure force with the high adhesion can damage the vascular wall: wall shear stress exceeds- 40N/m2. In cases of increased tensile and shear stress, the forming sclerotic site reducing vessel radius. **Conclusion:** The circular blood flow at the aortic arch in protodiastole promotes flow separation with the high adhesion and flow acceleration. High gradient pressure in flat flow profile can damage internal layer of the vessel. Arterial pulse fluctuation, low superficial tension and sclerotic changes of the vessel wall with the arterial stenosis defend the endothelial sheet from the subsequent functional and structural damage.

Funding: None

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ASSOCIATION OF LIPID ABNORMALITIES WITH CAROTID INTIMALMEDIAL THICKNESS IN INDIANS – THE CHENNAI URBAN RURAL EPIDEMIOLOGY STUDY (CURES)

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Objective: The aim of the study was to determine the association of lipid abnormalities with carotid intimalmedial thickness [IMT] **Methods:** The study subjects (n= 2350) were recruited from the Phase 3 of Chennai Urban Rural Epidemiology Study (CURES), a population based study on a representative population of Chennai. Fasting plasma glucose, glycosylated haemoglobin (HbA1c) , serum cholesterol, serum triglycerides and HDL cholesterol were measured using commercial kits, while LDL cholesterol was calculated. Lipids were classified by the NCEP ATP III guidelines. Mixed Hyperlipidemia was classified, if the subjects had both ; hypercholesterolemia and hypertriglyceridemia. Insulin resistance was assessed by Homeostasis Assessment Model (HOMA) . Carotid IMT was measured by B- mode high resolution ultrasound. **Results:** Mean IMT values in subjects with mixed hyperlipidemia (0.78 ± 0.28, P <0.0001), isolated hypercholesterolemia (0.76 ± 0.17, p<.0001), high non HDL cholesterol (0.74 ± 0.21, P < .0001) and high LDL cholesterol (0.73 ± 0.21 P < .0001) was significantly higher compared to subjects with normal lipid levels (0.68 ± 0.16). Linear regression analysis revealed LDL cholesterol (β:0.0002, P=0.016), non HDL cholesterol (β:0.0002, P=0.046) and total cholesterol / HDL ratio (β: 0.009, P=0.0061) to be associated with IMT after adjusting for age, smoking, hypertension, HbA1c, waist and HOMA IR. **Conclusion:** Non HDL cholesterol, LDL cholesterol and total cholesterol / HDL cholesterol ratio are strongly associated with Carotid IMT in Indians. **Key words:** IMT – intimalmedial thickness.

Funding: Chennai Willingdon Foundation, Chennai, India

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EFFECT OF STATIN THERAPY ON RECCURENCE RATE OF ATRIAL FIBRILLATION AFTER SUCCESSFUL CARDIOVERSION

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Objectives: There is limited data, that in patients with atrial fibrillation (AF) inflammation plays important role in its initiation and perpetuation. The antiinflammatory effect of statin therapy, on the other hand, is well established. It could be hypothesized, that statins have beneficial effect on prevention of AF. The aim of our retrospective study was to investigate whether statins reduce recurrence rate of AF after successful electrical cardioversion(EC). **Methods:** 249 consecutive patients (age , sex) with persistent AF who underwent successful EC were included. Treatment with statins (n = 55) was started prior to or immediately after EC and was continued during follow-up. **Results:** After a median follow up of 368 days 47% of patients with statin therapy and 33% of patients in control group were free of AF (p = 0.039, log rank test). Statin use significantly influenced EC outcome (RR 0.65, 95% CI 0.44 to 0.98, p = 0.04). This association remained significant after adjustment for potential confounders (RR 0.58, 95% CI 0.38 to 0.88, p = 0.01). **Conclusions:** Statins reduce recurrence rate of AF after successful EC.

Funding: None

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THE PROTECTIVE EFFECTS OF SIMVASTATIN ON ENDOTOXIN-INDUCED ACUTE LIVER AND RENAL TISSUE DAMAGE IN RATS

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We examined whether administration of simvastatin (SIMVA) inhibits endotoxin E. coli (LPS) induced production of proinflammatory mediators, prevents liver and renal tissue damage and improves survival rate. **Methods:** Female Wistar rats were randomized into control, LPS and SIMVA+LPS groups. The LPS group received single median lethal dose (LD_{50}) of LPS (22,15 mg/kg, i.p).Rats in SIMVA+LPS group received, SIMVA (20 mg/kg p.o, over 5 days) before LPS. Survival rate was monitored at intervals of 12 h during 24 h. For pathological examination liver and kidney were taken from the control, LPS and SIMVA+LPS rats, stained with haematoxylin and eosin. Serum TNF-α and IL-1β were measured in additional LPS and SIMVA+LPS groups at various time points after LPS treatment, determined by ELISA. **Results:** In these model of sepsis, SIMVA completely prevent LPS-induced death (p=0,04). Administration of LPS significantly increased TNF-α and IL-1β compared to baseline ($p<0,002$). SIMVA significantly reduced the peak levels of IL-1β at 120 min (mean 156 (5) vs. 752 (4) pg/mL, $p<0,01$) and at 180 min (mean 161 (7) vs. 520 (6) pg/mL, $p<0.05$) compared with LPS group. SIMVA showed maximal inhibitory effect on TNF-α only at 90 min (mean 15 (6) vs. 37 (6) ng/mL, $p<0.05$). SIMVA significantly attenuated the degree of liver and renal tissue damage, reducing inflammatory infiltration, edema and hyperaemia and restored tissue histology. **Conclusion:** SIMVA prevents organ failure in endotoxic shock and improves survival rate, by inhibition of IL-1β and partly of TNF-α.

Funding: Funded by Ministry of Science, Banja Luka, Bosnia and Herzegovina

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SOLUBLE CELLULAR ADHESION MOLECULES AND C-REACTIVE PROTEIN IN PATIENTS WITH INTRA-CARDIAC DEFIBRILLATOR: RELATION TO ARRHYTHMIC EVENTS AND HEART RATE VARIABILITY

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Objectives: To investigate a possible relation between markers of inflammation and ventricular arrhythmias and heart rate variability in patients with ischemic heart disease (IHD) and implantable intra-cardiac defibrillator (ICD). **Method:** 95 patients were recruited when attending the regular ICD follow-up. From the ICD-memory were noted episodes with ventricular tachycardia or ventricular fibrillation. Blood samples were analyzed for highly sensitive C-reactive protein (hsCRP), serum Intercellular Adhesion Molecule-1 (sICAM-1), Vascular Cellular Adhesion Molecule-1 (sVCAM-1), sP-selectin and sE-selectin. A 24-h Holter-recording was obtained from all subjects, and time-domain HRV indices (SDNN, SDNNi, and SDANN) were analyzed. **Results:** No significant differences in inflammatory markers were found between patients without arrhythmias and patients with one or more arrhythmic events. However, significant negative correlations were found between hsCRP, ICAM-1 or VCAM-1 and SDNN (r=-0.24, P=0.02; r= -0.28, P=0.006; r=-0.22, P=0.03), SDNNi (r= -0.21, P=0.04; r=-0.22, P=0.03, r=-0.18, not significant), and SDANN (r=-0.23, P=0.03; r=-0.23, P=0.03; r=-0.22, P=0.04). sP-selectin or sE-selectin did not correlate to HRV indices. **Conclusion:** Ventricular arrhythmias in patients with IHD were not associated with significantly elevated serum levels of inflammatory markers. However, hsCRP, sICAM-1 and sVCAM-1 were negative correlated to HRV indices, which may suggest a possible link between inflammation and cardiac autonomic dysfunction.

Funding: Obels Family Foundation, Denmark

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POSITIVE ASSOCIATION OF ADIPONECTIN WITH SOLUBLE VASCULAR CELL ADHESION MOLECULE-1 LEVELS IN PATIENTS WITH VASCULAR DISEASE OR DYSLIPIDEMIA

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Objective: The aim of our study was to evaluate the relationship of adiponectin with soluble forms of vascular cell adhesion molecule-1 (sVCAM-1) and intercellular cell adhesion molecule-1 (sICAM-1) in patients with cardiovascular disease or dyslipidemia. **Methods:** 264 patients (134 men/130 women, mean age 43.8±14.8/ 46.0±14.9 years) of Lipid Center**,** University Hospital Olomouc, off hypolipidemic therapy for at least 6 weeks, participated in the study. **Results:** In multiple regression analysis, adiponectin was independently positively associated with serum HDL-cholesterol ($p<0.0001$) and sVCAM-1 ($p<0.0001$), female gender ($p<0.0001$) and negatively with hs-CRP ($p=0.014$). Serum concentration of adiponectin and sICAM-1 did not correlate but sICAM-1 was independently, positively associated with sVCAM-1 ($p<0.0001$) and negatively with markers of insulin resistance and inflammation. Positive association of adiponectin with HDL-C and negative association with hs-CRP supports antiatherogenic properties of adiponectin. The finding of the positive association of adiponectin with sVCAM-1 in patients at risk is unexpected. **Conclusions:** We hypothesize that adiponectin may be involved (directly or indirectly) in shedding of ectodomains of VCAM-1 from endothelial surface and in this down-regulates their function. This process may be protective in the initial stages of atherosclerosis.

Funding: Supported by the grant IGA MZ CR NR/9068-3

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LIPID, APOLIPROTEIN E METABOLIC MAPS AND LIPOLYSIS EFFICIENCY ARE SENSITIVE TO APOE PHENOTYPE IN HYPERTRIGLYCERIDEMIA

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16 patients differing in plasma TG content were divided into E33, E23 and E34 groups by apoE phenotype. The lipid and apoE distribution between lipoproteins was followed by capillary isotachophoresis of plasma samples pre-stained with fluorescent NBD-C6-ceramide (C) or fluorescein-labeled apoE (E). The ceramide distribution among lipoprotein pools was insensitive to apoE phenotype while the higher level of apoE in VLDL was observed in E34 vs E33 patients. In a study of apoE displacement by apoC-III in vitro, apoE was found to bind more tightly to VLDL from E34 vs E33 patients. The plasma (P), VLDL (V) TG and apoB (B) clearance rates were measured as mass (k_1) or within the particle clearance (k_1*) at heparin-induced lipolysis in vivo. The k_1(V) values did not differ for three groups and were significantly higher the k_1(P) values. The k_1(P) values for E33 and E23 groups were 2-fold higher compared to E34. Two-fold increase in k_1*(V) value for E34 vs E23 group reflected lipolysis inhibition by apoE2. For E33 group, the k_1(V) value was negatively correlated to the level of non-displaceable apoE in 2E and the maximal apoE sorbtion capacity for 2E and 3E lipoproteins; the k_1(P) value was not associated with apoE binding parameters; the k_1(V) value was positively correlated to 4C level and the magnitude of apoC-III removal from VLDL; the k_1(P) value was positively correlated to the content of apoE, while negatively with apoC-III, in VLDL remnants. For E34 group, the k_1(V) value was positively correlated to 11C and 1-7C pool levels. ApoE structure may underlie apoE lipoprotein distribution and apoC-III interference in hypertriglyceridemia.

Funding: None

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INTRAVENOUS INFUSION OF PERFTORAN. INFLUENCE ON LIPID METABOLISM

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Objective: In the experiments on animals Perftoran a 10% emulsion of perfluorochemicals (PFC), has been shown to absorb FC, CE, FFA and PL during its persistence in the blood stream. But its influence on lipid metabolism after infusion in humans is still unknown. **Methods:** Perftoran was infused intravenously (100 ml in a day for 5 times) to 8 volunteers (2 women, 6 men). The lipid and lipoprotein content in serum and plasma were measured before and immediately after the course of the infusion by means of electrophoresis in agarose gel (lipoproteins), enzymatic methods (TC, TG, HDL-C), colorimetric methods (FFA, FC), gas-liquid chromatography (FA composition). **Results:** The TC content in plasma was elevated in 50%, the TG content – in 66% of the cases, the level of HDL-C did not change. There was a two fold increase of the VLDL content in serum of all patients while the LDL and HDL content decreased just slightly. The two fold decrease of the FFA and FC content in plasma of all volunteers was also revealed. The only change in the FA composition was observed concerning the 18:1/18:2 ratio that increased from 0,5 to 0,9. **Finding:** It is known that Perftoran emulsion consists of very small particles which being infused in the blood stream are captured predominantly by liver macrophages. The obtained results may indicate a phenomena of the fat substitution in hepatocytes by PFC in the form of induction of VLDL synthesis. Accordingly to that there is a decrease of the FFA content in blood. FC may be absorbed by the PFC particles.

Funding: None

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PLURONIC COPOLYMERS INHIBIT LDL AGGREGATION POSSIBLY VIA PREVENTION OF LIPOPROTEIN PARTICLES HYDROPHOBIC INTERACTIONS

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Objective: Hydrophobic interactions of modified LDL may underlay the mechanisms of aggregation and LDL-induced intracellular lipid accumulation. We have studied the effects of copolymers of propylene oxide and ethylene oxide (Pluronic) on LDL aggregation to test the hypothesis that amphiphilic molecules binding to hydrophobic surface domens of LDL can prevent aggregation. **Methods:** LDL aggregation was induced by incubation at 37°C at constant stirring. Pluronics F68, L61 and P85 were used to inhibit LDL aggregation. The degree of LDL aggregation and the average size of aggregates were measured by laser quasielastic scattering and light transmission fluctuations. **Results:** Hydrophilic/lipophilic P85 added to LDL before incubation inhibited its aggregation by 93% at concentration 0.1% and by 79% at concentration 0.01%, and was ineffective at concentration 0.005%. When added to LDL suspension after 1 or 2 hours of incubation at the effective concentrations, it completely arrested the ongoing formation of LDL aggregates. Lipophilic L61 at concentration 0.22% suppressed LDL aggregation totally, at concentration 0.022% inhibited LDL aggregation by 78 %, and at concentration 0.0022% it was ineffective. Hydrophilic F68 at all concentrations did not affect LDL aggregation. **Conclusions:** Highly and moderately hydrophobic Pluronics are able to suppress LDL aggregation. It confirmes the key role of hydrophobic interactions in LDL aggregation.

Funding: This study was supported by the Institute for Atherosclerosis Research

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MATERNAL FAMILIAL HYPERCHOLESTEROLEMIA: EFFECTS ON LIPID PROFILE AND ENDOTHELIAL FUNCTION IN THE OFFSPRING

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Objective: Maternal hypercholesterolemia has shown to be associated with enhanced fatty streak formation in fetal arteries, suggesting it may play a role in fetal lesion formation. In addition, the progression of atherosclerosis was markedly faster in offspring of hypercholesteromic mothers than in those of normocholesterolemic mothers. Here we investigated the effect of maternal hypercholesterolemia on lipid profiles and vascular function in children with parental familial hypercholesterolemia (FH). **Methods**: Lipid profile and endothelial function, as measured by flow mediated dilatation (FMD) in a subgroup, are compared between children with an FH mother and children with an FH father by multivariate regression analyses. **Results**: In FH children, aged 0.7-19.9 years, LDL-C and apoB levels did not significantly differ between subjects with an FH mother (n=439) or an FH father (n=594): 5.49 vs 5.48 mmol/L, p=0.57 and 1.48 vs 1.48 g/L, p=0.86, respectively. In non-FH children, LDL-C and apoB levels were slightly higher in subjects with an FH mother (n=83) than in subjects with an FH father (n=140): 2.60 vs 2.46 mmol/L, p=0.10 and 0.82 vs 0.80 g/L, p=0.23, respectively. FMD data are currently being measured, but data will be available at the DALM. **Conclusion**: So far, our data suggest that maternal hypercholesterolemia during pregnancy does not essentially influence the lipoprotein levels of the offspring during childhood.

Funding: None

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LIPOPROTEIN (a) LEVELS IN CORONARY AND CAROTID ATHEROSCLEROSIS IN PATIENTS FROM SERBIA

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Objective: Serum level of lipoprotein(a) Lp(a) is hereditarily constant throughout life within an individual. Increased concentrations of Lp(a) have been considered as risk factor for coronary artery disease but its role in carotid atherosclerosis and stroke has not been fully elucidated. The aim of study was to compare the plasma Lp(a) levels and frequency of subjects with elevated Lp(a) levels in coronary atherosclerosis, carotid atherosclerosis and healthy controls within the single population. **Methods:** Study sample consisted of 95 patients with carotid atherosclerosis, 110 patients with coronary atherosclerosis and 88 controls of Serbian origin. The Lp(a) concentration was measured in the fresh samples by immunonephelometric method. The intra- and inter assay coefficient of variation (CVs) were < 10 % and the lower assay limit was 2 mg/dl. **Results:** The mean Lp(a) values were significantly elevated only in coronary atherosclerosis group compared to carotid one and controls (42.7 mg/dl vs. 26.5 mg/dl vs. 20.9 mg/dl , respectively, $p< 0.001$, Kruskal-Wallis ANOVA). Patients with coronary atherosclerosis had 3 times higher relative risk for elevated Lp(a) levels (CI 1.6-5.4, $p<0.001$) compared to patients with carotid atherosclerosis. **Conclusion:** Our results suggest that elevated Lp(a) levels are independent risk factor for coronary, but not for carotid atherosclerosis in Serbian patients. Still, further analysis including genetic background should be performed.

Funding: Funded by Serbian Government Research Grant

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FRAMEWORK FOR MODELING AND SIMULATION OF LIPOPROTEIN DISTRIBUTION PROFILES IN HUMAN PLASMA

***Andreas P. Freidig**, Daan B. van Schalkwijk, Ben van Ommen. TNO Quality of Life, Zeist, Netherlands.*

Objective: To develop a model framework capable of simulating the steady state of ApoB100 containing lipoproteins (VLDL, IDL and LDL) in human plasma, based on general, physiological assumptions on production, metabolism and reabsorption of these particles. **Methods:** The model framework is based on a stochastic simulation engine (Particle Profiler) which predicts size, biochemical composition and age for up to 100.000 individual lipoproteins over time. Model output includes plasma steady state levels of clinically relevant parameters like LDL cholesterol, triglycerides and LDL size distribution. **Results:** The applicability of the model framework was confirmed with data from stable isotope studies. Individual data sets were correctly simulated. Furthermore, physiological hypothesis on the size dependence of lipoprotein metabolism were implemented and evaluated within the model framework. **Conclusions:** The model will be used to analyze data sets of individuals with different genotypic or phenotypic background and data sets from therapeutic intervention trials.

Funding: Funding of this research by the Dutch government (NBIC) is gratefully acknowledged

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COMPARATIVE BIOINFORMATIC ANALYSIS OF HUMAN SND1 AND RAT Snd1 GENE PROMOTERS

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Human SND1 gene and its rat homologue Snd1 encode, respectively, for p100 coactivator and SND p102, which are members of a plural family of proteins. p100, firstly described as a transcriptional coactivator, has been recently involved in different cellular processes including lipid metabolism. Our own studies have shown that SND p102 is associated to lipid bodies in a model of hepatocellular esteatosis and that adenovirus-driven differential expression of the protein modifies phospholipid secretion into lipoproteins in rat hepatocytes. Since nothing was known about transcriptional regulation of these genes, we firstly isolated, cloned and characterized the rat Snd1 gene promoter (AY957585), locating the origin of transcription 216 bases upstream its ATG codon and determining basal transcriptional activity of the cloned promoter region in both rat McA-RH7777 and human HepG2 hepatoma cells. In this study, we have isolated and analyzed a region of 3,492 pb flanking the 5' end of human SND1 gene by a gene walking method. Homology between these two sequences is greater than 80% in a region of 311 bases upstream rat Snd1 origin of transcription, which contains three CCAAT boxes and four GC boxes shown to bind, respectively, NF-Y and Sp1 and to be functional in rat Snd 1 gene transcription. Bioinformatic analysis of both promoter sequences has predicted the presence of a CpG island of 450-750 bp that includes the high-homology region, as well as additional common putative binding sites for transcription factors such as HNF-3beta, CREB and USF, suggesting a role for some other transcription factors in transcriptional regulation of the two genes.

Funding: Supported by Basque Government SA2005/00240.

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REDUCTION OF ATHEROSCLEROSIS BY VARIOUS FRACTIONS OF *CINNAMOMUM VERUM* IN RABBITS

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Objective: To determine the effects of *Cinnamomum verum* fractions in inhibition of atherosclerosis in cholesterol fed rabbits. **Methods:** Atherosclerosis is a disease of blood vessels, almost universally present in people of highly developed societies. Hypercholesterolemia results from circulation of rich lipoproteins. Cholesterol and atherodiet feeding to rabbits resulted in a significant increase in lipid levels. The present study was undertaken using oral ingestion of two fractions of *Cinnamomum verum* (Family- Lauraceae) i.e. 50:50 ($CHCl_3$: CH_3OH) and 25:75($CHCl_3$: CH_3OH) on rabbits for 60 and 120 days. All the animals were autopsied at the end of experimental period. **Result:** Body weights were not significantly affected in experimental animals. However, atherodiet feeding showed a significant increase in liver and aorta weight. Administrations of both the fractions of *Cinnamomum verum* showed a significant reduction in levels of serum cholesterol, triglycerides, phospholipids, LDL cholesterol and VLDL cholesterol whereas HDL cholesterol was improved significantly. **Conclusion:** This shows that the administration of *Cinnamomum verum* fractions reduces the hypercholesterolemia and may prevent atherosclerosis in rabbits.

Funding: Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India

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EFFECTS OF POST-MENOPAUSAL HORMONE REPLACEMENT THERAPY (HRT) ON OXIDATION OF LDL AND INTIMA MEDIA THICKNESS OF THE CAROTIDS

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Objective: To verify if HRT modifies lipoproteins and apolipoproteins, oxidized anti-LDL antibody titer, anti-D, anti-D2 and anti-A as well as the intima media thickness of the carotid arteries (IMT) in postmenopausal women and if the effects are pro or anti-aterogenic. **Methods**: 123 post menopausal women. 75% of them used conjugated estrogens -0.625mg/VO. Enzymatic methods were used for lipoproteins and nephelometric assay for apolipoproteins. Cholesterol ester transfer protein (CETP), phospholid transfer protein (PLTP), hepatic lipase (HL) and lipase lipoprotein (LPL) activities were radiometrically quantified. The ELISA assay was used to determine the autoantibodies using as antigens, highly oxidized LDL and oxidized Apo B proteic epitopes (anti-D, anti-D2 and anti-A).The IMT was determined using the *Doppler* ultrasound. The ANOVA and/or the Mann-Whitney test were used to compare the groups. **Results**: The HRT increased HDL-chol by 9% (without ↑ vs with, p= 0.053), reduced LH activity by 42% (with ↓ vs conjugated, p= 0,001) and by 50% (HRT estrogenic ↓ vs conjugated, p= 0.001). There was a 7% reduction in anti-D2 antibody titers (with↓ vs without RHT, p= 0.053). No change in IMT. **Conclusion**: This study demonstrates anti-aterogenic modulation by HL of HDL concentration in women treated with estrogenic or conjugated HRT and a reduction of one serum oxidizing marker, anti-D2 antibody, with the use of HRT, both without any repercussions on carotid intima-media.

Funding: CNPq

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THE TRIGLYCERIDEMIA PECULIARITIES DURING ALIMENTARY STRESS IN WOMEN OF REPRODUCTIVE AGE WITH POLYCYSTIC OVARIES SYNDROME (PSO) AND CORONARY HEART DISEASE (CHD) AND DIABETES MELLITUS (DM) IN ANAMNESIS

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Objective: Investigate peculiarities of triglycerides' utilization in plasma during alimentary fatty loading in women of reproductive age with PSO and CHD, DM in anamnesis. **Methods:** We questioned 89 women about CHD and DM in relatives, made alimentary fat loading in women of reproductive age with PSO. Test was made on an empty stomach. Patients took 20% fatty sour cream on the basis of 130 gram on 2 meters of body surface area. Then during 24 hours they only might drink water up to 1.5 liters. We estimated triglycerides' levels on empty stomach and in 3, 9, 24 hours after fat loading. The groups were divided depending on triglyceridemia increasing to the 3th hour. The 1 group 35 women had triglyceridemia growth more than 2 times, the 2 gorup was 54. **Results:** The 2 group had higher basal triglyceridemia (1.09±0.07 mmol/l compare with 0.84±0.05 mmol/l,p=0.018), plain type of triglyceride curve (1.09±0.07,1.56±0.09, 0.98±.0.81, 0.97±0.07 mmol/l on empty stomach, in 3,9,24 hours), and 3 times often DM type 2 in anamnesis. The 1 group had lower basal triglyceridemia(0.84±0.05 mmol/l), but higher increasing and triglycerides' elimination(0.84±0.05, 2.2±0.14, 0.98±0.08, 0.88±0.08 on empty stomach, in 3,9,24 hours), 4 times often CHD in anamnesis. Presence in differences in elimination of triglycerides in women with PSO and DM in anamnesis compare with women with PSO and CHD in anamnesis shows different pathogenic mechanisms of disease development and its treatment.

Funding: None

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PROTEOMIC ANALYSIS OF LIVERS FROM MICE WITH AN ATHEROGENIC LIPOPROTEIN PROFILE

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Elevated low density lipoprotein [LDL] and lipoprotein(a) [Lp(a)] levels are independent risk factors for the development of cardiovascular disease. We report here a proteomic analysis of liver proteins in Lp(a)/C57BL/6 transgenic mice [Lp(a) mice], with human Lp(a) and elevated LDL, compared to wild-type C57BL/6 mice [B6 mice]. We expected that the atherogenic lipoprotein profile of the Lp(a) mice would have an effect on the protein expression profile of the liver, given its central role in lipoprotein metabolism. Histopathological analysis revealed significant foam-cell adhesion in the coronary aortae of Lp(a) mice, which is an early indicator of atherosclerosis. Plasma lipid and lipoprotein analysis indicated that the plasma cholesterol in B6 mice was predominantly in the high density lipoprotein [HDL] fraction, whereas the plasma cholesterol in Lp(a) mice was 2-fold higher and mainly in the LDL and HDL fractions. Comparative two-dimensional [2D] gel electrophoresis identified fifty proteins that showed significantly different ($P < 0.05$) levels that were mostly higher in the livers of Lp(a) mice compared to B6 mice. Identification of these proteins by MALDI-TOF mass spectrometry revealed subsets of proteins associated with oxidative stress and energy metabolism. This study provides evidence that an atherogenic lipoprotein profile may alter the redox status and carbohydrate and fat metabolism in mouse hepatocytes.

Funding: This research was funded by The Marsden Fund of New Zealand and by The National Heart Foundation of New Zealand Postgraduate Scholarship

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EVALUATION OF A NOVEL PRECIPITATION METHOD FOR THE QUANTIFICATION OF SMALL DENSE LDL-CHOLESTEROL

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Objective: Several studies have demonstrated an association between a predominance of small dense LDL (sdLDL) particles and coronary artery disease. So far, quantitative determination of sdLDL has been laborious and not feasible for clinical application. Recently, a novel assay (Denka-Seiken sdLDL-C Kit) allowing rapid quantification of sdLDL concentrations in serum eluates has been introduced. **Methods:** In the present study we clinically evaluated the sdLDL assay in normolipidemic and dyslipidemic samples and tested the performances of Denka Seiken and Roche LDL-C reagents for their suitability with this assay. **Results:** The coefficients of variation using low- and high level sdLDL controls ranged from 3.6% to 6.3% for eluates measured with the Denka Seiken LDL-C reagent and from 3.9% to 7.5% for eluates measured with the Roche LDL-C reagent. Results from both tests were in good agreement as determined by Passing and Bablok regression analysis. In normolipidemic samples (n=33) sdLDL concentrations were 23.19 mg/dl ± 7.3 mg/dl for the Denka Seiken LDL-C reagent and 23.20 mg/dl ± 8.5 mg/dl for the Roche LDL-C reagent, with sdLDL representing 21.3% of total LDL-C. Samples from dyslipidemic patients (n=44) showed significantly higher sdLDL levels of 54.17 mg/dl ± 23.07 mg/dl for the Denka Seiken LDL-C reagent and 52.77 mg/dl ± 26.49 mg/dl for the Roche LDL-C reagent, with sdLDL representing 47.2% of total LDL-C. **Conclusions:** Our results demonstrate that the sdLDL precipitation method shows good analytical performance and can be used with homogenous LDL-C reagents from Denka-Seiken or Roche.

Funding: This study was supported by Roche Diagnostics and Denka Seiken

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PITAVASTATIN INHIBITS FOAM CELL FORMATION IN RAW264.7 MACROPHAGE AT CLINICAL CONCENTRATION

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[Objectives] Pitavastain is the first totally synthetic HMG-Co A reductase inhibitor in Japan that significantly reduces LDL cholesterol while raising HDL cholesterol. Clinical trail showed that pitavastain have a potent effect for LDL cholesterol lowering and is expected to have the prevention of atherosclerosis. In order to clarify the mechanism of reduction of atherosclerosis by pitavastain, we examined the effect of pitavastatin on the foam cell formation of RAW264.7 macrophages. **[Methods & Results]** Macrophages were cultured with pitavastain for 24 h, and exposed to oxidized LDL with pitavastain for 3 days. Pitavastain (10~100 nM) decreased the cellular cholesteryl ester content in a dose-dependent manner, and this effect was not via inhibition of HMG-CoA reductase because the concentration of pitavastain did not inhibit [14C]cholesterol synthesis from [14C]acetic acid and the effect did not affected by addition of mevalonic acid. Pitavastain increased neutral cholesterol esterase (NCEase) activity and did not affect ACAT activity, and decreased the expression of CD36 and ABCA1 mRNA. The mechanism of the increased of NCEase activity was that pitavastain directly modified the substrate state which was cholesterol oleate emulsified with lecithin. Our finging is that clinical blood concentration of pitavastain prevents foam cell formation of RAW macrophages by oxidized LDL, and this was not via inhibition of HMG-CoA reductase.

Funding: None

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EFFECTS OF NICOTINIC ACID ON MACROPHAGES: POTENTIAL IMPLICATIONS FOR ATHEROSCLEROSIS

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Objectives: Nicotinic acid (niacin, NA) has been shown in human subjects to lower LDL-cholesterol, increase HDL-cholesterol and improve survival after myocardial infarction. A G-protein coupled receptor for nicotinic acid, GPR109A, was recently identified and found to be expressed on adipocytes and immune cells. In these studies we have examined functional responses of human monocytes/macrophages to NA. **Methods and Results:** Calcium flux and chemotaxis in response to NA were monitored in primary human monocytes and monocyte-derived macrophages. Macrophages exhibited NA induced calcium flux and chemotactic responses that were enhanced by exposure to several pro-inflammatory cytokines known to be upregulated in atherosclerotic lesions. The responses to nicotinic acid occurred at concentrations consistent with GPR109A receptor-dependent responses in previously described biochemical and cell-based systems. Acute NA pretreatment of human macrophages desensitized responses by these cells to the pro-inflammatory chemokines CCL2/MCP-1 and CCL3/MIP-1α. CCL2/MCP-1 has been shown to be one of the key chemokines implicated in the progression of atherosclerosis in model systems. **Conclusion:** These studies indicate that monocytes/macrophages reproducibly respond to NA and raise the possibility that NA may impact atherosclerosis by direct modulation of the behavior and responsiveness of these cells to other inflammatory stimuli.

Funding: Merck & Co.

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MECHANISMS OF APOLIPOPROTEIN E GENE REGULATION IN MACROPHAGES UNDER INFLAMMATORY STRESS FACTORS

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The atheroprotective role of apolipoprotein E (apoE) is well established. During inflammation, expression of apoE in macrophages is reduced leading to enhanced atheromatous plaque development. In the present study, we explored the signaling pathways involved in the repression of apoE gene expression in response to lipopolysaccharide (LPS) treatment, in RAW 264.7 macrophages. We identified Tpl-2 and MEKK1 as the kinases that are primarily responsible for the downregulation of apoE promoter activity by LPS. Using a dominant negative form of IκB, we established that Tpl-2 and MEKK1 signaling pathways converge to NF-κB acting on the apoE core promoter -55/+73. In addition to NF-kB activation, LPS also activated c-Jun via its phosphorylation by JNK. The activity of the apoE promoter was repressed by c-Jun whereas siRNA-mediated inhibition of endogenous c-Jun expression reversed the inhibitory effect of Tpl-2 on the apoE promoter. Transfection experiments and DNA binding assays showed that the binding site for c-Jun is in the -55/+73 region of the apoE promoter. Finally, we showed that LPS inhibited apoE gene expression via activation of the Tpl-2/ MEK/ ERK pathway acting on a different apoE promoter region. In summary, LPS represses apoE gene expression in macrophages via signaling pathways that involve the upstream kinases Tpl-2 and MEKK1, the intermediate MAP kinases ERK and JNK and the downstream transcription factors AP-1 and NF-κB that inhibit the apoE promoter activity via distinct regions.

Funding: The research was supported by the Romanian Ministry of Education and Research "Excellence Research Program" grants 38/2005 and 130/2006

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IN VITRO STUDY OF PHOTODYNAMIC EFFECTS ON HUMAN MONOCYTE DERIVED MACROPHAGES

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Objectives: Chloroaluminum sulfonated phthalocyanine (Photosens (PS) - NIOPIK, Russia) is a photosensitizer that is widely used in photodynamic therapy (PDT) of tumor deseases. Recently it was proposed to use PDT in treatment of cardiovascular pathology like restenosis and atherosclerosis. Macrophages (MPH)are strongly involved in atherosclerotic lesion formation. This study was designed to characterize the effects of PDT on MPH viability and to define the cell-death pathway for PS. **Methods:** Human monocyte-derived MPH were obtained and cultured as described elsewhere. 24 h before PDT 2-100ug/ml of PS was added to culture medium. Then cells washed carefully and illuminated with 675-nm light (0.5-100 J/cm2). Cellular viability was measured with MTT test. Apoptosis/necrosis was assessed by use of annexin V-PI kit. Intracellular ROS was visualized with DCF-DA. **Results:** Fluorescence microscopy and spectrofluorimetry of MPH revealed time- and concentration dependent uptake of PS. 10ug/ml concentration was choosed for experiments because higher doses of PS induced cytotoxic effects. Either PS accumulation alone or laser illumination alone did not affect cells. Illumination of PS–loaded cells with 675-nm light ((0.5-100 J/cm2) impaired cellular viability in dose-dependent maner. Among dead MPH we did not reveal apoptotic with any fluence rate used. All dead MPH were necrotic. PDT was associated with interacellular ROS formation. **Conclusions:** PDT in the appropriate drug and light doses can eliminate MPH, including those responsible for lipid accumulation.

Funding: This research was supported in part by ISTC grant 2579 and RFBR grant 07-04-0150a

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PROTECTIVE EFFECT OF THE ACTIVATED PROTEIN C ON MAST CELLS

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Objective: The proteinases of haemostasis regulate blood coagulation and inflammation via proteinase-activated receptors (PARs). An anticoagulant proteinase - activated protein C (APC) binds with PAR1 and EPCR. APC have anti-inflammatory effects on endothelial cells and neutrophils. We hypothesised that APC can regulate the mast cell activity via PAR1. **Methods:** (1) analysis of beta-hexosaminidase release from purified rat peritoneal mast cells (PMC), obtained from Wistar male rats, and Student t-test for statistical treatment of data; (2) desensitization of PAR1 with thrombin (100nM) and other PAR1 agonists; (3) inhibition of APC proteolytic activity with phenilmethilsulphonil phtoride (PMSF). **Results:** In vitro experiments have shown that APC at very low concentrations (0.5-2.5nM) was able to reduce (6-11%) the rest mast cell beta-hexosaminidase (marker of cell secretion) release ($n=5$, $P<0.01$). Not only rest PMS but also cells, activated with not immune pro-inflammatory mediators, such as PAR1-agonist peptide (50mkM), duodenase (serine proteinase of must cells and duodenum, 80nM) and compound 48/80 (nonspecific PMS degranulator) responses to APC (0.5-2.5nM) with significant reduction of PMS mediator secretion ($n=5$, $P<0.01$). Desensitization of PAR1 and inhibition of APC proteolytic activity abolished APC effect and support our hypothesis about PAR1-mediated APC action on mast cells. **Conclusion:** Our data indicated that APC is an important endogenous PAR1-mediated regulator of mast cells degranulation and an anti-inflammatory agent.

Funding: This work was sponsored by RFBR (No. 05-04-48725)

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VARIATIONS IN PLATELET AGGREGATION DURING ASPIRIN AND CLOPIDOGREL INTAKE IN HEALTHY MEN

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Objectives: Normal responses and criteria for resistance towards Aspirin and Clopidogrel are still undefined. We examined the responses to these drugs in healthy men utilizing various agonists. **Methods:** In a randomized cross-over study of 20 healthy men, taking Aspirin (ASA) or Clopidogrel (Clo) for 10 days we performed platelet aggregation (PA) in platelet rich plasma with the agonists: Arachidonic acid (AA), ADP and collagen. Blood was drawn at baseline and at 4 times during treatment from 2 hours - 10 days. Reference intervals were defined by non-parametric analysis. **Results:** The 5-95th percentile ranges for PA at baseline were: 64-86% (AA), 62-87% (ADP) and 68-85% (collagen). During ASA therapy these limits decreased to: 0-9% (AA), 35-71% (ADP) and 15-66% (collagen). During Clo therapy we found: 30-76% (AA), 14-51% (ADP) and 61-80% (collagen). 2 out of 20 demonstrated < 10% change in PA induced by ADP compared to baseline, suggesting resistance to Clo. **Conclusions:** ASA therapy was demonstrated by a very low AA aggregation, and Clo clearly lowered ADP-induced PA in most persons. 10% of the men showed Clo resistance. While collagen induced platelet aggregation seem to (at least partly) depend on a functioning COX-1 enzyme, blockage of the P2Y12 receptor by Clo does not significantly affect this pathway for platelet activation. ASA therapy affects ADP induced aggregation and conversely Clo therapy affects AA induced aggregation, which may cause difficulty in analysing isolated drug effects in patients receiving dual antiplatelet treatment.

Funding: The Korning Foundation and the Research Foundation of the University of Aarhus have supported this study

Poster Session 2 "CLINICAL PRACTICE" - GROUP A

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THE BEST LIPID PREDICTOR

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Objectives: To compare the abilities of four lipid predictors to predict the population at risk of atherothrombotic disease (ATD). **Methods:** The lipid predictors are: LDL, CT, CT:HDL, and (LDL-HDL)/LDL. The author's general and ATD population databases demonstrate the frequencies and severity of each lipid predictor. **Results:** If each predictor is divided into sextiles, then as each predictor passes from lowest to highest sextile, the percentage of ATD patients per sextile increases progressively; however, the number of people in the general population per predictor sextile decreases progressively for LDL, CT, and CT:HDL—only for (LDL-HDL0/LDL does the number of people increase. The sextile of each lipid predictor in the ATD population in which the most patients are found is in the mid to lower sextile for LDL, CT, and CT:HDL. Only for (LDL-HDL)/LDL are the most patients found at the high end of the sextiles. At any level of LDL, CT, CT:HDL, knowledge of (LDL-HDL0/LDL allows further risk stratification in terms of age of ATD onset. A lower (LDL-HDL)/LDL is associated with an older age of ATD onset; a higher (LDL-HDL)/LDL, with a younger age. The reverse is not uniformly true. **Conclusion:** (LDL-HDL)/LDL is the best lipid predictor because, unlike LDL, CT, and CT:HDL, it predicts ATD risk progressively better in more patients as predictor sextiles range from lowest to highest and it has most ATD patients in its highest sextiles. Also, (LDL-HDL)/LDL can further risk-stratify any level of LDL, CT, and CT:HDL.

Funding: None

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PERSISTENTLY INCREASED HDL CHOLESTEROLEMIA AND REDUCED TRIGLYCERIDEMIA IN A LARGE LIPID CLINIC POPULATION TREATED WITH FENOFIBRATE FOR 15 YEARS OR LONGER

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Objective: Fenofibrate was recently evaluated in diabetics in the FIELD study. Drug treatment was associated with a modest reduction of cardiovascular events, but there was an apparent loss of activity of the drug over time, thus achieving only a +1.2% increase of HDL cholesterolemia at 5 years. **Methods:** Plasma lipids were investigated in a large series of patients followed at 5-year intervals up to 15 years or longer at the Lipid Clinic of the University of Milano (n= 124 for 5 years; n=65 for 10 years and n=45 for 15 years or longer). **Results:** The HDL-cholesterol raising properties (+ 24.6 % at 15 years) were well maintained over many years of treatment and tended to increase over time, particularly in diabetics. Fenofibrate also significantly reduced triglyceridemia and LDL-cholesterolemia (mean reductions of 54.9 and 28.5 % respectively at 15 years). Differences were, however, noted in individuals with predominant hypercholesterolemia and normal-high HDL-C levels. **Conclusions:** Long term fenofibrate treated patients show well maintained biochemical effects for a very long period of time. A prior study from the reporting investigators suggests that the inadequate activity of fenofibrate of the FIELD study might be due to bioavailability problems, as shown with some slow release formulations of the drug (Sirtori et al,. Eur J Clin Pharmacol 1985; 28:619--624).

Funding: The study was carried out as a spontaneous project of the Authors, without any specific funding

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THE EFFECTS OF ANTIPSYCHOTIC AGENTS ON LIPIDS AND OTHER CARDIOVASCULAR DISEASE RISK FACTORS

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Objectives: To assess effects on cardiovascular risk factors of a chronically mentally ill population before and after implementation of a comprehensive educational program for providers about metabolic risks associated with antipsychotic agents. **Methods:** Baseline data, including demographic information, weight, waist circumference, blood pressure, lipids, and glucose were obtained on 60 patients. Results were compared to a demographically similar group of 65 patients 2 years later. **Results:** While there was no change in BMI, weight (203.8 ± 48.3 lbs. vs. 205.0 ± 37.7 lbs.), waist circumference (41.6 ± 6.0 inches vs. 45.7 ± 6.0 inches), and the number of individuals overweight or obese were lower in the follow-up cohort. Improvements in lipid profiles were striking: more subjects in follow-up group had total cholesterol values < 200mg/dL (95% vs. 43%), triglycerides <150mg/dL (57% vs. 50%), and low density lipoprotein cholesterol <100mg/dL (61% vs. 24%). More patients (57%) had plasma glucose values less than 100mg/dL and fewer had levels greater than 126mg/dL (11%) than in the initial cohort (55% and 18%, respectively). Although more patients had hypertension, fewer (52% vs. 55%) had metabolic syndrome. A reduction in the use of olanzapine and an increase in ziprasidone prescribing was noted. **Conclusions:** Psychotic patients at high risk for cardiovascular disease should be assessed for general health status. Implementation of a comprehensive educational program for health care providers and regular metabolic monitoring of patients improved the cardiovascular health status of patients in our clinic.

Funding: Funding for the metabolic screenings conducted in the follow-up cohort was provided by Pfizer, Inc.

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MEDICATION USE IN OUTPATIENTS WITH ATHEROTHROMBOSIS IN GREECE

***Evangelos N. Liberopoulos**, Moses S. Elisaf. University of Ioannina, Ioannina, Greece.*

Objectives: Atherothrombosis is the leading cause of cardiovascular morbidity and mortality around the world. However, little is known about the use of medications among subjects with atherothrombosis in 'real' clinical practice. **Methods:** The Reduction of Atherothrombosis for Continued Health (REACH) Registry collected data on atherosclerosis risk factors and their treatment around the world. The present report presents data collected from Greece. **Results:** A total of 707 patients (179 were asymptomatic but had ≥ 3 atherosclerotic risk factors while 528 had symptomatic atherosclerotic disease) from 74 physician practices were included in the Greek REACH registry. Of these, 82% were taken at least one antiplatelet drug (52.6% aspirin, 48.8% a non-aspirin antiplatelet drug and 17.4% two antiplatelet drugs). Furthermore, 73.3% of participants were administered statins and 6% a non-statin hypolipidemic drug. Regarding antihypertensive medication, 37.6% of patients were taken calcium channel blockers, 36.6% beta-blockers, 38.3% diuretics, 46% angiotensin-converting enzyme inhibitors, 27% angiotensin receptor blockers and 4% other antihypertensives. Overall, 92.5% of subjects were receiving at least one antihypertensive drug. Of the total study population, 47.8% were diabetics; among these, insulin was used in 25%, biguanides in 30.2%, sulfonylureas in 58.6%, glitazones in 12.1% and other antidiabetics in 10.6% (93.7% of diabetics were receiving at least one antidiabetic medication). **Conclusions:** Antihypertensive and antidiabetic drugs are commonly used in outpatients with atherothrombosis in Greece. However, lipid-lowering and antiplatelet medications are not so widely used in these high-risk individuals.

Funding: The REACH registry is sponsored jointly by sanofi-aventis and Bristol-Myers Squibb

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NEW RISK SCORE FOR CARDIOVASCULAR EVENTS DEVELOPED FROM THE MEGA STUDY IN JAPANESE, A LARGE-SCALE PRIMARY PREVENTION TRIAL WITH PRAVASTATIN

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Objective: We developed a new risk score including statin treatment for coronary heart disease (CHD), stroke, and cardiovascular disease (CVD) based on the MEGA Study data, and compared the predicted incidence derived from Framingham risk equation with the new risk score (MEGA risk prediction score). **Methods:** We devised a risk prediction score based on the hazard ratios obtained by risk factor analysis, using the 5-year follow-up data in 7,832 hypercholesterolemic patients without CVD in the MEGA Study. The components of the MEGA risk prediction score are sex, age, high density lipoprotein cholesterol, fasting plasma glucose, hypertension, smoking, and treatment. Each component was scored for CHD, CVD, and stroke. The patients were divided into 5 levels of risk based on their MEGA risk prediction score, and the strata were compared to the predicted risks derived from Framingham risk equation, according to the treatment group. **Results:** The MEGA risk prediction score was well validated, with concordance between the predicted and actual incidence of events. The predicted 5-year risk derived from the Framingham risk equation for CHD and CVD were overestimated about 2-3 times in comparison with the risk as estimated by the MEGA risk prediction score. In contrast, the predicted risk of stroke by Framingham risk equation was underestimated in this population. **Conclusion:** The Framingham risk model for cardiovascular events is not applicable in Japanese hypercholesterolemic patients. The MEGA score is useful for more accurately estimating risk for CHD, stroke, and CVD in this low-risk population and similar risk profiles populations.

Funding: Daiichi Sankyo

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WHICH RISK FACTORS PREDICT FUTURE VASCULAR EVENTS IN CORONARY PATIENTS RECEIVING STATINS?

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Objective: Statins are a cornerstone in the treatment of patients with established coronary artery disease (CAD), but vascular risk in these patients remains high. Because it is not known which lipid parameters or other risk factors predict CAD progression despite statin treatment, we performed a prospective study on statin-treated angiographied coronary patients. **Methods:** We prospectively recorded vascular events over 6 years in 476 consecutive patients. At the baseline coronary angiography, lipids and lipoproteins were measured from fasting serum samples. **Results:** We recorded 146 vascular events (107 coronary and 39 non-coronary events). Among lipid parameters, in Cox regression analyses, low HDL cholesterol (standardized adjusted hazard ratio (HR) = 0.747 [0.618-0.904]; p = 0.003), low apolipoprotein A (HR = 0.743 [0.625-0.883]; p = 0.001) and a small LDL peak particle diameter (HR = 0.814 [0.693-0.956]; p = 0.012) significantly predicted vascular events, but not total cholesterol (p = 0.265), LDL cholesterol (p = 0.346), or apolipoprotein B (p = 0.533). After multivariate adjustment for non-lipid risk factors HDL cholesterol (HR = 0.766 [0.618-0.950]; p = 0.015), apolipoprotein A (HR = 0.771 [0.639-0.931]; p = 0.007), and the LDL peak particle diameter (HR = 0.801 [0.674-0.953]; p = 0.012) remained significantly predictive for the incidence of vascular events. **Conclusions:** We conclude that low HDL cholesterol, low apolipoprotein A, and small LDL particles are the main lipid risk factors for the incidence of vascular events in coronary patients on statin treatment.

Funding: There are no funding sources of commercial nature

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SIGNIFICANCE OF TRIGLYCERIDE RICH ATHEROGENIC LIPOPROTEINS IN PREDICTING CARDIOVASCULAR EVENTS

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Objective: Guidelines for cardiovascular (CV) disease prevention recommend triglyceride and non-HDL cholesterol as secondary targets of management after LDL. Despite proven benefits of reducing LDL cholesterol, event rates in treated individuals are substantial. We evaluated the prognostic significance of triglyceride rich atherogenic lipoproteins in predicting CV events in adults undergoing elective coronary angiography. **Methods:** Subjects were prospectively enrolled and excluded for age (men ≥ 55, women ≥ 65), prior diagnosis of coronary heart disease (CHD), or on statins. Subjects had fasting blood drawn for NMR analysis (Liposcience, Raleigh, NC) and were followed over time. Severe coronary artery disease (CAD) was defined as stenosis ≥ 50%. CV events included death, myocardial infarction (MI), and cerebral vascular accident (CVA). **Results:** 236 subjects had complete data (mean age 53 ± 8; 55% women). Mean cholesterol values were: total 207 ± 42; LDL 122 ± 30; HDL 51 ± 14 and triglyceride 171 ± 121 mg/dL. Severe CAD was diagnosed in 72. Median follow-up was 32 months. 12 events occurred in 10 subjects (death=3, MI =5, CVA=4). Mean values for total, LDL and HDL cholesterol did not differ in those with events compared to those without. Cox proportional hazard analysis revealed increased risk of events for triglycerides (HR:4.7,CI:1.3-16.6); VLDL (HR:4.8,CI:1.4-17.0) and LDL particle concentration (HR:4.7,CI:1.3-16.7). **Conclusion:** Atherogenic lipoproteins may play a role in improving CHD prevention. Compliance with guideline recommendations on triglyceride and non-HDL cholesterol may be crucial in making progress in CHD management.

Funding: Gundersen Lutheran Medical Foundation

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LIPID CHANGES IN HIV-POSITIVE PATIENTS ATTENDING A SPECIALISED LIPID CLINIC

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Objectives: The protease inhibitor (PI) class of antiretrovirals, used in combination regimens for the treatment of HIV, has been associated with detrimental lipid changes. Furthermore, this population has a high prevalence of traditional cardiovascular risk factors. The Royal Free Hospital, London provides a specialized lipid clinic for HIV-positive patients. **Methods:** We included all patients with a lipid clinic appointment in April 2002-April 2006. **Results:** 363/2583 (14%) HIV-positive patients under follow-up during the study period attended the clinic. 314 (87%) were male and 254 (71%) Caucasian. At 1st visit median age, CD4 count and time since first ART exposure was 42 years, 523 cells/μl and 6 years. 322 (89%) were receiving HIV treatment, of whom 284 (88%) had a HIV viral load<50 copies/ml (the lower limit of quantification of current assays, indicating successful treatment). 270 (74%) had ever and 211 (58%) were currently receiving PIs. Median (IQR) TC, LDL, HDL and TG were 6.3 (5.5,7.1), 3.5 (2.7,4.2), 1.3 (1.1,1.6) and 2.8 (1.6,5.2) mmol/l. Interventions prescribed included statins, fibrates, omacor and dietary advice. 274 (75%) patients had lipid measures 1 year after their 1st visit. Median (IQR) TC, LDL, HDL and TG at 1 year were 5.6 (5.0,6.4), 3.2 (2.5,3.7), 1.3 (1.1,1.6) and 2.3 (1.6,3.5) mmol/l. Median (IQR) changes over the 1st year were -0.7 (-1.6,+0.1;p<0.0001), -0.5 (-1.1,+0.2;p<0.0001), 0.0 (-0.1,+0.2;p=0.02) and -0.6 (-2.1,+0.1;p<0.0001) mmol/l. **Conclusions:** HIV-positive patients visiting a specialized lipid clinic had beneficial lipid changes. Further research will consider the association of lipid changes and specific interventions used.

Funding: There are no conflicts of interest for this study

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LIFESTYLE AND RISK FACTORS FOR CARDIOVASCULAR DISEASE IN THE ELDERLY

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Objectives: Lifestyle change is recommended before starting drug treatment. This study assessed CVD risk profile, diet, physical activity, smoking and drug therapy in elderly men and women in comparison to younger adults. **Subjects and Methods:** 590 women (42.4± 5.5 Vs 66.5± 4.0 y) and 486 men (44.1± 5.6 Vs 63.9± 7.0 y). Data on physical examination, fasting blood analyses, dietary protocols, questionnaires on physical activity, smoking, drug treatment were evaluated with SPSS 14.0. **Results:** In the elderly waist circumference, blood pressure, glucose, cholesterol were significantly higher than in the younger, BMI, LDL-C and TG only in women ; HDL-C was identical. Prevalence was higher in the elderly for abdominal obesity (by 29% in women, 28% in men), overweight (by7% in women, 3% in men) hyperglycemia (by 23% in women, 22% in men), hypertension (by 39% in women,15% in men), dyslipoproteinemia (TG by19% in women, 7 % in men and LDL-C by 42% in women, 12% in men, no difference in low HDL-C). Nonsmokers in the elderly: 96.8% women, 95.6% men, in the younger 82.4% women, 75.9% men. Physical activity was less in seniors: men by 13.2% no sports and 17.4% >twice a week, women by 7.2% no sports and 25.6% >twice a week. Seniors consumed significantly less carbohydrates (more mono-, less poly- saccharides), more alcohol and water; female seniors had a higher fat consumption (36.3 Vs 34.9 % energy). The elderly used more antihypertensives (33%) and lipid modifying drugs (12%) than th younger. **Conclusions:** The elderly are on higher risk for CVD, but were also aware of the importance of healthy lifestyle and of the importance of additionally drug treatment to control CVD risk.

Funding: Foundation for the prevention of Arteriosclerosis

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ABDOMINAL AORTIC ANEURYSM IN ELDERLY HYPERTENSIVE PATIENTS

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Objective: This study was undertaken to determine the utility of transthoracic echocardiography as a screening test for occult abdominal aortic aneurysm in hypertensive patients subjected to transthoracic echocardiography older 60 years. **Methods:** In hypertensive patients, longitudinal and transverse images of the abdominal aorta were obtained during the subcostal portion of the transthoracic echocardiography. Abdominal aortic aneurysm was defined as an abdominal aortic dimension (antero-posterior or lateral) over 3 cm. Exclusion criteria included prior abdominal aortic aneurysm repair, known abdominal aortic aneurysm and inadequate images of the abdominal aorta. **Results:** 196 patients met the study inclusion criteria (101 men; 95 women, mean age 65 years, range 61-85 years). An occult abdominal aortic aneurysm was identified in 40 patients (20.40%). 28 patients were men (70%), with a mean age of 68 years and a mean duration of hypertension of 12 years, 31 patients (77.50%) had a history of tobacco use and 9 patients (22.50%) had a positive family history of abdominal aortic aneurysm. All aneurysms were infrarenal in location; the mean diameter was 3.7 cm (range 3-4.8 cm). Laminated thrombus was present in 14 patients (35%). Imaging of the abdominal aorta during transthoracic echocardiography required an average of 6.5 minutes (range 5-8 minutes). **Conclusions:** The present study shows that incidence of occult abdominal aortic aneurysm detected by transthoracic echocardiography in hypertensive patients older 60 years of age is significant. Ultrasonography is highly accurate in the diagnosis of abdominal aortic aneurysm and the screening for abdominal aortic aneurysm can be readily incorporated into the transthoracic echocardiography examination of elderly hypertensive patients.

Funding: I did not receive any funding for this study

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INCREASED WAIST CIRCUMFERENCE IS ASSOCIATED WITH LOW GRADE INFLAMMATION IN FRAIL ELDERLY PEOPLE

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Objectives: Chronic low grade inflammation is associated with cardiovascular disease risk. The aim of this study is to investigate which anthropometric mesaures best correlates with the presence of the low grade inflammation in frail elderly people. **Methods:** Participants were 252 frail elderly (aged 65-99 years) people (76.6% women). Anthropometrics (body mass index - BMI, waist circumference - WC, hip circumference – HC, waist to hip ratio - WHR) and blood pressure (BP) were recorded and a fasting blood sample collected for lipid and inflammation status. Inflammation was assessed by levels of high sensitive C-reactive protein (CRP). Participants with CRP grater than 10 mg/L were excluded from study. The subjects were divided into three groups according to their CRP levels: a low (<1 mg/L, N=70), an average (1 to 3 mg/L, N=70), and a high (3 to 10 mg/L, N=72) CRP group. **Results:** The CRP level showed a significant positive correlation with WC (r=0.191, p=0.005), HC (r=0.173, p=0.012) and serum triglyceride level (r=0.151, p=0.028). In contrast, BMI, WHR, systolic and diastolic BP failed to correlate with the CRP level. There was significant negative correlation CRP with high density cholesterol (r=-0.170, p=0.013). WC and HC were significantly smaller in the low CRP group compared to the average (p=0.015, p=0.023, respectively) and the high CRP groups (p=0.012, p=0.047, respectively). **Conclusions:** Age-related changes in body composition should be taken into account when considering morbidity, but the same pattern of association exist between visceral obesity and low grade inflammation in frail elderly people as in younger adults.

Funding: None

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HIGH DENSITY LIPOPROTEIN CHOLESTEROL, CARDIOVASCULAR DISEASE AND PHYSICAL PERFORMANCE: THE InCHIANTI STUDY

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Objective: To examine the relationship between HDL-C and physical performance and the effect of CVD on this relationship. **Methods:** 836 older persons enrolled in the InCHIANTI study. Physical performance was assessed using 400-m walk speed and knee extension torque. Relationship between HDL-C sex-specific tertiles and performance measures was explored using multivariate regression models. **Results:** Independent of age and sex, and compared to subjects in the lowest tertile of the HDL-C distribution, participants in the highest HDL-C tertile were less likely to have hypertension, stroke, peripheral arterial disease, and diabetes. After adjusting for potential confounders (sociodemographic and life-style factors, body composition, clinical conditions, and biological markers) HDL-C levels were associated with knee extension torque in both men and women, and with 400-m walk speed in men (interaction term p<.01). Multinomial logistic regression analysis showed that men in the highest tertile of the HDL-C distribution had an almost four-fold higher probability of belonging to the highest tertile of 400-m walk speed (OR:3.7,95%CI 1.2-11.7) and knee extension torque (OR:3.6,1.4-9.3). Consistent results were found after excluding participants with prevalent CVD. **Conclusions:** In older people high levels of HDL-C are associated with better physical performance. The association is not explained by a lower prevalence of CVD.

Funding: None

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COMPARATIVE STUDY OF DIAGNOSTIC AND THERAPEUTIC STRATEGIES IN PATIENTS WITH DISLIPEMIA IN THE OUT-PATIENT CARDIOLOGY CLINIC OR LIPIDS CLINIC IN A CARDIOLOGY CENTER IN ARGENTINA

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Objective: Evaluate baseline characteristics, diagnostic procedures and treatment objectives according to ATP III, in patients (pts.) with dislipemia in the out-patient cardiology clinic (OPCCs), and the Lipids and Atherosclerosis Clinic (LAC) in a Cardiology Center in Argentina. **Methods:** Case reports of patients who attended OPCCs and LAC during the year 2004 for dislipemia. **Results:** 100 pts. were included in the LAC group and 101 in the OPCC group (age 56.7 ±.11.8, males, 46% vs. 63.5 ±11.8, males 43 %, p < 0.0001). 54 pts. in the LAC and 75 in the OPCC had hypertension (p = 0.00019) and family history of cardiovascular disease 14 and 4 pts. (p=0.014). No significant difference was observed in the number of diabetic patients (5 vs. 11). Intima media thickness was recorded in 57 pts. in the LAC and 15 pts. in the OPCC p<0,0001, and the presence of plaque was detected in 73% vs. 46% (p <0,0001), respectively. 44 pts. in the LAC and 14 pts. in the OPCC (p <0,0001) visited the nutrition. Lipid levels at baseline were higher among the pts. in the LAC group (p < 0,0001). No statistically significant differences were observed in the risk categories based neither on ATP III nor the LDL final goal. The LAC group required more statins (p 0,0034) and combined treatment (p 0,0001). **Conclusions:** Pts. in the LAC group had a worse baseline lipid profile; however, both groups reached the therapeutic goal based on ATP III, with the addition of more lipid lowering drugs in the LAC group. Lipid specialists investigated the presence of subclinical atheromatosis, with more plaques in said pts., which confirms the vascular involvement.

Funding: None

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LOW HDL-CHOLESTEROL IS A PROGNOSIS OR INCIDENCE DETERMINANT FACTOR OF RENAL FUNCTION IN THE PATIENTS WITH HYPERTENSION, DIABETES MELLITUS AND/OR HYPERLIPIDEMIA

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[Objectives] The multiple risk factors such as metabolic syndrome are associated with atherosclerotic desease. It is also considered that chronic kidney disease (CKD) have various dysfunctions of metabolism included dyslipidemia. It suggests that dyslipidemia exacerbates prognosis of renal function. **[Methods]** The subjects were 249 outpatients with hypertension, diabetes mellitus, hyperlipidemia. The patients were classified 5 groups by serum cholesterol concentration of high density lipoprotein fraction(HDL-C) : HDL-C<40mg/dl (n=46, group I),40≤HDL-C<50(n=62, group II), 50≤HDL-C<60(n=62, group III), 60≤HDL-C<70 (n=47, group IV), 70≤HDL(n=31,group V). We compared atherosclerotic parameters (BMI, BP, HbA1C, serum creatinine(Cre) and total cholesterol, triglyceride, HDL-C, apolipoprotein(Apo) A-I, Apo A-II for 3 years. **[Results]** The patients of group I tended to have more than two complications. The levels of Apo A-I and Apo A-II is low in the group I, and Apo A-I/ A-II ratio was lower in group I than the other groups. The serum levels of Cre at the beginning was higher in the group I than the other groups. After 3 years, newly incidence of chronic kidney disease (CKD) were observed 6.5% in group I, 4.8% in group II, 1.6% in group III, 2.1% in group IV, 0% in group V. **[Conclusion]** Dyslipidemia, especially HDL-C level seems to be an association with renal function. The low HDL-C level might be a signal of prognosis or incidence of CKD.

Funding: A salary and a research grants from Minami-kyusyu University

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SERUM PARAOXONASE ACTIVITY AND CARDIOVASCULAR RISK FACTORS IN END-STAGE RENAL DISEASES PATIENTS ON LONG-TERM HEMODIALYSIS

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Background: Hemodialysis patients (HD) are at high risk of atherosclerosis; an enhanced oxidant stress might have a major role. Paraoxonase (PON) is associated with HDL and may reduce LDL lipid peroxidation. We aimed to investigate the PON activity in HD patients and to evaluate the correlations of CV risk factors with PON activity. **Methods:** We enrolled 110 HD patients (63F/47M). Serum lipids were determined by colorimetric methods. HsCRP, apolipoproteins AI, B, E and Lp(a) were measured by nephelometry. LpAI was determined by immunoelectrophoresis. Paraoxonase activity was determined by using paraoxon like substrate. First, the rate of hydrolysis of paraoxon was measured by monitoring the increase in absorbance at 412 nm. The amount of p-nitrophenol generated was calculated from the molar absorptivity at pH 8.0 **Results:** HD patients showed an atherogenic dyslipidemia and elevated hsCRP. This one correlates inversely with apoAI and LpAI:AII and positively with apoB and Lp(a). PON activity was decreased in HD patients compared with controls. This activity decreased significantly in elderly and in patients with elevated LDL-c. PON activity correlates positively with LpAI:AII, and inversely with hsCRP, and apoE. **Conclusion:** We showed disturbed lipoprotein profile and inflammation in HD patients. The decrease in PON activity and thus the reduction of its anti-atherogenic properties could be a supplementary factor of premature atherosclerosis. The PON activity was decreased in HD patients especially in elderly and in patients with higher LDL-c.

Funding: None

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THE ROLE OF CHRONIC BACTERIAL-VIRAL INFECTION IN PERIPHERAL VASCULAR DISEASE ACTIVITY

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High frequency of Chlamydia pneumoniae(C.pn.), Cytomegalovirus(CMV),Epstein-Bar virus(EBV),I-type Herpes(HSV-I) among atherosclerotic(A) patients in Georgian population became the goal of performed study. Our objective was to evaluate if previous and recurrent infection can play a role in the development of peripheral vascular disease(PVD). 60 patients with PVD seropositive to C.pn.,antibodies to HSV-1, CMV and EBV with stable-Igroup(gr) and unstable(II gr) forms and 50 similarly infected patients withought evidence of A vessel ingery(III gr) along with plasma lipoprotein profile(LPL) underwent quantitative measurement of lipid hydroperoxide(LPO), acute phase proteins-CRP and fibrinogen(F), blood fibrinolytic activity(BFA) and immunohistochemical assays of the A plaque. Study revealed statistical difference between I-II, I-III gr in plasma concentration of C.pn. specific IgA(1,15±0,78 against 2,43±0,9 and 2,81±1,1), IgA+IgG(6,25% against 70%, 67,8%) antibody titers, LPO activity(6,65±2,15 against 10,67±2,35, 11,29±0,9), increase in CRP,F, considerable decrease of BFA on the background of almost identically disturbed PLP in I and II gr-s. Similar changes were found in II and III gr despite any abnormalities of PLP in non-A patients. In these gr-s LPO levels positively correlated with titres of antibodies, number of detected viruses, plasma LDL concentration. Significant growth of LPO activity in patients with and without A indicates that chronic infection activates free-radical reactions. In A patients it plays the role of precipitating or additional risk-factor leading to oxidative stress and contributing to PVD activity.

Funding: None

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LIPIDIC METABOLIC CHANGES IN PERIPHERAL ARTERIAL DISEASE OF DIABETIC PATIENTS

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Macrovascular complications affect the quality of life in patients with diabetes mellitus. Recognizing risk factors is important in order to prevent the development of atherosclerosis/atherothrombosis. **Objective:** evaluate lipidic risk factors in diabetics with atherosclerotic disease of lower extremities. **Methods:** retrospective study includes patients with peripheral arterial disease (group 1 - without diabetes and group 2 - with diabetes), admitted for symptoms of intermittent claudication, rest pain or foot ulcers. There have been noted noted the modifiable cardiovascular risk factors (hypertension, hypercholesterolemia, hypertrigliceridemia, smoking, obesity). The extent of atherothrombotic disease was evaluated by clinical examination and investigated by imaging methods, in order to evaluate atherosclerosis. **Results:** hypertriglyceridemia and low HDL-cholesterol are significantly associated ($p<0,05$) with a more severe evolution and a larger extent of arterial disease in diabetic patients, compared to non-diabetics; mean levels of LDL-cholesterol and total-cholesterol are higher in diabetic patients, but without any statistical significance. **Conclusions:** 1. diabetes mellitus aggravates arterial disease. 2. HDL-cholesterol and triglyderides signifficantly differ between diabetic and non-diabetic patients. 3. other lipidic fractions, although with higher levels in diabetic patients, are not statistically significant.

Funding: None

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CAROTID INTIMA-MEDIA THICKNESS IS STRONGLY ASSOCIATED WITH CORONARY ARTERY DISEASE

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Objectives: To predict angiographically defined coronary artery (CAD) disease by noninvasive method. **Methods:** We investigated the severity of coronary artery disease by coronary angiography (CAG), abdominal aortic sclerosis by abdominal aortic angiography, and carotid intima-media thickness (IMT) by ultrasound in 302 consecutive patients (214 men, 29-89 years old). We also investigated the other conventional coronary risk factors, such as age, diabetes mellitus, hypertension, lipid disorder, and smoking. **Results:** Carotid IMT was significantly greater in patients with angiographically defined CAD than those without CAD (1.3 ± 0.6 vs 1.1 ± 0.4, $p<0.05$). Higher carotid IMT, age, male sex, presence of diabetes, hypertension and lipid disorder were significantly associated with the existence of CAD ($P < 0.05$). The thickness of carotid IMT was increased depending on the severity of CAD(non CAD:1.0 ± 0.4, 1vessel disease:1.1 ± 0.3, 2 vessel disease:1.4 ± 0.7, 3 vessel disease 1.4 ± 0.7, $p<0.05$). Whereas aortic sclerosis was not associated with carotid IMT. A multiple logistic regression analysis revealed that carotid IMT was independently associated with CAD (odds ratio 3.2, 95% confidence interval 1.52 to 6.55; $p < 0.05$). **Conclusion:** These results demonstrate that carotid IMT is a useful noninvasive method to predict the existence of CAD.

Funding: None

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VALIDATING DIAGNOSES FROM HOSPITAL DISCHARGE REGISTERS CHANGE RISK ESTIMATES FOR ACUTE CORONARY SYNDROME

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Objectives Hospital discharge registers are cost-efficient data sources; however, their usability is highly dependent on the quality of the registered data. No previous studies have examined the effect of validating discharge diagnoses on relative risk estimates. We examined if a validation of acute coronary syndrome (ACS) diagnoses identified in a hospital discharge register changed the relative risk estimates of well-established risk factors for ACS. **Methods** All first-time ACS diagnoses (n=1138) in the Danish National Patient Registry were identified among male participants in the Danish cohort study "Diet, Cancer and Health" (n=26 946). Medical records were retrieved and reviewed using current European Society of Cardiology criteria for ACS. The ACS diagnosis was confirmed in a total of 781 participants. **Results** The relative risk estimates of ACS for a range of well-established cardiovascular risk factors appeared higher when using validated compared to crude hospital discharge data: smoking: 2.47 (2.13 - 2.87) vs. 2.06 (1.83 - 2.31), hypertension: 1.77 (1.57 - 1.98) vs. 1.74 (1.58 - 1.91), hypercholesterolemia: 1.74 (1.42 - 2.14) vs. 1.68 (1.43 - 1.90), diabetes mellitus: 1.57 (1.30 - 1.90) vs. 1.39 (1.18 - 1.64), respectively. **Conclusions** Use of hospital discharge diagnoses of ACS leads to underestimation of the impact of well established risk factors for ACS, compared to using validated diagnoses.

Funding: Support was given by MD Kopps Foundation, The Research Foundation of the Danish Medical Association in North Jutland and the Danish Heart Foundation

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INTERIM REPORT OF A LARGE-SCALE LONGITUDINAL PROSPECTIVE POST-MARKETING SURVAILLANCE STUDY OF PITAVASTATIN (LIVES STUDY)

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Objective: A large-scale prospective study involving 20,000 Japanese subjects was undertaken to evaluate the efficacy and safety of low dose(1-2 mg/d) pitavastatin. **Methods:** Subjects were enrolled to the study by a central registration method. Primary assessment were evaluation of liver dysfunction and myopathy. Efficacy was evaluated through analysis of lipid control in relation to risk categories for coronary artery disease. The present report is an interim analysis of the first year of treatment of 2 years-study. **Results:** Sixty seven % of 20,279 patients were female. The major associated diseases were hypertension (47%) and diabetes mellitus (26%). The initial pitavastatin dose was 1 mg/d and 2 mg/d in 39% and in 60% of the patients, respectively. The percent decrease in the LDL-C was 30% for the entire population, 31% for the low-risk group (no risk factor), 31% for the intermediate-risk group (1 or 2 risk factors), 29% for the high-risk group (3 or more risk factors), and 28% for patients with a history of CHD. LDL-C reduction was 29% for the 1 mg dose group. The percent decrease in non-HDL-C level was 29%. Among the 19,921 subjects evaluated, the overall incidence of adverse reactions was 9.7%, with 4.1% myopathy and 2.7% liver dysfuntion. No concerning safety signals were identified. **Conclusion:** Pitavastatin is a promising statin that is expected to allow lipid control tailored to the risk category, even at low dose levels (1-2 mg/d). The incidence of adverse reactions (myopathy and liver dysfunction) was low in up to a year of therapy.

Funding: This study was supported completely by Kowa Co. Ltd.

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TREATMENT WITH PIOGLITAZONE IN 2 PATIENTS WHO HAVE PREVIOUSLY EXPERIENCED A PARADOXICAL DECREASE IN HDL-C WHEN TREATED WITH ROSIGLITAZONE

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Paradoxical decreases in high density lipoprotein cholesterol (HDL-C) during treatment with rosiglitazone, although rare, are now a well recognised phenomenon. The paradoxical decrease in HDL-C with rosiglitazone occurs despite excellent improvements in HbA1c and is accompanied by hypoalphalipoproteinaemia. The mechanism by which this occurs is currently unknown. Rosiglitazone belongs to a class of drug known as the thiazolidinediones which are PPARgamma agonists. It is not known whether or not the paradoxical decrease in HDL-C observed is related to unique properties of rosiglitazone or is a class effect. We describe 2 patients who have previously shown paradoxical decreases in HDL-C during treatment with rosiglitazone and who have subsequently been treated with pioglitazone another member of the same class. In both patients the dramatic decreases in HDL-C observed with rosiglitazone was not observed with pioglitazone. This suggests that the paradoxical decrease in HDL-C seen with rosiglitazone is unlikely to be a class effect.

Funding: None

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SUBCLINICAL ATHEROSCLEROSIS IN HIV-INFECTED CHILDREN – PERI STUDY

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Methods: It is an observational, case-control study, comparing 37 HIV-infected children with high carotid intima-media thickness (cIMT) compared to 46 with normal cIMT from an outpatient ambulatory of infectology. A questionnaire, research of clinical records, physical examination, anthropometry, electrocardiography, chest X-ray, echocardiography and carotid ultrasound examination were performed and fasting blood sample were collected. **Results:** After bivariate analysis, higher cIMT were associated with lipodystrophy signals (bottom atrophy and trunk-limb ratio upper quartile), drug exposition (efavirenz and stavudine use), the AIDS control (CD8+ lymphocyte upper quartile and low levels of CD4+ lymphocyte), and lipid abnormalities (LDL-cholesterol and total cholesterol high levels). After multivariate analysis, high cIMT were positively associated with stavudine use [OR: 18.9(2.5-145.6), p = 0.005], high left atrium/aorta relation [OR: 15.6(1.6-154.6), p = 0.019], high suprailiac skinfold thickness [OR: 7.9(1.5-41.9), 0.015], tachypnea [5.9(1.2-29.3), p = 0.031], high CD8+ T cells count [OR: 5.7(1.1-28.3), p = 0.033], low CD4+ T cells count [OR: 5.5(1.2-24.6), p = 0.025], and negatively associated with total cholesterol [OR: 0.22(0.1-0.8), 0.1-0.8), p = 0.025] and zenith of CD8+ T cells [OR: 0.1(0.1-0.5), p = 0.025]. **Conclusions:** We found associations among cIMT and drugs, AIDS control and lipid profile. Further studies are required to elucidate the relationship among atherosclerosis, drug exposition and lipid abnormalities in HIV-infected children.

Funding: UNESC-WHO

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IMPACT OF FAMILY HISTORY OF STROKE AND MILD HYPERHOMOCYSTEINAEMIA ON THE HDL-Ch/ApoAI RATIO IN HEALTHY SUBJECTS

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The purpose of the study is to investigate the relationship between homocysteine, selected lipid parameters and inflammation markers in young healthy persons with a positive family history of stroke as compared to persons with a negative family history of stroke. The investigations were performed in 143 young healthy persons with a positive family history of stroke (71 women and 72 men), as well as in 201 young healthy persons with a negative family history of stroke (111 women and 90 men) who constituted the control group. All persons were aged 18-55 years. Systolic and diastolic blood pressure and body mass (BMI) were measured in all the subjects. The levels of homocysteine (HCY), folic acid (FA), C-reactive protein, fibrinogen, TG, Ch, HDL-Ch LDL-Ch, PL, ApoAI and ApoB, LP(a), glucose, uric acid, paraoxonase (PON1), aminotransferases: AlAT and AspAT were measured. The statistical analysis of results was conducted in subgroups of women and men, stratified by the family history of stroke (positive vs negative) and HCY concentrations by quartiles. The multiple regression analysis of the dependent variable showed that in the group with the positive history, HCY in women was described by BMI, TG and HDL-Ch and in men by TG, ApoAI, TG/HDL-Ch, folic acid and AlAT and AspAT. The discriminative function analysis showed that the HDL-Ch/ApoAI ratio is the discriminating parameter for the negative or positive history of stroke in the group of women and men.

Funding: Pomeranian Medical University

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EFFICACY AND SAFETY OF LONG-TERM ATORVASTATIN TREATMENT IN PATIENTS WITH ISCHEMIC STROKE WITH PAST HISTORY OF MYOCARDIAL INFARCTION

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The aim: of the study was to assess the effects of long- term atorvastatin treatment in patients (pts) with ischemic stroke with past history of myocardial infarction. **Methods:** This was a randomized, double blind, placebo controlled trial with atorvastatin (Liprimar, Pfizer, USA) (20 mg) administered once daily. 60 patients (aged 40- 79 years) were randomly assigned to one two treatment group, receiving either atorvastatin (n=30) or placebo (n=30). Duration of the study was 3 years. **Results:** At the end of the study there were significant and sustained decreases in plasma level of total(31%), LDL cholesterol(41.6%), triglycerides(25.09%), fibrinogen(30.12%) and Hs-CRP levels(24.1%) and increases in HDL cholesterol level(14.39%) in the atorvastatin group compared with the placebo group. In the atorvastatin group, intima- media thickness and occlusion degree of carotid arteries decreased by 24.1 and 46.1% respectively. Our study found that atorvastatin- treated pts actually showed improvement in cognition compared to placebo-treated pts. Fatal and non fatal myocardial infarction, fatal CHD was significantly lower by 38 % in the atorvastatin group than in the placebo group. There was also significant reduction in total cardiovascular events including revascularization procedures (24%), total coronary events (31%), fatal and non fatal stroke (29%). The number of serious adverse events did not differ between pts assigned atorvastatin or placebo. **Conclusion:** Our result suggests that atorvastatin should be prescribed for all ischemic stroke pts with a past history of myocardial infarction.

Funding: None

Poster Session 3 "DRUG EFFICACY AND SAFETY" - GROUP A

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LIPITOR'S EFFECT IN ALZHEIMER'S DEMENTIA (LEAD-E) STUDY: DESIGN AND BASELINE DATA

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Objectives: The LEADe study tests the hypothesis that adding a statin (atorvastatin 80 mg) to a cholinesterase inhibitor (donepezil-DPZ 10 mg) benefits cognition and global functioning more than DPZ alone in patients with mild-moderate Alzheimer's disease (AD). **Methods:** This is an international, multicenter (93 sites), double-blind, randomized (1:1), parallel-group study of patients with mild-moderate AD (MMSE 13-25). Inclusion criteria were: men/women 50-90 yrs, stable DPZ 10mg ≥3 months, LDL-C 95-195 mg/dL. Co-primary endpoints are changes in AD Assessment Scale-Cognitive subscale (ADAS-Cog) and AD Cooperative Study-Clinical Global Impression of Change (ADCS-CGIC) Scale scores. **Results:** Enrollment of 641 subjects is complete. The baseline mean data are: age 74±8 years, 52% women, MMSE 22±3, ADAS-Cog 32±11, Alzheimer disease Functional Assessment (ADFACS) 13±9, Neuropsychiatric Inventory (NPI) 10±11, Clinical Dementia Rating-Sum of boxes (CDR-SB) 6±3. Mean prior DPZ treatment was 409±407 days. Mean total cholesterol is 227±32 mg/dL, LDL-C 142±26 mg/dL, triglycerides 134±66 mg/dL, and HDL-C 64±18 mg/dL. **Conclusions:** LEADe will be completed by the end of 2007 and is expected to provide a more definitive evaluation of the potential for statins in the treatment of people with AD.

Funding: Pfizer Inc.

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COMPARISON OF EFFECTS OF PITAVASTATIN AND ATORVASTATIN ON PLASMA COENZYME Q10 LEVELS IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Background: Statins reduce serum LDL-C by inhibiting the synthesis of mevalonate, an intermediate in the cholesterol biosynthetic pathway. Under these conditions, production of coenzyme Q10 (CoQ10), which is also down stream of mevalonate, could be reduced, contributing to the side effects. **Subjects** & **Methods**: 19 patients with heterozygous familial hypercholesterolemia (7 male, mean age=58) were divided into 2 groups randomly, and took pitavastatin (4mg) or atorvastatin (20mg) for 8 weeks followed by 4 weeks of washout, and then took the other statin for another 8 weeks. **Results**: Overall, the changes of the LDL-C, HDL-C, and triglycerides before and after the treatments did not significantly differ between pitavastatin and atorvastatin (-42% vs. -41%, +12% vs. +11%, and -19% vs. -26%, respectively). No increases in AST, ALT, gamma-GTP, or creatinine kinase were seen. Atorvastatin significantly decreased plasma levels of CoQ10 (-26%, $p<0.001$), whereas pitavastatin did not (-8%). The changes of plasma CoQ10 before and after treatments were significantly different between these 2 statins ($p<0.03$). In atorvastatin treatment, the % changes of CoQ10 tended to have negative correlation with reduction rate of LDL-C ($r2=0.17$, $p=0.08$), which did not apply to pitavastatin treatment. **Conclusion**: The effects on lipids and short-term safety of both statin were similar. However, unlike atorvastatin, pitavastatin did not produce reductions in plasma CoQ10, which could be associated with long-term safety.

Funding: None

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STATIN-INDUCED MYOTOXICITY: LACTONE FORMS MORE POTENT THAN ACID FORMS IN HUMAN SKELETAL MUSCLE CELLS *IN VITRO*

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Objective: HMG-CoA reductase inhibitors (statins) are generally well tolerated, although a significant part of the patients have muscle-related adverse effects. The cause of the myotoxicity has not been fully elucidated, but clinical data indicate that elevated levels of the lactone form are present in patients with myopathy. The present study was designed to investigate the potency of acid and lactone forms of statins to induce myotoxicity *in vitro*. **Methods:** Primary cultures of human skeletal muscle cells were incubated for 24-72 hours with increasing concentrations of lactone or acid forms of atorvastatin, simvastatin, fluvastatin and pravastatin. Following incubation living myotubes were quantified by fluorescence. **Results:** The lactone forms were significantly more potent to induce myotoxicity, as compared to their acid forms, in human skeletal muscle cells. A 14-fold, 26-fold, 23-fold and 37-fold differences were seen between lactone and acid forms of atorvastatin, fluvastatin, pravastatin and simvastatin, respectively. Furthermore, differences between the myotoxic potential of the four statins were found; in ranked order simvastatin~fluvastatin>atorvastatin>pravastatin. **Conclusions:** For the first time the present study shows a significant difference between the lactone and acid forms of statins to induce myotoxicity in human skeletal muscle cells *in vitro*. These results clearly indicate the need to differentiate between acid and lactone forms in future elucidation of the mechanism of statin induced myotoxicity.

Funding: The study was supported by a grant from the Norwegian Pharmaceutical Society

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RELATIONSHIP BETWEEN BLOOD MAGNESIUM CONCENTRATION AND CLINICAL MARKERS OF MUSCULAR TOLERANCE TO STATIN THERAPY

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Statins are the most prescribed class of cholesterol-lowering drugs worldwide. Although well tolerated, they can be associated with a large spectrum of muscular side effects from unspecific complaint of myalgya to life threatening rhabdomyolysis. Magnesium (Mg2+) is an intracellular cation involved in energy metabolism and required for normal muscle function. Recent data suggest that Mg2+ might affect the lipid lowering response to statins. **Objective:** This study examined the association between serum Mg2+ levels and clinical biomarkers of muscular side effects to statin therapy. **Methods:** Creatine kinase (CK) and Mg2+ concentration were measured in a sample of 1,071 French-Canadians (698 men; 373 women) under statin treatment at the same lipid clinic. Multivariate analyses were performed to assess the association between Mg2+ level and markers of statin muscular tolerance (muscular complaint, serum CK level). Multivariate models included the effect of the type of statin received, daily dosage, concomitant drugs, age and gender. **Results:** Mg2+ concentration >0.9 mmol/L was associated with 1) an increased risk (odds ratio, OR) of elevated CK levels: CK>300 U/L: OR=1.90 [1.13-3.20], p=0.015; CK>600 U/L: OR=2.56 [1.01-6.47], p=0.047; CK>750 U/L: OR=5.17 [1.80-14.82], p=0.002 and 2) an increased risk of self-reported muscular complaints (OR=1.47 [0.97-2.21], p=0.067) following statin therapy. **Conclusions:** These results suggest an association between Mg2+ levels and the expression of muscular side effects among statin users. Further studies are needed to elucidate the underlying metabolic muscle impact of Mg2+.

Funding: This project was supported by the ECOGENE-21 project, CIHR Team #CTP-82941

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MARKERS OF HYDRATATION, ENERGETIC BALANCE AND THERMOREGULATION TO ASSESS THE RISK OF MYOTOXICTITY TO LIPID LOWERING DRUGS

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Although statins are well tolerated, they can be associated with muscular side effects, ranging from myalgia to rhabdomyolysis. These muscular symptoms are also observed in individuals exposed to high temperatures, dehydration or intense physical exercise. Athletes use oral glycerol supplements to improve body hydration and physical endurance. It raises the interest of studying biomarkers of glycerol metabolism in relation to statin muscular side effects. **Objective:** This study examined the relationship between fasting glycerol levels and clinical biomarkers of myotoxicity under statin therapy. **Methods:** Plasma creatine kinase (CK) and glycerol levels were measured in a sample of 1,512 French-Canadians under statin treatment. Self-reported information on muscular pain was collected. ANOVA were performed to assess the association between glycerol levels and markers of statin muscular tolerance. **Results:** A significant inverse relationship was observed between plasma glycerol, the occurrence of myalgia and post-treatment CK levels ($p<0.001$). This inverse association was not observed among those showing extreme plasma glycerol values (>0.2 mmol/L, 20 fold > normal) where a positive association of glycerol levels with myalgia and CK levels was observed ($p<0.05$), suggesting that glycerol resistance could be related to muscular statin intolerance. **Conclusion:** These results suggest an association between statin muscular tolerance and glycerol metabolism correlates, and raise the interest of studying determinants of muscle hydration or energetic balance to better understand or prevent statin side effects.

Funding: This project was supported by ECOGENE-21 project (CIHR TEAM grant CTP-82941)

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NEED TO OPTIMIZE HYPERCHOLESTEROLEMIA TREATMENT: RESULTS OF THE BELGIAN CEPHEUS SURVEY

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Objectives: Objectives of the Belgian Cepheus survey were to establish the proportion of patients on lipid-lowering drugs (LLD) reaching the LDL-C goals according to the Third Joint European Task force (TJETF) and to identify possible patient/physician characteristics associated with failure to be at the TJETF guideline LDL-C target. **Methods:** Cepheus Belgium was part of a European multi-centre cross sectional survey performed in 8 countries, including patients on LLD for at least 3 months, without a change of dose for a min. of 6 weeks. Fasting lipids were determined in a central lab. Physicians and patients filled in a questionnaire covering various aspects of hypercholesterolemia. **Results:** 445 Belgian physicians (94% GPs) recruited 5909 patients, treated with LLD. Mean age was 64 yrs (54% male). 92% had statin mono-therapy. 58% of the patients were at TJETF target for LDL-C. Only 28% of patients with coronary heart disease + diabetes reached the newest LDL<70mg/dl target (post-hoc analysis). The highest rate of controlled patients (68%) was reached in patients receiving rosuvastatin. Important patient characteristics of attaining LDL-C goal were: highly compliant patients (OR: 1.48; [1.32;1.67]) and cholesterol review every 3 months (OR: 1.32; [1.00;1.73]) Important negative predictors were: patient unawareness of cholesterol targets (OR: 0.76; [0.62;0.92]) and several drug changes in the past (OR: 0.69; [0.57; 0.85]). **Conclusion:** More than 40% of the patients using LLD are not on their target goal for LDL-C. Regular follow-up and measures to increase compliance may have an impact on reaching LDL cholesterol targets.

Funding: AstraZeneca

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THE EFFICACY OF SIMVASTATIN OR EZETIMIBE ON TISSUE FACTOR, VON WILLEBRAND AND C-REACTIVE PROTEIN

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Objective: The aim of this study was to determine and compare the pleiotropic effects of simvastatin (S) and ezetimibe (E). **Methods:** 44 patients with low-density lipoprotein cholesterol (LDL-C) > 130 mg/dl or LDL-C >100 mg/dl in pts with coronary artery disease or its equivalent, were treated with S (n=21) 10 mg daily or E (n=23) 10 mg daily. In both samples we measured the levels of total cholesterol (TC), high-density cholesterol (HDL-C), LDL-C, apolipoprotein (apo) A, apo B, lipoprotein (a) [lp(a)], homocysteine, tissue factor (TF), von Willebrand (vW) and C-reactive protein (CRP). **Results:** S and E decreased TC (262 mg/dl to 189 mg/dl, p<0.001 and 268 mg/dl to 220 mg/dl, p=0.001, respectively), LDL-C (177 mg/dl to 114 mg/ dl, p<0.001 and 196 mg/dl to 146 mg/dl, p<0.001, respectively) and CRP levels (1.145 mg/dl to 0.3 mg/dl mg/dl, p=0.001 and 2.8 mg/dl to 0.8 mg/dl, p=0.005, respectively). Also, S reduced apoB (125 mg/dl to 93 mg/dl, p<0.001). **Conclusions:** Both drugs improved the lipid profile and CRP levels. However no influence on TF and vW was found.

Funding: This study was conducted independently; no company or institution supported it financially

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CRP AND LDL-C RESPONSE TO EZETIMIBE: MONOTHERAPY AND ADD-ON TO BASELINE STATIN

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Objectives: To examine effects of ezetimibe (EZE) monotherapy or add-on to statin therapy on CRP and LDL-C. **Methods:**Results of randomized, placebo (PBO)-controlled trials of EZE 10 mg in hypercholesterolemic adults were pooled in 2 analyses: six 12-week monotherapy trials (n=1372); seven 6-8 week add-on to baseline statin therapy trials (n=3899). In patients with CRP≤10mg/L, ANOVA was used to evaluate treatment effects on mean % changes from baseline in CRP and LDL-C, and effects of subgroups (age, gender, race, BMI, diabetes, metabolic syndrome, CHD, baseline CRP or lipids, and statin potency). Spearman correlation coefficients between CRP and LDL-C were examined. **Results:**For both analyses, baseline characteristics were similar between groups. CRP reduction by EZE monotherapy was numerically greater than PBO (treatment difference 6.0%, P=0.094), and by EZE added to baseline statin therapy was significantly greater than PBO (treatment difference 10.4%, P<0.001). In both analyses, LDL-C reduction with EZE was significantly greater than PBO (P<0.001), treatment effects were consistent across subgroups, and correlation among baselines or % changes from baseline of CRP and LDL-C ranged from -0.007 to 0.126. **Conclusions:**Though the effect of EZE monotherapy on CRP is limited, when EZE is added to baseline statin treatment, CRP reduction is enhanced. Correlations between CRP and LDL-C are weak or non-significant. Effects of EZE on CRP were consistent across patient subgroups. The clinical relevance of changes in CRP to atherothrombotic risk still needs further investigation.

Funding: Merck & Co., Inc.

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INHIBITION OF INTESTINAL CHOLESTEROL ABSORPTION AND ENDOGENOUS CHOLESTEROL PRODUCTION BY EZETIMIBE/SIMVASTATIN IN MAN

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Objectives: To evaluate changes in cholesterol metabolism that accompany the large reductions in LDL cholesterol (LDL-C) seen with coadministration of an inhibitor of cholesterol production with an inhibitor of cholesterol absorption. **Methods:** This was a randomized, double blind, placebo controlled, 4-period crossover study to evaluate the effects of coadministering ezetimibe 10 mg (EZE) with simvastatin 20 mg (SIMVA) on cholesterol absorption and production relative to either drug alone or placebo in 41 patients. Each treatment period lasted 7 weeks. **Results:** Fractional cholesterol absorption was decreased by EZE and by EZE coadministered with SIMVA (EZE/SIMVA) by 65% and 59%, respectively ($p<0.001$ for both relative to placebo). SIMVA did not significantly affect cholesterol absorption. Fecal sterol excretion (corrected for dietary cholesterol) increased with EZE and EZE/SIMVA, by 109% and 79%, respectively ($p<0.001$). Cholesterol production assessed by plasma lathosterol concentration increased with EZE by 46%, but decreased with EZE/SIMVA by 56% and SIMVA alone by 65% (all $p<0.001$). LDL-C decreased by EZE, SIMVA and EZE/SIMVA, by 20%, 38% and 55%, respectively. Fractional absorption of sitosterol and campesterol decreased by EZE and EZE/SIMVA by an average of 72 to 66%, respectively ($p<0.001$). The coadministered therapy was well tolerated. **Conclusions:** Coadministered EZE and SIMVA yielded nearly additive reductions in LDL-C, in the face of decreases in net cholesterol absorption, and plasma cholesterol precursors, and increased fecal sterols.

Funding: Merck Schering Plough Joint Venture, North Wales, PA, USA

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CLINICAL STUDY ON THE EFFECT OF SIMVASTATIN ON PARAOXONASE ACTIVITY

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Introduction: The objective of this study was to determine changes in PON1 activity during treatment with simvastatin (CAS 79902-63-9) in patients with type IIa and/or IIb hyperlipoproteinemia. **Patients and Methods:** Thirty-two patients with type IIa (n=15) or IIb (n=17) hyperlipoproteinemia, whose plasma cholesterol level was ≥4.2 mmol/L, were included in the study. Laboratory parameters, including PON1 activity, were determined at baseline, 3 and 6 months after the beginning of therapy. **Results:** Statistically significant effects of simvastatin treatment on total cholesterol, HDL_2, LDL fraction and PON1 activity in all patients ($p<0.05$) were found. PON1 activity was statistically significantly increased until the third month of treatment with simvastatin. **Conclusion:** A statistically significant increase in PON1 activity observed, without significant changes in HDL cholesterol concentration, argues for an independent effect of treatment on the increase in the enzyme activity. No statistically significant correlation either between changes in HDL concentration and PON1 activity, or their first differences was observed. This indicates that the increase in PON1 activity is probably caused by a mechanism independent of apoAI-containing lipoprotein concentration.

Funding: None

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EFFECTS OF HIGH DOSE ATORVASTATIN ON CORONARY FLOW VELOCITY RESERVE IN PATIENTS AFFECTING ACUTE CORONARY SYNDROME

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Background: Acute coronary syndrome (ACS), accounts for 1-2 million hospital admission annually in U.S. Patients experience the highest rate of death and recurrent ischemic events during an acute coronary syndrome, but is not known whether early initiation of treatment with statin can reduce the occurrence of these events. Coronary flow velocity reserve (CFVR) has been considered an important diagnostic index of the functional significance of coronary artery disease (CAD), early in this setting of these patients. **Methods:** Thirty consecutive patients (group 1) with ACS, were assigned to receive atorvastatin (A) 80 mg/day for 12 weeks. Fourth pts were not taking any lipid-lowering drugs, served as control group (group 2). We studied the CFVR by TTDE defined as the ratio of myocardial blood flow after dipyridamole infusion to that at baseline. **Results:** CFVR was similar in the A treated group and control and did not reach statistical significance at baseline (p=NS). A significant increase in flow was obtained in A group before and after atorvastatin treatment ($p<0.01$) and in comparison to the control group by dipyridamole infusion ($p< 0.01$). Moreover, the atorvastatin group had a lower risk of symptomatic ischemia. **Conclusion:** Our data show that high dose atorvastatin is able to produce a beneficial effect for patients with acute coronary syndrome, reduces recurrent ischemic events in the first 12 weeks, mostly recurrent symptomatic ischemia requiring rehospitalization.

Funding: There is not funding in the work

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LOW-DOSE PIOGLITAZONE INCREASES NOT ONLY SERUM ADIPONECTIN LEVELS BUT ALSO HIGH-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS IN PATIENTS WITH HYPOADIPONECTINEMIA

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Objective: Hypoadiponectinemia is an emerging risk factor for coronary artery disease. This study assessed the effect of low-dose pioglitazone on serum adiponectin levels and other risk factors in Japanese patiens(pts) with hypoadiponectinemia. **Methods:** We randomly recruited 19 pts(14 men and 5 women) with hypoadiponectinemia(<4μg/ml in men and <5μg/ml in women). All pts had either impaired glucose tolerance or diabetes mellitus. Ten pts also had coronary artery disease. Patients received low-dose pioglitazone (15mg/day). We prospectively assessed at 1 and 3 months changes in adiponectin, hemoglobin A1c (HbA1c), high-sensitivity C-reactive protein (hs-CRP), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and lipoprotein(a) . **Results:** Serum adiponecin levels were significantly increased at one (3.37±0.74μg/ml to 7.30±3.06μg/ml, $p<0.001$) and three months (7.99±3.17μg/ml, $p<0.001$) respectively. Serum adiponectin levels were elevated in all pts after treatment. HbA1c levels at three months were significantly decreased (7.06±1.32% to 6.73±1.01%, $p<0.05$). No significant changes were found in the levels of serum hs-CRP, TG, LDL-C, lipoprotein(a) . HDL-C levels were also significantly ($p<0.05$) increased after treatment. **Conclusions:** The administration of low-dose pioglitazone effectively increased not only serum adiponectin levels but also HDL-C levels in pts with hypoadiponectinemia. Pioglitazone may represent a viable treatment option aimed at improving hypoadiponectinemia and low HDL-C in diabetic and other pts who are at high risk for coronary artery disease.

Funding: None

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EFFICACY AND SAFETY OF PITAVASTATIN COMPARED TO PRAVASTATIN IN ELDERLY PATIENTS WITH PRIMARY HYPERCHOLESTEROLEMIA OR COMBINED DYSLIPIDEMIA

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Pitavastatin is a new, potent, fully synthetic, non-CYP450-metabolized, HMG-CoA reductase inhibitor discovered and developed in Japan. It is in development in the EU and US following registration in Japan and Korea. This was a randomized, multicenter, double-blind, parallel group, active-controlled study with 12 weeks of treatment. Elderly patients (≥ 65) with primary hypercholesterolemia or combined dyslipidemia, whose LDL-C during a 6 - 8 week washout/dietary lead in was between 130 and 220 mg/dL, were randomized to 6 treatments in a ratio of 2: 2: 2: 1: 1: 1:- pitavastatin 1 mg QD, pitavastatin 2 mg QD, pitavastatin 4 mg QD, pravastatin 10 mg QD, pravastatin 20 mg QD or pravastatin 40 mg QD. 962 patients were randomized; baseline demographics were similar across groups. Mean baseline LDL-C was between 162.8 and 166.6 mg/dL; 1, 2 and 4 mg of pitavastatin and 10, 20 and 40 mg of pravastatin all lowered LDL-C significantly, by -31.4%, -39.0%, -44.3%, -22.4%, -28.8%, and -34.0%, respectively. Pitavastatin was statistically superior to pravastatin in all pre-planned dose comparisons (pitavastatin 1 mg vs. pravastatin 10 mg, pitavastatin 2 mg vs. pravastatin 20 mg and pitavastatin 4 mg vs. pravastatin 40 mg) ($P<0.001$). 83.1%, 88.8% and 91.0% of patients on pitavastatin 1, 2 and 4 mg achieved their LDL-C target (NCEP criteria), in comparison with 65.0%, 81.3% and 88.2% on pravastatin 10, 20 and 40 mg treatment. The adverse event profiles of pitavastatin and pravastatin were similar and treatment-emergent AEs did not increase with dose. In this study in elderly patients with hypercholesterolemia and mixed dyslipidemia, pitavastatin was as well tolerated as pravastatin at EU recommended doses but significantly more efficacious at reducing LDL-C.

Funding: Study sponsored by Kowa

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EFFICACY AND SAFETY OF PITAVASTATIN COMPARED TO SIMVASTATIN IN PATIENTS WITH PRIMARY HYPERCHOLESTEROLEMIA OR COMBINED DYSLIPIDEMIA

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Pitavastatin is a new, potent, fully synthetic, non-CYP450-metabolized, HMG-CoA reductase inhibitor discovered and developed in Japan. It is in development in the EU and US following registrations in Japan and Korea. This was a randomized, multicenter, double-blind, parallel group, active-controlled study with a 12-week treatment period. Patients with primary hypercholesterolemia or combined dyslipidemia, whose LDL-C during a 6 to 8 week washout/dietary lead in was between 160 and 220 mg/dL, were randomized to 4 treatment groups in a ratio of 3: 3: 1: 1:- pitavastatin 2 mg QD, pitavastatin 4 mg QD, simvastatin 20 mg QD or simvastatin 40 mg QD. 857 patients were randomized and baseline demographics were similar across treatment groups. Mean baseline LDL-C values were between 183.6 and 184.1 mg/dL: 2 and 4 mg of pitavastatin and 20 and 40 mg of simvastatin all lowered LDL-C significantly by -39.0%, -44.0%, -35.0%, and -42.8%, respectively. Pitavastatin, in the pre-planned statistical comparisons, was statistically superior to simvastatin in the lower dose comparison (pitavastatin 2 mg vs. simvastatin 20 mg) ($P=0.014$), and non- inferior in the higher dose comparison (pitavastatin 4 mg vs. simvastatin 40 mg). 70.0% and 79.3% of patients on pitavastatin 2 and 4 mg achieved their LDL-C target (NCEP criteria), respectively, in comparison with 64.5% and 78.2% on simvastatin 20 and 40 mg treatment. The adverse event profiles of pitavastatin and simvastatin were similar and there was no increase in the rate of treatment-emergent AEs with dose with either drug. In this study in patients with hypercholesterolemia and mixed dyslipidemia, pitavastatin 2 mg QD and 4 mg QD was as well tolerated and efficacious as simvastatin 20 mg QD and 40 mg QD at reducing LDL-C.

Funding: Study sponsored by Kowa

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ASSOCIATION BETWEEN BLOOD MAGNESIUM CONCENTRATION AND THE LIPID LOWERING EFFICACY OF STATINS

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Magnesium (Mg2+) is required for cell metabolism and acts as a cofactor for many enzymes. HMG-CoA reductase is the rate-limiting enzyme of cholesterol production within the cell. Statins and Mg2+ exhibit HMG-coA reductase inhibition properties. Through HMG-coA reductase inhibition and other mechanisms, statins reduce efficiently plasma LDL-cholesterol concentration, the atherogenicity of lipoproteins and prevent coronary artery disease, even in individuals with extreme phenotypes such as Familial Hypercholesterolemia (FH). However, important inter-individual variations are observed in the lipid-lowering reponse to statins in FH. **Objective:** The aim of this study was to evaluate the relationship between baseline Mg2+ concentration and the response to statin therapy in a sample of 119 FH subjects. **Methods:** Serum Mg2+ was measured before a lipid lowering therapy with a statin was prescribed. The effect of the lipid lowering treatment on the lipid profile was then compared after stratification of the patients in quartiles, according to baseline Mg2+. Patients with hypermagnesaemia due to renal failure or hypomagnesaemia due to alcohol abuse were excluded. **Results:** After adjustment for the age, gender, BMI, type and daily dosage of the statin received as well as pre-treatment cholesterol concentrations, FH patients in the fourth quartile of Mg2+ (>0,90 mmol/L) were significantly less responsive to the lipid lowering treatment (odds ratio=9.428, p=0.007). **Conclusion:** These results suggest a significant association between baseline blood concentration of Mg2+ and the response to the lipid lowering effect of statins.

Funding: Supported by the ECOGENE-21 project (CIHR TEAM grant # CTP-82941) and Astra Zeneca Canada

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THE INCREASED ABILITY OF FIBRATES TO IMPROVE THE LIPID PROFILE AMONG CARRIERS OF THE APOLIPOPROTEIN E2 ALLELE IS NOT RELATED TO THE ACCUMULATION OF IDL

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Apolipoprotein (apo) E2 carriers usually respond better to lipid lowering treatments than non carriers. Type III dysbetalipoproteinemia is a rare, extreme phenotype resulting from IDL accumulation; it correlates with the reduced capacity of apoE to interact with cell receptors for internalisation (apoE resistance). Type III is characterized by increased triglyceride (TG) and VLDL-cholesterol/TG ratio, beta VLDL, palmar xanthomas and atherosclerosis. ApoE resistance in type III is frequently, but not always, associated with apoE2. It is not known if the association of apoE2 with drug response is associated with apoE resistance (as in type III) or other apoE characteristics. **Objective:** This study aimed to examine the variations in plasma lipid following treatment with fibrates in type III and non-type III patients with various apoE genotypes. **Methods:** 108 hyperTG subjects (54 type III; 54 non-type III) were paired according to age, gender, baseline TG and BMI. Pre-post treatment lipid levels were compared with ANOVA. **Results:** Following treatment, type III presented a more significant improvement of most lipid-lipoprotein fractions than non-type III (p=0.001). The better response to fibrates in type III was related to the apoE2 allele and not to other type III characteristics. Type III without the apoE2 allele (n=11) showed a response similar to non-type III, whereas non-type III with the E2 allele responded better than non-E2. **Conclusion:** This study suggests that the effect of the apoE2 on the response to fibrates is not related to apoE resistance but to other characteristics of apoE.

Funding: Supported by ECOGENE-21 project, CIHR Team #CTP82941

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BEZAFIBRATE FUNCTIONS TO LOWER SERUM CHOLESTEROL LEVELS WITHOUT ACTIVATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS IN MICE

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Bezafibrate, a pan-activator for peroxisome proliferator-activated receptors (PPARs), has a hypocholesterolemic potential, but the contribution of PPARα activation to its effect remains unclear. In addition, there is a marked discrepancy in bezafibrate dosage between clinical use (<10 mg/kg/day) and conventional rodent experiments (>100 mg/kg/day). To investigate the association between bezafibrate-induced cholesterol-lowering effect and PPARα activation, wild-type and PPARα-null mice were treated with bezafibrate at low (10 mg/kg/day) or high dose (100 mg/kg/day) for 10 days. High-dose bezafibrate treatment markedly lowered cholesterol levels in liver but raised in serum, which occurred in PPARα-dependent manner. The hepatic cholesterol reduction on high-dose treatment, which was mediated by PPARα activation, seemed to be associated with a marked increase in expression of genes encoding transporters involved in bile secretion, such as Mdr2 and ABCG5/8. In contrast, low-dose bezafibrate treatment significantly reduced serum and liver cholesterol concentrations in both genotypes, which would be related to suppression of SREBP2-mediated cholesterogenesis and stimulation of cholesterol catabolism by enhancing expression of CYP7B1 gene. Interestingly, PPAR target gene analysis revealed that low-dose bezafibrate treatment did not activate all PPAR subtypes in mouse liver. Thus, our results demonstrated that the pharmacological action of bezafibrate on cholesterol metabolism was quite different in its dosages, and that bezafibrate-induced cholesterol-lowering effect in mice was not associated with PPAR activation.

Funding: None

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ANTI-ATHEROSCLEROTIC EFFECTS OF KY-382, A NOVEL ACAT INHIBITOR, IN COMPARISON WITH PACTIMIBE

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Objective: ACAT inhibitors have been expected to prevent and retard atherosclerosis. However, Pactimibe (Pac) was reported to fail to reduce plaque volume in CAD patients. In the present study, the anti-atherosclerotic effects of KY-382 (KY), a newly synthesized ACAT inhibitor, were examined in comparison with Pac. **Methods:** Physicochemical properties and biological activities of KY were determined and compared with Pac. KY was administered as a dietary admixture to KHC rabbits for 5 months, and the anti-atherosclerotic effects were examined. **Results:** KY is less lipophilic than Pac (logP 1.1 vs. 5.6), and thus has a lower plasma protein-binding ratio (60% vs. 98%). KY inhibited foam cell formation induced by acetylated LDL, glycated LDL, and beta-VLDL more strongly than Pac. KY was not accumulated in liver and was excreted in urine, while Pac was accumulated in liver and excreted in bile. In KHC rabbits, arterial concentration of KY after oral administration was higher than that of Pac. KY slightly reduced plasma LDL levels and the capability of LDL to form foam cells in KHC rabbits. KY had no effects on the atherosclerotic surface area, but significantly reduced arterial cholesterol ester. In the aorta from KY-treated rabbits, numbers of foamy macrophages and intracellular lipid droplets were reduced, smooth muscle cells were increased, and fibrosis was enhanced on microscopic observation. The anti-atherosclerotic effect of KY was greater than that of Pac on the basis of their plasma concentrations. **Conclusion:** It is suggested that KY is a safer and more potent anti-atherosclerotic agent than Pac.

Funding: None

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MEASURING NIACIN-INDUCED FLUSHING USING THE FLUSHING SYMPTOM QUESTIONNAIRE©

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We evaluated the utility of the Flushing Symptom Questionnaire© (FSQ), a quantitative tool to assess pt-reported flushing endpoints, to characterize the severity and bother of niacin-induced flushing (NIF) during initiation and persistent use of extended-release niacin (ERN). After a 1-wk placebo (P) run-in, pts were randomized to 1 of 4 groups (sequences of P and ERN given as NIASPAN® 1 g and 2 g). Pts reported daily flushing severity using the 11-question FSQ via a hand-held e-diary. The primary endpoint was based on the responses to one question in the FSQ, the Global Flushing Severity Score (GFSS), reported on a 0-10 scale (none=0, mild=1-3, moderate=4-6, severe=7-9, and extreme=10) to assess NIF in the initiation (Wk 1) and maintenance (Wks 2 to 8) phases of treatment. 175 pts were enrolled (P=33, ERN=142 at Wk 1). GFSS test-retest reliability and reproducibility coefficients were above 0.75. Construct validity of the GFSS was supported by moderate to strong correlations ($r>0.5$) with other FSQ scores. The GFSS discriminated between groups and demonstrated expected relationships with known groups: for all periods and doses, pts taking ERN reported greater flushing severity than those taking P. Flushing severity with ERN was greatest during wk 1 and remained consistently greater than P for the duration of the trial. These results support the measurement properties and validity of the FSQ; specifically, the GFSS was a sensitive and responsive measure that quantitatively characterized the severity of NIF. The FSQ will aid in the objective comparison of novel strategies intended to improve tolerability and adherence to niacin, an agent proven to reduce atherosclerotic cardiovascular risk.

Funding: Merck

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QUESTIONAIRE OF PERCEPTION OVER CLINICAL APPROACHES IN DISLIPEMIA. CHOLESTEROL SPAIN STUDY

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Background: A national survey was made, to know the performance rules in the handling of patient with dislipemia, to compare the opinion of the doctors that work at Primary and Specialized Attention, and to define treatment tendencies or opinion on the estatinas use. **Patients and methods:** A survey was designed where data on the treated population and the opinion about the control of the lipidic parameters , tolerance and risk of interactions medicamentosas of six estatinas, were completed. **Results:** There participated a total of 1998 doctors of 15 independent communities. 68,8% of the interviewed doctors works in Primary Attention , and 30,2% in centers of specialties or in hospitals. 91% follow the international consents on the control of the dislipemias. The therapeutic objective parameter to treat the used dislipemias is the LDL-cholesterol (83%), followed by the total cholesterol (62%), HDL-cholesterol (56%) and triglicéridos (51%). **Conclusions:** The performance approaches before the dislipemias are goodable. Important differences don´t exist in the opinions and performance between doctors of primary attention and specialists in the clinical and therapeutic approaches before the dislipemias.

Funding: Murcia Primary Health Care Management Unit

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FIBRATES POSITIVELY AND NEGATIVELY REGULATE THE EXPRESSION OF SEVERAL NUTRIENT/DRUG TRANSPORTERS IN MOUSE INTESTINE

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It has been well established that fibrates are agonists for PPARα and exert their hypolipidemic effects mainly in the liver. Recent our work, however, demonstrated that PPARα also plays a vital role in the intestine to induce drug metabolizing enzymes (Motojima & Hirai (2006) FEBS J. 273, 292). In this study, we carried out a systematic analysis to examine the effects of fibrates on the expression levels of all the nutrient/drug plasma membrane transporters in the mouse small intestine. We first identified the transporter mRNAs that were induced or repressed by feeding the diets containing two independent fibrates by a genome-wide microarray method using RNA isolated from the intestine. The changes were confirmed by real-time PCR using RNA isolated from the intestine and also from the liver of wild-type and PPARα-null mice fed test diets. Expressions of several nutrient/drug transporters in the intestine were up-regulated or down-regulated by fibrates. However, we could not replicate the previously published observation that the expression of PEPT1 was up-regulated by a fibrates and PPARα. We propose that at least several transport processes can be coordinately regulated with the intracellular metabolism by the nutrient nuclear receptors and that drugs and diet will also affects drug transport processes leading to secondary drug-drug interactions.

Funding: This work was supported in part by Meiyaku Open Research Project and grants-in-aid for Scientific Research from the Japan Society for the Promotion of Science

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IMPACT OF THE MTP-INHIBITOR, AEGR-733, ON THE SINGLE-DOSE PHARMACOKINETICS OF FENOFIBRATE

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Background: Fibrates effectively treat hypertriglyceridemia, but monotherapy is often insufficient, especially in patients that are also hypercholesterolemic. AEGR-733 is a microsomal triglyceride transfer protein (MTP) inhibitor that lowers LDL-C and triglycerides by a novel mechanism. Before conducting trials to assess safety and efficacy of the combination, we studied the potential for a drug-drug interaction between fenofibrate and AEGR-733. **Methods:** We enrolled 10 healthy volunteers to receive a single dose of micronized fenofibrate 145 mg on day 1 followed by a 7-day course of AEGR-733 monotherapy, 10 mg/day. On day 8, subjects took the last dose of AEGR-733 with another dose of fenofibrate. On day 1 and 8 we collected blood samples over 24 h to determine the mean (90% CI) area under the curve (AUC) and Cmax of fenofibrate. We also evaluated adverse events (AEs) and efficacy data. **Results:** Combination with AEGR-733 had little impact on fenofibrate levels. The change in serum fenofibric acid by AUC (0-inf) was 0% (-8 to +10%, p=0.7), by AUC (0-24) was -10% (-17 to -3%, p=0.03), and by Cmax was -29% (-16 to -40%, p=0.01). AEGR-733 monotherapy lowered LDL-C 20%, with a few mild GI AEs, and no clinically meaningful changes in liver enzymes. **Conclusion:** Combining AEGR-733 with fenofibrate was associated with minor decrease in fenofibrate levels. Though statistically significant, such changes are not considered clinically significant. We conclude that AEGR-733 10 mg can be combined with 145 mg of micronized fenofibrate without substantial attenuation of fibrate levels.

Funding: Aegerion Pharmaceuticals

Poster Session 4 "GENETICS AND CVD" - GROUP A

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PHYSIOGENOMICS OF STATIN SAFETY AND EFFICACY

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The post-market clinical efficacy and safety results for statins are less than those reported in industry-sponsored trials. LDL cholesterol reductions average 34% in clinical trials compared to 26% in naturalistic surveys. Similarly, myopathy prevalence ranges from 1-5% in pre- to 10% in post-market studies. Avoiding myositis (elevated serum creatine kinase) and myalgia (pain) while maximizing efficacy are keys to successful statin therapy. As no single mechanism explains the gamut of statin-associated neuromuscular complaints, we demonstrate pathway-specific explanations for myopathy and mylagia. In outpatients receiving statin therapy, SNPs rs2276307 and rs1935349 in the serotonin receptor genes HTR3B and HTR7, respectively, significantly associate with statin-induced myalgia (Ruano et al., Muscle and Nerve, 2007), and SNPs rs12695902 and rs1799983, in the AGTR1 (angiotensin receptor type I) and NOS (nitric oxide synthase) genes, respectively, significantly associate with myositis (Ruano et al., Pharmacogenomics 6:865, 2005). Here we report new associations linking the IL1R1 (interleukin-1 receptor 1), ADRB3 (adrenergic receptor β3), and PIK3CG (phosphoinositol kinase class III γ) genes to LDL cholesterol reduction to define efficacy. We thus have physiogenomics evidence that statin response phenotypes are modulated by separate gene pathways. The findings permit construction of a prototype of a physiogenomic-based safety/efficacy model that consolidates the myalgia, myositis, and LDL cholesterol reduction components. Pending further validation, we predict the existence of genotypes to help clinicians prescribe statins so as to minimize side effects and maximize efficacy.

Funding: None

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FAMILIAL DEFECTIVE APOLIPOPROTEIN B AND FAMILIAL HYPOBETALIPOPROTEINEMIA IN ONE FAMILY: TWO NEUTRALIZING MUTATIONS

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Objective: Hereditary disorders of lipoprotein metabolism can be characterized by hyper- as well as hypocholesterolemia. Here we describe a family in which two counteracting mutations in the apolipoprotein B-gene (*APOB*) are transmitted. **Methods:** Case report of a family in which 2 *APOB* mutations coincide. Lipids and lipoproteins as well as DNA analysis were evaluated. **Results:** The index case and two of his children had elevated low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B100 (apoB) levels, caused by a point mutation (R3500Q) in *APOB*, underlying familial defective apolipoprotein B (FDB). In contrast, his spouse and a third child had low LDL-C, apoB and triglyceride levels, caused by another mutation (11712delC) in *APOB*, leading to familial hypobetalipoproteinemia (FHBL). A normal lipoprotein profile was present in a fourth child, but DNA analysis revealed that this child carried both the R3500Q mutation and the 11712delC mutation, thus was diagnosed with FDB as well as FHBL. **Conclusion:** Mutations in the *APOB* gene, that disrupt its synthesis, can annihilate the consequences of delayed catabolism of the LDL-particle. These data offer hope for the future for inhibition of apoB synthesis that are currently in early development.

Funding: None

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METHIONINE ADENOSYL TRANSFERASE 1A (MAT1A) KNOCKOUT MICE EXHIBIT INCREASED HEPATIC VLDL PRODUCTION

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Objective: Mature liver expresses MAT1A that catalyze the formation of S-adenosylmethionine (SAM) the principal biological methyl donor. It is known that 3 month-old (3m) MAT1A-KO mice are more susceptible to a diet-induced fatty liver and at 8 months (8m) develop spontaneous non-alcoholic steatohepatitis. Both ages are encompassed by abnormal lipoprotein profiles, VLDL size changes and reduced hepatic SAM levels. Our aim was to assess whether MAT1A deficiency affects VLDL production in these two different stages of nonalcoholic fatty liver disease (NAFLD) **Methods:** 3m and 8m male MAT1A-KO mice and their WT littermates were injected Poloxamer (P-407) intraperitoneally, which inhibits clearance of triglyceride (TG)-rich lipoproteins from the circulation. Blood samples were drawn before and 6 h after injection and serum VLDL were isolated and analyzed for lipid, apoprotein content and size. Hepatic production was calculated by the subtraction of the 6 h vehicle-injected from the P-407-injected mice values. **Results:** The 6 h hepatic VLDL production in KO mice was increased 1.3 and 2.6-fold that of WT mice of 3 and 8 months, respectively. Both, apoB48 and apoB100-containing particles were overproduced by the two groups. However, while 3m KO mice secreted 24% less TG and 90% more TC, 8m KO mice secreted 90% more TG and 32% less TC in VLDL. **Conclusions:** Our findings indicate that MAT1A plays a decisive role in VLDL assembly in mice.

Funding: Supported by UPV 15942/2004 and GV IE05-147

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P325P, BUT NOT *XBAI* AND *PVUII* POLYMORPHISM OF ESR1 GENE INFLUENCE THE HYPOLIPEMIC EFFECT OF RALOXIFENE

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Objective: SERM–raloxifene, exerts hypolipemic properties partially through estrogen receptor (ESR) activation. We hypothesised that different genotypes for ESR1 gene may differently affect lipid lowering effect of raloxifen. **Methods:** We analysed effects of *P325P*, *Xba*I, and *Pvu*II polymorphisms of the ESR1 gene, on serum lipid levels among postmenopausal, non-smoking, osteoporotic women, not taking hypolpemic drug, treated for 12 months with raloxifene 60mg/d. The total-, LDL- and HDL-cholesterol, and triglycerides (TG) levels were determined before and after 6 and 12 months treatment. SSCP method was used to determine the *P325P* and RFLP to determine *Xba*I and *Pvu*II polymorphisms. **Results:** 53 women, (mean age 59.7 ±6.2), finished the 12 months of treatment. As there were only two women with GG genotype for *P325P* polymorphism, we combined the GG and CG genotype into the non-CC group. We were unable to find any relationship between ESR1 gene polymorphisms and serum lipids at baseline. When comparing lipid levels of women with non-CC- to CC-genotype, we found that after 6 and 12 months of treatment the total- ($p=0.015$ and $p=0.037$) and LDL-cholesterol levels ($p=0.008$ and $p=0.042$) were significantly lower in women with non-CC genotype, but no effect of *P325P* polymorphisms on HDL-cholesterol and TG levels was observed . Neither *PvuII* nor *XbaI* ESR1 gene polymorphisms affect lipid lowering effect of raloxifene. **Conclusions:** Our data suggests that *P325P* but not *Xba*I and *Pvu*II polymorphisms of ESR1 gene might intensify the cholesterol lowering effect of raloxifene.

Funding: Grant P3-0298 provided by the Slovenian Research Agency

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PHARMACOGENETIC STUDY OF CHOLESTERYL ESTER TRANSFER PROTEIN GENE AND SIMVASTATIN THERAPY IN HYPERCHOLESTEROLEMIC SUBJECTS

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Objective: The aim of this study was to examine whether the I405V and TaqIB polymorphisms of cholesteryl ester transfer protein (CETP), with major roles in lipid metabolism and homeostasis, are associated with variable response after simvastatin treatment. **Methods:** One hundred and eighty hypercholesterolemic patients were treated with simvastatin at such dose to achieve the ATP III treatment goal for low-density lipoprotein cholesterol (LDL) for at least 6 months. The I405V (I/V) and TaqIB (B1/B2) genotypes were determined. **Results:** Total, LDL cholesterol and triglyceride levels were significantly decreased after simvastatin administration (-31%, -39% and -20%, $p<0.001$ for each), while high-density lipoprotein cholesterol (HDL) concentration was increased (+7%, $p<0.001$). The triglyceride response was influenced by the I405V polymorphism with carriers of I allele showing the highest decrease compared to those with the V allele (-22% vs -16%, $p=0.04$). Additionally, carriers of the I allele were more prone to HDL increases than the V carriers after simvastatin treatment ($p=0.05$). **Conclusions:** Our study demonstrates that the response to simvastatin was affected by the CETP gene locus. The I allele of the I405V polymorphism seems to be more beneficial in triglyceride reduction and in HDL increase.

Funding: This study was conducted independently no company or institution supported it financially

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SOME ASPECTS OF GENETIC PREDISPOSES OF ATHEROSCLEROSIS

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Objective: The aim of the study was research of dyslipoproteinemia (DLP) in neonates, their parents and grandparents revealing genetically predispose lipid exchange disturbances. **Methods:** The levels of total cholesterol (TC), low density lipoprotein cholesterol (LDLC), high density lipoprotein cholesterol (HDLC) and triglycerides (TG) were investigated among 408 neonates and their parents. Blood groups of ABO system were also investigated in each case with the aim of studying the relationship between lipid levels and blood genetic markers. Methods we have used: CHOD-PAP, HDL-CHC, GPO-PAP; Enzymatic colorimetric test. **Results:** Examination have shown that neonatal umbilical blood revealed DLP in 12% (49 neonates). All indices (TC, LDLC, HDLC, and TG) were increased in each case. The character of the neonate's DLP coincided with the mother's one in 51,7%, with father's one-in 35% and the character of DLP of the both parents coincides with DLP of the neonates in 13,3%. Among 192 grandparents of above mentioned 49 neonates, 128 one (67%) had disease which could influence indices neonatal lipid exchange: myocardial infarction in early ages, hypertension, angina, stroke, diabetes mellitus. We found the high frequency of correspondence of hyperlipidemia with blood group A (in 93,1%), what should be considered as an important risk factor predisposing to the development of the atherosclerosis. **Conclusion:** we conclude that one part of neonates have the genetically predispose lipid exchange disturbances. It is possible to make a wide screening-examination of children with hereditary burden to atherosclerosis with the aim of revealing the DLP that will enable us carry out an early prevention of atherosclerosis.

Funding: None

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PLASMINOGEN ACTIVATOR INHIBITOR-1 PROMOTER POLYMORPHISM (4G/5G) PREDICTS RISK OF RECURRENT CORONARY EVENTS IN NON HYPERCHOLESTEROLEMIC, NON-HYPERTRIGLYCERIDEMIC POSTINFARCTION PATIENTS

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Objective: Non-hyperlipidemic postinfarction patients are at high risk for recurrent coronary events by virtue of incident MI; however, few studies assess the role of additional factors in establishment of risk beyond incident MI in these patients. **Methods:** We studied 846 non-diabetic postinfarction patients using outcome event mapping, a graphical exploratory data analysis tool, to characterize risk in terms of cholesterol and triglyceride levels and a set of genetic CVD-associated polymorphisms. **Results:** Results indicated risk associated with the 4G/5G insertion/deletion SNP in the PAI-1 gene promoter in a subgroup (21.5% of study population) of non-hypercholesterolemic/non-hypertriglyceridemic patients. Within-subgroup recurrent risk was further assessed using multivariable Cox regression analysis with a set of thrombogenic, inflammatory, and metabolic blood markers. Results adjusted for significant clinical covariates gave only the 4G/5G polymorphism (4G/4G versus 4G/5G plus 5G/5G patients) as significantly associated with risk with hazard ratio and 95% confidence interval of 4.02 (1.86 - 8.69), p = 0.00092. **Conclusions:** Only the 4G/5G polymorphism of the PAI-1 gene in a subgroup of non-hyperlipidemic postinfarction patients predicts risk beyond incident MI for recurrent coronary events from a set of CVD-associated genetic markers and from a set of thrombogenic, inflammatory, and metabolic blood markers.

Funding: Grant HL-48259, NIH, USA

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ASSOCIATION BETWEEN CORONARY ARTERY DISEASE AND PARAOXONASE-1 PROMOTER GENE POLYMORPHISM T(-107)C IN IRANIAN POPULATION

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Background: Increased Plasma LDLC level with enhanced LDL oxidation plays an integral role in CAD. Paraoxonase-1(PON1)protects LDL from oxidative modification. **Objective:** The present study tested the hypothesis that PON1 promoter polymorphism T(-107)C could be a risk factor for severity of CAD in Iranian population. **Methods:** Promoter genotypes were determined in 300 consecutive subjects (>40 years) with angiographic documentation(CAD^+=150&CAD^-=150)PON1 promoter genotypes were determined by PCR and RFLP. **Results:** CAD^+ subjects did not show any significant differences in the distribution of PON1 promoter genotypes as compared to CAD^- subjects(P=0.75) However the analysis of PON1 promoter genotypes distribution showed a higher percentage of (-107)TT among CAD^+compared with CAD^-(P=0.27) **Conclusion:** This data suggest that the TT genotypes may represent genetic risk factor for CAD in iranian population.

Funding: This work was supported by grant research from Tehran University of Medical Sciences

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CYS 311 SER POLYMORPHISM OF PARAOXONASE-2 GENE IS ASSOCIATED WITH THE RISK OF CORONARY ARTERY DISEASE (CAD) IN IRANIAN POPULATION

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Background: Oxidation of LDL plays an important role in initiation of coronary artery disease(CAD). Paraoxonase2(PON2) has antioxidant properties similar to PON1 and PON3. **Objective**: The aim of this study was to investigate the association between cys 311 ser PON2 polymorphism and CAD in iranian population. **Methods:** We assessed the frequency and genotype distribution of the cys 311 ser PON2 polymorphism in 300 subjects(>40 year)with angiographic documentation of coronary vessels(CAD^+=150;CAD^-=150)to determine the possible association between this mutation and susceptibility for CAD.The PON2 genotypes were determined by PCR and DdeI restriction enzymedigestion. **Results:** CAD^+ subjects showed significant differences in the distribution of cys311 ser PON2 genotypes as compared to CAD^-subjects(P=0.015).The analysis of PON2 genotypes distribution showed higher percentage of CC genotype among CAD^+compared with CAD^- (P=0.008).After controlling for other risk factors,the cys 311 derpolymorphism had not correlation with age,BMI,gender,smoking,diabetes,level of HDL-C,LDL-C,TG and TC.**Conclusion:**Our data indicated a major effect of the PON2 polymorphism on CAD risk in iranian population.

Funding: This work was supported by grant from Endocrinology and Metabolism Research Center, Tehran University

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ASSOCIATION BETWEEN APOLIPOPROTEIN(a) PHENOTYPES, PENTANUCLEOTIDE REPEAT POLYMORPHISM AND LIPOPROTEIN(a) LEVELS IN YOUNG CAD PATIENTS AND THEIR CHILDREN

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Objectives: High plasma lipoprotein(a) [Lp(a)] levels are a genetic risk factor for premature coronary artery disease (CAD). This work was planned to study the influence of apo(a) isoform size (K4 repeats) and pentanucleotide repeat (PNR) polymorphism on plasma Lp(a) levels in CAD patients and their children. **Methods:** Study was carried out in angiographically assessed CAD patients (age $\leq$ 40 yrs; n=46) their spouses and children (n=100). Plasma Lp(a) levels were determined by ELISA, apo(a) isoform size was determined by Western blotting and PNRP was determined by amplification of 5$^{'}$ control region followed by PAGE and silver staining. **Results:** Mean plasma Lp(a) levels were significantly higher in patients (father) as compared to their healthy spouses and children ($p<0.01$). The patients having a combination of $\leq$ 22 K4 repeats and $\leq$ 8 PNR had the highest plasma Lp(a) concentrations. Sons inheriting the same phenotype also had significantly higher Lp(a) levels as compared to daughters ($p<0.05$). These results demonstrate a significant intraclass correlation between father and sons (0.74, $p<0.001$). **Conclusions:** These results show that sons in particular, inherit atherogenic genes with respect to plasma Lp(a) levels from the affected father. Therefore, studies on Lp(a) levels and apo(a) polymorphism in offsprings may help to identify children having high risk of premature CAD.

Funding: Indian Council of Medical Research, Delhi, India

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CXCL5 IS AN ENDOTHELIAL TARGET AND PHARMACOGENETIC CANDIDATE FOR STATINS

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Objective: CXCL5 encodes the chemokine ENA-78. We have shown atorvastatin (AT) to lower basal ENA-78 production from endothelial cells (HUVECs), but its effects in an inflammatory state typical of atherosclerosis are unknown. We therefore investigated AT effects on IL-1β-stimulated ENA-78 in HUVECs. Also, since basal ENA-78 is modulated by AT, we hypothesized CXCL5 is a pharmacogenetic candidate and tested whether statin responses differ by CXCL5 genotypes in acute coronary syndrome (ACS) patients. **Methods:** HUVECs were treated with AT 1-50μM and IL-1β as previously described. A prospective cohort of ACS patients (n=704) with 3-year follow-up were assessed for the -156G>C polymorphism. Hazard ratios (HR) and 95%CI were calculated. Models were stratified by genotype and conducted separately by race. **Results:** AT decreased ENA-78 concentrations by 38% to 99% ($P<0.05$). Drug effect persisted over 48 hours ($P<0.05$). ENA-78 reduction was partially reversed by mevalonate, FPP, and GGPP, implicating the HMG-CoA reductase and prenylation pathways in the drug response. AT lowered basal CXCL5 mRNA expression by 87% ($P<0.005$) with no effect on IL-1β-induced CXCL5 expression. In the clinical cohort, statin therapy resulted in a significant reduction in mortality in G/G+G/C individuals (HR 0.53 95% CI 0.32-0.87). Patients with the C/C genotype did not gain statin benefit (HR 1.6 95% CI 0.40-6.4). **Conclusions:** AT lowers endothelial ENA-78 production during inflammation. Further, ACS patients with the CXCL5 -156C/C genotype did not gain the same benefit from statins as patients with other genotypes.

Funding: AHA SDG 0435278B, NIH CO6 Grant RR17568, NIH P50HL077113

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APOLIPOPROTEIN E GENOTYPE AND CIRCULATING INTERLEUKIN-10 LEVELS IN PATIENTS WITH STABLE AND UNSTABLE CORONARY ARTERY DISEASE

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Objectives: To assess the relation between apolipoprotein E (apoE) genotype and serum interleukin (IL)-10 levels, in patients with acute coronary syndrome (ACS) and chronic stable angina (CSA). **Methods:** ApoE genotypes were assessed in 166 consecutive ACS patients and 70 CSA patients. Serum IL-10 and CRP were assessed at study entry. **Results:** Analysis of co-variance showed that genetic variation in apoE gene locus significantly influences serum IL-10 levels both in ACS ($p=0.009$) and CSA patients ($p=0.013$). Among ACS patients IL-10 levels were lower in E3/E4 carriers compared to E3/E3 carriers ($p=0.01$), and marginally lower compared to E2/E3 carriers ($p=0.065$). Among CSA patients IL-10 levels were lower in E3/E4 carriers compared toE2/E3 carriers ($p=0.004$), and marginally lower compared to E3/E3 carriers ($p=0.086$). **Conclusions:** IL-10 concentrations differ in ACS and in CSA patients with different apoE genotypes. The e4 allele was associated with a trend towards lower IL-10 serum levels. Our results may provide an explanation to findings in previous studies that cardiovascular risk is higher in e4 carriers despite the presence of low CRP levels.

Funding: We have received funding from in-faculty research budget

Poster Session 5 "HDL" - GROUP A

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HDL SIZE AND HDL PARTICLE NUMBER INDEPENDENTLY PREDICT CORONARY ARTERY DISEASE RISK IN APPARENTLY HEALTHY INDIVIDUALS. THE EPIC-NORFOLK PROSPECTIVE POPULATION STUDY

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Background: HDL subpopulations are heterogeneous and this heterogeneity is not adequately reflected by measuring the concentration of the total HDL fraction. Measuring the number of HDL particles (HDL-P) and size may provide additional insight in the associations between HDL with cardiovascular risk. **Methods:** We conducted a case-control study nested in the prospective EPIC Norfolk study. Cases (n= 822) developed fatal or nonfatal coronary artery disease (CAD) during 6 year follow-up. Controls (n=1401) were matched for age, sex and enrollment time. Odds ratios for future CAD were calculated by quartile of each HDL variable. **Results:** HDL-P and HDL size were independently associated with CAD risk (OR in highest quartile: 0.60; 95% CI 0.46-0.79 and 0.52; 95% CI 0.40-0.69, respectively). The association for HDL size was lost after adjustment for the metabolic parameters triglycerides and apolipoprotein B (1.00; 95% CI 0.71-1.39) whereas these parameters had limited effect on the risk estimate for HDL-P. Adjustment for inflammatory parameters like myeloperoxidase (MPO), paraoxonase (PON) and C-reactive protein had limited effect on both risk estimates. **Conclusion:** HDL size and HDL-P are independently associated with cardiovascular risk. HDL size was associated with characteristics of the metabolic syndrome. These findings may contribute to understanding the relationship between HDL-c and cardiovascular risk.

Funding: Medical Research Council United Kingdom, Cancer Research United Kingdom, support from the European Union, Stroke Association, British Heart Foundation, Department of Health, Food Standards Agency and the Wellcome Trust

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HIGH DENSITY LIPOPROTEIN SUBCLASSES DISTRIBUTION AND COMPOSITION IN MEXICAN ADOLESCENTS WITH LOW HDL CHOLESTEROL AND/OR HIGH TRIGLYCERIDE CONCENTRATIONS

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Objective: We tested whether low high density lipoprotein cholesterol (HDL-C) and/or high triglycerides (TG), are associated to alterations in HDL subclasses distribution and composition, and their relationships with fasting insulin and C-reactive protein (CRP) in plasma. **Methods:** Four groups (G) of adolescents were studied: G1 (n=18) low HDL-C (≤35mg/dL) and high TG (≥150mg/dL). G2 (n=33) isolated low HDL-C. G3 (n=21) isolated high TG, and G4 (n=39) normolipidemic subjects. **Results:** HDL_{2b}, HDL_{2a} were lower, and HDL_{3b}, HDL_{3c} were higher in G1 and G2 vs. G4. HDL particle size in G1 (9.3±0.15nm) and G2 (9.36±0.15nm) was lower than in G4 (9.52±0.18nm; $p<0.001$). HDL cholesteryl ester content (%) was lower in G1 (17.3±3.6) and G2 (17.6±2.7) compared to G4 (20.8±2.5; $p<0.001$). G1 also showed the highest HDL TG proportion (6.7±2.2 vs 5.1±1.4 in G2, 5.0±1.0 in G3, and 3.6±1.1 in G4; $p<0.01$, for all). Compared to G3 (0.61) and G4 (0.25), median values of CRP (mg/L) were significantly ($p<0.01$) higher in G1 (1.18). Insulin concentration (μU/mL), was highest in G1, but significant difference was observed only with G4 (13.1±7.9 vs 5.3±4.1; $p<0.001$). HDL subclasses distribution and composition were independently associated only with HDL-C and waist circumference. **Conclusions:** Adolescents with high TG and low HDL-C have abnormalities in HDL subclasses distribution and lipid composition, which may render their HDL dysfunctional. In addition, these subjects have high CRP and insulin levels suggesting the presence of chronic low grade inflammation.

Funding: None

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HIGH DENSITY LIPOPROTEINS ARE ABNORMAL IN WOMEN WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Objective: Little is known about qualitative abnormalities of high density lipoproteins (HDL) in systemic lupus erythematosus (SLE). We studied distribution and composition of HDL subclasses in SLE and healthy women. **Methods:** In 30 premenopausal women with uncomplicated SLE and 18 controls matched for age and sex, plasma and HDL lipids by colorimetric enzymatic assays, HDL size distribution by native gradient polyacrilamide gel electrophoresis (PAGE), and apolipoproteins in HDL by sodium dodecyl sulfate denaturing PAGE, were determined. **Results:** Compared with controls SLE patients had significantly ($p < 0.05$) lower proportions of HDL_{2b} (-14.7 %), and higher proportions of HDL_{3b} (+8.8 %) and HDL_{3c} (+23.3 %). Cholesteryl ester (-18 %) and apolipoprotein AI (-9 %) were lower, while triglycerides (+32 %) and apolipoprotein E (+27 %) were higher in SLE HDL ($p < 0.05$; for all). In patients, multiple linear regression analyses showed that prednisone treatment was independently associated with HDL_2 ($r = -0.483$; $p = 0.019$) and HDL_3 ($r = 0.479$; $p = 0.021$); while in controls, plasma triglycerides were related to HDL_2 ($r = -0.811$; $p < 0.001$) and HDL_3 ($r = 0.810$; $p < 0.001$). **Conclusions:** This study demonstrates that HDL distribution and composition are abnormal in SLE patients. Some studies have shown that these HDL abnormalities are associated to prevalence of CHD and impaired atheroprotective properties of HDL. Therefore, they may contribute to the premature atherosclerosis observed in women with SLE.

Funding: None

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HDL-CHOLESTEROL METABOLISM IN HIV-INFECTED CHILDREN EXPOSED TO HIGHLY ACTIVE ANTI-RETROVIRAL DRUGS – PERI STUDY

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Methods: It is an observational, case-control study, comparing 49 HIV-infected children with low levels of HDL-cholesterol (HDL-C) with 34 with normal levels of HDL-C from an outpatient ambulatory of infectology exposed to highly active anti-retroviral therapy. A questionnaire, research of clinical records, physical examination, anthropometry, electrocardiography, chest X-ray, echocardiography and carotid ultrasound examination were performed and fasting blood sample were collected. **Results:** After bivariate analysis, HDL-C low levels were associated with diffuse alterations of ventricular repolarization (27% versus 11%, p=0.020), dyspnea (10% versus 1%, p=0.021), positive D-dimer (14% versus 3%, p=0.028) and glycated hemoglobin (27% versus 11%, p=0,041). After multivariate analysis, HDL-C low levels were associated with Abacavir use [OR: 0.11 (0.026-0.47), p=0.003], Nelfinavir use [OR: 0.03 (0.09-0.86), p=0.027], and exposition of more than 3 anti-retroviral drugs [OR: 15.15 (1.30-176.32), p=0.030]. **Conclusions:** We found associations among HDL-C and drugs, that need to be take into account when this lipid abnormality is present in this disease. Further studies are required to elucidate the relationship among this lipid abnormality and several aspects of AIDS disease and its therapy, to determine atherosclerotic risk control in this population.

Funding: UNESC-WHO

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NEW PPAR ß/δ AGONIST (CER002) INCREASES PRE-ß HDL LEVELS IN NORMAL MONKEYS

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The protective effect of high density lipoprotein (HDL) particles against atherosclerosis is usually attributed to their central role in "reverse cholesterol transport" (RCT) inducing regression of atherosclerotic plaques in various animal models including humans. We have studied the effects of CER-002, a highly selective PPAR ß/δ agonist compound, on plasma lipoprotein levels in normal 2 year old Macaca fascicularis monkeys fed a standard chow diet . Various doses of CER-002 were administrated orally once a day for 2 weeks in an escalating dose escalation study. Blood samples were collected at one week intervals and analyzed for lipoprotein profiles using online HPLC chromatography. Results demonstrate an increase of the HDL and apoA-I levels (+ 27 % and 30 % respectively), but more importantly, a remarkable increase of pre-β HDL particles (> 70 %). Furthermore, we observed a significant decrease of the LDL, VLDL and TG level in those animals. In conclusion, we have demonstrated that CER-002 is a potent enhancer of pre-β HDL particles in normal monkeys. Pre-ß HDL particles are well known to be the primary acceptors of cholesterol from peripheral cells (e.g. macrophages) for further elimination by the liver (after different maturation processes in the circulation). The recent finding in humans of plaque regression measured by IVUS technique following administration of using artificial small HDL particles highlight the critical role of these type of HDL particles in RCT. Here, we have a small orally bioavailable compound that is able, for the first time, to increase this critical type of HDL particle, the pre-ß HDL.

Funding: None

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NOVEL OXIDATION CHARACTERISTICS OF LCAT-DEPLETED LIPOPROTEINS

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Introduction & **Objectives:** Lecithin:cholesterol acyltransferase (LCAT), an enzyme involved in lipoprotein metabolism, is typically found associated with HDL, and to a lesser extent LDL. The central aim of this project was to assess the possible role of LCAT in a phenomenon previously reported by our group, whereby HDL acts as a pro-oxidant during VLDL oxidation. **Methods:** LCAT was removed from plasma by ion exchange chromatography, with lipoproteins (VLDL, LDL & HDL) being subsequently isolated from the resultant eluant by ultracentrifugation. Oxidation of various lipoprotein combinations was mediated by $CuCl_2$ and the production of conjugated dienes monitored at l=234nm. The kinetic parameter "time at ½ max." ($t_{½max}$) was used for comparison of treatments. **Results:** When VLDL was oxidised alone, $t_{½max}$ was significantly prolonged compared to VLDL oxidised in the presence of native-HDL (230.1±3.6 *vs*. 38.2±2.0 min; $P<0.05$, $n=6$). Furthermore, when VLDL was co-oxidised with LCAT-deplete-HDL, a subtle yet significant restoration of $t_{½max}$ was observed (38.2±2.0 *vs*. 51.3±2.6 min; $P<0.05$, $n=6$). We also found that LCAT-deplete-LDL oxidised more readily compared to native-LDL (46.7±2.6 *vs*. 65.3±1.1min; $P<0.05$, $n=6$), and similarly, LDL oxidised more readily in the presence of LCAT-deplete-HDL, than in the presence of native-HDL (54.2±2.1 V's 90.1±3.3 min; $P<0.05$, $n=6$). **Conclusions:** We have demonstrated involvement of LCAT in the processes of lipoprotein oxidation, whereby it demonstrated pro-oxidant activity during VLDL+HDL oxidation, and antioxidant properties during LDL and LDL+HDL oxidation. These findings have identified the need to further investigate the role of lipid transfer proteins in the intricate processes of atherosclerosis.

Funding: None

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CHOLESTERYL ESTER INCORPORATED INTO RECONSTITUTED DISCOIDAL HDL: COMPOSITIONAL AND STRUCTURAL FEATURES

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The reconstituted discoidal HDL with saturated (DPPC) or unsaturated (POPC, PLPC) phosphatidylcholines (PC) with fluorescent cholesteryl ester analogues differing in chain length (cholesteryl 1-pyrene-butyrate, -hexanoate and -decanoate (CPD)) were prepared by cholate sorbtion and isolated by gel chromatography. The U-shaped elution profiles by both probe/PC ratio and excimerization parameter were left- and right-side upward for saturated and unsaturated PCs, respectively, while an inflection point with a minimal probe incorporation corresponded to top fraction with apoA-I:PC ratio expected for discs. The increase in initial CPD concentration for POPC resulted in probe redistribution, relative to top fraction, from smaller to larger structures. The CPD saturation levels in DPPC and POPC discs were 1.4 and 1.8 mole %, respectively. The concentration dependence of excimerization parameter, sensitive to both probe concentration and lateral diffusion, positively deviated from linearity for all probe/PC combinations evidencing the contribution of disc occupancy by probe molecules. The difference in quenching of CPD monomer and excimer fluorescence by acrylamide in DPPC complexes increased at the decrease of complex dimensions suggesting the lack of equilibrium between monomer and excimer populations. The concentration dependence of fluorescence resonance energy transfer from apoA-I tryptophan residue(s) to probe molecule(s) at 37 °C was more prominent for unsaturated vs saturated PCs suggesting the different CPD distribution between boundary lipid close to apoA-I molecules and the rest of bilayer. The structure of matrix lipid seems to determine both incorporation efficiency and distribution mode of cholesteryl ester in discoidal HDL.

Funding: None

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ADIPONECTIN ACCELERATES REVERSE CHOLESTEROL TRANSPORT BY INCREASING BY HIGH DENSITY LIPOPROTEIN ASSEMBLY IN THE LIVER

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Background: Plasma high density lipoprotein (HDL)-cholesterol levels are negatively correlated with the incidence of coronary artery disease. HDL plays an important role in protecting against atherosclerosis by removing cholesterol from atheroma and transporting it back to the liver. The ATP-binding cassette transporters (ABCA1 and ABCG1) and scavenger receptor BI (SR-BI) are thought to be one of the rate-limiting factors to generate HDL in the liver. Adiponectin (APN) secreted from adipocytes is also one of the important molecules to inhibit the development of atherosclerosis. Recently it has been reported that plasma HDL-cholesterol levels are positively correlated with plasma APN concentrations in humans. Therefore, we investigated the association of APN with HDL assembly in the liver. **Methods:** Human hepatoma cell line, HepG2 cells, were incubated for 24 hours in the culture medium with the indicated concentrations of recombinant APN. **Results:** APN enhanced the mRNA level of apolipoprotein A-I (apoA-I) in HepG2 cells and increased the secretion of apoA-I from the cells to the medium. Furthermore, APN increased both mRNA and protein levels of ABCA1, but not ABCG1 and SR-BI, in HepG2 cells. **Conclusions:** APN might protect against atherosclerosis by increasing HDL assembly through enhancing ABCA1 pathway and apoA-1 synthesis in the liver.

Funding: None

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INDUCTION OF ABCA1 INHIBITS TRIACYLGLYCEROL BIOSYNTHESIS VIA REPRESSION OF 1,2-DIACYL-*SN*-GLYCEROL ACYLTRANSFERASE-1 GENE EXPRESSION IN GROWING BABY HAMSTER KIDNEY CELLS

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Objective: ATP binding cassette A1 (ABCA1) transporter induction in BHK cells increases phosphatidylserine (PS) in the plasma membrane exofacial leaflet. We examined if this increase in PS was due to an alteration in glycerolipid synthesis. **Methods**: BHK cells expressing a mifepristone (MFI)-inducible ABCA1 (ABCA1 cells) were incubated for 24 h with 10 nM MFI then incubated for 4 h with [1,3-^{3}H]glycerol or [1-^{14}C]oleate or [^{3}H]serine and label incorporated into lipids determined. 1,2-Diacylglycerol acyltransferase-1 (DGAT-1) and DGAT-2 activities and DGAT-1 mRNA expression were determined. **Results**: Radioactive glycerol and oleate incorporated into PS were elevated 2.4-fold ($p<0.05$) and 54% ($p<0.05$), respectively, only in ABCA1 expressing cells compared to controls. MFI inhibited [^{3}H]serine uptake 41% ($p<0.05$) and incorporation into PS in both mock and ABCA1 cells indicating PS synthesis in BHK cells is dependent upon serine uptake. [1,3-^{3}H]Glycerol incorporated into triacylglycerol (TG) was reduced 42% ($p<0.05$) and into diacylglycerol (DG) elevated 55% ($p<0.05$) and [1-^{14}C]oleate incorporated into TG reduced 21% and into DG elevated 21% ($p<0.05$) only in ABCA1 expressing cells. This was due to a 34% decrease ($p<0.05$) in DGAT-1 activity and a 38% decrease ($p<0.05$) in DGAT-1 mRNA expression. **Conclusions**: The results indicate that PS synthesis from glycerol and oleate precursors are elevated in BHK cells expressing ABCA1. In addition, expression of ABCA1 may regulate TG biosynthesis via repression of DGAT-1 gene expression.

Funding: CIHR and the Canada Research Chair Program

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ADIPONECTIN PROMOTES CHOLESTEROL EFFLUX FROM MACROPHAGES THROUGH ABCA1-DEPENDENT PATHWAY

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Background: Recent researches have demonstrated that ABCA1 in macrophages has been identified as an important membrane receptor for removing cholesterol and phospholipids from macrophage foam cells in the arterial wall. On the other hand, adiponectin (APN) secreted from adipocytes is one of the important molecules to inhibit the development of atherosclerosis. Epidemiological studies have revealed APN levels are decreased in obesity, patients with type 2 diabetes or coronary artery disease. In the current study, we have investigated the role of APN on cholesterol efflux from macrophages. **Methods:** Human monocyte-derived macrophages were isolated from whole blood of health volunteers and incubated with the medium containing 10 % type AB serum for 7 days. The indicated concentrations of recombinant human APN were added to the medium for 24 hours. The expression of ABCA1 was measured by real-time quantitative PCR and western blot analyses. ApoA-1 mediated cholesterol efflux from [^{3}H]-cholesterol loaded macrophages was measured with or without APN and expressed by a percentage of radioactivity of the medium to total radioactivity (cells plus medium). **Results:** The mRNA and protein levels of ABCA1 in human macrophages were significantly increased by APN by 3.6- and 2-fold, respectively. APN enhanced apoA-1-mediated cholesterol efflux by 8.1-fold. **Conclusions:** APN might act as a protective factor against atherosclerosis by increasing cholesterol efflux from macrophages through ABCA1-dependent pathway.

Funding: None

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SREBP-2- AND LXR-DRIVEN DUAL PROMOTER REGULATION OF HEPATIC ABCA1 GENE EXPRESSION: MECHANISM UNDERLYING THE UNIQUE RESPONSE TO CELLULAR CHOLESTEROL STATUS

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ABC transporter A1 (ABCA1) mediates and rate-limits biogenesis of HDL, and hepatic ABCA1 plays a major role in regulating plasma HDL levels. HDL generation is also responsible for release of cellular cholesterol. In peripheral cells ABCA1 is up-regulated by the LXR system when cell cholesterol increases. However, cholesterol feeding has failed to show significant increase in hepatic ABCA1 gene expression and its expression is up-regulated by statins, suggesting distinct regulation. In this study, we investigated the mechanism of regulation of the rat hepatic ABCA1 gene and identified two major ABCA1 transcripts and two corresponding promoter regions. Compactin activated the novel liver-type promoter in rat hepatoma McARH7777 cells by binding the sterol regulatory element binding protein-2 (SREBP-2). In contrast, compactin repressed the previously identified peripheral-type promoter in an LXR-responsive element-dependent manner. Thus, compactin increased the liver-type transcript and decreased the peripheral-type transcript. The same two transcripts were also dominant in human and mouse livers, whereas the intestine contains only the peripheral-type transcript. Treatment of rats with pravastatin and a bile-acid-binding resin (colestimide), which is known to activate SREBP-2 in the liver, caused a reduction in the hepatic cholesterol level and the same differential responses in vivo, leading to increases in hepatic ABCA1 mRNA and protein and plasma HDL levels. We conclude that the dual promoter system driven by SREBP-2 and LXR regulates hepatic ABCA1 expression, and may mediate the unique response of hepatic ABCA1 expression to cellular cholesterol status.

Funding: None

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ATP-BINDING CASSETTE TRANSPORTER G1 PROMOTER -257T>G POLYMORPHISM PROVIDES AN ANTI-ATHEROSCLEROTIC EFFECT OF STATIN THERAPY

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Background: ATP-binding cassette transporters G1 (ABCG1) is membrane cholesterol transporter and has been implicated to mediate cholesterol efflux from cells in the presence of high-density lipoprotein (HDL). We have reported that an activity of ABCG1 gene transcription was significantly low in -257T>G missense mutation *in vitro* reporter assay. To appear the role of ABCG1 transporter on the beneficial effect of statin therapy, hyperlipidemic patients were analyzed the relationship between changes of lipid profiles after treatment with statin and the promoter variants on ABCG1 gene. **Methods:** Sixty patients were given statin daily during 3 to 6 months period, and they were analyzed -257T>G polymorphism assessed by MS-PCR methods. We classified into T/T genotype and G allele in -257T>G on ABCG1 gene and compared the polymorphism with lipid profile characterized by analytical capillary isotachophoresis (cITP). **Results:** Plasma levels of total, LDL and HDL cholesterol were changed after treatment with statin. There were no significant differences in these cholesterol levels between 2 groups of ABCG1 genotypes. However, the change of the ratio in fast migrating-HDL to slow migrating-HDL assessed by cITP by treatment with statin in G allele group were significantly lower than those in T/T genotype (G allele, 1.026; T/T genotype, 1.055, $p<0.05$). **Conclusion:** The characterization and activation of HDL particles might be modified by ABCG1 transporter. ABCG1 transporter activity contributes to lipid metabolism and may activate the anti-atherosclerotic effect of statin therapy.

Funding: None

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PDZK1 IS A CRITICAL MODULATOR OF HEPATIC SR-BI EXPRESSION IN HUMAN SR-BI (CLA-1) TRANSGENIC MOUSE

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Background: SR-BI is an HDL receptor that mediates the selective cholesterol uptake from HDL. We have reported that human SR-BI (CLA-1) does not influence the HDL cholesterol metabolism in CLA-1 transgenic mouse. PDZK1 is an associate protein with SR-BI in the liver stabilizing the receptor in the membrane. To clarify the regulatory mechanism of CLA-1's expression in the liver, we generated human PDZK1 BAC transgenic mouse. **Results** & **Conclusion:** In CLA-1 transgenic mouse CLA-1 is transcribed in the liver with a corresponding level to that of the endogenous SR-BI. However, the protein level of CLA-1 was low in the liver compared with that of the endogenous mouse SR-BI. As a result CLA-1 transgene does not impact on the plasma total / HDL cholesterol levels.To investigate the regulatory mechanism of the molecule's expression in the liver CLA-1 transgenic mouse with SR-BI-/- background was crossbred with human PDZK1 BAC transgenic mouse. Whereas the PDZK1 transgene does not impact on the mRNA levels of CLA-1 nor of endogenous SR-BI, human PDZK1 increases the protein expression of CLA-1 in the liver. PDZK1 is proved to be an important molecule regulating the CLA-1 expression in the liver impacting on the HDL mediated reverse cholesterol transport in human.

Funding: This work was partially supported by a research grant from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan) for Y. Ueda. This work was supported by a Grant-in-Aid for Scientific Research (c) to Y. Ueda (NO. 18590984) from the Ministry of Education, Science, Sports, and Culture of Japan

Poster Session 6 "NEW CHALLENGES IN HYPOLIPIDEMIC THERAPY" - GROUP A

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LIPID PROFILE IMBALANCE, HEPATIC STEATOSIS AND BILIARY DYSFUNCTION: FACETS OF THE SINGLE PATHOLOGIC PROCESS

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Objective: To examine lipid blood profiles in patients with hepatic steatosis depending on biliary dysfunction type. **Methods:** there were including in study 36 patients with non-alcoholic hepatic steatosis and 34 healthy volunteers (control). Hepatic steatosis was diagnosed by standard ultrasonography. Biliary motility was studied by dynamic ultrasonography with evaluation of gallbladder contractility (hypo-, normo-, hyperkinetic) and Oddi sphincter tone (hypo-, normo-, hypertonic). Body mass index (BMI) was calculated. Blood samples were analyzed for level of cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very little density lipoprotein (VLDL), triglyceride. Standart statistical methods were used. **Results:** All patients had biliary dysfunction with next distribution in types: 24 patients (66.7%) had hypokinetic-hypertonic, in fours (in 11.1%) – hypokinetic-hypotonic, hypokinetic-normotonic and normokinetic-hypertonic type. BMI showed valid increase only by combined dysfunction: hypokinetic-hypertonic ($p<0.05$) and hypokinetic-hypotonic ($p<0.001$). There were determined significant increment of cholesterol (5.9+/-0.2 mmol/l, $p<0.001$ to control 4.5+/-0.1 mmol/l), LDL (4.3+/-0.1 mmol/l, $p<0.001$ to control 3.2+/-0.2 mmol/l) and triglyceride (2.1+/-0.1 mmol/l, $p<0.001$ to control 1.3+/-0.1 mmol/l) by hypokinetic-hypertonic; cholesterol (5.7+/-0.1 mmol/l, $p<0.001$) and LDL (4.2+/-0.1 mmol/l, $p<0.001$) by hypokinetic-hypotonic type. All groups had not valid difference in HDL and VLDL to control. **Conclusions:** Availability in patients with hepatic steatosis combined biliary dysfunction, mostly – hypokinetic-hypertonic type, associate with higher BMI and significant lipid profiles imbalance, which are aggravating factors for hepatic steatosis course.

Funding: None

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NAFLD AND T2D: A GENETIC OR METABOLIC ISSUE?

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Background: Type 2 diabetes (T2D) seems to be a risk factor for the development of Non Alcoholic Fatty Liver Disease (NAFLD) and for its progression to fibrosis. The pathogenesis of the NAFLD-T2D association is not known. Hyperinsulinemia and Insulin resistance may be the primary phenomenon. Aim of the study was to evaluate: -the prevalence of NAFLD in T2D patients; -the relation with the Metabolic Syndrome (MetS) features; -the relation between NAFLD and PC-1 K121Q and IL-6 –174 C/G polymorphisms. **Methods:** We enrolled 80 diabetics who underwent ultrasound (US) to establish the presence of bright liver. Steatosis was defined as mild, moderate or severe according to Fatty Liver Indicator (FLI). **Results:** Diabetics were overweight with BMI=28.60 (25°÷ 75°=25.35÷ 32.95), had normal lipid profile and had higher GPT levels =28 U/L (GPT: 25°÷ 75°; 21.00÷38.00). 1) 77.5% of diabetics had different degree of Fatty Liver. FLI did correlate with BMI ($p< 0.01$),Waist circumference($p< 0.01$), Total-cholesterol($p< 0.01$) and TG ($p<0.01$). BMI and Total-cholesterol resulted to be indipendent risk factors of steatosis. 2) No significant difference in polymorphisms prevalence was observed when NAFLD subjects were compared to a control group. **Discussion:** Our data show that T2D patients have a very high prevalence of NAFLD which is probably related to hyperinsulinism and Insulin resistance. This is further supported by the positive correlation of NAFLD with BMI, W Tot-cholesterol and TG. The lipogenic effects of insulin may underlie such relationship. In our population NAFLD associates with some features of MetS whereas no significant genetic component is present.

Funding: Supported by COFIN-PRIN grant 2004 067491

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A COMPARATIVE METABOLOMIC ANALYSIS OF PPAR ACTIVATORS FOR EVALUATING DRUG EFFICACY AND SAFETY

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Effective selection of lead candidates early in drug discovery optimally requires the use of broad analytical approaches that can globally evaluate efficacy and safety. Application of metabolomic analysis to lysates of drug-exposed cultured cells provides a novel and comprehensive analysis of changes in their biochemical profile. We tested PPAR activators selective to PPAR- gamma (Troglitazone, Ciglitazone), or –alpha (GW7647) in adipocytes. Primary human pre-adipocytes were obtained following liposuction and treated with drugs for 3 or 8 h. The following pathways were affected: purine metabolism, urea cycle and metabolism of amino groups, phenylalanine, tyrosine, and tryptophan biosynthesis, pyrimidine metabolism, several glycerolipid pathways, hexosamine metabolism, glutathione metabolism and aspartate metabolism. Within pathways, there were distinct differences between drugs acting on the 2 PPAR receptor subtypes. Differences were also noted between the 2 PPAR gamma ligands. To further evaluate the metabolomic approach for assessing safety, in a follow up experiment, HepG2 hepatocytes were treated with 3 PPAR gamma ligands including Troglitazone, which has been discontinued due to toxicity; and Rosiglitazone and Pioglitazone, two currently marketed PPAR drugs. Full biological interpretations of results will be presented.

Funding: Funded internally

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THE NUCLEAR RECEPTOR LXRa CONTROLS BILIRUBIN HOMOEOSTASIS

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Recently, the remarkable antioxidant capacity of bilirubin, a heme degradation product, has been associated with a number of protective functions, including anti-atherosclerotic effects. Bilirubin is synthesized by heme oxygenase (HO-1) and biliverdin reductase (BVR) and eliminated from the body as a glucuronide conjugate. The latter is formed by the hepatic UDP-glucuronosyltransferase (UGT)1A1 and transported by the apical transporter MRP-2. The oxysterol-activated transcription factor liver X receptor (LXR)a is a central regulator of lipid homeostasis which prevents atherosclerosis *in vivo*. In the present study we tested the hypothesis that LXRa positively regulates bilirubin homeostasis. HUVEC, HepG-2 cells and primary hepatocytes were treated with the synthetic LXRa agonist T091317 or endogenous oxysterols. Cells were then analyzed for mRNA and protein levels of relevant genes by real-time RT-PCR and Western blotting, respectively. In HUVEC, T091317 strongly up-regulated the rate limiting enzyme HO-1 at mRNA and protein levels in a time- and dose-dependent manner, whereas UGT1A1 was not expressed in vascular cells. On the other hand, LXRa agonists only moderately affected HO-1 expression in hepatic cells, but induced UGT1A1 as well as MRP-2. These observations suggest that LXRa exerts double actions on bilirubin homeostasis: First, it increases bilirubin synthesis in the vascular wall, thus favouring its anti-oxidant actions locally for controlling atherosclerosis. Second, LXRa stimulates hepatic bilirubin conjugation and excretion in the liver to protect the body against a toxic accumulation of this metabolite. Such coordinate regulation of bilirubin homeostasis may be considered as an additional mechanism by which LXRa exerts its athero-protective effects.

Funding: Heart & Stroke Foundation, IRSC

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22-HYDROXYCHOLESTEROLS REGULATE LIPID AND GLUCOSE METABOLISM IN HUMAN MYOTUBES

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The nuclear liver X Receptors (LXR)s are sensors of cholesterol metabolism and important regulators of lipid and glucose homeostasis. Oxysterols are known LXR ligands, but the functional role of oxysterols is unknown. This study showed that treatment with a synthetic LXR agonist (T0901317) increased lipogenesis, and that this effect could be counteracted by 22-S-hydroxycholesterol (22-S-HC). When incubated with 22-S-HC alone, de novo synthesis of diacylglycerol, cholesteryl ester and free cholesterol from acetate was reduced below baseline values. 22-S-HC also reduced fatty acid uptake and oxidation at the same time as glucose uptake and oxidation were increased. At the regulatory level, T0901317 and 22-R-hydroxycholesterol (22-R-HC) increased gene expression of LXRalpha and sterol regulatory element-binding protein 1c, while 22-S-HC had little effect. The expression of fatty acid synthase (FAS) and stearoyl-CoA desaturase-1 was increased by T0901317 and unchanged by 22-R-HC, while 22-S-HC markedly reduced mRNA expression of these genes. Both 22-S-HC and 22-R-HC decreased the expression of genes involved in cholesterol synthesis, hydroxymetylglutaryl-coenzym A synthase 1 and reductase, but only 22-R-HC increased the expression of the reverse cholesterol transporter ABCA1. Transfection studies confirmed that the FAS promoter was activated by T0901317, that 22-S-HC reduced reporter activity and counteracted the effects of T0901317. An LXR modulator with properties similar to 22-S-HC might therefore have a potential as model-substance for drugs modifying skeletal muscle lipid synthesis and accumulation and thus the development of insulin resistance.

Funding: This work was funded by the Norwegian Diabetes Foundation and the Nansen Foundation

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ACTIVATION OF LIVER X RECEPTORS IN THE BRAIN: REGULATION OF TARGET GENE EXPRESSION WITHOUT DISTURBING STEADY-STATE LIPID HOMEOSTASIS

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Objective: The present study was aimed at clarifying the impact of in vivo activation of LXRs on the brain lipid metabolism. **Methods**: C57Bl6 mice were treated orally with vehicle or TO901317, a synthetic LXR agonist which crosses the blood-brain barrier. Ex-vivo analyses included brain expression of the relevant genes (qRT-PCR, western blot), as well as brain and plasma lipid composition (HPLC, GC-MS). **Results**: Expression analysis revealed elevated levels of Abca1 and Abcg1 in the brains of TO901317 treated mice as compared to mice treated with vehicle, but decreased levels of SR-BI and no changes in the Abcg4 expression. Administration of TO901317 resulted also in increased brain expression of Apo-AI and ApoE, while no changes were observed in the expression of Cyp46a1, Lipg, Lpl, NPC2 and APP. Interestingly, Pltp expression in the brain appeared to be down-regulated by TO901317, and moreover, negatively correlated with brain ApoE and liver Pltp expression. Similarly, negative correlation was observed between APP and Abcg1 expression in the brain. Analysis of plasma lipid composition demonstrated elevated levels of total cholesterol, HDL-cholesterol and phospholipids in TO901317 treated mice versus mice receiving vehicle. Despite alternations in plasma lipid composition and in brain expression of genes involved in cholesterol turnover, no changes were observed in the brain levels of cholesterol, a-tocopherol and total fatty acids. **Conclusions**: Obtained results suggest that LXR activation in vivo promotes cholesterol recycling in the brain, without disturbing cerebral steady-state lipid homeostasis.

Funding: FWF P17474

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BILE DUCT LIGATION AMELIORATES HEPATIC STEATOSIS AND HYPERTRIGLYCERIDEMIA BY INHIBITING LIPOGENIC GENE EXPRESSION INDEPENDENTLY OF FXR-SHP ACTIVATION

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Non-Alcoholic Fatty Liver Disease is characterised by accumulation of triglyceride in the liver due to imbalance of fat metabolism likely related to insulin resistance. Little is known about the effect of bile acids and related nuclear receptor on these metabolic alterations. Aim of the present study was to analize the effects of cholestasis on NAFLD and on the expression of nuclear receptors involved in bile acid and lipid metabolism. **Methods:** 55 male Sprague-Dawley rats received either a high fat (HFD), a choline deficient (CDD) or control diet (CTR). Aftere 4 week treatment, bile duct ligation (BDL) or sham operation were performed. Serum lipids, leptin and insulin were assayed. Liver histology was scored for NAFLD and liver lipids were extracted with Folch metod. mRNA levels of nuclear receptors and lipogenic genes were analyzed by real-time RT-PCR **Results:** steatosis score was markedly higher in CDD (>50%) than in HFD (15-20 %) and CTR. HFD group displayed characteristics of insulin resistance, such as increased cholesterol, triglyceride, insulin and leptin levels ($p<0.05$, HFD-BDL vs sham). In BDL rats, expression of lipogenic genes (FAS, SCD1, ACC) were reduced and so were FXR and SHP. **Conclusion:** endogenous bile acids ameliorate diet induced hepatic steatosis and metabolic alterations via reduction of lipogenesis. Activation of FXR/SHP independent pathways is likely. The data may bring relevant implications on pathophysiology and treatment of NAFLD and hyperlipidemia associated with insulin resistance.

Funding: This work was supported by COFIN-PRIN grant 2004067491

Poster Session 7 "LIPID LOWERING THERAPY" - GROUP B

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THE INFLUENCE OF 2-YEAR SIMVASTATIN TREATMENT ON CAROTID ARTERY INTIMA MEDIA THICKNESS AND SOME BIOCHEMICAL PARAMETERS

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Objective: The objective of the work was to estimate the 2-year Simvastatin(Zokor) treatment effectiveness and its influence on the parameters of blood lipids, CRP, interleukins and D dimers as well as that on carotid arteries(CA) intima-medial thickness (IMT). **Methods:** 204 patients with hypercholesterolemia(7.3±0.06mmol/l) were investigated. Seropositivity to *Chlamidia Pneumoniae* IgG and IgM was fixed in 31 (15.2%) patients. **Results:** 2-year treatment with Simvastatin (40mg per day) showed that along with the Tch normalization, HDL cholesterol increased significantly and LDL cholesterol decreased. A well-defined tendency to Apo-A and Apo-B changes was observed as well. The CRP, IL-1, IL-6 values were noted to decreased, while IL-10 and TNF-α increased . In patients with seropositivity to Chlamidial infection, the IgG and IGM titers decrease was established. In 149 patients (73.5%), CA IMT decreased as well. **Conclusion:** The considerable improvement of the Interleukins, CRP and D-dimer values took plase as the result of the 2-year Simvastatin treatment under the blood lipid normalization. One should note as well the decrease seropositive titer to *Chlamidia Pneumoniae*. It is of utmost importance to establish the decrease of CA IMT under the treatment.The above studies enable us to think that within prolonged Simvastatin treatment(2 years), along with a stable hypolipidemic effect, the antiinflammatory and antitrombotic influence of the given medicine seems to take place.

Funding: None

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THE INFLUENCE OF ONE-YEAR TREATMENT WITH ATORVASTATIN, ROSUVASTATIN AND SIMVASTATIN ON INTIMA MEDIA THICKNESS IN PATIENTS WITH HYPERCHOLESTEROLEMIA

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Objective: To study the effect of one-year treatment of Atorvastatin, Rosuvastatin and Simvastatin on Intima media Thickness (IMT), interleukins, CRP. **Methods:** 146 patients with hyperlipidemia were investigated for studying this problem. The mean age of the patients varied within 55.4±10.7. They were divided into 3 groups. Group I (N=84) was treated with 20 mg Atorvastatin(Liprimar); Group II (N=10) with 10 mg Rosuvastatin (Krestor); Group III (N=52) with 40 mg Simvastatin (Zokor). B mode ultrasound scanning of carotid arteries was perfomed for each subject. The interleukins(IL-1, IL-6,) lipids and CRP were defined as well. **Results:** Under the treatment perfomed, the Tch and LDL were noted to decrease in the three groups, while HDL increased. The same tendency to decrease the mean values of IL-1,IL-6 and CRP is obsrrved. IMT decreased by 24.1, 26.1% and 23.4% . One should mention that despite a well-defined positive dynamics in the above three groups, it was more expressed in group III. **Conclusion:** One-year treatment of hypolipidemic patients with Atorvastatin, Rosuvastatin and Simvastatin resulted in the normalization of lipids, as well as in that of interleukins and CRP, but IMT decreased by 24-26%. Due to high hypolipidemic and unti-infammatory effects of the therapy, it is necessary to use them for a long time in the primary and secondary prophilaxis of the heart ischemic diseases.

Funding: None

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ATORVASTATIN INCREASES SOLUBLE ADIPONECTIN PLASMA LEVELS IN SUBJECTS AT HIGH CARDIOVASCULAR RISK

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Objective: Adiponectin can suppress atherogenesis by inhibiting the adherence of monocytes and the accumulation of modified lipoproteins in the vascular wall. Resistin mechanistically links metabolic syndrome and insulin resistance to atherosclerotic burden. **Methods:** Adiponectin and resistin plasma levels were measured in 102 statin-free subjects from the Spanish population of the Achieve Cholesterol Targets Fast with Atorvastatin Stratified Titration (ACTFAST) study, a 12-week, prospective, multi-centre, open-label trial which enrolled subjects with coronary heart disease (CHD), CHD-equivalent or a 10-year CHD risk > 20%. Subjects were assigned to atorvastatin (10-80 mg/day) based on LDL-C at screening. **Results:** Adiponectin levels were diminished in patients at high cardiovascular risk compared with age and gender-matched healthy subjects (N=40) [4166 (3661-4740) vs 5806 (4764-7075) ng/mL respectively; geometric mean (95% CI); p<0.0001]**.** No changes were noted in resistin levels [9.4 (8.5-10.5) vs 9.9 (8.9-11) pg/mL; respectively; N.S.]. In the whole population, atorvastatin treatment increased adiponectin levels [9.7 (3.2-16.7); % Change (95% CI); p=0.003]. This increment was in a dose-dependent manner; maximal effect with atorvastatin 80 mg/d [24.7 (5.7-47.1); p=0.01]. No effect was observed in resistin levels after atorvastatin treatment. Adiponectin concentrations were associated with total cholesterol/HDL-C before and after treatment with atorvastatin, but not with CRP. **Conclusions:** Atorvastatin increases adiponectin plasma levels in subjects at high cardiovascular risk, probably indicating novel anti-inflammatory effects of these drugs.

Funding: Study supported by Pfizer, Inc.

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DECREASED PLASMA LEVELS OF SOLUBLE TWEAK IN SUBJECTS AT HIGH CARDIOVASCULAR RISK ARE AUGMENTED BY ATORVASTATIN TREATMENT

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Objective: Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a novel member of TNF superfamily that may have evolved to guard against development of potentially harmful excessive inflammatory response. We reported that soluble TWEAK (sTWEAK) plasma levels are inversely associated with intima/media thickness in subjects with atherosclerosis (ATVB 2007;27:916-22). Now, we have evaluated the effect of atorvastatin treatment on sTWEAK levels in subjects at high cardiovascular risk. **Methods:** sTWEAK plasma levels were measured in 102 statin-free subjects from the Spanish population of the Achieve Cholesterol Targets Fast with Atorvastatin Stratified Titration (ACTFAST) study, a 12-week, prospective, multi-centre, open-label trial which enrolled subjects with coronary heart disease (CHD), CHD-equivalent or a 10-year CHD risk > 20%. Subjects were assigned to atorvastatin (10-80 mg/day) based on LDL-C at screening. **Results:** sTWEAK levels were decreased in patients at high cardiovascular risk compared with age and gender-matched healthy subjects (N=40) [266 (242-292) vs 458 (413-508) pg/mL respectively; geometric mean (95% CI); p<0.0001]**.** In the whole population, sTWEAK levels were increased by atorvastatin treatment [18.1 (8.3-28.8); % Change (95% CI); p=0.0003]. All doses of atorvastatin augmented sTWEAK concentrations. sTWEAK levels were not associated with different lipid parameters analyzed or CRP values before or after treatment with atorvastatin. **Conclusions:** Atorvastatin increased sTWEAK plasma levels in subjects at high cardiovascular risk, probably indicating a novel anti-inflammatory effect of statins.

Funding: Study supported by Pfizer, Inc.

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ATORVASTATIN POSSESS HYPOLIPIDEMIC AND ANTI-INFLAMMATORY EFFECTS IN ApoE/LDLR DOUBLE-KNOCKOUT MICE

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The statins represent golden standard in dyslipidemia treatment in humans. However, its effects in animal models of atherosclerosis are very inconsistent. Thus, the aim of this study was to evaluate whether atorvastatin has hypolipidemic and inflammatory effects in apoE/LDLR deficient mice. Female ApoE/LDL-deficient mice at 8 weeks of age were divided into 2 groups. The control group was fed with the atherogenic diet for 8 weeks. In atorvastatin group atorvastatin was added to the diet at the dosage of 100 mg/kg per day. Biochemical analysis of blood cholesterol fractions, ELISA analysis of MCP-1 in blood, and immunohistochemical and western blot analyses of VCAM-1 and ICAM-1 expression in aortic sinus were performed. The biochemical analysis showed that administration of atorvastatin significantly decreased level of TC, VLDL, LDL, TAG, and moreover significantly increased level of HDL. ELISA analysis showed that atorvastatin significantly decreased levels of MCP-1 in blood. Atorvastatin treatment resulted in a significant reduction of VCAM-1 and ICAM-1 expression in the vessel wall. In conclusion, this study demonstrates that atorvastatin has hypolipidemic and anti-inflammatory affects in apoE/LDLR deficient mice suggesting these mice might represent animal model for the study of statins effects in experimental atherogenesis.

Funding: This work was supported by Alltracel Pharma Ltd. and Zentiva a.s.

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ATORVASTATIN 80MG REDUCES RISK OF CARDIOVASCULAR EVENTS BEYOND OCCURENCE OF THE FIRST EVENT: ANALYSIS OF THE IDEAL TRIAL

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Background: Endpoints of statin trials generally refer to time to occurrence of first event but a significant number of all events occur subsequent to the first. In the IDEAL trial, which compared intensive statin therapy (atorvastatin 80 mg, A80) to standard therapy (simvastatin 20-40 mg, S20-40) in 8888 CHD patients, the primary endpoint of major coronary event (MCE, time to first occurrence of nonfatal myocardial infarction, CHD death, or resuscitated cardiac arrest) was reduced by 11%, p=0.07, with A80. The secondary endpoint of any cardiovascular (CV) event (time to first occurrence of MCE, stroke, unstable angina, hospitalization for congestive heart failure, or peripheral artery disease) was significantly reduced by 16%, p<0.0001, with A80. We hypothesized that A80 also resulted in reduction of risk of any CV event that occurred subsequent to the first event. **Methods:** Posthoc time-to-event analysis to estimate the treatment hazard ratio separately for the time to 1st, 2nd, 3rd, 4th, and 5th any CV event. **Results:** 1048 patients had a 2nd CV event, 416 had a 3rd, 192 had a 4th and 93 had a 5th event. Patients randomized to A80 compared to those to S20-40 had reduction in the relative risk of 2nd event by 24% (p<0.0001), of 3rd event by 19% (p=0.03), of 4th event by 24% (=0.06) and of 5th event by 28% (p=0.12). **Conclusions:** Over time, beyond the reduction in risk of first CV event, A80 continued to reduce risk of any CV event more than standard therapy with S20-40.

Funding: Pfizer

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PERSISTENCE AND COMPLIANCE AMONG PATIENTS WITHOUT PRIOR CARDIOVASCULAR EVENTS INITIATING ATORVASTATIN OR SIMVASTATIN THERAPY

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Objectives: To examine differences in persistence and compliance in patients without prior CV events initiating atorvastatin (ATV) or simvastatin (SIM) therapy, using integrated medical and pharmacy claims data, a nationally representative data of more than 90 managed care plans **Methods:** Patients were new statin users, ≥18 years of age, treated with ATV or SIM from 1/2003-9/2005, who were continuously enrolled in a covered plan for ≥12 months before and after therapy initiation. Patients included did not have a CV event during the one year pre-treatment period. Persistence was the days of therapy between the first and last prescription; the maximum gap allowed between prescriptions was 60 days. Compliance using Medication Possession Ratio compared days of medication supplied while on therapy to the total number of days persistent on therapy. Differences in the persistence and compliance were tested by univariate techniques **Results:** Median persistence was approximately 50 days longer for patients initiating therapy with ATV versus SIM (207 days vs 157 days, respectively; p<0.0001). Median compliance was similar between the ATV (86%) and SIM (85%) treatment groups. Among patients ≥65 years of age enrolled in a Medicare Advantage plan, ATV patients remained on therapy approximately 45 days longer than SIM patients (150 days vs 104 days; p<0.0001), although compliance rates were similar for ATV (82%) and SIM (83%) **Conclusion:** In patients without prior CV events, treatment persistence was longer with ATV versus SIM, although compliance was similar for ATV and SIM.

Funding: This study was funded by Pfizer Inc., New York, NY

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STATIN UTILIZATION AMONG PATIENTS WITH PRIOR CARDIOVASCULAR EVENTS

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Objectives: To examine statin utilization among patients with prior CV events who initiated atorvastatin (ATV) or simvastatin (SIM) therapy, using integrated medical and pharmacy claims data, a nationally representative data of ≥90 managed care plans. **Methods:** Patients were ≥18 years, newly-treated with ATV or SIM from 1/2003-9/2005, and continuously enrolled in a covered plan for ≥12 months before and ≥30 days after therapy initiation. Persistence was days of therapy between first and last prescription; the maximum gap allowed between prescriptions was 60 days. Compliance using Medication Possession Ratio compared days of medication supplied while on therapy to the total number of days persistent on therapy. Differences in persistence and compliance were tested by univariate techniques. **Results:** A total of 16,827 patients were selected; mean age was 56 years, 70% were male. The majority of patients had a prior CV event of heart attack (42%) or angina/CAD (50%). Two-thirds of patients initiated therapy with ATV 10-20mg and, of the patients initiating therapy with simvastatin, 93% began treatment with SIM ≤40mg. Among patients with 12 months of follow-up available (n=11,331), median persistence was <9 months, but was significantly longer among ATV patients (266 days vs 181 days; p<0.0001). Median compliance was similar for ATV (91%) and SIM (92%), (p=0.5613). **Conclusions:** These data suggest that higher risk patients may be under-dosed for statin therapy. There is also need to improve the persistence with statin therapy among this population, although persistence was greater for ATV patients compared to SIM patients.

Funding: This study was funded by Pfizer Inc., New York, NY

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RELATIONSHIP BETWEEN CD40 LIGAND UPREGULATION AND SUPEROXIDE ANION PRODUCTION IN HYPERCHOLESTEROLEMIA. EFFECT OF ATORVASTATIN

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Introduction: We investigated if in hypercholesterolemia overexpression of platelet CD40 ligand (CD40L), may depend on enhanced intraplatelet formation of superoxide anion(O2-) and therefore if atorvastatin could directly affects platelet CD40L expression via O2- production downregulation. **Methods:** We compared 40 patients with hypercholesterolemia and 40 sex- and age-matched controls. Hypercholesterolemics were then randomized to either a diet or atorvastatin 10 mg/day for three days. Lipid profile, CD40 ligand platelet expression. and O2- production were measured at baseline and after 3 days of treatment. **Results:** Compared with controls hypercholesterolemics had enhanced production of O2- and higher platelet expression of CD40L . Platelet CD40L significantly correlated with platelet O2- (r=0.79) Both groups (diet and atorvastatin) did not show any changes in lipid profile after 3 days of treatment . In diet group , no changes in platelet CD40L expression and O2- production was observed. In atorvastatin group, a significant decrease in platelet CD40L (48.6 ± 15.2 vs 34.1 ± 7,3 AU) and platelet O2- (4.2±0.5 vs 2.5±0.3 SI) was observed after three days. Atorvastatin dose-dependently significant decreased in vitro platelet O2- production and CD40L expression. **Conclusion:** This study provides first evidence that in hypercholesterolemia, platelet CD40L overexpression may be mediated by enhanced platelet O2- production.Atorvastatin has a direct antioxidant effect that may accounts for reduced platelet CD40L downregulation.

Funding: MURST 06

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COADMINISTRATION OF LAPAQUISTAT ACETATE (TAK-475, LAPA), A NOVEL SQUALENE SYNTHASE INHIBITOR, WITH ATORVASTATIN INCREMENTALLY LOWERS NONHDL CHOLESTEROL

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Objective: Although statins (HMG-CoA reductase inhibitors) are effective agents for treating hypercholesterolemia, many patients fail to achieve target LDL cholesterol levels. The objective of this study was to determine whether the combination of lapaquistat acetate (LAPA), a potent squalene synthase inhibitor, with a statin might prove superior to either drug alone in lowering nonHDL-C in the guinea pig, a useful animal model of lipoprotein metabolism. **Methods:** Guinea pigs fed with high fat diet were administered LAPA (30mg/kg), atorvastatin (ATV, 10mg/kg), or both agents for 14 days; plasma nonHDL-C levels were then measured. The LDL fractional catabolic rate (FCR) was calculated following injection of fluorescence-labeled LDL. **Results:** Each agent significantly decreased nonHDL-C levels compared with control (LAPA: -37%, ATV: -33%). LAPA- and ATV-treated guinea pigs exhibited 53% and 37% increases in LDL FCR, respectively. The combination of LAPA plus ATV resulted in greater reductions in nonHDL-C (-48%), concurrent with a 74% increase in the LDL FCR, compared with control. LAPA, ATV, and the combination treatment increased mRNA expression of hepatic LDL receptor by 1.5-, 1.7-, and 2.2-fold, respectively. In HepG2 cells, the combination treatment incrementally enhanced ^{125}I-LDL binding, compared with LAPA or ATV alone. **Conclusions:** The combination of LAPA and ATV produced a greater reduction of nonHDL-C levels than either agent used alone, effects that are most likely mediated by enhanced LDL receptor expression and activity. These results demonstrate that lapaquistat acetate holds promise for use in combination with statins for the management of hypercholesterolemia.

Funding: None

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STATIN AND FIBRATE CHRONIC TREATMENTS: MOLECULAR MECHANISMS INVOLVED IN THE ALTERATION OF CLC-1 CHLORIDE CONDUCTANCE IN RAT SKELETAL MUSCLE

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Objective: Lipophilic statins and fibrates cause a reduction of the membrane resting chloride conductance (gCl) in rat skeletal muscle (Pierno et al., Br J Pharmacol. 149: 909, 2006). Resting gCl, sustained by ClC-1 chloride channel, plays an important role in the membrane repolarisation and is negatively regulated by a Ca2+-dependent protein kinase C (PKC). Here we investigate on the mechanism responsible for the reduction of gCl that probably causes muscle damage. **Methods**: Two-intracellular microelectrode technique, patch clamp and RT-PCR were used. **Results**: The *in vitro* application of chelerythrine, the PKC inhibitor, partially restored gCl toward the control value in muscle dissected from rats treated with 5 and 20mg/kg/day fluvastatin suggesting the involvement of PKC in statin action. In contrast, chelerythrine did not antagonize the reduction of gCl induced by 60 mg/kg/day fenofibrate chronic treatment. Accordingly, cytosolic Ca2+ increased in muscle of chronically fluvastatin treated rats, but not in those treated with fenofibrate (Liantonio et al., J Pharm Exp Ther. 321:626, 2007). Preliminary data showed that the reduction of gCl paralleled a decrease in ClC-1 mRNA expression in both fluvastatin and fenofibrate treated animals. Fenofibric acid, but not fluvastatin reduced chloride current recorded from ClC-1 expressed in Xenopus Oocytes. **Conclusion**: Fluvastatin can affect PKC regulatory pathway finally modulating gCl, while fenofibrate is responsible for a direct block of the ClC-1 channel.

Funding: FIRB-RBAU015E9T

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ANALYSIS OF THE RENAL SAFETY OF LONG-TERM ATORVASTATIN USE IN A BROAD SPECTRUM OF PATIENTS

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Objectives: In recent years, there has been increased scrutiny on all safety aspects of long-term statin therapy, including renal adverse events (AEs). In the current analysis, the long-term renal safety of atorvastatin (ATV) was investigated using data from placebo (PBO)-controlled clinical trials **Methods:** A broad range of patients at varying risk of CV events from 4 long-term (≥1 year in duration), PBO-controlled clinical trials of ATV were pooled for this analysis. The incidence of hematuria and albuminuria was analyzed in 10,533 patients (mean age 61.5 years , 58.2% male, 55.2% with diabetes, 40.4% with CKD) treated with ATV 10-80 mg (n=5467, of whom 2479 received ATV 80 mg) or PBO (n=5066) for a median follow-up of 4.0 years. The incidence of overall AEs was analyzed in patients with or without chronic kidney disease (CKD) at baseline. **Results:** Renal AEs were rare and the incidence was similar between the ATV and PBO groups. Albuminuria occurred in 1.5% of ATV patients and 1.3% of PBO patients, and hematuria occurred in 1.9% of ATV patients and 1.7% of PBO patients. Reports of serious AEs were similarly low for both albuminuria (0.6% ATV, 0.5% PBO) and hematuria (0.8% ATV, 0.9% PBO). In patients with CKD and patients with normal eGFR, overall incidences of AEs, serious AEs, and discontinuations due to AEs were similar between the ATV and PBO groups. **Conclusions:** In patients treated with ATV, renal AEs were experienced infrequently, with an incidence that was comparable to PBO. Patients with CKD were not at any increased risk of treatment related AEs compared with patients with normal eGFR. These data demonstrate that long-term treatment with ATV 10-80 mg does not present any renal safety concerns in a broad spectrum of patients across a range of renal function.

Funding: This study was funded by Pfizer Inc., New York, NY

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EFFECT OF EZETIMIBE/SIMVASTATIN VS ATORVASTATIN ON ATTAINMENT OF OPTIONAL RECOMMENDED LEVELS OF LDL-C, APOB, NON-HDL-C AND HSCRP IN TYPE 2 DIABETES PATIENTS: VYTAL STUDY

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Objective: In addition to LDL-C, non-HDL-C, ApoB and hsCRP are considered to be predictive risk factors for cardiovascular disease (CVD). This study assessed the proportion of type 2 diabetes (T2DM) patients treated with ezetimibe/simvastatin (E/S) versus atorvastatin (A) who attained the optional target of LDL-C (<70 mg/dL) and one of the following: ApoB<90 mg/dL, hsCRP<2 mg/L or non-HDL-C<100 mg/L (patients with TG ≥200 mg/dL). **Methods:** Post-hoc analysis of a multicenter, randomized, double-blind, 6 wk parallel study in T2DM patients with hypercholesterolemia (HC). % of patients who attained optional target levels at E/S(10/20, 10/40mg) vs A(10, 20, 40mg) was assessed by logistic regression. **Results:** A significantly higher % of patients treated with E/S than A attained optional recommended levels of LDL-C and ApoB, LDL-C and non-HDL-C, and LDL-C and hsCRP, as well as single target levels for LDL-C, ApoB and non-HDL-C at all doses compared. % attainment of hsCRP<2 mg/L was also higher at E/S 10/20mg vs A10 or 20mg, and was comparable at E/S 40mg and A40mg. **Conclusion:** Significantly higher proportions of patients attained optional recommended levels of LDL-C and targets set for ApoB, non-HDL-C, and hsCRP with E/S compared to A among T2DM patients with HC. Thus, E/S provides a therapeutic option to T2DM patients for reaching treatment goals in the reduction of CVD risk.

Funding: Merck/Schering-Plough Pharmaceuticals, North Wales, PA, USA

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EFFECTS OF EZETIMIBE/SIMVASTATIN VS. ROSUVASTATIN ON LOWERING LDL-C AS WELL AS APOLIPOPROTEIN B, NON-HDL-C, OR C-REACTIVE PROTEIN

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Objectives: To evaluate the % patients who attained LDL-C<70mg/dL and specific levels of other or emerging risk factors (ApoB<90 mg/dL, CRP<2.0 mg/L, or non-HDL-C<100 mg/dL). **Methods**: This double-blind, 6-week, parallel group trial randomized hypercholesterolemic patients to ezetimibe/simvastatin (E/S:10/20, 10/40, 10/80mg) or rosuvastatin (R:10, 20, 40mg); treatment comparisons were between usual starting, next highest, maximum, and pooled doses. Non-HDL-C was examined in patients with triglycerides (TG) ≥200mg/dL. **Results**: Groups had similar baseline characteristics. At most dose comparisons, a significantly higher % patients receiving E/S vs. R achieved LDL-C<70mg/dL and specified levels of other risk factors, both individually and in combination with the LDL-C goal. For example, in pooled dose comparisons, % patients achieving (LDL-C<70mg/dL + CRP<2.0mg/L): E/S 29.1% vs. R 17.3%; (LDL-C<70mg/dL + ApoB<90mg/dL): E/S 41.2% vs. R 27.5%; (LDL-C<70mg/dL+non-HDL-C<100mg/dL): E/S 30.7% vs. R 22.6% (P<0.001 for all three). Exceptions were no significant treatment differences for ApoB<90mg/dL alone and for (LDL-C<70mg/dL + non-HDL-C<100mg/dL) comparing E/S 10/40mg vs. R 20mg, and none for CRP<2.0mg/L alone at all dose comparisons. **Conclusion**: The potentially greater efficacy of E/S compared with R extends to modifications of some other and emerging risk factors. Ultimate clinical implications of these findings still need to be defined.

Funding: Merck & Co., Inc.

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EZETIMIBE/SIMVASTATIN MORE FAVORABLY INFLUENCES LIPOPROTEIN/APOLIPOPROTEIN RATIOS THAN ATORVASTATIN IN PATIENTS WITH TYPE 2 DIABETES

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Objectives: Lipoprotein and apolipoprotein ratios may reflect the balance of cholesterol delivery and removal at the arterial wall and provide assessment of CHD risk that is supplemental to LDL-C, the primary guide for cholesterol-lowering therapy. In this study comparing ezetimibe/simvastatin (E/S) with atorvastatin (A) in patients with Type 2 Diabetes Mellitus(T2DM), changes in the ratios LDL-C/HDL-C, total-C/HDL-C, non-HDL-C/HDL-C, and Apo B/Apo A-1 were assessed. **Methods:** This randomized, double-blind, parallel-group study enrolled T2DM patients with LDL-C ≥100 mg/dL for 6-week treatments with either usual daily starting doses (A 10 or 20mg, E/S 10/20mg) or next highest doses (A 40mg, E/S 10/40mg). Changes in the above ratios were prespecified exploratory endpoints and were analyzed using ANOVA. **Results:** Efficacy results were based on 1198 patients with sufficient data among 1229 patients randomized. Baseline characteristics of treatment groups were comparable. E/S produced significantly greater reductions compared with A in each lipoprotein or apolipoprotein ratio at each dose comparison (P<0.001). For example, reductions from baseline in total-C/HDL-C were A 10mg, -30.2%; A 20mg, -34.9%; E/S 10/20mg, -41.6%; A 40mg, -37.9%; E/S 10/40mg, -43.5%. Tolerability of the two treatments was similar. **Conclusions:** For the doses assessed, E/S was more effective compared with A in lowering lipoprotein and apolipoprotein ratios that might be considered secondary measures of CHD risk.

Funding: Merck & Co., Inc.

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EFFECTS OF ORLISTAT-EZETIMIBE COMBINATION THERAPY ON SERUM LIPID PROFILE IN OVERWEIGHT AND OBESE PATIENTS WITH HYPERCHOLESTEROLEMIA

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Objective: The aim of our study was to evaluate in an open-label randomized trial the effects of treatment with orlistat, ezetimibe and orlistat plus ezetimibe on serum lipid profile in overweight and obese patients with hypercholesterolemia (LDL-C>160 mg/dl). **Methods:** Sixty three patients were enrolled. All patients received a low fat low-calorie diet and were randomized to receive orlistat 120 mg three times/day (n=21, group O), ezetimibe 10 mg/day (n=21, group E) or a combination of orlistat 120 mg three times/day and ezetimibe 10 mg/day (n=21, group O+E). Anthropometric and lipid parameters were assessed before and after three months of treatment. **Results:** Body weight, body mass index (BMI) and waist circumference were significantly reduced in O and O+E groups (p<0.05). The levels of total cholesterol (TCHOL), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were significantly decreased in all treatment groups (p<0.05).The reduction in TCHOL was greater in the combination group (-28%) compared to O and E monotherapy (-16% and -18%, respectively),{p<0.05 vs O and E groups}. In the O+E group a more pronounced reduction in LDL-C (-34%) was observed compared with that in the O and E groups (-18% and -19%, respectively),{p<0.05 vs O and E groups}. **Conclusion:** Orlistat plus ezetimibe administration can substantially improve anthropometric characteristics and serum lipid parameters in overweight and obese patients with hypercholesterolemia.

Funding: None

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EZETIMIBE: FROM NON-RESPONDER TO HYPER-RESPONDER IN THE MORE JUDICIOUS MANAGEMENT OF PATIENTS

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Ezetimibe, the cholesterol transport inhibitor of the Niemann-Pick C One Like One Protein (NPC1L1), is the first of a new class of drugs for lipid lowering. A practical approach to lipid lowering would focus on either absorption or synthesis as the primary physiology for increased lipids. The purpose of the present study was to determine if management decisions could be based on the extent of response to ezetimibe alone as a guide to mono or combination therapy. Ezetimibe, either as a single drug or in addition to previous therapy, was evaluated in 146 patients at the Victoria Lipid Clinic from 06/2003 to 10/2006 to determine the scope of change on various lipid parameters. Total cholesterol, HDL-C,LDL-C, and triglycerides with ratios were evaluated. The follow-up at <28d, >28-56d and >56d was assessed. The triglycerides and HDL-C were virtually unchanged in both men and women, however there was marked individual variation in the response to total cholesterol and LDL-C lowering (increases of 10% to decreases of 70%). The variation in lipid lowering is consistent with our present knowledge of the NPC1L1 protein polymorphisms. Individual LDL-C patient response to ezetimibe is evaluated as non-effective or non-responsive (<15%), minimally responsive (60%), and moderately or hyper responsive(>25%). Ezetimibe may be a non-strategy for some 15%, used as part of combination therapy in 60% and as monotherapy in 25% of patients. In summary, ezetimibe provides an opportunity to assess the NPC1L1 protein status, categorize the response and provide a therapeutic approach that is tailored to the individual patient.

Funding: Support of Merck, Schering, Astra Zeneca, Pfizer, and Fournier for the Victoria Lipid Clinic is acknowledged

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ASSOCIATION BETWEEN BASELINE LIPID LEVELS AND LIPID-ALTERING AND HSCRP EFFICACY OF EZETIMIBE/SIMVASTATIN VS ATORVASTATIN IN TYPE 2 DIABETES PATIENTS: VYTAL STUDY

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Objective: Baseline LDL-C, TG and HDL-C levels are important factors to consider in treating dyslipidemia in type 2 diabetes (T2DM) patients. Association of these factors with % changes from baseline in LDL-C, HDL-C, TG, non-HDL-C, ApoB, ApoA-I, and hsCRP, and % attainment of LDL-C<70 and <100mg/dL was assessed in hypercholesterolemic, T2DM patients treated with ezetimibe/simvastatin (E/S) vs atorvastatin (A). **Methods:** Association of baseline levels with efficacy measures was assessed in a multicenter, randomized, double-blind, 6 wk parallel study at E/S(10/20, 10/40mg) vs A(10, 20, 40mg). **Results:** Greater % reductions in LDL-C, non-HDL-C and ApoB, and lower LDL-C target attainment were generally associated with increasing LDL-C stratum. Baseline TG≥150 mg/dL was associated with greater % increases in HDL-C and decreases in TG vs TG<150 mg/dL; hsCRP reductions were greater at TG<150 mg/dL. Larger % increases in HDL-C and ApoA-I, smaller % decreases in non-HDL-C, and generally greater % decreases in TG were observed at baseline HDL-C<40 and <50 vs ≥40 and ≥50 mg/dL (men and women respectively). Subgroup treatment differences were consistent with the significantly greater effects of E/S vs A in the whole cohort. **Conclusions:** Baseline LDL-C, TG and HDL-C levels may impact lipid treatment in T2DM patients and should be considered in the management of dyslipidemia in this setting.

Funding: Merck/Schering-Plough Pharmaceuticals, North Wales, PA, USA

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EZETIMIBE TOGETHER WITH ANY STATIN: CHOLESTEROL ENHANCEMENT AND SAFETY IN CLINICAL PRACTICE

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Objective: Adding ezetimibe to ongoing statin therapy leads to substantial additional reduction in LDL-cholesterol (LDL-C) levels. We report the results of an open, cluster randomized trial evaluating the impact of ezetimibe and counseling on diet and/or physical exercise. **Methods:** The trial enrolled high cardiovascular risk patients with LDL-C ≥ 3 mmol/L (115 mg/dL) despite statin therapy. Physicians were randomly assigned to counsel their patients regarding diet (group 1), physical activity (group 2) or both (group 3) and ezetimibe, 10mg once daily, was added to the ongoing statin in all patients during 6 to 10 weeks. **Results:** A total of 1496 patients, recruited by 428 physicians, received ezetimibe. 1411 patients were included in the Intent To Treat analysis. At baseline, LDL-C was 149 ± 35 mg/dL. At end-point, the mean LDL-C lowering efficacy was -30.4 ± 19.3% (group 1: -29.6%; group 2: -31.6%; group 3: -29.8%; p=0.59 between groups) and 61.6% achieved target goals (total cholesterol < 190mg/dl; LDL-C < 115mg/dl). CK ≥ 5 times the upper limit normal (ULN) were observed in 1 patient at baseline and in 2 patients on statin+ezetimibe, transaminases ≥ 3 x ULN in 4 patients at baseline and in 1 patient on statin + ezetimibe. No rhabdomyolysis was reported. Myalgia or cramps were recorded in 38 (2.6%) patients on statin+ezetimibe. Treatment was stopped due to side effect in 50 (3.3%) patients. **Conclusion:** These results confirm the good efficacy, short term tolerability and safety of ezetimibe added on lifestyle changes and statin treatment.

Funding: This work was supported by a grant of MSP France

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EFFICACY AND SAFETY OF THE MTP-INHIBITOR, AEGR-733, AS MONOTHERAPY AND IN COMBINATION WITH EZETIMIBE

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Patients that cannot tolerate statin therapy have few options for robust LDL-C reduction. Microsomal triglyceride transfer protein (MTP) represents a potential new target for lipid-lowering therapy. No human studies have documented the safety and additive efficacy of combining a MTP-I with a cholesterol absorption inhibitor (CAI), a potential option for the statin intolerant. In this randomized, double-blind, active-controlled study, we studied the efficacy and safety of the MTP-I AEGR-733 alone to ezetimibe (EZE) alone and in combination with EZE. 84 patients were randomized to one of 3 arms: AEGR-733 alone, EZE 10 mg alone, or the combination of AEGR-733+EZE 10 mg. Patients randomized to AEGR-733 were titrated from 5 mg to 7.5 mg to 10 mg at 4-week intervals. Patients randomized to EZE maintained 10 mg throughout the 12-week study. The primary endpoint was the % change in LDL-C from baseline. Secondary endpoints included changes in TC, TGs, HDL-C and non-HDL-C. All adverse events (AEs) were captured. Patients receiving AEGR-733 had reductions in LDL-C of 18%-30% at 5-10 mg as compared to 20-22% for EZE 10 mg and 35-46% for AEGR-733+EZE. Discontinuations due to AEs were 4, 8 and 4 patients in the EZE, AEGR-733 and combination arms, respectively. These results demonstrate the potential for MTP-I as a new treatment option for dyslipidemia. In particular, the high degree of additivity and good tolerability of AEGR-733+EZE offers statin-intolerant patients robust statin-like efficacy.

Funding: This study was funded by Aegerion Pharmaceuticals

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NON-ABSORBABLE CHOLESTEROL INHIBITOR AVE5530 IS MORE EFFECTIVE IN PREVENTING ATHEROSCLEROSIS THAN EZETIMIBE IN APOE*3LEIDEN MICE

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Objectives: Cholesterol uptake inhibitor ezetimibe (EZE) is used as a cholesterol lowering drug. EZE is nearly 100% absorbed in the intestine and is active on cholesterol transport in macrophages. The consequence of this systemic activity is unclear. Novel cholesterol absorption inhibitor AVE5530 (AVE) is not absorbed. We compared the anti-atherosclerotic activities of AVE and EZE. **Methods:** APOE*3Leiden mice were fed a cholesterol-raising diet alone or supplied with AVE or EZE (both 0.3 mg/kg bw/day) for 20 weeks. Effects on plasma lipids, levels of pro-inflammatory markers and atherosclerosis were assessed. **Results:** AVE and EZE lowered plasma cholesterol (-62% and -39%, respectively; $p<0.001$) and both had positive effects on inflammation markers, as indicated by reduced plasma levels of SAA (-69% and -60%), VCAM-1 (-28% and -24%), E-selectin (-32% and -30%), MCP-1(-38 % and 43%,) and IL-1β (all $p<0.002$, respectively). Also, AVE reduced fibrinogen levels (-32%; $p = 0.009$). AVE strongly inhibited atherosclerosis development, as indicated by reductions in lesion size (-93%), percentage of severe lesions (-58%) lesion number (-61%) and by an increase of undiseased segments (7.3-fold) (all $p<0.001$). EZE only reduced atherosclerosis by 59% ($p<0.001$). AVE differed significantly from EZE in all atherosclerosis parameters. **Conclusions:** We show that AVE has strong anti-inflammatory effects, most likely caused by its strong cholesterol-lowering properties and is clearly more potent in preventing atherosclerosis development as compared to EZE.

Funding: Sponsored by a grant from sanofi-aventis Pharma

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IMPACT OF THE MTP INHIBITOR AEGR-733 ON PHARMACOKINETICS OF STATINS

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Objective: Statins are first-line therapy for hypercholesterolemia, but high-risk patients may not reach LDL-C goals on statins alone. AEGR-733 is a microsomal triglyceride transfer protein (MTP) inhibitor, which reduces LDL-C by a novel mechanism. In vitro studies suggest that AEGR-733 may inhibit the cytochrome p450 3A4 isoenzyme. We performed a pharmacokinetic (PK) study to determine if AEGR-733 affects the metabolism of statins. **Methods:** Healthy volunteers received a single dose of either atorvastatin 20 mg (n=16), simvastatin 20 mg (n=15), or rosuvastatin 20 mg (n=10) on day 1 followed by a 7-day course of AEGR-733 10 mg daily. On day 8 subjects received the final dose of AEGR-733 and another dose of the day 1 statin. On days 1 and 8 blood samples were collected over 24 hours to determine the single-dose PK profile of the statin. Primary PK measures were area under the curve (AUC) and Cmax. Adverse events (AEs) and efficacy data were also collected. **Results:** AEGR-733 led to a modest increase in AUC(0-24) and Cmax for simvastatin [+39% (90% CI, +10%-+76%) and +35% (90% CI,+11%-+64%)], but did not significantly impact AUC(0-24) and Cmax for atorvastatin [+14% (90% CI, -4% - +36%) and +32%, (90% CI, -5% - +81%)], or rosuvastatin [+2% (90% CI, -14%-+21%) and +6% (90% CI -24%-+48%)]. AEGR-733 resulted in LDL-C reductions of ~30%, few mild GI AEs and no clinically meaningful change in liver enzymes. **Conclusions:** AEGR-733 did not have clinically significant effects on the PK of the statins tested, which supports the potential for combined use of AEGR-733 and statins.

Funding: Aegerion Pharmaceuticals

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METABOL: NEW MECHANISMS OF LIPID LOWERING INTERVENTION

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In experiments *in vitro* and on Zucker Fatty rats it was demonstrated that the dietary supplement product Metabol could effectively interfere in the expression of genes involved in the control of lipid metabolism by: - suppressing the insulin signalling pathway at phospho-AKT and insulin receptor substrate-2 (IRS-2) levels; - suppression of the transcription of the major genes regulating biosynthesis of cholesterol and triglycerides in liver homeostasis – SREBP-1 and SREBP-2; - enhancement of the transcription of the major genes regulating biosynthesis of cholesterol; - repression of transcription of major cholesterol sensing genes – HMG-CoA reductase, insulin-inducible gene1 and sterol cleavage activating protein (SCAP). The suppression of insulin-induced hepatic lypogenesis, combined with increased expression of LDL-receptors in rats fed by Metabol only for 8 days resulted in: - lowering of insulin level in plasma from 16.3 ± 2.4 to11.2 ± 0.8; - lowering of body weight from 420 ± 13.5g to 368 ± 32.3g, and liver weight from 19.0 ± 2.8g to 14.2 ± 1.3g, suggesting a positive effect of Metabol on steatosis; - reduction of plasma total cholesterol from 236 ± 122.7 to 83.7 ± 16.2, and reduction of plasma triglycerides (TG) from 1,974 ± 328 to 333 ± 107. These results and the fact that there is an absence of any known side-effects from the active ingredients of Metabol, indicate that this product could be safely recommended for further clinical lipid-lowering trials in patients with Metabolic Syndrome and Insulin Resistance.

Funding: CamMEDICA Ltd.

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SLX-4090-REPEAT DOSE TREATMENT WITH A NOVEL ENTEROCYTE SPECIFIC MTP INHIBITOR REDUCES THE POST-PRANDIAL TRIGLYCERIDE RESPONSE AND LOWERS LDL-C IN HEALTHY VOLUNTEERS

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Introduction: SLx-4090 is a small molecule designed to inhibit microsomal triglyceride transfer protein (MTP) in enterocytes without being systemically available so avoiding toxicity such as fatty liver associated with other drugs active against MTP. Inhibiting MTP in enterocytes reduces the formation and secretion of chylomicrons lowering the absorption of triglyceride and cholesterol. Intestinal specific MTP inhibitors should have benefits in dyslipidemia, obesity and the metabolic syndrome. **Design:** 10 cohorts of 12 healthy male volunteers were studied in an inpatient setting with controlled dietary intake. Randomized to placebo (n=3) and active (n=9) all subjects received placebo on day 1. Cohorts 1-4 took 50, 100, & 200 mg tid, and 200 mg bid from days 2-15. Cohorts 5-10 took 100, 50, & 25 mg bid and 200, 100, & 50 mg qd from days 2-6. Primary endpoints were safety and tolerability, secondary endpoints were PK, changes in lipid parameters and bowel function. **Results:** SLx-4090 produced significant reductions of ≈50% in AUC for post-prandial triglycerides compared to placebo and a reduction in LDL-C. Cohorts 9 & 10 were no different from placebo. SLx-4090 was well tolerated in all groups. There were no serious AE's and no withdrawals for drug related reasons. All AE's were mild or moderate. No SLx-4090 was detected in the plasma at any dose (LOQ 10 ng/ml) and there were no clinically relevant increases in LFT's. **Conclusion:** Repeat doses of SLx-4090 were well tolerated up to 200 mg tid for 14 days and reduced LDL-C and post-prandial triglyceride rises after 5 days of dosing down to 25 mg bid and 200 mg qd. SLx-4090 is currently in phase 2a patient studies.

Funding: Surface Logix Inc.

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PHARMACOKINETICS AND PHARMACODYNAMICS OF SINGLE DOSES OF MK-0859, A POTENT CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) INHIBITOR, IN HEALTHY SUBJECTS

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Objective: The pharmacokinetics (PK) and pharmacodynamics (PD) of MK-0859, a potent CETP inhibitor in development for dyslipidemia, was evaluated. **Methods**: Double-blind, randomized, placebo-controlled study in healthy subjects included a rising single oral dose design with 2-1000 mg doses of MK-0859 and a 3-period, randomized, crossover design to evaluate the effect of food on the PK of a single oral 125 mg dose of MK-0859. Safety, tolerability, MK-0859 PK and CETP activity were evaluated. Blood pressure was carefully assessed. **Results**: MK-0859 was rapidly absorbed with peak concentrations occurring at ~4 hours post-dose. Plasma AUC and C_{max} increased in less than an approximately dose-dependent manner in the fasted state, with an apparent plateau at higher doses. Co-administration with meals increased MK-0859 exposures by up to ~6-8 fold. MK-0859 markedly and dose-dependently inhibited serum CETP activity with peak effects of ~90% inhibition at T_{max} and ~58% inhibition at 24 hours post-dose. MK-0859 was well tolerated and was not associated with any increase in blood pressure. **Conclusions**: This study provided proof of pharmacologic characteristics for MK-0859 with respect to CETP inhibition in humans. MK-0859 was well tolerated in the study, had no effect on blood pressure, and exhibited PK and PD characteristics that support a once-daily dosing regimen.

Funding: Supported by Merck & Co., Inc.

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A_1 ADENOSINE RECEPTOR PARTIAL AGONISTS AS ANTI-LIPOLYTIC AGENTS AND THEIR THERAPEUTIC POTENTIAL IN INSULIN RESISTANCE AND DIABETES

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Elevated levels of circulating free fatty acids (FFA) play an important role in the pathogenesis of insulin resistance and diabetes. Thus, lowering FFA levels by reduction of lipolysis in adipocytes is of potential benefit in treatments of dyslipidemia and type II diabetes. A_1 adenosine receptor (A_1AdoR) agonists are potent anti-lipolytic agents that inhibit adipose tissue lipolysis and lower circulating FFA levels. However, A_1AdoR agonists have potential side effects due to the presence of A_1AdoR in many tissues in addition to the adipose tissue. Functional selectivity can be achieved by exploiting the differential receptor-effector coupling between adipose tissue and other tissues. The undesired effects of A_1AdoR in non-adipose tissues can be further minimized by use of low-efficacy agonists or partial agonists. We have discovered a partial agonist, CVT-3619, selective for the A_1AdoR that inhibits adipose tissue lipolysis (IC_{50} =44 nM). In in-vivo studies, CVT-3619 decreases both FFA and triglyceride (TG) levels in a dose-dependent manner in normal and insulin resistant rats and does not have any significant effect on heart rate and blood pressure at the doses that have maximal anti-lipolytic effects. CVT-3619 also increases the potency of insulin to decrease lipolysis by 3-fold, suggesting that CVT-3619 increases insulin sensitivity. ZDF rats treated with CVT-3619 (10 mg/kg twice daily) for 5 consecutive days had significantly lower fasting plasma glucose, insulin, FFA and TG concentrations as compared to the vehicle treated group. In conclusion, CVT-3619 is a novel, partial A_1AdoR agonist that lowers circulating FFA and TG levels resulting in improved insulin sensitivity with minimal cardiovascular effects.

Funding: Sponsored by CVT

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A SQUALENE SYNTHASE INHIBITOR (SSI), LAPAQUISTAT ACETATE (TAK-475, LAPA): ITS HEPATOPROTECTIVE AND MYOPROTECTIVE EFFECTS IN STATIN-TREATED GUINEA PIGS

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Objective: HMG-CoA reductase inhibitors (Statins) can be associated with myo- and hepatotoxicities. These may be due to reduced mevalonate-derived isoprenoids. SSIs may spare the synthesis of these products in the cholesterol biosynthetic pathway. Accordingly, we investigated the effects of LAPA on cholesterol-lowering activity and toxicities caused by cerivastatin (CEV) and atorvastatin (ATV) in guinea pigs. **Methods:** Drugs were orally administered to guinea pigs for 1 or 2 weeks. Plasma parameters were enzymatically measured. Ubiquinone (CoQ10) levels were measured by liquid chromatography. Histological changes in muscle and liver were evaluated after H&E staining. **Results:** LAPA coadministered with CEV or ATV induced incremental reductions in plasma TC. With the addition of mevalonolactone (MVL) to CEV, TC remained at control levels. CEV (1 mg/kg) increased plasma CPK (10 fold) and Mb (89 fold), and induced development of muscle lesions. These changes were abrogated by coadministration of LAPA or MVL with CEV. ATV (50 mg/kg) induced significant elevations in plasma ALT (1.6 fold) and AST levels (3.9 fold); coadministration of LAPA (30 mg/kg) or MVL with ATV ameliorated these elevations. Compared with untreated controls, ATV (30 mg/kg) decreased hepatic CoQ10 levels by 17%, whereas LAPA+ATV increased CoQ10 by 26%. ATV-induced hepatic lesions occurred in animals with marked decreases in hepatic CoQ10 content. **Conclusions:** Lapaquistat acetate not only enhanced the cholesterol-lowering effects of the statins, but also protected against statin-induced myo- and hepatotoxicity in guinea pigs.

Funding: None

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EVALUATING COMORBIDITIES AND HOSPITALIZATION RATES OF MEDICARE OR COMMERCIAL STATIN USERS IN A HEALTH PLAN

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Objectives: To assess the prevalence of cardiovascular (CV) comorbidities and hospitalization rates among statin users in Medicare and Commercial populations of a large health plan. **Methods:** This study retrospectively assessed pharmacy and medical claims of Medicare and Commercial health plan members between 4/1/2005 and 3/31/2006. All observations were over this 1y period. **Results:** Out of populations of 238,900 Medicare and 3,258,266 Commercial members, 51,818 (21.7%) and 285,820 (8.8%) were statin users, classified with dyslipidemia (DL) and were included in the analysis. The mean age of Medicare and Commercial statin users was 73.1y and 54y, respectively, and 59.2% and 39.3% were female. The prevalences of CV comorbidities were: coronary heart disease (CHD)– Medicare 35.2% (4.0% with acute coronary syndrome [ACS]), Commercial 19.7% (2.6% with ACS); diabetes (DM)– 35.6%, 24.9%; hypertension (HTN)– 81.4%, 60.4%; renal impairment– 16%, 8.3%; stroke/transient ischemic attack (S/TIA)– 15.5%, 5.0%; CHD or DM or S/TIA– 61.8%, 40.6%, respectively. In addition to DL, 19.7% of Medicare and 32.7% of Commercial patients had both DM and HTN. In contrast, the proportion of statin users with only DL but none of the observed other CV risk factors was 9.2% of Medicare and 29.2% of Commercial members. Hospitalizations occurred in 20.3% of Medicare and 10.3% of Commercial statin users, with 14.2% and 3.1% hospitalized for CHD. **Conclusions:** This large study demonstrated that statin users within the Medicare and Commercial populations of a health plan have a high prevalence of CV comorbidities and hospitalizations, particularly in Medicare where only 9.2% of patients had no CV comorbidities and 14.2% were hospitalized for CHD.

Funding: This study was funded by Pfizer Inc.

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A NEW THERAPEUTIC PROPOSAL FOR LIPID LOWERING TREATMENT: ASSOCIATION OF TRANSIALIDASE AND ANTI-OXIDANT ELEMENTS. AN EXPERIMENTAL STUDY IN RABBITS

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Oxidized lipoproteins are the main causative factor for atherosclerosis. Also, high serum level of sialic acids (AS), N-acetylated derivates of neuraminic acid, with numerous biological functions and participating in cell-to-cell and cell-to-matrix interactions, is a determinant of atherosclerosis. Patients with Chagas disease usually do not present atherosclerosis and present Trypanosoma cruzi (TC)-produced transialidase (TS) in their blood. Here are promising results of a new lipid-lowering treatment in an atherosclerosis experimental model, associating TS with anti-oxidant elements: metal chelant Phyrrolidine- dithiocarbomate (PDTC) and plant-derived anti-oxidative nanoparticles. We compared six groups of rabbits. GI - normal diet; GII - 1% cholesterol diet for 12 weeks; GIII - 1% cholesterol diet for 12 wks and TS plus PDTC in the last 4 wks. Groups IV, V and VI received the same scheme of GIII plus nanoparticles derived from extracts of: Allium sativum (AL); AL+ Ginko biloba (GB) and AL+GB + Zingiber officinale (ZO), respectively. **Results:** Mean (SD) of % total area plaque was 0 (0), 75 (9), 50 (3), 67 (14), 42 (8) and 11 (1); % plaque fat area 0 (0), 89 (5), 50 (3), 61 (10), 40 (14), 17 (10) and LDL mg/dl serum levels 33 (24), 775 (227), 743 (92), 635 (60), 335 (29) and 18 (6), respectively in GI, GII, GIII, GIV, V and GVI. **Conclusion:** The use of TS from T.cruzi reduced atherosclerotic lesions in experimental atherosclerosis. TS efficacy was enhanced when associated with PDTC and anti-oxidative plant-derived nanoparticles. Our results indicate a new potential treatment to combat atherosclerosis and reduce lipid levels.

Funding: H&S Ciencia e Biotecnologia Ltda

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THE HYPOLIPIDEMIC EFFECT OF A NEW DIETARY SUPPLEMENT

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Aim of the study: To evaluate the efficacy of a new food supplement (Armolipid) containing natural hypocolesterolemic and antioxidant agents (Policosanols, Red Yeast, Coenzyme Q10, Astaxantine, Folic Acid) combined to the diet (A_D) in a randomised multicenter study in hyperlipidemic patients versus diet alone (D). **Methods**: 2400 hyperlipidemic patients were enrolled in 266 italians units: 1008 cases treated with A-D and 894 with D alone. The remaining 498 were treated with A-D in an open design. Admission criteria were: serum chol>200 mg/dl or LDL >150 mg/dl; all patients were on a diet regimen, whilst the AD group added one tablet/day of supplement for 16 wks. Lipid pattern, and vital signs at baseline and weekly were measred. **Results**: The two groups were homogeneous: mean age 58 yrs (A-D) vs 57.5 yrs (D) almost equally distributed between males and females; BMI 27 kg/m^2 for both; Total Chol 257 mg/dL in A-D, 247 in D; LDL-Chol 159 in A-D, 166 in D; HDL-Chol 48 mg/dL in A/D, 41 mg/dL in D. The total cholesterol gradually decreased up to 18% in the A_D group versus 9% in D group. LDL-Chol reduction in the two groups was similar to that observe in tot chol. HDL-levels showed a moderate increase: almost 10% in AD and 5% in D group. Body weight and triglycerides decreased similarly and moderately in both groups. **Conclusions**: Food supplement and diet allow a more effective reduction of lipid levels suggesting that natural products could be easily used for controlling lipid profile with a positive impact on CHD prevention.

Funding: None

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SAFETY & ACTIVITY OF ISIS 301012 IN HETEROZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA

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Background: ISIS 301012 promotes dose-dependent reductions in LDL-C. In subjects with primary hypercholesterolemia, LDL-C reductions >70% and >50% have been observed with monotherapy and add-on to statin therapy, respectively. In subjects with homozygous familial hypercholesterolemia (FH), unparalleled LDL-C reductions >50% have been demonstrated. The drug has been well tolerated. Painless erythema at the sc injection site has been the most common adverse event. **Objectives:** A randomized, double-blind, placebo-controlled, sequential dose-escalation study has been conducted to evaluate the efficacy and safety of ISIS 301012 in subjects with heterozygous FH. **Methods:** This study has involved 44 subjects with baseline LDL-C ≥130 mg/dL on stable, maximally tolerated therapy. Doses of 50, 100, 200 and 300 mg of ISIS 301012 or placebo (4:1 randomization) have been administered by sc injection for 6 wks or 13 wks for the 300-mg cohort. **Results:** This study is in progress. Final efficacy and safety results will be available for presentation at the meeting. Based on the impressive performance of the drug to date, we anticipate reporting significant dose-dependent reductions in LDL-C and other atherogenic lipoproteins in the heterozygous FH population.

Funding: This trial was sponsored by Isis Pharmaceuticals

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EFFECTS OF ADDING FENOFIBRATE ON ATHEROGENIC LIPOPROTEIN SUBFRACTIONS IN HIGH RISK PATIENTS WITH MIXED HYPERLIPIDEMIA NOT CONTROLLED BY PRAVASTATIN

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Objective: To determine the effects of the fenofibrate/pravastatin (FENO/PRA) fixed combination on atherogenic lipoprotein subfractions in high risk patients with mixed hyperlipidemia. **Methods:** After a 8-week pravastatin (PRA) 40mg and diet run-in period, high risk patients (n=248) with LDL-cholesterol ≥ 100mg/dl and triglycerides ≥ 150 and ≤ 400 mg/dl, were randomized to a double-blind, multicenter, 2 parallel arms, 12-week comparison of FENO/PRA 160/40 mg versus PRA 40mg alone followed by an open-label, 52-week safety phase of the combination therapy. This study reports the effects on lipoprotein subfractions at the end of the double-blind phase. **Results:** After 12 weeks of double-blind treatment, the ApoB/ApoA1 ratio was significantly ($p<0.0001$) decreased with FENO/PRA (- 16.2 %) compared with PRA (- 6.1 %). LpB:E, LpB:CIII and RLP-cholesterol were significantly reduced with FENO/PRA (respectively – 23.8 %, - 12.4 % and – 30.3 %) compared with PRA (respectively – 0.2 %, + 7.4 % and – 13.7 %). The mean LDL size was increased in the FENO/PRA group (+ 1.54 %) compared with the PRA group (- 0.16 %) ($p<0.0001$). The FENO/PRA fixed combination was generally well tolerated. **Conclusion:** The combination of fenofibrate 160mg and pravastatin 40mg in patients with mixed hyperlipidemia provided significant improvements in atherogenic lipoprotein parameters compared with pravastatin 40mg monotherapy.

Funding: This study was sponsored by Laboratoires SMB, Brussels, Belgium

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THE SOLUBLE IMMUNOADHESIN CD68-FC: AN INNOVATIVE ANTI-ATHEROSCLEROTIC STRATEGY FOR THE SPECIFIC INHIBITION OF FOAM CELL FORMATION

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Objective: We cloned and characterized a new fusion protein consisting of the extracellular domain of CD68 and a human Fc domain (CD68-Fc). Its binding and blocking properties were studied *in vitro*, *ex vivo* and *in mice.* **Methods and Results:** First, an ELISA with anti-human-Fc antibodies was created at human plaque specimens. CD68-Fc binding was significantly higher at lipid-rich plaques compared to control-Fc. This was confirmed by immunohistochemistry at paraffin embedded plaque sections. Further, binding of radioactively labeled CD68-Fc was studied in $ApoE^{-/-}$-mice. After i.v. injection of ^{124}I-CD68-Fc, specific binding at lipid-rich lesions in $ApoE^{-/-}$ vs. wildtype mice was detected by autoradiography and lipid stainings. Previously, we published an *in vitro*-atheroscreen model in which cocultivation of human platelets with $CD34^{+}$-progenitor cells or monocytes for 5d induces foam cell formation expressing the scavenger receptor CD68 and taking up oxidized LDL. This was prevented efficiently by CD68-Fc, as also MMP-9 activity, an important foam cell function. To clarify the mechanisms, FACS was performed after incubation of platelets and macrophages with oxidized LDL. Both cell types took up Dil-OxLDL and this could be prevented by CD68-Fc. Interestingly, also platelet-mediated phagocytosis of Dil-OxLDL into macrophage foam cells was inhibited by CD68-Fc. **Conclusions:** We identified CD68-Fc as a promising new tool to accumulate at local plaque sites and inhibit foam cell formation. Thus, CD68-Fc may influence a central feature in primary atherosclerosis or transform vulnerable lipid-rich plaques into stable fibrous plaques in patients with advanced atherosclerosis.

Funding: None

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HYPOLIPIDEMIC EFFECT OF *VERNONIA AMYGDALINA*, A TROPICAL VEGETABLE IN RATS FED ON HIGH CHOLESTEROL DIET

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Objective: This study was designed to evaluate the hypolipidemic effect of *Vernonia amygdalina* (VA) in rats fed on high cholesterol diet, while Questran (Qu), a standard hypolipidemic drug served as the reference. **Materials and Methods:** 42 male Wistar rats were distributed into 7 groups of 6 animals each. Group A served as control, groups B and C were given cholesterol (CH) and supplemented diets (1% and 2% VA, respectively). Group D was given CH. Group E was given 1% VA and, group F received CH and Qu. Finally rats in group G were given Qu. The protocol conforms to the guidelines of NIH for laboratory animal care and use. CH and Qu were given at doses; 30 mg / animal and 0.26-g/ kg body weight, respectively. **Results:** Administration of CH for 9 weeks resulted in a significant increase ($P<0.05$) in the plasma and postmitochondrial fraction (PMF) CH levels of rats by 33% and 55%, respectively. Diets containing 1% and 2% VA reduced the plasma and PMF CH levels by 20%, 23% and 23%, 29%, respectively. Similar reduction in CH level was obtained in Qu-treated rats. Furthermore, diet with 2% VA caused a significant ($p<0.05$) decrease in plasma and PMF low-density lipoprotein (LDL) CH levels by 23% and 49%, respectively and also decreased plasma and PMF triglyceride levels by 29% and 28%, respectively. There were also significant decreases ($p<0.05$) in lipid peroxidation (LPO) levels of animals on supplemented diets. Specifically, 1% and 2% VA diets decreased plasma and PMF LPO by 38%, 35% and 42%, 45%, respectively. Furthermore, 1% and 2% VA diets modulated the CH-induced decrease in glutathione levels. **Conclusion:** The results suggest that VA exerts hypolipidemic effect by interfering with lipid metabolic pathways in the hypercholesterolemic rats.

Funding: Self-sponsored project

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MTP-INHIBITOR, AEGR-733, REDUCES BODY WEIGHT IN PATIENTS WITH HYPERCHOLESTEROLEMIA

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Obesity is a driver of dyslipidemia and type 2 diabetes. AEGR-733 is an inhibitor of microsomal triglyceride transfer protein (MTP) that alters the assembly/secretion of apo B-containing lipoproteins including intestinal chylomicrons. Through MTP-I, AEGR-733 could also produce weight loss.We report our observations on the effects of AEGR-733 on body weight in a study designed to test the lipid-lowering effects in hypercholesterolemic (HC) subjects. In this randomized, double-blind, active-controlled study, we studied the efficacy and safety of AEGR-733 alone to ezetimibe (EZE) alone and in combination with EZE. 84 patients were randomized to one of 3 arms: AEGR-733, EZE 10 mg, or AEGR-733+EZE 10 mg. Patients receiving AEGR-733 were titrated from 5 mg to 7.5 mg to 10 mg at 4-week intervals. Patients receiving EZE maintained 10 mg through the 12-week study. The mean baseline BMIs across the 3 groups were 28.6-29.6 kg/m^2. All subjects were instructed to follow a low fat diet without emphasis on weight loss. At 12 weeks, weight changes of -0.2 ±1.9 kg, -0.7 ± 2.0 kg and -1.4 ± 2.6 kg were seen in the EZE, AEGR-733, and AEGR-733+EZE groups, respectively. The change from baseline was statistically significant for the combination group (p=0.013). Effects were more pronounced in obese subjects. These initial data suggest that the MTP-I, AEGR-733 reduces body weight in patients with HC, particularly with EZE. Ultimately, a drug that causes weight loss and reduces LDL-C could be useful in treating obese, HC patients.

Funding: This study was funded by Aegerion Pharmaceuticals

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OUR CONSIDERATION ABOUT THE COMBINED TREATMENT WITH ATORVASTATIN AND EZETIMIBE FOR THE SECONDARY PREVENTION OF CHD IN PATIENTS WITH TYPE 2 DIABETES

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Background: The risk of progression of the atherosclerotic process to coronary heart disease (CHD) in patients with type 2 diabetes increases progressively with increasing levels of low density lipoprotein cholesterol (LDL-C). **The aim** of the study was to study the efficacy of the combined treatment with Atorvastatin and Ezetimibe in patients with (CHD) and type 2 diabetes(42 patients). **Materials and methods:** We studied 42 patients (31 males/11 females, age range from 40 to 80 years), who were treated with 10 mg Ezetimibe and 20 mg Atorvastatin, during 8 weeks. **Results:** Target levels of the lipid profile were reached in 75% of patients. Clinical improvement of patients' condition was evaluated via the decrease of angina pectoris attacks and nitro-glycerine consumption, and the decrease was statistically evident. **Conclusion:** Hence, in order to reach the target levels of cholesterol combined therapy with Ezetimibe+Statins gives chance atherosclerosis progression to be stopped and maintain these improvements in the longer term. Therefore, every patient with compensative type 2 diabetes must be treated with the above mentioned scheme in the early period of the disease, and our treatment will be forwarded not only against dyslipidemia, but against the risk factors of type 2 diabetes and arterial hypertension, because these diseases create vicious circle and aggravate each-other.

Funding: National Center of Therapy

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RELATIVE REDUCTIONS IN C-LDL AND IN HS-CRP CORRELATE DIRECTLY IN HYPERCHOLESTEROLEMIC PATIENTS TREATED WITH FLUVASTATIN XL ALONE OR COMBINED WITH EZETIMIBE

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Compare efficacy and safety of fluvastatin (FL) XL administered alone or combined with ezetimibe (EZE) for 12 weeks on plasma lipid levels and hs-CRP in patients with primary hypercholesterolemia. Randomized, multicenter, open-label, parallel-group study n=82. The FL XL/EZE combination provided significantly greater reductions than FL XL in LDL-C levels (50% vs 35%, $p<0.001$) and in total cholesterol ($p<0.001$), triglycerides ($p=0.02$) and apo B ($p<0.001$). A greater proportion of patients on combination arm achieved LDL goals compared with patients receiving FL XL (87%vs67%, $p=0.04$). Only the combination significantly lowered hs-CRP levels in patients with either ≥ 1 risk factor $p<0.05$ or HT $p=0.01$; which had higher hs-CRP levels than those without additional CVRF (2.8±2.4 and 1.2±1.2mg/L, $p<0.001$) or normotensive (3.0±2.5 and 1.8±1.8mg/L , $p<0.01$). There was a direct correlation between the relative reduction in hs-CRP and levels of LDL-C ($r=0.476$, $p<0.001$) and total cholesterol ($r=0.399$, $p<0.001$). Safety and tolerability profiles were comparable among groups. The results suggest that FL XL/EZE is a well-tolerated, effective lipid-lowering regimen that allows the majority of patients with primary hypercholesterolemia to achieve treatment goals. The combination was more effective than monotherapy in reducing hs-CRP levels in patients at higher CV risk. This beneficial effect may be due to the larger lipid-lowering effect produced by the combination.

Funding: Novartis Farmacética

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TWELVE YEARS OF SIMVASTATIN (S) PLUS FENOFIBRATE (G) COMBINATION TREATMENT IN PATIENTS WITH FAMILIAL COMBINED HYPERLIPIDEMIA (FCH)

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During the 90's, in our Lipid Clinic, patients with FCH resistant to low-fat, low-cholesterol diet and to physical aerobic activity, were treated with statines or fibrates in relationship to baseline total cholesterol (TC) and triglycerides (TG) values. In 1995, 45 selected patients (below 65 years old, without heart, hepatic, renal or muscular diseases, endocrine disorders and impaired Na and K balance, not taking cyclosporine or erythromycin, and without strenuous muscular work), that under monotherapy showed a TC value still ≥240 mg/dL and a TG value still ≥200 mg/dL, S 20 mg/day and F 200 mg/day have been associated. In the 34 patients who reached the 12 years of combination treatment, compared to monotherapy, S+F produced a statistically higher improvement in TC (-17%), LDL-C (-21%), in HDL-C (+8%), in TG (-23%), in ApoB (-17%) and ApoAI (+11%). During the 12 years of follow-up, 2 patients dropped-out because gastrointestinal distress, 2 for high transaminases and 7 for reasons other than side effects. None of the patients exhibited myopathy (muscle pain, weakness, creatine-kinase levels ≥3 the upper normal limit) or rhabdomyolisis. In conclusion, S+F can be suggested in selected patients with FCH who are resistant to monotherapy. However, a strict monitoring and patient counselling on the risks and warning signs of myopathy are highly recommended in achieving a rapid diagnosis of myopathy, a possible adverse effect of this combination therapy.

Funding: None

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LONG-TERM EFFICACY OF FIBRATE-STATIN COMBINATION THERAPY

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Objectives: To determine the long-term efficacy of statin-fibrate combination therapy. **Methods:** A retrospective cohort survey comprising > 5 years therapy with combination compared with LDL-lowering therapy was conducted. **Results:** The combination therapy cohort comprised 254 patients with initial total cholesterol (TC) was 6.77 mmol/L; triglycerides (TG) 2.79 mmol/L; HDL-C 1.04 mmol/L. 146 patients with polygenic hypercholesterolaemia had a presentation TC of 7.03 mmol/L ; TG 1.75 mmol/L and HDL-C 1.47 mmol/L. The statin-fibrate cohort had an average 8.3 (±2.98) years follow-up and the parallel cohort 6.88 (±3.88) years . With combination fibrate statin therapy TC decreased by 29%, triglycerides by 34% and HDL-C increased by 24% giving final values of TC 4.55 (±1.18) mmol/L, TG 1.67(0.46-35.9) mmol/L and HDL-C 1.25 (±0.40) mmol/L. In the parallel cohort TC decreased by 22%, TG by 9.5% and HDL-C increased by 8%. 16% of patients on statin-fibrate had cardiovascular events compared to 18% in the parallel cohort. Prognosis in statin-fibrate patients was associated with lower age (p=0.003), absence of cardiovascular disease (CVD) (p=0.01), greater changes in TG (p=0.001) and less strongly with changes in TC (p=0.01) and HDL-C (p=0.02). In contrast in the parallel cohort only the presence of CVD was significant. In all the patients successfully initiated on combination therapy only 4 cases of myalgia and 2 cases of raised creatine kinase (> 5 x ULN) were seen. **Conclusions:** This study suggests that in patients with mixed hyperlipidaemia it is likely that reducing triglycerides, raising HDL-C and reducing LDL-C with a combination therapy leads to improved prognosis.

Funding: None

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EFFECTS OF PITAVASTATIN AND ATORVASTATIN ON HDL CHOLESTEROL LEVELS IN PATIENTS WITH HYPER-LDL CHOLESTEROLEMIA AND GLUCOSE INTOLERANCE: THE PIAT STUDY

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Objective: A randomized trial was implemented to evaluate effects of two strong statins on HDL cholesterol and glucose tolerance status in Japanese men and women. **Methods**: Study subjects were those at the age of 20-74 years who had LDL cholesterol of 140 mg/dL or greater and also glucose intolerance or type 2 diabetes mellitus. Individuals were randomly allocated to either pitavastatin (2 mg/day) or atorvastatin (10 mg/day) treatment, and follow-up measurements were done in 2, 6 and 12 months after the initial measurement. The primary endpoint was the change in HDL cholesterol levels at 12 months or 6 months (if 12-month data were not available) after treatment. Initially, 103 and 104 patients were allocated to pitavastatin and atorvastatin, respectively. However, 34 persons did not either start or continue the treatment for 2 months or longer, and numbers of subjects remained in the analysis were 88 in the pitavastatin group and 85 in the atorvastatin. **Results**: HDL cholesterol increased by 8.2% in the pitavastatin group and by 2.9% in the atorvastatin group. The difference in increase was statistically significant (P = 0.03). More evident was a greater increase in apolipoprotein(apo) AI in the pitavastatin treatment. On the other hand, the decrease in LDL cholesterol as well as in apos B and E was slightly greater in the atorvastatin treatment. There was no difference in the rate of deterioration in glucose tolerance between the two treatments. **Conclusions**: The findings add to evidence that pitavastatin has high potential in increasing HDL cholesterol.

Funding: None

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INHIBITION OF CHOLESTERYL ESTER TRANSFER PROTEIN BY TORCETRAPIB ATTENUATES THE ATHEROGENICITY OF POSTPRANDIAL TRIGLYCERIDE-RICH LIPOPROTEINS IN MIXED HYPERLIPIDEMIA

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To evaluate the impact of torcetrapib, a specific inhibitor of cholesteryl ester transfer protein (CETP), on atherogenic triglyceride (TG)-rich lipoprotein subfractions in the postprandial phase in mixed hyperlipidemia. The quantitative and qualitative features of the postprandial profile of TG-rich lipoproteins were determined at baseline, and after treatment for 6 weeks with 10 mg/day atorvastatin, and subsequently with an atorvastatin/ torcetrapib combination (10/60 mg/day) in 18 men with mixed hyperlipidemia. After ingestion of a standardised mixed meal (1200 kcal), TG-rich lipoprotein subfraction profiles were evaluated over 8 hours following each experimental period. On a background of atorvastatin, torcetrapib significantly attenuated the incremental postprandial area under the curve (iAUC 0-8 h) for VLDL-1 (-40%), and the AUC 0-8 h for VLDL-2 (-53%), with minor effect on chylomicron iAUC (-24%); concomitantly, the ratio of neutral core lipids in both VLDL-1 and VLDL-2 was significantly reduced (-27% to -42%). Such reduction was due to Torcetrapib-mediated attenuation of postprandial CE transfer to Chylomicrons (-17%) and VLDL-1 (-33%). Marked reduction in postprandial VLDL-1 levels was associated with apoE enrichment. On a background of atorvastatin, torcetrapib attenuated the quantitative and qualitative features of the atherogenic postprandial profile of chylomicrons, VLDL-1 and VLDL-2. Such changes reflect the sum of torcetrapib-mediated effects on TG-rich lipoprotein production, intravascular remodelling and catabolism.

Funding: We are indebted to Pfizer and to INSERM for generous support of these studies

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NIACIN DECREASES PLASMA CHOLESTEROL AND TRIGLYCERIDES AND INCREASES HDL IN APOE*3LEIDEN.CETP TRANSGENIC MICE. REDUCED CETP LEVELS AND HEPATIC LIPASE ACTIVITY

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Objectives: Niacin (nicotinic acid, vitamin B3) potently decreases plasma TG and LDL-cholesterol and is also the most potent HDL-cholesterol increasing drug used in the clinic. In the present study, we aimed at elucidation of the mechanism underlying its HDL-raising effect. **Results:** Hetero, female APOE*3-Leiden transgenic mice with the human CETP transgene, under control of its natural flanking regions, were fed a Western-type diet with or without niacin (up to 1 g/kg bw). Niacin dose-dependently decreased plasma total cholesterol (TC) (up to -69%, $P<0.05$) and TG (up to -79%, $P<0.05$). At the same time, niacin dose-dependently increased HDL-cholesterol (up to +87%, $P<0.05$) and plasma apoAI (up to +72%, $P<0.05$). Interestingly, niacin did not increase HDL-cholesterol in APOE*3-Leiden mice that do not express CETP. In fact, niacin appeared to dose-dependently decrease both the CETP activity (up to -49%, $P<0.05$) and CETP mass (up to -45%, $P<0.05$) and hepatic lipase (-47%, $p<0.05$) in plasma. **Conclusions:** Niacin decreases TC and TG levels and increases HDL levels, the latter related to decreased levels of CETP in plasma and reduced activity of hepatic lipase. Since, unlike CETP inhibitors, niacin does not result in accumulation of CETP protein in plasma, the CETP reducing properties of niacin may add to its therapeutic benefit.

Funding: Study was not funded

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ATORVASTATIN INCREASES HDL-CHOLESTEROL BY REDUCING CETP EXPRESSION IN CHOLESTEROL-FED APOE*3-LEIDEN.CETP MICE

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In addition to lowering LDL-cholesterol (LDL-C), statins modestly increase HDL-C and decrease cholesteryl ester transfer protein (CETP) mass and activity. Our aim was to determine if the increase in HDL-C is secondary to the effect of statins on CETP expression. APOE*3-Leiden (E3L) mice, with a human-like lipoprotein profile, were crossbred with mice expressing human CETP under control of its natural flanking regions resulting in E3L.CETP mice. Male E3L and E3L.CETP mice were fed a Western-type diet with or without atorvastatin. Atorvastatin (0.01% in the diet) reduced plasma cholesterol in both E3L and E3L.CETP mice (-26% and -33%, $P<0.05$), mainly in VLDL, but increased HDL-C only in E3L.CETP mice (+52%). Hepatic mRNA expression levels of genes involved in HDL metabolism (PLTP, ABCA1, SR-BI, apoAI), were not differently affected by atorvastatin in E3L. CETP mice as compared to E3L mice. However, in E3L.CETP mice, atorvastatin down-regulated the hepatic CETP mRNA expression (-57%, $P<0.01$), the total CETP level (-29%) and CE transfer activity (-36%, $P<0.05$) in plasma. In female E3L.CETP mice, atorvastatin (0.001% and 0.01%) caused a dose-dependent effect on plasma cholesterol (-34% and -71%, $P<0.01$), HDL-C (+118% and +176%) and CETP activity (-31% and -61%, $P<0.05$). We conclude that atorvastatin increases HDL-C in E3L.CETP mice by reducing the CETP-dependent transfer of cholesteryl esters from HDL to (V)LDL, as related to lower hepatic CETP expression and a reduced plasma (V)LDL pool.

Funding: NHS 2003B136

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EFFECTS OF 12 MONTH TREATMENT WITH LOW-DOSE SIMVASTATIN ON PLASMA CHOLESTEROL AND OXIDIZED LDL IN 3 LDL SUBFRACTIONS

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Objective: We reported the effect of 3 month treatment with simvastatin on plasam lipoprotein subfractions in Atherosclerosis, 1995. We examined the effects of 12 month simvastatin treatment on plasma cholesterol and ox-LDL in 3 ultracentrifugally separated LDL subfractions in this study. **Methods:** Simvastatin was administered to 30 patients with hypercholesterolemia. Initial daily dose was 5 mg and the dose was increased to 20 mg via 10 mg for reducing plasma LLDL-C to 130 mg/dL or less and the dose was fixed at 3 months of treatment. Total test duration was 12 months. Plasma lipoprotein subfractionation into VLDL, IDL, low-density (ld)-LDL($1.019<d<1.035$), medium-density (md)-LDL ($1.035<d<1.045$), high-density (hd)-LDL ($1.045<d<1.063$), HDL2, and HDL3 was made every 3 months. **Results:** 12 month treatment with low-dose simvastatin highly significantly reduced plasma cholesterol in ld-LDL, md-LDL, and hd-LDL from 60 mg/dL, 71 mg/dL, and 20 mg/dL to 41 mg/dL, 49 mg/dL, and 15 mg/dL. Simvastatn treatment reduced plasma ox-LDL in ld-LDL and md-LDL from 42 U/L, and 70 U/L, to 39 U/L ($p == 0.007$), 56 U/L ($p = 0.018$), respectively. However, ox-LDL in hd-LDL was not decreased after 12 month treatment with simvastatin. **Conclusions:** Twelve month treatment with low-dose simvastatin reduced plasma cholesterol and ox-LDL in ld-LDL and md-LDL. It reduced plasma cholesterol but not ox-LDL in hd-LDL.

Funding: None

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PERCENT OF PATIENTS ATTAINING JOINT BRITISH SOCIETIES' GUIDELINES LDL-C LEVEL <2 mmol/L IN A 6-WEEK STUDY TO EVALUATE EZETIMIBE/SIMVASTATIN VS ATORVASTATIN IN PATIENTS WITH TYPE 2 DIABETES AND HYPERCHOLESTEROLEMIA

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Objective: The Joint British Societies' guidelines (JBS2) now specify an LDL-C target of <2 mmol/L (~77 mg/dL). A post-hoc analysis evaluated the % of patients attaining LDL-C of <2 mmol/L in a 6-week study to assess the efficacy and safety of ezetimibe/simvastatin (E/S) vs atorvastatin (A) in patients with type 2 diabetes and hypercholesterolemia. **Methods:** The primary study endpoint was % change from baseline in LDL-C and the secondary endpoint was % of patients attaining LDL-C <70 mg/dL. JBS2 includes a target LDL-C of <2 mmol/L, prompting the comparison of E/S to A at reaching this target. The pre-specified dose comparisons in the main study were for the recommended usual starting doses, E/S 10/20 mg vs. A 10 mg and E/S 10/20 mg vs. A 20 mg, and the next highest doses, E/S 10/40 mg vs. A 40 mg. This post-hoc analysis also compared E/S 10/20 mg to A 40 mg. A logistic regression model with terms for treatment group and LDL-C strata was used for the analysis (significant at P <0.05). **Results:** Patients attaining JBS2 goal of LDL-C <2 mmol/L: 73.9% (176/238) E/S 20 mg; 86% (208/242) E/S 40 mg; 33.3% (79/237) A 10 mg; 50.8% (122/240) A 20 mg; 69.3% (167/241) A 40 mg. **Conclusions:** For the pre-specified dose comparisons, a higher % of patients administered E/S attained the JBS2 LDL-C level <2 mmol/L (P<0.001) than those administered A at each of the treatment comparisons . The difference between E/S 10/20 mg and A 40 mg favored E/S 10/20 mg numerically, but was not significantly different (P=0.203).

Funding: Merck & Co., Inc.

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NCEP LDL GOAL ATTAINMENT WITH EZETIMIBE/SIMVASTATIN, ATORVASTATIN, OR ROSUVASTATIN IN PATIENTS WITH DIABETES, METABOLIC SYNDROME, OR NEITHER

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Objective: To evaluate the consistency of LDL lowering, comparing patients with diabetes mellitus (DM), metabolic syndrome (MS), or neither disease (N). **Methods:** Post-hoc analysis of two multicenter, double-blind, randomized, 6-week studies comparing ezetimibe/simvastatin (E/S) 10/10, 10/20, 10/40, or 10/80 mg with either atorvastatin (A) 10, 20, 40, or 80 mg (Study 1), or rosuvastatin (R) 10, 20, or 40 mg (Study 2). This analysis compares treatments by pooling across all doses for the percentage of patients attaining individual NCEP LDL goals in the subgroup of patients with DM, MS without DM, or N. **Results:** LDL goal attainment was greater with E/S than A in the DM (78.3% vs 64.2%), MS (75.7% vs 63.6%), and N (82.3% vs 74.5%) subgroups. Attainment was also greater with E/S than R in the DM (84.3% vs 81.2%), MS (89.9% vs 82.7%), and N (93.3% vs 91.4%) subgroups. These subgroup results were consistent with those of the full patient cohorts, in which goal attainment was significantly greater with E/S than A (79.2% vs 68.7%; p<0.001) or R (91.2% vs 87.4%; p<0.001). The N subgroup was significantly more likely to achieve NCEP LDL goal than the DM or MS subgroups in Study 1 (p<0.001) and 2 (p=0.027). **Conclusions:** NCEP LDL goal attainment was greater with E/S than either A or R in patients with DM, MS, or neither, consistent with the full study cohorts. Attainment was significantly more likely in patients with neither DM nor MS, though the greater effect with E/S was consistent across subgroups.

Funding: The original studies and secondary analyses were funded by Merck/Schering-Plough Pharmaceuticals

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ACTIVATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-α IN MICE INDUCES EXPRESSION OF THE HEPATIC LOW DENSITY LIPOPROTEIN RECEPTOR

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The LDLR plays a pivotal role in the maintenance of cholesterol homeostasis. Mutations in the LDLR gene cause familial hypercholesterolemia, high serum cholesterol levels and premature coronary heart disease. Genetic deletion of the LDLR also induces atherosclerosis in hypercholesterolemic mice. LDLR expression is predominantly regulated by SREBP2. Fibrates, a drug class that includes fenofibrate, are used in patients with elevated triglyceride levels. These drugs are anti-atherogenic and exert their actions through activation of the nuclear receptor, PPARα. We have investigated the effects of the fenofibrate on LDLR expression in mouse hepatocytes and livers. Treatment of hepatocytes with fenofibrate induced expression of LDLR mRNA and protein and increased LDL binding. Fenofibrate also restored hepatic LDLR expression inhibited by LDL and 25-hydroxy-cholesterol. Mechanistic studies demonstrated that induction of LDLR expression by PPARα was SRE-dependent. Activation of PPARα increased the maturation of SREBP2 without affecting SCAP expression. PPARα ligands increased phosphorylation of Akt to activate SREBP2 and induced LDLR expression. In vivo, a high-fat diet suppressed LDLR expression in mouse liver and elevated total/LDL cholesterol levels in plasma. However, fenofibrate restored the high-fat diet-inhibited LDLR expression in the liver and reduced LDL cholesterol levels in plasma. Our data suggest that PPARα activation by fenofibrate increases hepatic LDLR expression by a mechanism involving Akt phosphorylation and SREBP2 mediated LDLR gene transcription.

Funding: NIH P01HL072942

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LIPID LOWERING AND ANTI-ATHEROSCLEROTIC EFFECT OF AVE5530; A POORLY- ABSORBED CHOLESTEROL ABSORPTION INHIBITOR IN SYRIAN HAMSTER

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Objective: Inhibition of cholesterol absorption is a successful approach to the treatment of hypercholesterolemia. The drug available at present with this mechanism of action is ezetimbe. Ezetimibe is fully absorbed, – as its glucuronide - is secreted via the bile back to the small intestine where it inhibits cholesterol absorption. AVE5530 is a non-systemic inhibitor of intestinal cholesterol absorption. The pharmacological effect of AVE5530 and ezetimibe was investigated in hamster. **Methods:** Pharmacological effect on cholesterol turnover was investigated in male Syrian hamster. The animals were treated once daily by gavage or compounds were mixed with chow. Atherosclerosis study was conducted in male atherosclerosis prone Syrian hamster (Bio F_1B) on a high fat diet for 24 weeks. **Results:** AVE5530 inhibited cholesterol absorption and decreased LDL cholesterol dose dependent in hamster on cholesterol free or cholesterol enriched diet. The ED_{50} on LDL was 0.14 mg/kg. The efficacy and ED_{50} was nearly the same when AVE5530 was administered once daily by oral gavage or with each meal time by mixing into chow. In the hamster atherosclerosis study AVE5530 was well tolerated, reduced serum cholesterol and atherosclerotic lesion dose-dependent. The anti-atherosclerotic effect of AVE5530 was related to cholesterol lowering in the same order of magnitude than with ezetimibe. **Conclusion:** AVE5530 is new minimally absorbed cholesterol absorption inhibitor, in hamster being at least as effective as ezetimibe; in addition the results indicate that absorption and enterohepatic circulation of ezetimibe has no additional benefits on anti-atherosclerotic effects of ezetimibe.

Funding: Study was funded by sanofi-aventis

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PCSK9-DEPENDENT LDL RECEPTOR REGULATION: EFFECTS OF PH AND LDL

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Objective: PCSK9 over-expression is associated with LDL-receptor (LDLr) downregulation, and mutations within PCSK9 are associated with familial hyper- and hypo-cholesterolemia. To investigate mechanisms governing plasma LDL levels, we studied the regulation of LDLr by PCSK9. **Methods:** We measured changes in cellular LDL-uptake following the addition of PCSK9 to the medium of HEK293, HepG2 and CHO cells. We also used surface plasmon resonance (SPR) to determine the affinity of the LDLr ectodomain for LDL and PCSK9 proteins at different pH levels. **Results:** We found that PCSK9 decreases cellular LDL-uptake in a dose-dependent manner. The relative potencies of PCSK9 mutants (S127R and D374Y, associated with hypercholesterolemia; and R46L, associated with hypocholesterolemia) correlate with LDL levels in humans with such mutations. Using SPR, we found that (i) wild type PCSK9 binds LDLr ectodomain with ~ 150-fold higher affinity at an acidic, endosomal pH compared to neutral pH; (ii) PCSK9 D374Y mutant binds LDLr with higher affinity than wild type PCSK9; (iii) LDL and PCSK9 compete for LDLr binding. **Conclusions:** We propose a model for PCSK9-dependent down-regulation of LDLr, in which the LDLr/PCSK9 complex is shuttled to the endosomal compartments, where low pH stabilizes the complex, preventing LDLr recycling and leading to its lysosomal degradation. Molecules interfering with formation of the PCSK9–LDLr complex represent a strategy for PCSK9 inhibition that could be developed to modulate plasma LDL levels.

Funding: All research was performed at Merck Research Laboratories

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PHARMACOLOGICAL AND HEMATOLOGICAL IMPACTS OF NON-SUBTYPE SELECTIVE PPAR MODULATORS IN DOGS

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Peroxisome Proliferator Activated Receptor α regulates the expression of genes involved in lipid metabolism and its interactions with some modulators display species-selectivity. The activation of PPARγ has been related to hematological modifications and increased plasma volume in humans and various animal species such as dog. This study aimed at evaluating the effects of the dual PPARαγ modulators F16505 and F16482 in normolipemic dogs after a 14-day oral treatment. In transactivation assays F16505 and F16482 are 92- and 7-fold more potent on hPPARα than hPPARγ, respectively, and have EC_{50} values 33-fold higher with murine than human PPARα suggesting a human selectivity. F16505 (1 mpk) lowers total plasma cholesterol by 24 and 32% after 7 and 14 days, respectively; F16482 (1 mpk) has comparable effects (-23%), as well as tesaglitazar at 2 mpk. LDL-C and HDL-C decrease, and despite the very low VLDL level in dogs F16482 also reduces plasma triglycerides. In a parallel study F16482 and F16505 decrease LDL-C and HDL-C at the respective lowest active dose 0.03 and 0.01 mpk. Discrete reductions of the red blood cell number (11%, 12%, 12%) and the hematocrit (11%, 12%, 13%) without alteration of the mean red blood cell volume were observed for F16482, F16505 (1 mpk) and tesaglitazar (2 mpk), as already described for the latter. No clinical adverse event or electrocardiographic modification were detected. In conclusion, F16482 and F16505 show hypolipidemic properties in dogs; the reduction in hematocrit after treatment with a supra-maximal dose could be related to their PPARγ properties.

Funding: Centre de Recherche Pierre Fabre

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LIPID-REGULATING PROPERTIES OF NON-SUBTYPE SELECTIVE PPAR MODULATORS

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Peroxisome Proliferator Activated Receptors are ligand-activated transcription factors which regulate the expression of genes related to lipid and glucose metabolism. From a drug discovery program F16141 and F16347 emerged as dual PPARαγ modulators with PPARα-dominant properties. In human recombinant cell-based reporter gene assays, respective EC_{50} for PPARα and γ are 71 and 2070 nM (F16141), 427 and 3040 nM (F16347). When compared to fenofibric acid and pioglitazone, F molecules display full efficacies for PPARα activation and a partial agonism for PPARγ which may suggest reduced lipogenic effects. In HepG2 cells, they up-regulate CPT-1 mRNA in a concentration-dependant manner with a 30-fold higher potency than fenofibric acid. In Zucker fa/fa rats both compounds given orally for 7 days reduce fasting plasma triglycerides dose-dependently; F16347 alleviates glucose intolerance and improves insulin sensitivity at 10 mpk in OGTT and IPITT. When administered for 9 days to db/db mice, both agents also decrease fasting plasma glucose dose-dependently up to 40% with a threshold dose at 2.5 mpk for F16347. Since apoA1 synthesis may be a key issue for the antiatherosclerotic effect of HDL, the molecules were administered to human apoA1 transgenic mice for 14 days; at 10 mpk F16141 and F16347 respectively increase plasma human apoA1 by 404 and 238% above control, despite species differences to activate murine PPARα (at least a 33-fold less potency than for human PPARα). In conclusion, F16141 behaves as a more dominant PPARα agonist over the γ isoform than F16347; both molecules which are in regulatory development may control diabetic dyslipidemia and glucose intolerance.

Funding: Centre de Recherche Pierre Fabre

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TRANSFER OF LIPIDS TO HIGH DENSITY LIPOPROTEIN IN CORONARY ARTERY DISEASE PATIENTS: EFFECTS OF SIMVASTATIN

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Objective: The relationships of lipid transfers and coronary artery disease (CAD) are complex and yet unclear.Lipid transfers are specially important for HDL metabolism and function. In this study, we aimed to clarify whether statin use modifies the ability of HDL to receive cholesteryl esters (CE), triglycerides (TG), phospholipids (PL) and free cholesterol (FC) in patients with CAD, since statins may diminish CETP action. **Methods:** We studied 56 patients with CAD (56±5yo) confirmed by cineangiocoronariography performed in the last 6 months; 28 patients were being treated with 20mg/day simvastatin and 28 were not treated. An artificial nanoemulsion (LDE) was used as lipid donor to HDL. LDE labeled with ^{3}H-TG and ^{14}C-FC or ^{3}H-CE and ^{14}C-PL was incubated with plasma samples for 1h. After chemical precipitation, the supernatant containing HDL was counted for radioactivity. HDL size was measured by laser-light-scattering. **Results:** There was no difference in plasma lipid values between the two groups. The transfer of all the lipids to HDL was smaller in patients treated with simvastatin than in those without statin therapy (*CE*: 1.9±0.8, 3.2±0.8; *PL*: 19.4±1.6, 21.4±1.3; *TG*: 3.1±0.7, 5.3±0.9; *FC*: 5.0± 1.1, 6.5± 1.2, respectively, $p<0.001$). In the other hand, the subjects using statin and those without statin were not different in HDL size (9.0±1.0 and 9.0±0.9, respectively). **Conclusion:** Our results show that simvastatin modify the lipid flux to HDL. The diminished ability to receive lipids, which is consistent with diminished CETP action elicited by statin use, may increase the stability of the lipoprotein particles.

Funding: FAPESP

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SELF-MEDICATION WITH STATINS: THE UK EXPERIENCE

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Introduction: Cardiovascular disease is the commonest cause of premature death in the UK but physician-directed treatment with statins is targeted at high risk patients. In 2004 simvastatin 10mg became available for self-medication from pharmacies in the UK for people at moderate risk of CHD (10-15% 10 year risk of coronary events). The rationale, delivery model and impact of this move is reported. **Rationale and Model:** Reducing LDL reduces CHD risk in endpoint studies across the range of risk severity. The predicted efficicacy of simvastatin 10mg was extrapolated from the known effect on LDL (30% reduction) and risk reductions observed in similar risk populations. The moderate-risk population was defined based on self-reported key risk factors: age, gender, family history, smoking status, obesity and ethnicity. LFTs were not deemed necessary and cholesterol measurement was encouraged but not mandated. Pharmacists used a consumer questionnaire completed in store or on-line and printed off. Strong advice on risk improvement was also given with tailored lifestyle advice (on line and print). **Impact:** Self-medication is growing slowly from a small base. No safety issues have emerged. The user base is educated and behaving appropriately. Key factors limiting usage are: low level of UK consumer knowledge of CHD risk; pharmacists with low threshold to refer even when unnecessary; physicians who were treating much higher risk at the time were not supportive. **Conclusions:** The switch of simvastatin to self-medication status in the UK set a global precedent in addressing risk of future disease. Public health impact has been limited so far by factors specific to the UK environment and this experience should be extrapolated to other populations and health-care systems only with caution.

Funding: Author is a consultant to Merck & Co. Inc.

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COUNTERACTING EFFECTS OF STATINS AND CIGARETTE SMOKE ON POLYUNSATURATED FATTY ACID METABOLISM

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Statins enhance the production of long chain polyunsaturated fatty acids (LC-PUFA) from short chain precursors of the n-6 and n-3 series, both in vitro and in vivo. In particular, an increased conversion of [^{14}C] linoleic acid (18:2n-6, LA) and alpha linolenic acid (18:3n-3) to their LC derivatives, mainly arachidonic (20:4n-6, AA), eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids, is observed in different cell lines, with a different incorporation of products in various lipid classes. On the contrary, toxic compounds, as oxysterols and products of cigarette smoke (CS) negatively and dose-dipendently affect the LC-PUFA production in vitro. **Objective:** To evaluate the effects of CS alone or in association with simvastatin (S), on the incorporation and conversion of [^{14}C]LA to LC-PUFA in THP-1 cell line. **Results:** Conversion of [^{14}C]LA decreases dose-dependently after exposure of cells to CS, from 47% in controls to 22% at CS highest concentration (0.02 cigarettes), while the incorporation of LA in the different lipid classes is not affected. After co-incubation (48h), S nullifies the effects of CS, maintaining LA conversion comparable to controls. However, at the highest CS concentration, S doesn't counteract the effects of CS on LA conversion. Moreover, S is unable to revert the effects of CS when added 24h after CS. Changes of LA conversion reflect the modulation of the desaturase activities by S and CS. **Conclusion:** CS decreases PUFA conversion both in the n-3 and n-6 series in different cell lines and its effects on PUFA metabolism are modulated by the opposite effect of statins. It may be speculated that statin treatments in smoking patients may provide some beneficial effects on PUFA metabolism in addition to lower cholesterol levels.

Funding: None

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EFFECTS OF INTENSIVE ATORVASTATIN AND ROSUVASTATIN TREATMENT ON APOLIPOPROTEIN B-48 AND REMNANT LIPOPROTEIN CHOLESTEROL LEVELS

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Objective: Atorvastatin and rosuvastatin at maximal doses are both highly effective in lowering low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels. Rosuvastatin has been shown to be more effective than atorvastatin in lowering LDL-C and in raising high density lipoprotein (HDL) and its subclasses. This study was carried out in order to examine the effects of these two statins at maximal doses on lipoproteins of both intestinal and liver origin. **Methods:** We compared the effects of daily oral doses of rosuvastatin 40 mg with atorvastatin 80 mg over a 6-week period on serum apolipoprotein B-48 (the only valid marker of intestinal lipoproteins) and remnant lipoprotein cholesterol (RemL-C) levels using novel assays in 271 hyperlipidemic men and women. **Results;** Both atorvastatin and rosuvastatin caused significant reductions in LDL-C(-50.1%,-52.3%), which were significantly greater for rosuvastatin, but the reductions in triglycerides(-24.4%,-25.9%), RemL-C(-47.9%,-54,8%), and apoB-48(-25.2%,-24.8%) were similar for two statins. **Conclusions;** Our findings are indicate that intensive statin therapy lowers LDL-C and RemL-C about twice as much as TG and apoB-48 levels.

Funding: Kyowa Medex Co., (Otokozawa and Dr.Tanaka), Denka-Seiken Co., (Drs. Ai and Tanaka)

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ROSUVASTATIN RAPIDLLY AMELIORATES LIPIDS AND LIPOPROTEIN PROFILE AND INSULIN RESISTANCE IN JAPANESE PATIENTS WITH DYSLIPIDEMIA

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Rosuvastatin is a new highly efficacious statin that has been shown to produce large dose-dependent reductions in low-density lipoprotein cholesterol (LDL-C) in western hypercholesterolemic patients. However, there are few reports with rosuvastatin treatment in Japanese. We determined the lipids and metabolic factors in 41 patients (mean 61.7 years) before and after 6 months of rosuvastatin (2.5 to 20 mg/day) treatment. LDL-C (160.2 to 93.7 mg/dl, $p<0.001$) and triglyceride (145.7 to 111.0 mg/dl, $p<0.05$), significantly decreased, but not high-density lipoprotein cholesterol, low-density lipoprotein particle concentration significantly decreased. Moreover, insulin resistance (HOMA-R) also significantly improved. HbA1c and plasma homocysteine concentrations did not changed. Thus, rosuvastatin significantly improved in dyslipidemia and insulin resistance, and tended to exert the beneficial effects other metabolic factors in Japanese hypercholesterolemic patients.

Funding: There are no relationship to disclose

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EFFECTS OF LOW-DOSE SIMVASTATIN ON PLASMA LIPIDS, APOPROTEINS, OXIDIZED LDL, AND HIGH SENSITIVITY-CRP

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Objective: Low-dose statins are effective for reducing plasma LDL-C in Japanese people. We tried to examine the dose of simvastatin for reducing plasma LDL-C to 130 mg/dL or less. And, we also studied its effect on plasma ox-LDL and hs-CRP in 12-month open-label trial. **Methods:** Simvastatin was administered to 30 patients with hypercholesterolemia. Initial daily dose was 5 mg and the dose was increased to 20 mg via 10 mg for reducing plasma LLDL-C to 130 mg/dL or less and dose was fixed at 3 months of treatment. Total test duration was 12 months. Plasma lipids, apoproteins, ox-LDL, and hs-CRP were measured every 3 months. **Results:** Daily simvastatin dose at 12 months of treatment was 5 mg for 23 patients, 10 mg for 4 patients, and 20 mg for 3 patients. Plasma levels of LDL-C, TG, HDL-C, apo A1, apo B , and RLP-C were highly significantly changed from 182 mg/dL, 137 mg/dL, 55 mg/dL, 141 mg/dL, 144 mg/dL, and 5.4 mg/dL to 117 mg/dL, 114 mg/dL, 61 mg/dL, 154 mg/dL,103 mg/dL, and 3.8 mg/dL, respectively. Plasma ox-LDL was decreased from 162 U/L to 134 U/L (p=0.029). Plasma hs-CRP was also decreased from 1050 ng/mL to 844 ng/mL (p=0.019). **Conclusions:** Twelve months of treatment with low-dose simvastatin significantly reduced plasma levels of LDL-C, apo B, ox-LDL, and hs-CRP.

Funding: None

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EFFECT OF SIMVASTATIN AND EZETIMIBE ADMINISTRATION ON SERUM LEVELS OF LIPIDS AND OXIDATIVE STRESS MARKERS

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Objectives: One of the most effective combination of hypolipidemic drugs currently used for hypercholesterolemia treatment is simvastatin combined with ezetimib. The only monitoring of previously known atherosclerosis risk factors became insufficient nowadays because they have been neglecting newly discovered risk factors. One of those newly approved risk factors for IHD is oxidative stress. For that reason we examined and evaluated it while using effective combination of drugs: simvastatin and ezetimib. **Methods:** 20 patients with hypercholesterolemia were treated with simvastatin (20 mg/day) and ezetimib (10 mg/day). Lipid parameters (Total cholesterol, HDL, LDL, TAG) and oxidative stress markers (oxLDL, IgoxLDL, SOD, MDA, AOC) were examined at the beginning and at the end of the period. **Results:** Following main changes were registered: total cholesterol level decreased by 48 %, HDL cholesterol raised by 21 %, LDL cholesterol decreased by 50 %, SOD by 15 %, oxLDL by 42 %, IgoxLDL by 56 %, MDA by 42 %. **Conclusions:** Simvastatin dosage causes the decrease of atherogenic lipids and of some parameters associated with metabolism of free radicals and oxidative stress. The administration of combination of statins and ezetimib is suitable for further decrease of atherogenic lipids and oxidized LDL. Both cholesterol synthesis in liver and its absorbtion in intestinal mucosa was affected by this combination of drugs.

Funding: IGA Grant, Ministry of Health, Czech Republic

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THE IMPACT OF LIPID LEVELS ON CHOICE OF FIRST LINE ANTIRETOVIRAL REGIMEN IN HIV-POSITIVE PATIENTS

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Aim: First-line antiretroviral (ART) regimens to treat HIV typically contain 2 nucleoside reverse transcriptase inhibitors (NRTI) with either a protease inhibitor (PI) or a non-NRTI (NNRTI). The PI class, including the drug lopinavir/ritonavir (LPV/r), has been linked to lipid increases. We studied the association of lipids with ART regimen choice in HIV-positive patients. **Methods:** We studied ART-naive patients at the Royal Free Hospital, London starting ART in July 2003-July 2005 with TC measures recorded. All lipid measures are in mmol/l. **Results:** 114 started NNRTI+NRTIs (NNRTI); 66 started LPV/r+NRTIs (LPV/r); 32 started other regimens (Other). There was no association between pre-ART lipids and regimen choice: median TC levels were 3.9 for LPV/r, 3.9 for Other and 4.0 for NNRTI (p=0.97). For TG these were 1.3, 1.3, 1.2 (p=0.74), for LDL 2.2, 2.3, 2.3 (p=0.59) and for HDL 1.0, 1.0, 1.1 (p=0.36) Changing the initial PI/NNRTI in the ART regimen was not associated with current (time-updated) TC, LDL or HDL. However, higher current TG was associated with an increased chance of PI/NNRTI change (hazard ratio=1.50 per 1 mmol/l higher;1.29,1.73;p<0.0001). The maximum TC in the 1st year of ART was a mean 1.3 higher than pre-ART values. Compared to the NNRTI group, LPV/r had on average a 0.38 greater TC increase (95% CI 0.10,0.67) and Other had a 0.38 greater increase (-0.18,0.57;p=0.03). 13 (20%) of LPV/r, 4 (13%) Other and 7 (6%) NNRTI visited a lipid clinic for HIV-positive patients (p=0.02). **Conclusions:** We found no association between pre-ART lipid levels and initial regimen choice. The LPV/r group had greater average TC increases in the 1st year of ART.

Funding: There are no conflicts of interest

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A NOVEL CHOLESTEROL LOWERING EFFECT OF DISSOLVED ORGANIC MATTER FROM DEEP SEA WATER IN HYPERLIPIDEMIC RABBITS

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Background: Recently research for bioreactive components from natural resources has been intensified. We focused our attention to dissolved organic matter (DOM) from desalted deep sea water (dDSW) which comprises the largest reservoir of organic carbon at $700x10^{15}$g . DOM was isolated by ultrafiltration technique, by passing original DSW through 10 micron and Octadecyl C18 filter and the filtered substance was extracted in ethanol. Here, we demonstrated the anti-atherogenic effect of DOM. **Materials and Methods:** Balloon injury was performed in common carotid artery of Japanese white rabbits, followed by feeding with 1% high cholesterol diet and atherosclerotic lesions were assessed after 4 weeks. Neointimal thickness in Groups fed with DOM containing dDSW for 4 weeks or 6 weeks (2 weeks pre-operatively) reduced drastically in contrast to control group with p<0.01. In addition, cholesterol fed WHHL rabbits were evaluated for its atheroscelrotic lesions. Group1: fed with 50 ml/day normal drinking water, Group 2: fed with DOM containing dDSW, Group 3: fed with high dose of DOM for 2 months. Oil red-O stained en face preparation of aortae of WHHL rabbits revealed that atherosclerotic plaques decreased markedly in Groups 2 and 3 when compared to Group 1 (47.2%, 35.7% vs 60.3%) and moreover significant reduction in total cholesterol levels (TCHO) was observed in Group 2 and 3 with p<0.05 vs Group 1. To elucidate the cholesterol lowering effect of DOM, we conducted spectrophotometric analysis for HMG- CoA reductase. DOM attenuated the enzyme activity by 51.8%. **Conclusion:** These datas represent that DOM exerts anti-atherosclerotic effect in hyperlipidemic rabbits possibly by modulating lipid profile.

Funding: None

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EFFECTS OF PIOGLITAZONE ON LIPID LEVELS IN PATIENTS RECEIVING A STATIN AT BASELINE IN PROACTIVE

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PROactive showed that pioglitazone (PIO) reduced the risk of death, MI, or stroke *vs* placebo (PBO). This benefit was consistent across 56 subgroups, including baseline statin use. We performed a *post-hoc* analysis of lipid changes in the baseline (BL) statin-user subgroup in PROactive. PROactive was a randomized, double-blind, PBO-controlled study in 5238 patients with type 2 diabetes and macrovascular disease who received PIO (up to 45 mg) or PBO on top of other glucose-lowering and CV therapies. Mean follow-up was 34.5 mos. Fasting plasma lipid levels were assessed every 6 mos. At BL, 2245 (43%) patients were on statins (>90% continued on statins until final visit). Within this subgroup, triglycerides (TG) decreased with PIO (12.3%; BL: 1.82 mmol/L), but increased with PBO (3.1%; BL: 1.81 mmol/L; $p<0.0001$ between-group difference). HDL-C increased more with PIO (20.3%; BL: 1.08 mmol/L *vs* 10.0% from 1.10 mmol/L with PBO; $p<0.0001$); there was no difference in LDL-C changes (+8.20% from 2.50 mmol/L with PIO *vs* +7.09% from 2.46 mmol/L with PBO; $p=0.3387$). Accordingly, there was a more favorable shift in the LDL-C/HDL-C ratio with PIO (-9.13% *vs* -1.44% with PBO; $p<0.0001$). The favorable effects of PIO on markers of diabetic dyslipidemia noted in the total PROactive population were also noted in the baseline statin-user subgroup.

Funding: The PROactive study was funded by Takeda Pharmaceutical Company and Eli Lilly & Company

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DUAL PPARα/γ AGONIST TESAGLITAZAR BLOCKS PROGRESSION OF PRE-EXISTING ATHEROSCLEROSIS IN APOE*3LEIDEN.CETP TRANSGENIC MICE

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Objective: To evaluate the effect of the PPARα/γ agonist tesaglitazar (TESA) on the progression of pre-existing atherosclerotic lesions in APOE*3Leiden. CETP transgenic mice. **Methods:** E3L.CETP mice were fed an atherogenic diet for 11 weeks to induce atherosclerosis, resulting in plasma cholesterol (TC) levels of about 20 mM. Then the cholesterol in the diet was reduced to obtain human like TC levels (±10mM). After 4 weeks the mice were matched into 3 groups: a progression control group (PC), which was sacrificed, a regression control (RC) and a TESA-treated group, which were treated for 8 weeks and then sacrificed to assess atherosclerosis development. **Results:** TESA reduced triglycerides by 71% ($p<0.001$), TC by 55% ($p<0.001$), mainly in the VLDL/LDL, and CETP mass by 42% ($p=0.001$) and increased HDL. In the PC group 136± 87 *1000 μm^2 lesion area per cross section was developed, of which 47± 23% were severe lesions. After 8 weeks the RC group showed lesion progression (210± 84 *1000 μm^2 and 69± 17% severe lesions; $p<0.01$), whereas TESA totally blocked further lesion development (140± 97 *1000 μm^2) and progression of severity (59 ± 24%), and also stabilized the lesions, by increasing their collagen content (65± 9% vs 33± 11% in PC and 54± 10% in RC; $p<0.01$). **Conclusions:** Dual PPARα/γ agonism with TESA markedly reduced VLDL/LDL and increased HDL levels which may be the mechanism for the observed complete inhibition of progression and stabilization of pre-existing atherosclerotic lesions.

Funding: This study was sponsored by a grant from AstraZeneca

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ANTIATHEROGENIC ACTION OF ISOFLAVONOID-RICH BOTANICALS: AN IMPLEMENTATION FOR ATHEROSCLEROSIS PREVENTION IN POSTMENOPAUSAL WOMEN

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Objective: Isoflavonoids are thought to prevent atherosclerosis development in postmenopausal women, but little is known about their direct effects on atherogenesis. We have studied the effect of isoflavonoid-rich plants on the ability of blood serum to induce lipid accumulation in cells. **Methods:** Blood was taken from apparently healthy postmenopausal women before oral intake of plant pills and 2, 4 and 6 hours after it. Atherogenicity was assessed by serum-induced cholesterol accumulation in cultured human monocyte-macrophages. **Results:** At the baseline, serum induced 1.3-1.5-fold increase in intracellular cholesterol content in cultured cells. Grape seeds extract (100 mg) lowered serum atherogenicity integrally by 76±3%. Similar effects were observed for garlic (150 mg), hop cones (250 mg), sage leafs (100 mg), green tea leafs (250 mg), sea kelp (500 mg), fucus (250 mg) and carrot (1000 mg) (the most effective single doses are given). The effects exceeded that of soya beans extract (35 mg) where serum atherogenicity lowered integrally by 32±6% after single dose administration. Isoflavonoid-rich composition of garlic, green tea, grape seeds and hop cones extracts possessing highest antiatherogenic potency has been developed. The long-term placebo-controlled trial has been started to estimate direct effect of this composition on natural history of carotid atherosclerosis in postmenopausal women. **Conclusions:** The reduction of serum atherogenic potential with isoflavonoid-rich plants may be the effective way for the prevention of cholesterol deposition in vascular cells, therefore inhibiting atherogenesis.

Funding: This study was supported by grant from the Institute for Atherosclerosis Research

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HUMAN BLOOD SERUM-INDUCED INTRACELLULAR CHOLESTEROL ACCUMULATION: THE RELATIONSHIP TO ATHEROSCLEROSIS DEVELOPMENT

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Objective: The deposition of intracellular cholesterol is a key feature of atherogenesis at the level of arterial wall. The ability of blood serum to induce lipid accumulation in cultured cells (atherogenicity) is highly correlated with the severity of atherosclerosis. To elucidate the relationship between serum atherogenicity and atherosclerosis progression, the two-year placebo-controlled study has been performed in men with subclinical atherosclerosis. **Methods:** Time-released garlic powder tablets (Allicor, INAT-Pharma, Russia) were used for serum atherogenicity reduction. Intima-media thickness (IMT) of common carotid arteries was evaluated by B-mode ultrasound. Atherogenicity was assessed by serum-induced cholesterol accumulation in cultured human monocyte-derived macrophages. **Results:** In 41 patient with initially non-atherogenic serum the increase of atherogenicity during follow-up correlated with the increase of IMT ($r=0.345$; $p=0.027$). Spontaneous uprise of serum atherogenic potential was contingent with the progression of early atherosclerotic lesions ($p=0.008$). In 165 patients blood serum induced 1.2-3.9-fold increase in cholesterol content of cultured cells, i.e. was atherogenic at the baseline. The overall changes in serum atherogenicity correlated with the dynamics of IMT ($r=0.155$, $p=0.047$). The complete removal of serum atherogenic potential in Allicor-treated patients was contingent with atherosclerosis regression ($p=0.014$). **Conclusion:** The uprise of blood serum atherogenicity is related to atherosclerosis progression, and the elimination of atherogenicity induces the regression of atherosclerotic lesions.

Funding: This study was supported by grant from the Institute for Atherosclerosis Research

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THE REDUCTION OF PROINFLAMMATORY CYTOKINE EXPRESSION BY NATURAL COMPONENTS: A NEW APPROACH TO THE PREVENTION AND TREATMENT OF ATHEROSCLEROSIS AT THE CELLULAR LEVEL

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Objective: To evaluate anti-inflammatory and anti-atherogenic activity of natural drug Inflaminat based on Black Elder berries, Calendula and Violet flowers. **Methods:** Blood serum was obtained from atherosclerotic patients before drug administration and in 2, 4 and 8 hours after it. Its ability to induce cholesterol accumulation and expression of IL-1 and TNF-α was investigated in primary culture of human blood-derived macrophages. Anti-inflammatory activity was confirmed in animal model and in the pilot open-label clinical trial in patients with inflammatory diseases of joints. **Results:** Oral administration of Inflaminat led to decrease of serum ability to induce overexpression of inflammatory cytokines: IL-1 expression was lowered integrally by 23.5±4.0% ($p<0.05$), and TNF-α by 16.8±5.0%, ($p<0.05$) for 8 hours. At the same time blood serum atherogenicity was lowered integrally by 63.9±7.8%, ($p<0.05$). In animal model, the significant reduction of the number of mast cells and oedema was observed. The results of clinical trial demonstrated anti-inflammatory potential of Inflaminat with the respect to total number of injured joints, disease-specific complaints and pain syndrome. **Conclusions:** Natural anti-inflammatory drug Inflaminat provides both anti-cytokine and anti-atherogenic effects. Double mechanism of action allows to suggest novel non-lipid-lowering clinical implications for long-term atherosclerosis prevention and treatment.

Funding: This work was supported with grant from the Institute for Atherosclerosis Research

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REGRESSION OF THE INTERNAL CAROTID ARTERY ATHEROSCLEROTIC PLAQUES AFTER LONG-TERM STATIN THERAPY: A CASE REPORT

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Dyslipidaemia, especially elevated levels of LDL cholesterol and low levels of HDL cholesterol, is strongly associated with the risk of cardiovascular disease, including cerebrovascular disease. Statins are recommended as first-line therapy for patients with dyslipidaemia. It has been shown that for every 1,0 mmol/l reduction in LDL cholesterol, statin therapy reduces the risk of major vascular event by 21%. Body of available evidence points to "lower is better" when it comes to LDL cholesterol and atherosclerosis. A number of different techniques, including ultrasound, can be used to image the arterial wall and evaluate the extent of atherosclerotic plaques and the associated risk of cardiovascular disease. Sonography has successfully been used to investigate the effects of lipid-affecting therapy on the state of atherosclerosis. We present the case of a 55-year old asymptomatic male who was referred to our lipid clinic due to mixed dyslipidaemia (C 7, LDL 4,5, TG 4) and sonographic signs of 50% left internal carotid artery stenosis (due to hypoechoic, 'soft' plaques) in its proximal part in 1993. For few months, he was treated with diet only, afterwards with lovastatin for a year, fluvastatin 40 mg for 4 years, simvastatin 20 mg for 4 years and atorvastatin 20 mg for 4 years (for 2 years in combination with fibrate), with therapy mostly achieving recommended lipid targets at the time. At sonographic follow-ups, we have been observing the gradual regression of the internal carotid artery atherosclerotic plaques. The last imaging revealed borderline intima-media thickness only. Long-term statin therapy has been associated with the near-full resolution of carotid atherosclerotic stenosis in our patient.

Funding: Our work has not been funded in any way

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CO-MORBIDITY OF SEVERE HYPERTRIGLYCERIDEMIA (>1000 mg/dl): ANALYSIS OF 893 CASES

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Severe Hypertriglyceridemia (SHTG) is believed to be a rare condition difficult to treat, but associated with a low risk of atherosclerosis. We performed a morbidity survey of SHTG followed up for at least 2 years in the lipid clinics of the German Working Group. Evaluation was based on patient chart review. The data of 893 SHTG cases (689 males,204 females) were compiled. As an admission criterion to the study SHTG patients had to have a documented TG >1000 mg/dl prior to follow-up. Mean age was 50 ±12 years, mean BMI was 28.7 ±4.5. Upon admission to the lipid clinics mean TG was 2800 mg/dl (1000–16000 mg/dl) and mean TC was 480 mg/dl (162-15920 mg/dl). Even after long term treatment mean TG and TC were still highly elevated (1190mg/dl and 322mg/dl, respectively). Regarding co-morbid conditions the following prevalence data were obtained: episodes of pancreatitis 24% (21% in males (m),35% in females (f)), gallstones 21%(17% m, 32% f), hypertension 70%, diabetes 51%, hyperuricemia 72%. The prevalence of clinical manifestations of cardiovascular disease was as follows: CHD by angiography 24% (27% m, 16% f), MI 14% (16% m, 5% f), angina 21% (23% m, 17% f) , PVD 15 % (14% m, 17% f), cerebrovascular disease 20% (19% m, 24% f). A total of 36% of all SHTG patients (37% m, 33% f) showed at least one clinical manifestation of atherosclerotic cardiovascular disease. After excluding other risk factors 19% of remaining SHTG cases (20% m, 16% f) still had clinical CVD. In conclusion, we were able to demonstrate a significant cardiovascular disease burden in both male and female SHTG patients. SHTG itself seems to be an independent CVD risk factor.

Funding: None

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ALGAL-DERIVED DOCOSAHEXAENOIC ACID SUPPLEMENTATION LOWERS FASTING TRIGLYCERIDE LEVELS IN NORMAL AND IN HYPERTRIGLYCERIDEMIC SUBJECTS

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Elevated triglycerides (TG) represent an independent risk factor for cardiovascular disease. For TG lowering, omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are recommended by the American Heart Association (AHA) at 2-4 g/day. **Objective:** To determine the independent effect of DHA supplementation on TG lowering. **Methods:** Studies with outcomes related to Martek algal-derived DHA and TG levels were identified. Data from these studies were extracted and expressed as daily dose of DHA *versus* % change in baseline TG level. **Results:** Sixteen studies were identified: 12 studies supplemented subjects with algal oils from *Crypthecodinium cohnii* and 4, from *Schizochytrium sp.* (both oils contain 35-40 % DHA, <3 % EPA). Twelve studies investigated normal subjects. The remaining four studies investigated hypertriglyceridemic subjects (TG >150 mg/dL) of which two were conducted with concomitant statin therapy. DHA supplementation significantly reduced fasting TG in a dose-dependent fashion, regardless of type of algal oil or baseline TG level. Supplementation of 1-2 g/day of DHA, with or without concomitant statin therapy, effectively lowered TG by 15 - 20 %. The absolute decrease (mg/dL) in TG in the hypertriglyceridemic subjects was markedly greater than that observed in normals. **Conclusions:** Algal-derived DHA alone (essentially devoid of EPA) is effective in lowering fasting TG levels whether as the sole agent or co-administered with a statin. In the studies reviewed, TG reductions were achieved using DHA alone at a dose lower than that currently recommended by AHA for the combination of DHA and EPA.

Funding: Martek Biosciences Corporation

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STRUCTURAL MODIFICATION OF THE AMIDE REGION OF BEZAFIBRATE RESULTS IN ENHANCED FIBRATE ACTIVITY AND ACAT INHIBITION

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The amide containing fibric acid derivative, bezafibrate, has been widely utilized as an antihyperlipidemic agent for over two decades. We have previously shown that the lipid lowering properties of novel N-alkyl/aryl ureido phenoxyisobutyrates are associated with potent PPAR dependent effects but not with their ACAT inhibitory activity (J. Lipid Res. 1997: 38, 1189). To further investigate the mechanism(s) of action of the fibrate class of antilipemic agents, we examined several analogues of bezafibrate that incorporated selected key structural features of the N-alkyl/aryl ureido phenoxyisobutyrates. The *in vitro* fibrate (lauric acid hydroxylase induction) and ACAT inhibitory activities of the N-n-butyl analogue of bezafibrate were 2.7 and 4.0 times more potent than bezafibrate itself, respectively. Reversal of the amide group in bezafibrate and the addition of an n-heptyl group to the carbon alpha to the carbonyl group resulted in a number of analogues with much greater fibrate and ACAT inhibitory activities. For example, replacing the chloro substituent of bezafibrate with 2 and 4 position fluoro substituents, reversing the amide and adding a heptyl substituent alpha to the carbonyl, resulted in an agent with 27 times more fibrate activity and 20 times more ACAT inhibitory activity. Our results indicate that the antilipemic activity of bezafibrate can be greatly enhanced by the structural modifications to the amide region noted above.

Funding: Funding graciously provided by Glaxo Smith Kline

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PROBABLE METABOLITES OF POTENT ANTIHYPERLIPIDEMIC N-ALKYL/ARYL UREIDO FIBRATE ANALOGUES: SYNTHESIS AND *IN VITRO/IN VIVO* ACTIVITIES

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Highly potent N-alkyl/aryl ureido fibrates with significant ACAT inhibitory activity and noteworthy *in vivo* antihyperlipidemic activity in rodents have previously been reported (J. Lipid Res. 1997, 38, 1189). In an attempt to ascertain the degree to which metabolic products may contribute to the overall activity of these agents, we have synthesized a number of probable (reported for structurally similar ACAT inhibitors) ω-oxidation and benzylic oxidation metabolites. These agents with ω- hydroxyl or carboxyl groups within the N-alkyl chain or alternatively with a benzylic position hydroxyl or carbonyl group, generally demonstrated dramatically reduced lauric acid hydroxylase inducing activity, correlating with minimal PPAR agonist activity. ACAT inhibitory activity was slightly decreased in many of these compounds but was eliminated in the dicarboxylic acids tested. Several of these probable metabolites were also tested for antihyperlipidemic activity in rodents and found to have no effect at a dose of 10mg/kg/day. These experiments indicate that the potency of the N-alkyl/aryl ureido fibrate parent compounds is highly dependent upon the reduced state of these agents and ω-oxidation or benzylic oxidation metabolites are not likely to contribute to their overall *in vivo* antihyperlipidemic activity.

Funding: Funding graciously provided by Glaxo Smith Kline

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EFFECT OF EZETIMIBE ON PLATELET AGGREGATION AND LOW-DENSITY LIPOPROTEIN TENDENCY TO PEROXIDATION IN HYPERCHOLESTEROLEMIC PATIENTS ON SIMVASTATIN TREATMENT

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Objective: To investigate the effect of LDL lowering by ezetimibe on platelet activity and LDL tendency to peroxidation in hypercholesterolemic patients on simvastatin therapy. **Methods:** Twenty two hypercholesterolemic patients on fixed dose of simvastatin with LDL>130 or >100 mg/dL (in according to ATP III goal) and triglycerides<300 mg/dL without active coronary heart disease enrolled. The patients continued on their treatment with simvastatin 20 mg/day for 6 weeks, and then the patients treated by the same dose of simvastatin combined with ezetimibe 10 mg/day for other 6 weeks. Blood tests for lipids, liver and renal function tests, LDL tendency to peroxidation in the presence of $CuSO_4$ measured by lag time in minutes required for the initiation of $CuSO_4$-induced LDL oxidation and by LDL oxidation at the maximal point (plateau) which was analyzed by the thiobarbituric acid reactive substances assay, which measures malondialdehyde (MDA) equivalents and platelet aggregation were done before and after ezetimibe treatment. Student's t-test is used for comparing the two means. Data are presented as mean ± standard deviation. **Results:** Serum levels of total cholesterol and LDL-C were reduced significantly by 15% and 23%, respectively, on combined simvastatin-ezetimibe therapy. Lag times in minutes required for the initiation of LDL oxidation in the presence of $CuSO_4$ were 55.9±16.5 minutes on simvastatin therapy alone and 82.7±11.6 minutes on combined simvastatin-ezetimibe therapy, n=22, $p<0.0001$. MDA equivalents content in LDL measured at the maximal point (plateau) were 112.5±67 on simvastatin therapy alone and 74.8±56 on combined simvastatin-ezetimibe therapy], n=22, $p=0.049$. There were no differences in platelet aggregation after activation of platelets by collagen, ADP or epinephrine. **Conclusions:** Treatment with ezetimibe on top of simvastatin have additive anti-oxidative effect on LDL.

Funding: None

Poster Session 8 "METABOLIC SYNDROME" - GROUP B

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INFLAMMATORY CYTOKINES IN THE DEVELOPMENT OF METABOLIC SYNDROME IN MALE OBESE SUBJECTS

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It is postulated that inflammatory cytokines are involved in the development of metabolic syndrome (MS). We examined serum levels of inflammatory cytokines in 47 male subjects (of an average age of 42±7) with visceral fat obesity (waist circumference over 85cm). They were subdivided into 3 groups; simple obesity without other metabolic abnormalities (n=15), and those with one metabolic disorder (n=16), and those with MS (n=16, according to the criteria of Jpn. Soc. Int. Med. 2005). Serum adiponectin levels were 7.2±2.6μg/ml, 6.4±2.5μg/ml, and 5.8±3.0μg/ml, respectively, in the above described order. Serum IL-6 levels increased from 1.1±0.5pg/ml, 1.2±0.4 pg/ml to 1.7±0.8pg/ml ($p<0.05$) in the same order. TNF-α were 1.2±0.3 pg/ml, 1.3±0.3 pg/ml, 1.5±0.3 pg/ml, respectively and h-CRP were 502±393ng, 822±935ng, 1463±1350ng/ml ($p<0.01$), respectively. These results indicated that a low-grade inflammatory process might be responsible for the development of MS in obese subjects.

Funding: There is no funding sources of commercial nature for the work done in the abstract

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THE PREVALENCE OF METABOLIC SYNDROME AMONG FILIPINO COLLEGE STUDENTS AGED 18-25 YEARS. FINAL REPORT

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Objectives: The study determined the prevalence of metabolic syndrome (MS) among Filipino college students aged 18-25 years, and those *at risk* for MS using the NCEP ATPIII Modified and WHO Asian Modified criteria. Another aim was to identify the risk factors associated with MS in this subpopulation. **Design:** Cross-sectional descriptive prospective study. **Setting:** The study was conducted in 6 collegiate schools,3 were private and the other 3 were government schools. **Participants:** A total of 505 (M=129 and F=376) Filipino college students were enrolled, 27 students had no blood analysis and/or incomplete anthropometric data. A total of 478 students completed the study. **Outcome:** Anthropometric measures were obtained. Blood analyses for FBG, lipid profile, SGOT, SGPT and serum insulin were extracted, and urine microalbumin was determined. Prevalence rates for MS and *at risk* for MS were obtained. The most commonly associated risk factors for MS were also determined. **Results:** Mean BMI, WC, SBP,DBP,FBS, triglyceride,HDL-C,SGOT/SGPT, HOMA-IR and urine microalbumin were reported. By NCEP ATPIII Modified Criteria, the prevalence of MS was 2.3%, and 7.5% were at risk for MS. By WHO Asian Modified Criteria, MS was prevalent in 14.2%, and 8.2% were at risk for MS. **Conclusions:** The prevalence for MS among Filipino college students was 14.2% by WHO criteria, and 2.3% by NCEP criteria. The risk factors most commonly associated were abnormal HDL-C, blood pressure, FBS, urine microalbumin, insulin resistance and waist-to-hip ratio. These data support early intervention for management of cardiovascular and metabolic risk factors even at an early age.

Funding: Solvay Pharma Phils. Inc.

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USEFULNESS OF THE CORONARY ARTERY CALCIFICATION SCORE BY MULTI-DETECTOR ROW COMPUTED TOMOGRAPHY FOR METABOLIC MARKERS

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Background: Metabolic diseases which may be due to dysfunction of adipocytokine are risk factors of coronary artery disease (CAD). Association between coronary artery calcification (CAC) score determined by multi-detector row computed tomography (MDCT) and medications or metabolic markers are not well known. **Methods:** Subjects included 88 consecutive patients (male/female=53/35, age=63±11 years) who underwent coronary angiography using MDCT. We quantified CAC score and coronary stenotic (CS) score by MDCT and measured blood pressure (BP), ankle-brachial index (ABI) and pulse wave velocity (PWV). We also analyzed plasma levels of adiponectin, tumor necrosis factor (TNF)-α, bone morphogenetic protein (BMP)-2, lipid profile and blood glucose. **Results:** Plasma adiponectin levels were negatively correlated with body mass index, while plasma adiponectin or BMP-2 levels were not correlated with CAC score. Since we previously reported that a higher number of coronary stenosed vessels was associated with a higher CAC score, CAC score were divided into three groups [lower (L) group, CAC score=0-12; intermediate (I) group, score=13-445; higher (H) group, score>445]. No significant differences among three groups were observed in medications or metabolic markers except for PWV. CS score and PWV in the I and H group were significantly higher than those in L the group. In addition, CS score was positively correlated with PWV. **Conclusions:** The higher CAC score associated with higher values of PWV can be useful for predicting CAD independent of other medications or metabolic markers.

Funding: None

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AMELIORATING EFFECT OF EXERCISE TRAINING ON SERUM LIPIDS AND ADIPONECTIN: CLINICAL SIGNIFICANCE OF MONITORING VLDL CHOLESTEROL

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Objective: Recently, increasing evidences have demonstrated favorable effects on serum lipid profile and good clinical outcomes in patients with cardiovascular diseases. The present study was performed to investigate effects of exercise training (ET) on serum lipids and adiponectin. **Methods:** Hyperlipidemic patients (N= 26) were enrolled and they performed ET (60 min/ day, 8 times/ month; fitness training) for 16 weeks. Serum cholesterol levels of each lipoprotein (HDL, LDL, IDL, VLDL, chylomicron) were measured by the HPLC method as we previously reported (J Lipid Res 2003; 44: 1404-12). Other parameters were measured conventionally. **Results:** ET significantly decreased body weight, cholesterol levels of LDL and IDL and particular reduction in VLDL cholesterol (-56%), but significant changes in triglyceride and cholesterol levels of HDL, chylomicron, and RLP were not found. VLDL cholesterol reduction (-52%) was markedly observed as early as 8 weeks after beginning of ET. Adiponectin significantly increased by 53% at week 16 although no major change was observed at week 8, and HOMA-R eventually came to decrease at week 16 as well. **Conclusions:** These results suggest that VLDL cholesterol measurement is likely to be useful for assessing effect of ET, and that VLDL cholesterol reduction during ET may take place before the increased adiponectin.

Funding: The present research was supported by the Jikei University Research Fund

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METABOLIC BACKGROUND OF INCREASED CARDIOVASCULAR RISK CAUSED BY OBSTRUCTIVE SLEEP APNEA SYNDROME

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Background: Obstructive sleep apnea syndrome (OSAS) causes higher cardiovascular morbid-mortality: hypertension,heat failure,increased risk of myocardial infarction,stroke.OSAS can result in early dementia,traffic and working injuries with daytime sleepiness.Average prevalance is 2-4%,most of patients are hypertensive and obese.Although diagnostic gold standard is polysomnography in special sleep labs,apnograph is a new method pre-diagnosing OSAS.Device has 3 parts,ABPM,full-day 3-lead ECG-Holter (with HRV analysis) and pulzoxymetry. **Method and Results:** By help of mentioned diagnostic tools pts (all had signs of metabolic syndrome) were divided into 2,by demographic features similar groups:OSAS group-proved sleep apnea (27 pts), control group-habitual snorers (24 pts).Aim was to compare metabolic values.OSAS-group had significantly worse parameters in total cholesterol (5,26$\pm$0,67,and 4,48$\pm$0,86 mmol/l - p 0,03), triglyceride (2,28$\pm$1,49,and 1,49$\pm$0,67 mmol/l - p 0,01), LDL-cholesterol (3,56$\pm$0,73,and 3,03$\pm$0,83 mmol/l - p 0,02).In blood glucose (6,21$\pm$1,14,and 5,72$\pm$1,14 mmol/l – p 0,17),HDL-cholesterol (1,16$\pm$0,2,and 1,15$\pm$0,16 mmol/l – p 0,75) uric acid (394$\pm$135,and 370$\pm$119 umol/l – p 0,4) no significant difference was proven between 2 groups. **Conclusion:** Sleep apnea syndrome is independent cardiovascular risk factor with high prevalence of hypertension and obesity.OSAS pts had worse metabolic parameters (LDL-cholesterol,triglycerid and total cholesterol) compared with habitual snorers.Although these results need reenforcement in higher population,mentioned data can explain cause of metabolic background of elevated cardiovascular risk among sleep apnea patients.

Funding: None

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LEPTIN: ADIPONECTIN RATIO IS AN INDEPENDENT PREDICTOR OF INTIMA-MEDIA THICKNESS OF THE COMMON CAROTID ARTERY

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The evaluation of the leptin:adiponectin ratio (L:A) has been suggested as an atherosclerotic index in patients with type 2 diabetes and a useful parameter to assess insulin resistance in patients with and without diabetes. We investigated, therefore, the relationship between L:A ratio and IMT in 110 healthy males. L:A ratio was significantly correlated to BMI waist, hip waist-hip ratio, SBP, IMT, HDL, apolipoprotein A-I, glucose and HOMA-R. No significant correlation was observed with age, DBP, LDL, tryglicerides, apolipoprotein B, ApoB/ApoA-I ratio, insulin and resistin. In addition, when the relationship between IMT and adiponectin or leptin alone was analysed, only leptin plasma levels significantly associated with IMT ($r=0.301$, $p<0.01$). In a multiple regression analysis including in the statistical model the risk factors known to affect IMT we observed that only age, L:A and glucose were independent predictors of IMT (Table 2). As expected, obese subjects (BMI>30 Kg/m^2) showed a significantly higher L:A ratio compared to non-obese subjects (1.20 vs 0.42 respectively, $p<0.001$); in addition, subjects with the metabolic syndrome showed a significantly higher L:A ratio level (0.79) compared to subjects without (0.52) $p<0.01$. In summary, we show here that the L:A ratio is a powerful independent predictor of IMT in healthy subjects and correlates with several anthropometric, metabolic and clinical parameters better than each single adipokine.

Funding: This work was supported by grants from the Ministry of University and Research

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METABOLIC SYNDROME – COMPOSITION OF FATTY ACIDS AND LIPOPEROXIDATION

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Objective: The aim of this study was to examine the fatty acid (FA) profile in the main lipid classes of plasma with relation to insulin resistance, some polymorphisms of candidate genes for insulin resistance and to the composition of lipoproteins as well as the parameters of lipoperoxidation. **Materials and Methods:** The study involved 95 patients with metabolic syndrome (MS) (56M/39F) and 195 controls (99M/96F). We examined basic clinical data, the parameters of glucose homeostasis, the concentrations of plasma lipids, FA profile in lipid classes, and conjugated dienes in LDL. Polymorphisms of apoE, intestinal isoform of FABP (Ala54Thr), and g2 isoform of PPAR (Ala12Pro) were assayed with the combination of methods of PCR and RFLP. **Results:** The patients with MS had higher concentrations of CRP and conjugated dienes in LDL. In all lipid classes of plasma, we observed decrease in n-6 polyunsaturated FA and increase in saturated FA. In detail, we found reduced content of linoleic acid and raised content of palmitic and well as palmitoleic acids. It could be inferred that the activities of D9 desaturase, D6 desaturase and elongase are all increased. The concentrations of conjugated dienes in LDL negatively correlated with linoleic acid. No relationship of clinical or laboratory parameters with homozygosity of analysed polymorphisms was found. **Conclusions:** In MS, the changes in FA composition are caused by enhanced lipogenesis and increased oxidative stress.

Funding: Supported by the Research Project MSM 0021620820, Ministry of Education, Youth and Sports, Czech Republic

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PLASMA FATTY ACIDS AND LIPOPEROXIDATION IN THE DEPRESSIVE DISORDER AND METABOLIC SYNDROME

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Objectives: Supposed relationship of depressive disorder (DD) to metabolic syndrome (MS) can contribute to increased cardiovascular morbidity in patients with DD. **Methods:** We observed anthropometric indexes, plasma lipoproteins, glucose, insulin, C-peptide, non-esterified fatty acids, fatty acid (FA) composition in plasma phosphatidylcholine (PC), cholesteryl esters (CE), and triacylglycerols (TG) and conjugated dienes (CD) in LDL in 27 persons (20M/7F) with MS (mean age 55.4 yrs), 8 probands (2M/6F) with DD (mean age 63.1 yrs), and of 12 persons (7M/5F) of the control group (CON) (mean age 50.6 yrs) . **Results:** Probands with DD differed from CON by lower content of n-6 FA in PC (37.5 vs 40.2 mol%), CE (60.9 vs. 66.2 mol%), linoleic acid in CE (53.0 vs 57.9 mol%), alpha-linolenic acid in TG (0.7 vs. 1.2 mol%) and CE (0.4 vs. 0.6 mol%) and higher one of saturated FA in CE (12.8 vs. 11.3 mol%), monoenoic FA in PC (12.7 vs. 11.0 mol%), CE (25.0 vs. 21.3 mol%); all $P < 0.05$; oleic acid in PC (10.5 vs. 8.9 mol%; $P < 0.01$), CE (19,8 vs. 16.9 mol%) and palmitoleic acid in TG (3.7 vs. 2.9 mol%) and CE (3.6 vs. 2.7 %; all $P < 0.05$). The concentrations of CD were higher in DD (88.7 vs. 52.9 μmol/l; $P < 0.05$). These changes were similar to those of the MS group, although MS had higher BMI, TG, waist, insulin, systolic and diastolic blood pressure. **Conclusions:** Persons with DD in our study were similarly to those of MS characterized by signs of increased lipogenesis and lipoperoxidation despite of absence of several indices of insulin resistance.

Funding: Supported by the research grant of Ministry of Health Czech Rep., NR-8806-03

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THIOCTIC ACID AND HORMONAL REPLACEMENT TREATMENT IN MEN AND WOMEN WITH 2 TYPE DIABETES AND OBESITY

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Aim: To estimate the thioctic acid influence in monotherapy and in combination with hormonal replacement treatment (HRT) in middle aged men and women with 2 type diabetes mellitus (Type 2 DM) and obesity on weist circumference (WC), body weight index (BWI), lipid levels, basal and postprandial insulin, HOMA index, total testosteron and sex binding globulin (SBG). **Methods**: 30 men with androgen deficit syndrome (hypothyroidism and hyperprolactinemia were excluded) and 30 women in menopause (53±14 years) were treated. All of them intravenously received thioctic acid 600 mg once a day during 3 weeks and after that they received thioctic acid 600 mg per os during 45 weeks. By 11 weeks from the thioctic acid beginning, Androgel to men and Femoston 1/10-1/5 to women were added and used for 45 weeks. The WC, BWI, lipid levels, basal and postprandial insulin, HOMA, testosterone, SBG were researched before and 11 and 48 weeks after the treatment start. 20 men with androgen deficit syndrome and 20 women in menopause with obesity but without Type 2 DM were as a control. For statistic analysis a program Statistica 6.0 was used. **Results**: In 11 weeks of thioctic acid treatment there were decreasing all of parameters besides of testosteron level (it was increasing – statistically validity). In 48 weeks of combined thioctic acid and HRT the efficiency of treatment was better. All patients noticed the improvement of their life: they were in better mood; they wanted to move and to do physical exercises. **Conclusions:** Thioctic acid has a positive influence on insulin resistance. Testosterone level increase and decrease of SBG level under thioctic acid were shown by us earlier. The combined therapy of thioctic acid and HRT improves the life of middle aged men and women.

Funding: None

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EFFECTS OF CO-ADMINISTERED EZETIMIBE/ SIMVASTATIN AND FENOFIBRATE IN MIXED HYPERLIPIDEMIC PATIENTS WITH METABOLIC SYNDROME

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Background: Metabolic syndrome (MetS) is characterized by a clustering of atherosclerotic CHD risk factors including abdominal obesity, high triglycerides (HTG), low high-density lipoprotein cholesterol (HDL-C), and increased proportion of small, dense LDL particles (Pattern B). This post-hoc analysis examined the treatment response of ezetimibe/simvastatin (EZE/SIMVA) co-administered with fenofibrate (FENO) in 609 mixed hyperlipidemic patients with or without MetS. **Methods:** Patients (20-79 years), with LDL-C 3.4-5.7 mmol/L [2.6-4.7mmol/L for patients with type 2 diabetes] and TG 1.7-5.7 mmol/L, were randomized (1:3:3:3) to one of four treatments for 12 weeks: placebo; EZE/SIMVA (10/20mg); FENO (160mg); or EZE/SIMVA + FENO. MetS status was determined using NCEP ATP III criteria. **Results:** At baseline, patients with MetS had higher TG, a higher BMI, lower HDL-C, and more had the LDL size pattern B than patients without MetS. Treatment with EZE/SIMVA + FENO significantly reduced LDL-C by 46%, TG by 50%, non-HDL-C by 51% and increased HDL-C by 19% in the whole cohort. Similar results were observed when these mixed hyperlipidemic patients were stratified by the presence or absence of MetS. In both subgroups, treatment with EZE/SIMVA + FENO and FENO alone shifted patients away from the smaller, more dense LDL size pattern B to pattern A. **Conclusions:** In patients with mixed hyperlipidemia, co-administration of EZE/SIMVA + FENO produced consistent benefits on the lipid profiles of patients with or without the presence of MetS.

Funding: This study was funded by Merck/ Schering-Plough

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THE EFFECT OF INSULIN SENSITIZERS, EZETIMIBE AND VALSARTAN (EACH ALONE OR IN COMBINATIONS) ON PLASMA PARAOXONASE ACTIVITY AND mRNA EXPRESSION IN EXPERIMENTAL RAT NON-ALCOHOLIC FATTY LIVER DISEASE

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Objective: The aim of this study is to investigate the effect of several medications on plasma paraoxonase (PON) activity and mRNA expression in the non-alcoholic fatty liver disease (NAFLD) induced by methionine choline deficient diet (MCDD). **Methods:** 60 male Sprague–Dawley rats were included: MCDD only, MCDD with either metformin (M) (200 mg/kg), Rosiglitazone (R) (3 mg/kg), M+R, ezetimibe (E) (2 mg/kg), valsartan (V)(2 mg/kg), or combination of R+M+V or of R+M+V+E for a total of 15 weeks. Serum PON activity and PON2 mRNA expression in the liver. **Results:** PON activity in serum and liver tissue were decreased in MCDD rats.PON activity in serum was increased significantly in all treatment groups. PON activity in liver tissue was increased significantly in the groups R, E, V, R+M+V and R+M+V+E. PON2 mRNA expression in the liver was increased significantly in MCDD, R+M, E, V, R+M+V and R+M+V+E. **Coclusions:** PON2 mRNA expression in the liver is increased but inactivated in experimental NAFLD. Treatment with ezetimibe, valsartan or their combination with rosiglitazone or with rosiglitazone and metformin increased hepatic PON activity of NAFLD rats.

Funding: None

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DIFFERENT ACTION FOR ADIPONECTIN AND LEPTIN ON INSULIN SENSITIVITY OF OVERWEIGHT TYPE 2 DIABETICS WITH PLURIMETABOLIC SYNDROME

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Introduction: Insulin sensitivity (IS) can be partitioned into basal (hepatic) and peripheral (muscle, fat) and measured respectively by QUICKI = 1/(log(Gb)+log(Ib)), Gb Ib fasting glucose and insulin, and OGIS: OGTT-derived post-prandial glucose clearance. Lipid overaccumulation product (LAP) = (waist–k) × triglycerides (TG) is considered. Adipocytokines are mediators of glucose homeostasis in overweight diabetic patients (T2D). Relationships of abdominal fat with the above components of IS are unclear. **Aim:** Evaluation of adipocytokines relationships with IS components in T2D with plurimetabolic syndrome. 75g OGTT was performed in 16 T2D (5F/11M; age 59±2(SE) y; HbA1c 6.7±0.1 %; Gb 134±5 mg/dl; Ib 9.4±1.2 µU/ml; BMI 29.5±1.1 kg/m^2; waist 104±2 cm; LAP 66±8 cm mM/l; total cholesterol 204±8, HDL 53±3, LDL 119±8, TG 168±27, all mg/dl) and in 16 controls (CTN). **Results:** In T2D adiponectin (ADI 7.4±0.5, 7.8±0.9 µg/ml), leptin (LEP 13±3, 13.3 ng/ml), QUICKI (0.39±0.01, 0.39±0.01) were the same ($p>0.2$) that CNT, but OGIS (317±11, 406±12.6 ml min^{-1}m^{-2}) was markedly reduced ($p=0.0001$). LAP inversely correlated with OGIS ($r=-0.57$, $p=0.026$) and QUICKI ($r=-0.56$, $p=0.022$) only in T2D. Both cytokines were normalized to BMI (normalized ADI 0.25±0.02; LEP 0.42±0.08). LEP inversely correlated with fasting QUICKI ($r=-0.45$, $p=0.009$), ADI directly with post-prandial OGIS ($R=0.43$, $p=0.015$), in all subjects. **Conclusions:** Lipid overaccumulation plays a major role in IS impairment of T2D. In mediating insulin resistance, adipocytokines have different sites of action: ADI seems to dynamically operate at peripheral tissue level, while LEP seems active mainly on basal fasting (liver) condition and it is not relevant in diabetic state.

Funding: None

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OBESITY-RELATED DISORDERS (THE METABOLIC SYNDROME) AND ADIPONECTIN

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The MetS is characterized by a concurrence of interrelated cardiovacular risk factors including abdominal obesity, insulin resistance, hypertension, dyslipidemia and glucose intolerance.The aim of our study was to evaluate prospective association of Adiponectin with the MetS as defined according to guidelines of the NCEProgram. **Methods:** 126 patients with Body mass index (BMI) 34.6+/- 1.7 kg m^2 , age 66 +/- 4 yrs were compared with 239 BMI and age mached controls. **Results:** The prevalence of MetS was statistically significantly higher in patients (55/126, 44%) than in controls (52/239, 22% $p= 0.012$). The prevalence of atherogenic risk factors as hypertension (81%), alcohol abuse (68%), dyslipidemia (59%), type 2 diabetes (50%) and smoking (29%) was high in the obese persons. After adjusment for waist circumference, alcohol intake and smoking, adiponectin was significantly inversely associated with insulin resistance, triglyceride, and positively associated with high density lipoprotein cholesterol (HDL-cholesterol).Risk of the metabolic syndrome decreased significantly with increasing adiponectin. **Conclusions:** Adiponectin was a significant independent predictor of the MetS.Decreased plasma level of adiponectin (Hypoadiponectinemia) is an independent biomarker of the MetS and may play important role in the pathophysiology of the disorder in obese persons. The prevalence of MetS was statistically significantly higher in patients (55/126, 44%) than in controls (52/239, 22% $p= 0.012$).Risk of the MetS decreased significantly with increasing adiponectin.

Funding: None

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THE CLINICAL CHARACTERISTICS AND ITS RELATIONSHIP WITH IMPARED GLUCOSE REGULATION IN SUBJECTS WITH METABOLIC SYNDROME

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Previous studies have shown that insulin resistance and abdominal obesity, as a part of metabolic syndrome(MS), are associated with high incidence of impaired glucose regulation(IGR). **Objective:** To study the clinical characteristics of IGR in subjects with MS (95 males and 81 females average 45.2±12.7). **Methods:** The exploration of IGR in 176 citizens of Tbilisi with MS (average 45.2±12.7) was done by OGTT in a cross-section study. NGT, IGR and DM were grouped based on the1999 diagnosis standard of WHO. IGR was composed IFG, IGT and both of which. **Results:** The prevalence of IGR was 42.05% , among which IGT was 66.2% . Compared with the NGT group, the IGR group had higher age, BMI (p=0.021*), BP, TG , T-Chol, LDL- chol and HOMA-IR , lower HDL-chol and QUICKI (p=0.047*). The IGR group had lower BP, TG and HOMA-IR, and higher QUICKI (P=0.037*) than the DM group. When each subgroup of IGR was compared with each other, both IFG plus IGT subgroup and IFG subgroup had higher BMI and HOMA-IR, and lower QUICKI than IGT subgroup. The prevalences of hypertension, lipid disorder, obesity/overweight in each subgroup of IGR were statistically higher than that of the NGT group. The incidence of IGR was high in people with MS in citizens of Tbilisi. There were various metabolic disorders in the subgroups of IGR. The IFG plus IGT and IFG group had higher BMI, hypertension, microalbuminuria and HOMA-IR, but lower QUICKI than the IGT group.

Funding: None

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SERUM ADIPOCYTE FATTY ACID-BINDING PROTEIN-4 IN OBESE ADOLESCENTS

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Objective. Fatty acid binding protein-4 (FABP-4) is a member of the fatty acid binding protein family, expressed in adipocytes, which is known for the ability to bind fatty acid. Recent studies demonstrated FABP-4 expression in macrophages, in which FABP-4 can modulate inflammatory response. The aim of this study was to determine plasma FABP-4 concentrations in obese pubertal children and to establish the possible associations with components of metabolic syndrome. **Methods.** The study group consisted of 54 obese adolescents (25 boys and 29 girls), aged 13-18 y. The control group comprised 36 lean children (18 boys and 18 girls) in similar age and pubertal status. Plasma insulin, glucose, lipids, C-reactive protein (CRP) were determined using commercial kids. Serum adipocyte FABP-4 concentrations were measured, using ELISA method by BioVendor Inc. **Results.** Mean ciculating concentration of FABP-4 was significantly higher in obese than in lean persons (35.3±13.6 vs 21.1±9.8 μg/L, P<0.001). Age-, sex- and pubertal stage adjusted serum FABP-4 concentrations correlated positively with waist circumference (P<0.001), fasting insulin (P<0.001), total cholesterol (P<0.05), triglycerides (P<0.001) and CRP (P<0.01). **Conclusion.** Adipocyte FABP-4 is a serum marker closely associated with obesity and parameters of the metabolic syndrome in children and might be the useful for clinical diagnosis of obesity-related metabolic complications.

Funding: None

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INFLUENCE OF SIMVASTATIN ON URINE ALBUMIN EXCRETION IN PATIENTS WITH DIABETES MELLITUS AND MICROALBUMINURIA

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Objective: Dyslipidemia is implicated in the pathogenesis of diabetic nephropathy. However, there is little information concerning the possibility to halt the progression of diabetic nephropathy by correction of dyslipidemia by use of statins. Therefore, the aim of this study was to investigate the influence of simvastatin on urine albumin excretion in subjects with diabetes mellitus and microalbuminuria. **Methods:** We examined 24 patients with type 1 (9 subjects) and type 2(15 patients) diabetes mellitus aged 54,8±2,5 years (data are presented as mean ± SEM). Duration of diabetes was 14,7±1,48 years. Simvastatin was prescribed in dose of 20 mg once daily for 3 months. The levels of microalbuminuria were assessed before and after the treatment with simvastatin by photometric method. Statistical analysis was performed using Student's test for paired variables. **Results:** Treatment with simvastatin resulted in significant decrease of plasma levels of total cholesterol (6,4±0,26 and 5,5±0,21 mmol/L, $p<0,05$, before and after treatment, respectively), LDL(4,5±0,27 and 3,9±0,21 mmol/L, $p<0.05$). We found the significant decrease of microalbuminuria at the end of the treatment period - 158,5±18,27 vs.108,9±15,35 mcg/ml, $p<0,001$ before and after the treatment. **Conclusions:** The treatment with simvastatin resulted in the decrease of urine albumine excretion in patients with diabetes mellitus. The positive effects of simvastatin on albumin excretion could be attributed to correction of plasma lipids or to pleiotropic effects of statins. The revealed decrease of albumin excretion in patients with diabetic mellitus and microalbuminuria could indicate the need for the larger trials to examine the possibility to prevent and reverse the progression of diabetic nephropathy using statins.

Funding: None

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CRP REDUCTION AND GOAL ATTAINMENT WITH EZETIMIBE/SIMVASTATIN, ATORVASTATIN, OR ROSUVASTATIN IN PATIENTS WITH DIABETES, METABOLIC SYNDROME, OR NEITHER

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Objective: To evaluate the effect on CRP by ezetimibe/simvastatin (E/S) compared with atorvastatin (A) or rosuvastatin (R) in patients with diabetes mellitus (DM) or metabolic syndrome (MS), which may be considered pro-inflammatory states. **Methods:** Post-hoc analysis of 2 multicenter, double-blind, randomized, 6-wk studies comparing E/S 10/10, 10/20, 10/40, or 10/80 mg with either A 10, 20, 40, or 80 mg (Study 1) or R 10, 20, or 40 mg (Study 2). This analysis compares treatments by pooling across all doses for the change from baseline in CRP and the percentage of patients reaching CRP<2.0 mg/L in the subgroup of patients with DM, MS without DM, or with neither disease. **Results:** The percentage change from baseline CRP was similar for E/S vs A or R. There were non-statistically significant treatment differences, but no consistent trends were observed. There was no interaction between treatment and DM/MS subgroup in either study, indicating that the relative treatment differences among E/S, A, and R were consistent across the DM/MS subgroups. A similar percentage of patients reached CRP<2.0 mg/L for E/S vs A or R. The neither group was significantly more likely to achieve this level in Study 1 ($p=0.045$) and 2 ($p<0.001$). There was no interaction with treatment and subgroup. **Conclusions:** In both studies, E/S had CRP reductions similar to A and R, and a similar proportion of patients attaining CRP<2.0 mg/L. The neither subgroup was significantly more likely to reach CRP<2.0 mg/L.

Funding: The original studies and secondary analyses were funded by Merck/Schering-Plough Pharmaceuticals.

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COLESEVELAM HCL IS AN EFFECTIVE AND SAFE ADD-ON TO SULFONYLUREA-BASED THERAPY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Objective: To investigate the effects of the bile acid sequestrant colesevelam HCl on lipid levels and glycemic control in patients with type 2 diabetes mellitus (T2DM) inadequately controlled on a sulfonylurea (SU) alone or combined with other oral antidiabetic agents. **Methods:** Patients with T2DM receiving SU-based therapy with HbA_{1c} 7.5%–9.5%, LDL-C ≥60 mg/dL and triglycerides ≤500 mg/dL were randomized to receive double-blind treatment with colesevelam HCl 3.75 g/day (n=230) or placebo (n=231) for 26 weeks. **Results:** For the colesevelam HCl/placebo groups, respectively, at baseline: mean age 56.6/57.0 years, body mass index 33.1/32.5 kg/m^2, fasting plasma glucose (FPG) 176.6/181.0 mg/dL and HbA_{1c} 8.2/8.3%. Colesevelam HCl significantly (P<0.001) reduced mean LDL-C (–16.7%), non-HDL-C (–6.7%), total cholesterol (–5.0%) and Apo B (–6.7%) vs placebo. Colesevelam HCl also significantly reduced mean total cholesterol/HDL-C, LDL-C/HDL-C, non-HDL-C/HDL-C and Apo B/Apo A-I ratios vs placebo (P≤0.003). Median triglyceride levels increased vs placebo (+17.7%; P<0.001), as did Apo A-I (+3.8%; P<0.001). Median change in high-sensitivity C-reactive protein was marginally significant (–11.2%; P=0.056). Colesevelam HCl was associated with significant mean reductions in HbA_{1c} (–0.54%; P<0.001), FPG (–13.5 mg/dL; P=0.009), and fructosamine (–21.4 µmol/L; P<0.001) vs placebo. Colesevelam HCl was well tolerated with no serious treatment-related adverse events. **Conclusions:** Colesevelam HCl is an effective and safe adjunct to SU-based therapy for improving lipid levels and glycemic control in patients with T2DM.

Funding: Daiichi Sankyo, Inc.

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COLESEVELAM HCL IS AN EFFECTIVE AND SAFE ADD-ON TO INSULIN-BASED THERAPY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Objective: To investigate the effects of the bile acid sequestrant colesevelam HCl on lipid levels and glycemic control in patients with type 2 diabetes mellitus (T2DM) inadequately controlled with insulin alone or in combination with other oral antidiabetic agents. **Methods:** Patients with T2DM receiving insulin-based therapy with HbA_{1c} 7.5%–9.5%, LDL-C ≥60 mg/dL and triglycerides ≤500 mg/dL were randomized to receive double-blind treatment with colesevelam HCl 3.75 g/day (n=147) or placebo (n=140) for 16 weeks. **Results:** Relative to placebo, colesevelam HCl was associated with significant mean reductions in LDL-C (-12.8%; P<0.001), Apo B (-5.3%; P=0.040), mean LDL-C/HDL-C ratio (-0.33; P<0.001), and Apo B/Apo A-I ratio (-0.06; P=0.004), in addition to a significant median increase in triglycerides (+21.5%; P<0.001). Colesevelam HCl also produced numerical reductions in non-HDL-C, total cholesterol and HDL-C and an increase in Apo A-I, but these changes were not statistically significant. Median change in high-sensitivity C-reactive protein was of marginal significance (-12.2%, P=0.069). Colesevelam HCl produced significant mean reductions in HbA_{1c} (-0.50%; P<0.001) and fructosamine (-21.7 µmol/L; P<0.001), relative to placebo. Fasting plasma glucose levels were not significantly reduced (-14.6 mg/dL; P=0.082). Colesevelam HCl was well tolerated with no serious treatment-related adverse events. **Conclusions:** Colesevelam HCl is an effective and safe adjunct to insulin-based therapy for improving lipid levels and glycemic control in patients with T2DM.

Funding: Daiichi Sankyo, Inc.

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COLESEVELAM HCL REDUCES CHOLESTEROL AND GLUCOSE LEVELS WHEN ADDED TO METFORMIN-BASED THERAPY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Objective: To investigate the effects of the bile acid sequestrant colesevelam HCl on lipid and glucose levels in patients with type 2 diabetes mellitus (T2DM) with inadequate glycemic control on metformin (MET) alone or in combination with other oral antidiabetic agents. **Methods:** Patients with T2DM receiving MET-based therapy with HbA_{1c} 7.5%–9.5%, LDL-C ≥60 mg/dL and triglycerides ≤500 mg/dL were randomized to receive double-blind treatment with colesevelam HCl 3.75 g/day (n=159) or placebo (n=157) for 26 weeks. **Results:** Colesevelam HCl was associated with significant (P<0.001) placebo-corrected reductions in mean LDL-C (-15.9%), non-HDL-C (-10.3%), total cholesterol (-7.2%) and Apo B (-7.9%). Median change in triglycerides (+4.7%) and mean changes in HDL-C (+0.9%) and Apo A-I (+1.8%) were not significant vs placebo. Colesevelam HCl was associated with significant reductions in mean total cholesterol/HDL-C, LDL-C/HDL-C, non-HDL-C/HDL-C and Apo B/Apo A-I ratios compared with placebo (P<0.01). Median percent high-sensitivity C-reactive protein level was also reduced vs placebo (-14.4%; P=0.016). Colesevelam HCl also produced significant mean reductions in HbA_{1c} (-0.54%; P<0.001), fasting plasma glucose (-13.9 mg/dL; P=0.014) and fructosamine (-23.2 μmol/L; P<0.001) compared to placebo. Colesevelam HCl was generally well tolerated. **Conclusions:** Colesevelam HCl is an effective and safe adjunct for improving both lipid and glucose levels in patients with T2DM having inadequate glucose control on MET monotherapy or MET in combination with other oral antidiabetic agents.

Funding: Daiichi Sankyo, Inc.

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RISK FOR CARDIOVASCULAR EVENTS IN A LOCAL POPULATION OF DIABETIC PATIENTS

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Diabetes mellitus (DM) is a major risk factor for cardiovascular (CV) events. Many algorithms have been devised to assess CV risk, some of which specific for diabetics. Most of them, however, are based on data which can hardly be extrapolated to Mediterranean countries. **Aim** of the present study was to analyze CV risk and the incidence of CV events in a local cohort of patients with type 2 DM. **Methods.** Charts of Diabetes Clinics of Modena in the period 1991-1995 were analyzed. Patients aged 35-65 with type 2 DM and no history of CV disease were eligible. Global CV risk was computed according to Framingham, RISCARD, Progetto Cuore and UKPDS algorithms and compared with the actual rate of CV events over the following 10 years. **Results.** 1880 patients were screened; 829 of them (44.09%) were eligible on the basis of defined criteria and data completeness. In such population an absolute 10-yr risk rate of 10.8% was observed. When comparing the estimated risk rate according to the different functions, a high degree of variability was present; Italian algorithms were more consistent with the observed data even if only 23.8% of patients with CV events had a risk > 20% at initial observation. **Conclusions.** Estimation of CV risk is largely dependent on the algorithm adopted and on the baseline risk of the reference cohort. The overall performance of such functions is however low. The algorithm derived from the present study will be utilized for a prospective evaluation of CV risk in our local cohort.

Funding: Supported by COFIN PRIN grant n. 2004 067491

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HORMONAL-METABOLIC PATTERNS OF DYSLIPIDAEMIAS IN NON-OBESE TYPE II DIABETIC PATIENTS WITH ATHEROSCLEROSIS

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Objective: The aim was to evaluate the hormonal-metabolic patterns of dyslipidaemias in non-obese patients (pts) with type II diabetes mellitus (DM) and documented atherosclerosis (ATH). **Methods:** Group 1 (gr1) was composed of 30 normal subjects (age = 54.50±2.03; means±SEM). Group 2 (gr2) consisted of 20 non-obese type II DM pts with hypertriglyceridaemia (type IV HLP) and ATH (age = 53.19±1.91). Group 3 (gr3) consisted of 23 non-obese type II DM pts with mixed hyperlipidaemia (type IIB HLP) and ATH (age = 56.73±1.96). Following have been determined in serum, in fasting state: total cholesterol (CH), HDL-cholesterol (HDL-CH), atherogenicity coefficient (HAC), triglycerides (TG), lipolytic activity (LA), lipoprotein fractions, prostaglandins A1 and E1, prostaglandin F2α(PGF). Following have been determined in plasma, during standard OGTT: glucose, insulin, insulin/glucose index (IGI), glucagon, C-peptide, STH, somatostatin, ACTH, cortisol, aldosterone, β-endorphin. **Results:** Both gr2 and gr3 pts, compared to gr1, had higher body mass, CH, TG, HAC, and lower HDL-CH, LA, insulin (at OGTT hour 1), IGI, STH (hour 2), basal aldosterone. Gr2 pts, compared to gr1, had lower STH (hours 1 and 2). Gr3 pts, compared to gr1, had higher glucagon (hour 2), somatostatin (hours 0 and 1), cortisol (hours 1 and 2), PGF, and lower C-peptide (hour 1), STH (hours 0 and 2). **Conclusions:** Altered hormonal-metabolic patterns have been observed in non-obese type II DM pts with ATH and dyslipidaemias, including decreased STH and elevated cortisol.

Funding: Academy of Sciences of Moldova and Medical University, Chisinau, Moldova

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EFFECTS FOR METABOLIC FACTORS BY RECONSTITUTED HIGH-DENSITY LIPOPROTEIN IN GOTO-KAKIZAKI RATS

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Low plasma level of high-density lipoprotein (HDL) and high plasma glucose (PG) are both diagnostic criteria for metabolic syndrome (MetS) associated with dysfunction of adiponectin secretion and insulin sensitivity. Recently, pharmacological intervention using reconstituted (r)-HDL in atherosclerosis has been the subject of growing interest. Therefore, we examined whether rHDL improved metabolic factors using Goto-Kakizaki rat, a genetic model of type 2 diabetes mellitus. Fifteen weeks old rats were divided into two groups: control and rHDL groups that received intravenously infusions of saline and rHDL [containing POPC (1-palmitoyl-2-oleoyl-phosphatidylcholine) and 6 mg/kg apolipoproteinA-I] twice/week for 5 weeks, respectively. We performed an oral glucose tolerance test (2 g/kg) and measured PG and plasma immunoreactive insulin (IRI) before and after 5 weeks. We also measured body weight (BW), plasma adiponectin, lipid profile, urinary volume (UV) and electrolyte. The rHDL group significantly decreased BW and increased UV compared with control group. In addition, rHDL group showed a tendency for improvement of HOMA-R compared with control group, while there were no differences in HOMA-β, ΔIRI/ΔPG and plasma adiponectin. The finding represents an exciting new area in treatment for MetS. The rHDL-based therapy associated with BW reduction and improvement of insulin resistance may be useful for MetS.

Funding: This work was supported by funds from the Central Research Institute of Fukuoka University, Japan

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BLOOD FLOW IN SKELETON MUSCLES IN PATIENTS WITH INSULIN DEPENDENT DIABETES MELLITUS BY RADIOACTIVE 133Xe METHOD

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Aim: To investigate skeleton muscles blood flow in patients (pts) with insulin dependent diabetes mellitus (IDDM) by radioactive 133Xe method. **Material and method:** 11 healthy people and 60 pts with IDDM were investigated skeleton muscles blood flow in two lower extremities by means of radioactive 133Xe method of Ketty S.S. in modification of Lassen N.A. at all. There were examined time of half rezorption from muscle depot (T1/2), muscle flow (MF) in rest. After physical load by occlusive pressure in cuff about 250 mm Hg above knee there was registered reactive hyperemia. **Results:** Data received testify to time half rezorption from muscle depot (T1/2) 133Xe in healthy people was equal 11.423+/- 0.309 min, MF in rest – 5.63+/-0.319 ml/100g/min. After lood in reactive hyperemia MK was equal 19.82+/- 0.271 ml/100g/min. In IDDM pts time half rezorption from muscle depot from MF consisted from 22.333+/- 1.103 min to 23.198+/- 0.811 min. More significant differences were in IDDM pts with mean grad stage, when time half rezorption formed 19.494+/- 0.812 min and in heavy grad- 25.824+/- 0.84 min ($p<0.001$). MF in rest consisted in mean grad 4.187+/-0.135 ml/100g/min and in heavy grad – 3.43+/- 0.172 ml/100g/min ($p<0.002$). In reactive hyperemia MF formed 15.23+/- 0.213 ml/100g/min in mean grad IDDM, in heavy grad -14.065+/- 0.374 ml/100g/min. T1/2 and reactive hyperemia were reduced also depending on duration IDDM and presence of angiopathyes. **Conclusion:** Pts with IDDM suffered from disturbance of blood flow in skeleton muscles in rest and in conditions of reactive hyperemia in lower extremities. T1/2 and reactive hyperemia by 133Xe method decreased depending on heaviness, duration IDDM and presence angiopathyes.

Funding: None

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CHRONIC HYPERGLYCEMIA IMPAIRS FLEXIBILITY RELATED TO FATTY ACID METABOLISM IN HUMAN MYOTUBES

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Skeletal muscle of insulin resistant individuals is characterized by inflexible metabolism. We examined if metabolic inflexibility could be induced by hyperglycemia (20 mM glucose for 4 days) in healthy myotubes. Metabolism of oleic acid (OA) was studied using a new multiwell assay. Acute glucose (5 mM) increased cell associated OA from 60.3 nmol/mg protein to 94.7 nmol/mg protein in normoglycemic (NG) cells. This effect of glucose was reduced in hyperglycemic (HG) cells, from 50.7 nmol/mg protein to 63.5 nmol/mg protein. Oxidation of OA was significantly lower in HG cells than in NG cells, 1.2 nmol/mg protein and 2.6 nmol/mg protein, respectively. Acute glucose suppressed OA-oxidation by 50 % in NG cells, whereas HG cells were much less sensitive (25 %). The fraction of oxidized OA was 4.7 % in NG cells and 4.4 % in HG cells in absence of glucose. Glucose suppressed this fractional OA oxidation by 68 % NG cells and by 41 % in HG cells. Distribution of oleic acid to intracellular lipids was also suppressed by glucose, but there were no obvious differences between NG and HG cells. The suppressive effect of glucose was not obtained with deoxyglucose. The glucose 6-phosphate dehydrogenase activity was not changed by hyperglycemia neither were expression of the genes CD36, FAS, SCD1, ACC or CREBP, nor phosphorylation of AMPK or ACC. We have shown that hyperglycemia reduces OA oxidation and its metabolic flexibility in myotubes. The mechanism is not known, but accumulation of some signalling metabolite is more likely that altered gene expression.

Funding: None

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CLASSICAL AND NOVEL CARDIOVASCULAR RISK FACTORS IN RELATION WITH HYPERGLICEMIA

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Objectives: The aim of this sudy was to evaluate the prevalence of classical and new cardiovascular risk factors and it´s distribution according to glucose metabolism. **Methods**: Prospective study of consecutive patients admmited with acute coronary syndrome, stratified according glucose disturbances. Blood lipids were done within the first 24 h after admission and in patients without known diabetes an OGTT was done on days 4 or 5, after clinical stabilization. CRP was evaluated at admission (days 1/2) and on peak (days 4/5). Statistical anlysis was performed with SPSS program for windows. **Results**: 191 patients were evaluated, 63 (33%) with and 128 (67%) without a previous diagnosis of diabetes.In these the OGTT identified 1 patient with IFG (1%), 40 with IGT (21%), 22 with diabetes mellitus (12%) and 65 (33%) with normal glucose tolerance (NGT). There were no significant differences among these groups according to the prevalence of high cholesterol, low HDL, hypertriglyceridemia, high Lp(a), high Apo B, homocistein and fibrinogen levels. At admission there was a trend for higher CRP levels in patients with new diabetes melitus, and at days 4/5 CRP is significantly higher in patients with new diabetes mellitus than in those with NGT (48,8 vs 30 ng/ml; p=0,04). There was a significant correlation of CRP with markers of myocardial necrosis but there were no significant differences of these markers between the several groups. **Conclusions:** In this population there was a high prevalence of cardiovascular risk factors (high total and LDL cholesterol, low HDL, hypertriglyceridemia, elevated Lp(a)) but no differences were seen in it´s prevalence according to disturbances of glucose metabolism.

Funding: None

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ANALYSIS OF THE LIPID COMPONENTS AND THE INFLUENCE OF GENDER IN PATIENTS WITH METABOLIC SYNDROME

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A strong association between Metabolic Syndrome (MS) and Insulin Resistance (IR) was described. There have been postulated that MS is the main clinical manifestation of IR. Some doubts have emerged about the importance of dislipemia and its relation with the degree of IR. **Objectives**: Evaluate in men and women the correlation between high density lipoprotein cholesterol (HDL), triglicerides (TG) and the ratio TG/HDL with IR measured by Homeostasis model assessment for IR (HOMA-IR). **Materials and Methods:**Primary prevention patients (p) who underwent a cardiovascular check up were included in the analysis. Patients with secondary dyslipidemia, diabetes and those in treatment with niacin or fibrates, were excluded. HOMA IR was calculated to quantify IR, and subsequent correlation with HDL, TG and TG/HDL index were verified. **Results:** 264 patients were studied (age 51±13 years), of whom 116 were women. Hypertension was present in 187 p, 120 p had dyslipidemia, 40 p were active smokers and 36 had familiar antecedents of coronary heart disease. In the whole population, a low correlation between IR and lipid parameters were observed (HDL vs. HOMA-IR, $r=0.28$, $p<0.05$; TRIG vs. HOMA-IR, $r=0.26$, $p<0.05$; TG/HDL vs. HOMA-IR, $r=0.29$, $p<0.05$). Particularly in women, the correlation was higher (HDL vs. HOMA, $r=0.34$, $p<0.05$; TRIG vs. HOMA, $r=0.36$, $p<0.05$; TG/HDL vs. HOMA, $r=0.41$, $p<0.05$) than in men (HDL vs. HOMA, $r=0.20$, $p<0.05$; TRIG vs. HOMA, $r=0.18$, $p<0.05$; TG/HDL vs. HOMA, $r=0.21$, $p<0.05$). **Conclusion:** These results confirm the relation between lipid parameters in MS and IR, yet it seems to be weaker in relation with other variables such as hypertension. This relation appears also influenced by others co variables, specially gender.

Funding: None

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METABOLIC OUTCOME OF A 9-MONTH TREATMENT WITH ATYPICAL ANTIPSYCHOTIC AGENTS ON ADIPONECTINEMIA AND HOMA HYPERBOLIC PRODUCT IN LEAN SUBJECTS WITHOUT METABOLIC SYNDROME

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Atypical antipsychotic drugs (AAD) induce truncal adiposity and risk of metabolic syndrome (MetS), impaired glucose tolerance or diabetes. AAD effects in lean schizophrenic patients free of MetS is poorly documented, especially the relationship between weight gain, adiponectinemia, insulin sensitivity (S) and β-cell function (B). We prospectively determined the outcome of 9 month therapy with AAD on anthropometrics, adiponectinemia, gluco-lipidic metabolism, HOMA-modeling of (S), (B), and on the hyperbolic product (BxS), a precise means to assess an individual's β-cell function adjusted for (S). 36 schizophrenic subjects [M:F 24:12], 35±9 years (mean±SD) free of MetS (ATPIII) were evaluated before and after quetiapine (n=12), olanzapine (n=3), risperidone (n=10) or aripiprazole (n=11) therapy. At 9 months, BMI rose from 22±3 to 25±2 kg/m^2 and waist circumference from 82±9 to 91±11 cm ($p<0.0001$). Blood pressure and lipids were unaffected, while adiponectinemia decreased: 8.7±5.0 to 7.4±4.0 µg/ml, $p=0.0001$. (S) decreased from 134±50 to 110±58%, (BxS) decreased from 108±22 to 91±27%, despite an increase in (B) from 89±30 to 100±40% (all $p<0.005$). Consequently, fasting glycaemia increased (88±6 to 95±9 mg/dl, $p=0.002$). **Conclusions:** Long-term use of AAD in lean schizophrenic subjects free of MetS induces rapid weight gain as well as abdominal fat accretion, alongside a decrease in adiponectinemia. The fall in HOMA hyperbolic product (BxS) underlies the rise in fasting plasma glucose.

Funding: None

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REDUCTION OF A-FABP VALUES UPON THE STATIN THERAPY. THE NEW PLEIOTROPIC EFFECT OF ATORVASTATIN?

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Background: Experimental evidences suggest that statins appear to display additional pleiotropic effects. Recently it has been suggested that serum A-FABP might be a new marker of metabolic syndrome. **Aim:** examination of serum A-FABP value in patients with dyslipidemia treated by atorvastatin. **Methods:** 27 high-risk probands with hyperlipidemia and atherosclerotic complications were investigated. All subjects were tested initially and 3 months after the statin therapy (20 mg of atorvastatin daily). Serum samples were analyzed for cholesterol, HDL, LDL, triglycerides, ALT, AST, creatinine, CK, glucose, uric acid and A-FABP. **Results:** Mean of A-FABP value in patients with dyslipidemia before the therapy was 54.3±28.1 ug/L, women had higher A-FABP value then men ($P=0.011$). After 3 months therapy, significant reduction of total cholesterol, LDL-cholesterol, glucose, AST, uric acid, glucose and A-FABP values were observed (40,9±16,7; $P<0.05$). No difference was found in BMI, CK, ALT, or HDL values. We next adjusted serum A-FABP value to total cholesterol value. Significant difference in serum A-FABP level before and after the therapy remains after the correction in both men and women, and likewise in overall study group (-1,7±4,1 vs. -5,4±3,9; $P<0.001$). **Conclusion**: Novel pleiotropic direct mechanism for the antiatherosclerotic actions of statins was presented.

Funding: The authors declare that they have no conflicts of interest

Poster Session 9 "NUTRITION AND CVD" - GROUP B

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COMPARISON OF ARTERIAL WALL ELASTICITY BETWEEN TWO ETHNIC GROUPS DETERMINED BY PULSE WAVE VELOCITY

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Purpose: To compare arterial wall elasticity of normal subjects with normal BMI in two ethnic groups with different dietary habits.**Method:** Arterial wall elasticity was determined indirectly by Pulse Wave Velocity (PWV) with an electronic device which recorded the left external carotid and left dorsalis pedis arteries with a single lead ECG. The time delay between the two pulses is computed.A shorter time of PWV indicates decreased arterial wall elasticity. Dietary history was taken.**Materials:** A total of 115 clinically asymptomatic female South African Caucasians and 54 female Greeks were examined. Of these groups, only 28 South Africans and 29 Greeks, with BMI less than 25 kg/m², were included in the study. All had normal BP, blood sugar, lipid profile and ECG. Mean age: South Africans - 30.8 yrs.; Greeks - 40.2 yrs. Mean BMI: South Africans- 21.4 kg/m²; Greeks - 19.8 kg/m².**Results:** Mean PWV time of South Africans - 0.157 sec.; Greeks- 0.177 sec. The difference between the two mean times of PWV, corrected for age, was statistically significant. **Discussion:** PWV time is shorter, indicating stiffer arterial walls, among Caucasian South Africans who have high daily intake of meat. Greek females on Mediterranean diet show more elastic arteries as shown by a longer PWV time. Decreased arterial wall elasticity predisposes individuals to develop cardiovascular disease (CVD). **Conclusion:** A diet high in meat predisposes individuals to have stiffened arteries making them higher risks to develop CVD while a Mediterranean diet lessens this predisposition.

Funding: Partly supported by the Northwest University, Potchefstroom

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ASSESSMENT OF THE LIPID AND DIETARY TRENDS IN THE MALE POPULATION OF TALLINN, ESTONIA: YEARS 1984-2001

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Objective: Three independent random samples of the male population of Tallinn aged 30-54 were examined in 1984/1987 (Survey I), 1992/1994 (Survey II) and 1999/2001 (Survey III). The total number of examinees was 3111. The aim was to assess the lipid profile and some nutrition indices during the study period. **Methods**: The screening procedure included standard epidemiological investigation methods. The lipid profile was assessed by the EAS classification of hyperlipidemia. The nutrition structure was investigated by means of the 24-h recall method. **Results:** At Survey III the TC mean values (212.1 mg/dl) were significantly lower than at Surveys I and II (223.2 and 221.9 mg/dl). Changes of the TG mean values were less pronounced. The HDLC mean values increased significantly in men by Survey III. The percent of men with normal lipid values increased from 30.1% to 36.0% by Survey III ($p<0.05$). The proportion of patients belonging to group A and B were almost similar for all surveys. A slight decreasing tendency was observed concerning the prevalence of mixed hyperlipidemia forms (D+E) - 12.3, 9.7 and 7.5%, accordingly. Positive changes were also found in diet: the energy, fat, saturated fatty acid and cholesterol consumption decreased while the P/S ratio increased. This was in accordance with favorable CVD mortality trends in the Estonian population in the late 1990s. **Conclusion:** Favorable nutrition changes were accompanied by positive changes in lipids. Despite favorable trends in the lipid profile, 2/3 of the male population aged 30-54 has elevated lipid values and need correction of hyperlipidemia.

Funding: The study was carried out as a part of the basic research project from the Ministry of Science and Education of Estonia

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REDUCED HDL CHOLESTEROL IS NOT ASSOCIATED WITH CARBOHYDRATE-INDUCED HYPERTRIACYLGLYCEROLEMIA IN A YOUNG CHINESE HAN POPULATION

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Objective: To examine the relationship of LF-HC diet with body composition and its effects on serum lipid profile in young Chinese Han population. **Methods:** 56 healthy subjects (22.89±1.80 years) were given control diet of 31% fat and 54% carbohydrate for 7 days, followed by LF-HC diet of 15% fat and 70% carbohydrate for 6 days, without total energy restriction. **Results:** After LF-HC diet, subjects experienced weight loss and decrease of BMI, waist circumference (WC), and percent body fat (BF %) (P <0.05). Triacylglycerol increased by 13.63% (P<0.01). Total cholesterol (TC) and LDL cholesterol decreased by 6.95% and 17.69% respectively (P <0.001). HDL cholesterol (P <0.01) and apolipoprotein (apo) A-I (P <0.05) were increased. When BMI and WC are taken into account, the change of triacylglycerol for subjects with high BMI or high WC, and the changes of HDL cholesterol and apo A-I for subjects with medium or low BMI/WC were not significant. When analyzed based on BF %, the change of HDL cholesterol for subjects with high or low BF %, and the change of triacylglycerol for subjects with high BF % were not significant. **Conclusions:** Subjects with different BMI, WC, or BF % have different triacylglycerol and HDL cholesterol responses to LF-HC diet, suggesting that other factors need to be considered for understanding the mechanism of the hypertriacylglycerolemic effect of LF-HC diet. Furthermore, dietary intervention needs to be individualized based on the factors each subject bears.

Funding: Supported by the Program for New Century Excellent Talents in University (NCET-04-0863) and the Program for Key Issues of Sciences and Technology (03SG022-002-3)

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NUTRITION AND CVD

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Cardiovascular diseases are the most often consequence of the atherosclerosis and the increased level of a blood pressure. Preventive Services Center Sombor has researched risc factors for chronically noninfective diseases on 101 patients, 24 younger than 50 and 77 older than 50. 88% of younger and 83% of older had a heart disease history in their families. 29% of younger and 38% older had an irregular diet and more than 50% from both groups was physically inactive.46% of younger and 69% of older had an increased level of blood cholesterol; 41% of younger and 81% of older had a Body Mass Index over 25. Blood presure was regular in group of younger, but more than 50% of older an increased level of blood pressure; 25% of them had a heart disease. Obesity, increased blood fats, disease history in family and physically inactive life are conected with high level of blood pressure, heart disease and the age of patient.

Funding: Health Center,Center of Prevention, Sombor, Serbia

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THE EFFECT OF A LOW CARBOHYDRATE VEGAN DIET ON WEIGHT REDUCTION AND SERUM LIPIDS

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Objective: To determine the effect on weight loss and serum lipids of a low carbohydrate vegan diet high in soy protein and vegetable oil, under metabolic and *ad libitum* conditions. **Method:** Forty four hypercholesterolemic subjects (18M, 26F; 56.2±7.5y; 31.1±2.6kg/m^2) were instructed to take a low carbohydrate (26% of energy), high protein vegan diet (test) or a low saturated fat National Cholesterol Education Program (NCEP Step 2) diet (control). During the first month metabolic phase, subjects consumed 60% of their estimated energy requirements, and for an additional 6 months thereafter, subjects were counseled to follow their respective diet *ad libitum*. **Results:** Twenty three subjects completed both phases. On the metabolic phase, changes in body weight were significantly reduced on both the vegan and NCEP diets (-4.9±0.4%, p<0.005; -5.1±0.2%, P<0.005; respectively) as were the total:HDL-C levels (-19.2±6.5%, P=0.002; -6.4±2.8%, P=0.046; respectively). On the *ad libitum* phase, reductions in body weight on the vegan and NCEP diets were -7.3±1.2% (P<0.005) versus -6.6±1.0% (P<0.005) and total:HDL-C reductions were -10.1±.3.2% (P=0.005) versus -3.4±2.6% (P=0.127), respectively. **Conclusion:** Despite similar weight reductions in the metabolic and real-world settings, the low carbohydrate vegan diet, high in soy protein and vegetable oil, showed improvements in serum lipids beyond that seen with the NCEP diet.

Funding: The Solae Company, Loblaws Ltd.

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BARLEY PROTEIN AND BLOOD LIPIDS

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Objective: To assess the effect of high protein barley flour baked into bread on lowering lipid risk factors for heart disease. **Method:** 33 hypercholesterolemic adults were randomly assigned to 2 groups. Each group received either 30g of barley protein (intervention group) or calcium caseinate (placebo) per day per 2,000 kcal energy requirements in a randomized controlled crossover design with a 2 week washout. Body weight, blood pressure and serum lipids were measured at 0, 2, and 4 weeks on each treatment. **Results:** Preliminary data were available on 12 subjects. There was no evidence that barley protein supplementation improved low density lipoprotein cholesterol (LDL-C) and total cholesterol to high density lipoprotein cholesterol ratio (TC:HDL-C) at week 4 expressed as the difference from baseline (-0.16 mmol/L, P=0.393 and -0.03 mmol/L, P=0.787, respectively). The corresponding data for the calcium caseinate group also showed no overall improvement (LDL-C, -0.08 mmol/L, P=0.507 and TC:HDL-C, 0.08 mmol/L, P=0.372). Furthermore, barley protein did not significantly improve the lipid profile in direct comparison to dairy protein (LDL-C, 0.07 mmol/L, P=0.724 and TC:HDL-C, 0.12 mmol/L, P = 0.507). **Conclusion:** 30 g/day per 2,000 kcal energy requirement of barley protein was not shown to be more beneficial than dairy protein supplementation on serum lipids.

Funding: Natural Sciences and Engineering Research Council of Canada (NSERC); Loblaws Ltd.

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EFFECTS OF THERAPEUTIC NUTRITION WITH ANTIOXIDANT FUNCTIONAL FOODS IN OBESE PATIENTS WITH METABOLIC SYNDROME

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Objective: To test the effects of antioxidant functional foods on reversibility of metabolic syndrome in obese patients. **Methods:** Nutritional trial testing functional foods in 300 obese patients with metabolic syndrome randomized for intervention or non-intervention. Duration of intervention:6 months. Functional foods used: balsamic vinegar from apples and honey, grape juice with polyphenolic compounds from seeds and skins of grapes, bakery products with a nutraceutic mix. Multivariate analysis will assess associations between: gender, age, diet, Δ body weight reduction and HOMA-IR insulin resistance, lipid profile, adiponectin; intima-media thickness in the common carotid artery; oxidative stress. Determinations at inclusion, 3 and 6 months. **Preliminary results:** The study is presently recruiting. Sixty patients, mean age 54.25±11.08, 30M, 30F have been included, BMI=35.65±9.96 kg/m2, %BF=40.8±8.1. HOMA-IR was increased in 63.6% patients (mean 4.08±3.34) and radicalic activity was >310uFORT in 60% patients. Adiponectin was 9.37±7.28 microg/ml. IMT LCCA was 0.590±0.138 mm and in the RCCA 0.570±0.155 mm. In 12 patients determinations were repeated after 3 months. Mean weight loss was 6.5±1.7 kg. There was a significant decrease of lipid parameters, HOMA-IR and radicalic activity in 7 patients most compliant to diet. No significant changes in IMT or adiponectin were noted so far. **Conclusions:** The long-term effects of this diet, still under study, could lead to promising results to reach target values together with lipid-lowering drugs.

Funding: Grant CEEX 10/2005 funded by the Romanian Ministry of Research, program BIOTECH

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ARTEMISIA AUCHERI AND REGRESSION OF AORTA WALL ATHEROSCLEROTIC LESION IN ATHEROSCLEROTIC RABBITS

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Regression and suppression of atherosclerotic lesion is one of the candidate approach for prevention of atherosclerotic complication. Despite the number of clinical agents developed by the pharmaceutical industry a large majority of people use herbal medicine for treatment disease. *Artemisia aucheri* (A.a) is a native-growing plant which is widely used in Iranian traditional medicine. This study was designed to evaluate the effects of A.a on development of atherosclerotic in hypercholesterolemic rabbits. Twenty five rabbits were randomly divided into four groups of five each and treated 3-months. Groups were treated as follows: 1: normal diet, 2: Hypercholesterolemic diet (HCD), 3: HCD+ A.a (100 mg. kg^{-1} .day^{-1}), 4 and 5: HCD for 60 days and then normal diet and normal diet + A.a respectively for an additional 30 days(regression period). Dietary use of A.a in groups 3 and 5 significantly decreased LDL-cholesterol, total cholesterol and triglyceride, whereas, HDL-cholesterol was significantly increased. The atherosclerotic area was significantly decreased in the same animals whereas, the animals that in regression period received only normal diet showed no regression but rather progression of atherosclerosis. These finding suggest that dietary A.a may causes the regression and suppression of atherosclerotic lesion.

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STRUCTURAL DAMAGE ON HUMAN HDL IN OBESITY: OVER LIPOPROTEINS LEVELS

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Objective: We investigate relationship between HDL oxidative stress and paraoxonase (PON) activity.PON, enzyme associated with HDL exert a protective role against oxidative damage and atherogenic modifications such glycation, homocysteinylation. **Methods**: PON activity and levels of hydroperoxides on HDL from obese males and healty controls (HC) were compared. We investigated effect of homocysteine-thiolactone (Hcy) on HDL from obeses, complicated (OB-C)and not (OB-NC)compared to HC.Structural damage were evidenced by spectrofluorymetric study. **Results:** Obeses HDL-PON activity was lower respect to controls ($p<0.001$).Results showed a increase of hydroperoxides in HDL from obeses ($p<0.001$). Negative correlations between HDL-PON activity and hydroperoxides levels on HDL confirm relationship betweenPON activity and lipid peroxidation.HDL were incubated with Hyc-thiolactone;levels of –SH in Hcy-HDL demonstrated that homocysteinylation of HDL even occurred at physiological concentrations. Hcy-HDL of OB-C had higher levels of –SH than Hcy- HDL of OB-NC.Time-dependent increases of-SH in Hcy-HDL of OB-NC and OB-C were significantly higher than Hcy-HDL of HC ($p<0.05$). Negative correlations between HDL-PON activity and levels of hydroperoxides associated with Hcy-HDL confirm relationship between paraoxonase activity and lipid peroxidation.Obeses HDL are more susceptible at homocysteinylation of –NH2 than HC. **Conclusion**: Increase of oxidative stress in HDL of obeses and structural and functional damage increasing risk for CV disease also in normolipemic subjects.

Funding: None

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ENHANCED CHOLESTEROL LOWERING EFFECT OF SOY IN CONJUNCTION WITH FRUCTO-OLIGOSACCHARIDES IN EQUOL PRODUCERS

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Objective: Can fructo-oligosaccharides (FOS) enhance the hypocholesterolemic effect of soy foods by increasing colonic soy isoflavone metabolism (i.e. equol production). **Methods:** 23 hyperlipidemic subjects (11 M, 12F; 58±7 y; 4.06±0.70 mmol/L) completed three 4-week treatments consisting of FOS (10 g/d) and low-fat dairy (control), and soy protein (30 g/d) foods with or without FOS (10 g/d) in a randomized controlled crossover study. Fasting blood lipids and anthropometrics were measured biweekly, with urine collected at baseline and at the end of each treatment. **Results:** A stepwise reduction in TChol:HDL-C was seen for FOS (0.25±0.13, P=0.08), soy (-0.07±0.08, P=0.41) and soy plus FOS (-0.36±0.14, P=0.02), compared to baseline. Preliminary analysis indicated that the observed relationship was attributable to the equol producers (n=7). Changes in TChol:HDL-C for equol producers were 0.09±0.16 on FOS, -0.23±0.14 on soy and -0.72±0.42 on soy plus FOS, compared to baseline. Whereas for non-equol producers, changes in TChol:HDL-C were 0.41±0.17, 0.04±0.09 and -0.17±0.08, respectively. **Conclusions:** Greater improvements in the lipid profile were observed in individuals consuming soy foods with FOS compared to their intake alone, suggesting a possible synergistic effect. This improvement was more pronounced in equol producers, indicating that colonic soy isoflavone metabolism may play a role in the cholesterol lowering effect of soy.

Funding: Heart and Stroke Foundation of Canada, ORAFTI Group

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THE EFFECTS OF ADDING STRAWBERRIES TO A CHOLESTEROL-LOWERING DIETARY PORTFOLIO

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Objective: We assessed the effects of adding strawberries to improve the palatability of an effective cholesterol-lowering diet (dietary portfolio). **Methods:** Twenty-eight hyperlipidemic subjects who had followed a cholesterol-lowering dietary portfolio, consisting of soy, viscous fiber, plant sterol and nuts, for a mean of 2.5 years were randomized to take strawberries (454 g/d, 112 kcal) or additional oat bran bread (65 g/d, 112 kcal, ~2g β-glucan) (control) each for one month in a cross-over design separated by a two-week washout. **Results:** Palatability of the dietary portfolio with the addition of strawberries was significantly higher compared to additional oat bran bread ($P<0.001$). Reductions in LDL-C and T-C:HDL-C from the original study baseline were maintained at -13.4±2.1% and -15.2±1.7%, respectively ($P<0.001$), at the end of the strawberry phase, and -13.9±2.3% and -14.2±2.0%, respectively ($P<0.001$), at the end of the oat bran bread phase. These results were similar to lipid reductions seen at one year on the dietary portfolio. Blood pressure reductions on both strawberry and oat bran bread treatments were also similar and comparable to one-year. **Conclusion:** Palatability of the dietary portfolio was improved with the addition of strawberries without compromising the cholesterol-lowering effectiveness of the diet.

Funding: Canada Research Chair Endowment of the Federal Government of Canada; California Strawberry Commission

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AFFECT OF FOOD FIBERS ON LIPID METABOLISM IN PATIENTS WITH ATHEROSCLEROSIS

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Objective: We have studied the affect of Citron – food fiber obtained from the crust of tangerine – on the lipid metabolism in patients with atherosclerosis. **Methods:** Treatment with citron had been carried out on 116 patients. Georgian patent #170 has been obtained. Treatment course lasted for 6 weeks, with the dose – 3 g. a day. Lipid had been studied using spectrophotometer SF-46LOMO. **Results:** The treatment course resulted in reduction of total cholesterol in blood by 17%, triglycerides by 22%, b-cholesterol by 19%, pre- b-cholesterol by 29%; level of a-cholesterol remained unchanged. Sharply reduced the atherogenobe coefficient. **Conclusions:** They are not character effects and are much cheaper in comprasion with other anti-atherosclerotic drugs. **Finding:** Citron can be considered as a preventive and curative mean in the cases of atherosclerosis.

Funding: None

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ROLE OF GENISTEIN IN THE REGULATION OF LIPID EXCHANGE IN THE CASES OF ATHEROSCLEROSIS

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Objective: We have studied the affect of genistein – extracted from soybean - on experimental animals and patients with atherosclerosis. **Methods:** Affect of the genistein on the total level of cholesterol in the blood of experimental animals (100 white rats) in conditions of atherosclerosis have been studied and its medico-biological value assessed. Clinical study has been carried out on 150 patients with atherosclerosis accompanied by hyperlipidemia. In difference from the control group, the patients of experimental group were additionally given 200 mg genistein supplement during 6 weeks. In the observation period we had been measuring the arterial pressure; lipid metabolism indicators were studied by photospectrometer; level of nitric oxide in the blood had been determined using the electronic paramagnetic resonance method. **Results:** Clinical studies showed that affect of genistein resulted in: well manifested normalization of arterial hypertension; reduction of heart construction rate; increase of total cholesterol by 17.5%, triglycerides by 20.7%, low and very low density lipoproteins by 22.1% and 26.9% respectively, high density lipoproteins by 22.1%; concentration of nitric oxide increased by 8%; total peripheral resistance reduced by 14.7%. **Conclusions:** It was determined that this biologically active supplement is well accepted by and completely safe for the animal organism. Genistein is a preventive and curative mean, which regulates the lipid metabolism and improves hemodynamic indicators.

Funding: None

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ANTIOXIDANTS IN CARDIOVASCULAR DISEASE: RELEVANCE OF GENDER AND AGE

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Objective: Low levels of antioxidants are associated with an high risk for cardiovascular disease, but clinical trials with antioxidant supplementation yielded contrasting results on the progression of the atherosclerosis. Aim of this study was to investigate whether the depletion of antioxidants in coronary artery disease (CAD) is modulated by gender and/or age. **Methods**: 168 coronary patients (CAD) and 107 healthy controls were assayed for plasma antioxidants: reduced glutathione (GSH), alpha- and gamma-tocopherol (-T), and for a damage score (DS), representative of oxidative stress status. **Results**: DS was significantly higher in CAD than in Controls while the antioxidants exhibited a complex behavior. Low GSH levels were associated with CAD status (mean ± SEM: 0.812±0.059 vs. 0.264±0.074 in Controls, $p<0.0001$), in male gender and older age. Conversely, no difference were found in alpha- and gamma-T contents, comparing CAD and Controls. Low levels of alpha-T in CAD were limited to female gender ($p=0.003$ for interaction of CAD status and gender). **Conclusions:** Our study showed a different involvement of antioxidants in CAD, being sensitive to patients' characteristics. This findings should be considered in planning antioxidant treatments and could suggest suggests a new strategy focused on specific supplementation of the deficient antioxidant factors in relation, for instance, to age and gender of the single patients.

Funding: None

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A NOVEL CELLULAR ACTIVITY OF RESVERATROL AND ITS POTENTIAL EFFECTS IN DEGENERATIVE DISEASES

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Introduction and Objectives: Resveratrol, a natural polyphenol, causes several health-protective effects: antioxidant, anti-cancer, anti-atherogenic, and life-extending. It has been claimed as a beneficial components of the Mediterranean diet. Many of the resveratrol's activities overlap with those of p66Shc, a redox enzyme that generates ROS and induces apoptosis. Ablation of p66Shc extends life span and prevents diet-induced atherosclerosis in mice. We investigated whether resveratrol would activate p66Shc and influence growth in cultured human prostate cells (EPN and EPN-PKM3). **Methods:** Immunuprecipitation of cell lysates were analyzed by Western Blotting. Growth curves were obtained by cell counting. **Results:** We observed a dose- and time-dependent increase of p66Shc Ser36 phosphorylation reaching a peak at 200 μM resveratrol and 30 min of treatment. Resveratrol inhibits the growth of both EPN and PKM3 cells in a dose-dependent manner and consistently with the level of p66Shc activation. **Conclusion:** This is the first evidence linking resveratrol and p66Shc and may provide insight into the mechanisms underlying resistance to oxidative stress, pathogenesis of degenerative diseases, and longevity.

Funding: Partially supported by Regione Campania, Assessorato alle Attività Produttive, Italy

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ATERONON AS NEW INHIBITOR FOR LIPID OXIDISING ATHEROGENIC ANTIBODIES: PHASE II CLINICAL TRIAL

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ATERONON, a new inhibitor of lipid oxidising atherosclerotic catalytic antibodies, AtheroAbzymes, was tested on 150 patients with Coronary Heart Disease, CHD, who were positive on the presence of these antibodies. A previous study on ATERONON predecessor, CT002, which, due to its potential contraindications, lasted only for two months until abzymes were completely inhibited, demonstrated that even after this relatively short trial in 1 year follow up there was a significant reduction in the number of secondary cardiac events. Oral administration of ATERONON resulted in a complete inhibition of AtheroAbzymes also in the period of less than 2 months. Withdrawal of this product led to re-activation of these antibodies, and in 1 or 2 months after termination of its administration their activity reached the level of the pre-treatment period. ATERONON is a new generation of anti-abzyme products, which, due to its safe nature, could be taken, in a dose–dependant manner, as a dietary supplement for continuous inhibition of pro-atherogenic antibodies for indefinite period because of its highly safe profile.

Funding: Cambridge Theranostics

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EFFECT OF DIETARY ZINC ON THE EXPRESSIONAL CHANGES OF COPPER/ZINC SUPEROXIDE DISMUTASE IN AORTA, MYOCARD, KIDNEY, LIVER AND PANCREAS OF SPONTANEOUSLY HYPERTENSIVE RATS AND WISTAR-KYOTO RATS

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The aim of the present study was to examine the effects of feeding different Zn containing diets (50, 150, 250 mg Zn/kg lab chow) on the activity and expression of Cu/ZnSOD in aorta, myocard, kidney, liver and pancreas of SHR and Wistar-Kyoto (WKY) rats. Cu/ZnSOD expression was analyzed by immunohistochemistry and the activity of Cu/ZnSOD was measured by RANSOD kit. Atomic-absorption spectrometry was used to determine Zn and Cu in the rat sera. Cu/ZnSOD was expressed mainly in medial smooth muscle cells in aorta. Cu/ZnSOD had only weak immunoreactivity in the endothelium. In the group with zinc supplementation (250 mgZn/kg lab chow) Cu/ZnSOD staining was more enhanced than on the group with Zn diet (50 mg/kg lab chow) and systolic blood pressure was significantly decreased compared with SHR fed a standard diet. There was no significantly changed in Cu/ZnSOD expression between groups in WKY. The expression of Cu/ZnSOD was significantly increased in SHR in comparison with WKY fed a Zn supplmentation diet (250 mgZn/kg lab chow). The results of expression of Cu/ZnSOD in myocard, kidney, liver and pancreas are not present because of the limited space. The expression of Cu/ZnSOD changes depending on Zn content in the diet. Zinc supplementation increases the expression of Cu/ZnSOD in aorta of SHR more than WKY. The present data suggest that Zn concentration in the diet may play an important role in the expression of Cu/ZnSOD.

Funding: Medical University Pleven Bulgaria

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THE EFFECT OF ZINC DIETS ON THE PROCESSES OF LIPID PEROXIDATION AND GLYCATION OF PROTEINS IN SERUM OF SPONTANEOUSLY HYPERTENSIVE RATS AND WISTAR-KYOTO RATS

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Oxidative stress and non-enzymatic glycation of proteins are one of the most important pathogenetic factors in the development of hypertension and atherosclerosis. The aim of present study was to investigate the role of Zn suplementation in process of lipid peroxidation and non-enzymatic glycation of proteins in SHR and Wistar Kyoto (WKY). We examined the effects of feeding different Zn diets (diet 1-50, diet 2-150, diet 3-250 mg Zn/kg lab chow) in both SHR and WKY. The diets were introduced at 2 months after birth and the animals were fed for 8 weeks. Cu/ZnSOD activity was measured by RANSOD kit (RANDOX). Anti-AGE antibodies were investigated via method based on the main principles of direct ELISA. The lipid hydroperoxide concentration (LHC) was significantly decreased in SHR with Zn suplementation (diet-3) compared to rats fed a diet 1, ($p=0.016$) and diet 2, ($p=0.005$). In WKY LPC was no significantly changed between groups. It was found a significantly increased Cu/ZnSOD activity in groups with Zn suplementation in SHR, compared to WKY groups. Zinc supplementation (250 mg/kg) affects lipids profile by decreasing LDL and increasing HDL in SHR. At this stage of investigation it was established significant decrease of anti-AGE antibodies in SHR with Zn suplementation. In WKY was not found such decrease of anti-AGE antibodies. We conclude that zinc supplementation affects the serum oxidative status as decreases lipid hydroperoxide concentration and increases Cu/ZnSOD activity. Decreasing of anti-AGE antibodies is significant in SHR than WKY.

Funding: MU Pleven

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NATURAL POLYAMINE AS A POSSIBLE INHIBITOR OF AGE-RELATED DISEASES

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Objective: Recent investigations have revealed the involvement of polyamines in lipid metabolism. Polyamine-rich foods, such as cheese and soybeans, as well as dietary fibers that enhance polyamine synthesis in the gut, have inhibitory effects against atherosclerosis and other age-related diseases. We therefore hypothesized that natural polyamines, spermine and spermidine, inhibit the progression of these diseases. **Methods:** We examined the effects of an increased intake of polyamines on blood polyamine levels and the effects of an increased blood polyamine levels on the immune function. **Results:** On the 26th week of breeding with laboratory chow using three different polyamine densities, the mean blood spermine concentration in mice bred with high polyamine chow was 10.1 ± 2.4 μM and higher than those of mice bred with normal polyamine chow (5.2 ± 0.9 μM) and low polyamine chow (4.7 ± 0.5 μM). A two-month daily intake of 50 to 100 gram of the polyamine rich food " Natto = fermented soybeans" increased the blood spermine concentration by a factor of 1.36 ± 0.09 (n=10), while among volunteers who were asked not to eat polyamine-rich foods, the blood spermine concentration did not change. Spermine and spermidine decreased the adherent capacities of human blood mononuclear cells, and exclusively suppressed the expression of CD11a (=LFA-1). Among 42 healthy volunteers, blood spermine levels inversely correlated (r=-0.48, p=0.001) with CD11a intensities. **Conclusion:** LFA-1 is involved in the inflammatory process and the pathogenesis of several age-related diseases. We consider polyamines to be a natural substance inhibiting the progression of age-related diseases.

Funding: Grants-in-Aid for Exploratory Research (grant No. 18659186) from the Japan Society for the Promotion of Science

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DIETARY INTAKE AND FACTORS DETERMINING LATER CARDIOVASCULAR RISK IN GERMAN SCHOOL CHILDREN AND ADOLESCENTS

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Objectives: Primary prevention of caronary heart disease (CHD) should begin at early age. Established intervention programs in adults include modifications in lifestyle and nutrition to prevent hypercholesterolemia, hypertension, diabetes, smoking, thus reducing the risk for premature coronary heart. Information on frequency of early coronary risk factors in the young is rare. The main objective of the present study was to evaluate dietary and lifestyle factors in school children and young adolescents to establish effective new strategies to prevent early development of CHD in adulthood. **Methods:** The study was performed at 6 schools in Leipzig/ Germany and included 725 children (14-18 years, 303 males). Cholesterol, HDL-Cholesterol, anthropometry and blood pressure were determined. Dietary and lifestyle factors were evaluated using standardized questionaires. Evaluated 7 d diet diaries were used to assess the composition and the energy of the daily dietary intake. **Results:** The composition of nutrients was 53% carbohydrate, 33% fat , 14% protein. The mean intake of calories from sucrose was >13%, reflecting high sucrose intake. In contrast, intake of fibre was < 30 g/d, which was below normal recommendations.The low fibre intake was inversely correlated with cholesterol. Overweight was significantly related to elevated non-HDL-cholesterol and higher blood pressure, indicating already the disposition for glucose intolerance. **Conclusions:** Metabolic factors determining increased cardiovascular risk at later age are already present in adolescents. The findings emphasize the necessity to implement early and specific CHD prevention strategies before adulthood.

Funding: Techniker Health Insurance Company Hamburg/Germany

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FEMALE SPRAGUE-DAWLEY RATS DEVELOP HYPERINSULINEMIA EARLIER THAN MALE ON A LIQUID FRUCTOSE DIET

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Fructose (F) ingestion as a liquid solution induces hepatic leptin resistance in male Sprague-Dawley rats (MSD). Epidemiologic studies in humans indicate that women are more affected by fructose ingestion than men. We sought to determine whether such a gender-related difference is present in fructose-fed rats. To this end, two groups of 10 MSD each and two groups of 10 female SD rats (FSD) each had free access to water (control group CM and CF) or to a 10% F solution (F group, FM and FF). After 14 days, plasma and liver samples were obtained for determining plasma analytes and liver triglycerides and fatty acid β-oxidation activity (βOX). Statistical analysis was done by the unpaired t-test, $P<0.05$. FM versus CM had hyperleptinemia (1.9 Fold Induction), hypertriglyceridemia (1.3 FI), increased liver triglycerides (1.6 FI) and decreased βOX (0.8 FI), with no significant changes in plasma glucose and insulin concentrations. On the contrary, although FF had hypertriglyceridemia (1.45 FI) and reduced liver βOX (0.57 FI) versus CF, they present no significant changes in plasma leptin and hepatic triglyceride concentrations, and had hyperglycemia (1.1 FI) and hyperinsulinemia (1.65 FI). Thus, under ingestion of fructose as a liquid solution, FSD develop manifestations of insulin resistance earlier than MSD.

Funding: Supported by FPCNL, IBUB, REDIMET, FIS PI 060247, SAF2004-03045, and SGR05-00833

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SELECTED LIPID AND NON-LIPID MARKERS OF CARDIOVASCULAR RISK IN HEALTHY VEGETARIANS AND SUBJECTS ON A WESTERN MIXED DIET

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In group of long-term vegetarians in comparison to non-vegetarians were evaluated selected markers of cardiovascular risk in relation to consumption of protective or risk food. **Methods:**Lipid and non-lipid risk parameters for cardiovascular disease (Cholesterol, triacylglycerols, hsCRP, insulin resistance–IR/HOMA/) were measured in 400 adult non-smoking subjects aged 21-76 years of two different nutritional habits. **Results:** Values of total cholesterol, LDL-cholesterol, triacylglycerols, hsCRP and IR/HOMA/ were significantly reduced in vegetarians with low incidence of risk-related values (10.1 % vs. 47.2 % of subjects for total cholesterol, 4 % vs. 32 % LDL-cholesterol, 9.6 % vs. 27.6 % triacylglycerols, 0 % vs. 11.8 % hsCRP, 0 % vs. 8.9 % IR/HOMA/). Beneficial values of cardiovascular risk markers in vegetarians are a consequence of significantly higher intake of polyunsaturated fatty acids, linoleic and a-linolenic acids, fiber, vitamin C, vitamin E, arginine, glycine, serine, plant fats, plant proteins, legumes, barley, oat, nuts, fruit and vegetables and on the other hand they are a consequence of significantly reduced intake of total fats, cholesterol, saturated fatty acids, methionine and lysine if the intakes were compared with non-vegetarians. **Conclusion:** The results of low values of cardiovascular risk markers document a beneficial effect of vegetarian nutrition in prevention of cardiovascular disease.

Funding: This work was supported by Slovak Grant Agency of the Ministry of Health

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OMEGA-3 POLYUNSATURATED FATTY ACIDS IMPROVE ERYTHROCYTE CHARACTERISTICS IN CORONARY ARTERY DISEASE

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Objectives: Red blood cells (RBC) play an important role in thrombotic complications in coronary artery diseased (CAD) patients (pts). Abnormal changes in erythrocyte membrane are associated with the decrease of polyunsaturated fatty acids (PUFA) level and lead to platelet aggregation and atherosclerosis progression. The study aimed to examine changes of RBC properties in CAD pts taking Omega-3 PUFA. **Methods:** 22 CAD pts aged 57±7 years and 20 healthy subjects (HS) aged 48±3 years were included in the study. All pts were administrated Vitrum Cardio Omega-3 "Unipharm. Inc" (USA) in daily dose 2 g for 6 month. RBC aggregation and fragility was investigated before and after the treatment by laser aggregation analyzer BIOLA, Russia (analysis of fluctuations of optical transmission and turbidometric method). Morphologic control was conducted by light and scanning microscopy. **Results:** In 16 pts (73%) RBC aggregation was elevated (5,6±2,6 ru vs 1,9±0,4 ru, $p<0,05$) and enlargement of the aggregates was detected by microscopy. In 13 of 16 pts (81%) with the baseline higher aggregation this parameter decreased after the treatment (6,1±2,6 ru vs 3,3±1,0 ru, $p<0,05$). In 3 of 16 pts (19%) the level of aggregation did not changed (3,5±0,8 ru vs 4,2±0,9 ru, $p>0,05$). RBC fragility was reduced in 8 pts (36%), hemolysis began at the 234,1±32,2 s vs 344,1±40 s in HS ($p<0,05$). After the treatment this parameter rised up to normal range (347,9±54,6 s, $p<0,05$). There were no changes in RBC aggregation or fragility in subjects who have these parameters initially normal. **Conclusions:** Omega-3 PUFA lower the elevated RBC aggregation and improve the fragility in CAD pts because of the stabilizing effect on the cell membrane.

Funding: Federally funded

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THE EFFECT OF MARINE N-3 POLYUNSATURATED FATTY ACIDS IN DIFFERENT DOSES ON LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A_2

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Objective: There is growing evidence that plasma lipoprotein-associated phospholipase A_2 (Lp-PLA_2) represents a novel and independent marker of the risk for cardiovascular disease. Marine n-3 polyunsaturated fatty acids (PUFA) may reduce vascular disease and the objective of this study therefore was to examine the effect of dietary supplementation with marine n-3 PUFA on plasma Lp-PLA_2. **Methods:** Sixty healthy subjects (25 women and 35 men, mean age 38 years, range 21-57 years) were randomly assigned to different doses of daily fish oil supplementation or control oil for 12 weeks: 1) high-dose n-3 PUFA (6.6 g), 2) low-dose n-3 PUFA (2.0 g and 4,9 g of olive oil) or 3) control (7.0 g of olive oil). Lp-PLA_2 was measured by a commercially available ELISA (PLAC™ – diaDexus Co, South San Francisco, USA). **Results:** The three intervention groups were comparable at baseline regarding gender, age, body mass index, plasma lipids and lipoproteins. Plasma Lp-PLA_2 at baseline and after supplementation (mean±SEM) were: 1) high-dose n-3 PUFA: 199±13 ng/ml and 206±38 ng/ml; 2) low-dose n-3 PUFA: 197±17 ng/ml and 218±28 ng/ml; and 3) control: 316±56 ng/ml and 277±50 ng/ml. There were no significant changes in Lp-PLA_2 after dietary supplementation within any of the three groups or between groups. **Conclusion:** The present study does not suggest any beneficial effect of marine n-3 PUFA on plasma Lp-PLA_2.

Funding: The authors would like to thank diaDexus for providing Lp-PLA_2 reagents

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RELATIONSHIP OF PLASMA FATTY ACIDS COMPOSITION TO INFLAMMATORY CYTOKINES AND SERUM LIPIDS IN MALE OBESE SUBJECTS

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To explore the cause for a low-grade inflammation and dyslipidemia in the development of metabolic syndrome, we examined the relation of plasma fatty acid composition to serum inflammatory cytokines and serum lipids in 16 male subjects (of an average age of 42±9) with visceral obesity (waist circumference over 85cm). They ware not taking hypoglycemic or lipid lowering drugs(s). Though LDL-cholesterol revealed no correlation with polyunsaturated fatty acids / saturated fatty acid (P/S) ratio, triglycerides had a significant negative correlation ($r=-0.867$, $p<0.0001$) with P/S ratio. HDL-cholesterol also had a significant positive correlation ($r=0.642$, $p<0.001$) with P/S ratio. IL-6 showed a significant negative correlation (=-0.412, $p<0.001$), while TNF-α tended to decrease as P/S ratio increased, though not statistically significant. These data suggested that fatty acid composition might be playing a causative role in genesis of a low-grade inflammatory process and dyslipidemia in the development of metabolic syndrome in male obese subjects.

Funding: There is no funding sources of commercial nature for the work done in the abstract

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THE RELATION BETWEEN N-3 FATTY ACIDS AND CORONARY RISK MARKERS IN PATIENTS WITH DIABETES MELLITUS

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Objective: The risk of coronary heart disease is increased in patients with diabetes mellitus compared to non-diabetic subjects. Apolipoprotein B (apoB), lipoprotein(a) (Lp(a)), and high sensitivity C-reactive protein (hsCRP) are independent predictors of coronary heart disease. The marine long chain n-3 polyunsaturated fatty acids (PUFA) have beneficial effects in coronary heart disease. Our aim was to investigate the relation between the content of n-3 PUFA in platelets and apoB, Lp(a), and hsCRP in a diabetic population. **Methods:** In a cross-sectional study, 43 patients with type 1 diabetes and 38 patients with type 2 diabetes were recruited from the outpatient's diabetic clinic at Hjørring Hospital, Denmark. Blood was drawn after an overnight fast. The content of n-3 PUFA in platelets was measured by gas chromatography, and hsCPR, apoB and Lp(a) was measured by standard laboratory methods. **Results:** The type 1 diabetics were older than the type 2 diabetics (mean age 40 ± 11 yrs vs. 57 ± 10 yrs). There was no difference in HbA1c (8.0 ± 1.3 % vs. 8.1 ± 1.2 %) between groups. The content of n-3 PUFA in platelets, hsCRP and apoB was significantly higher in type 2 diabetics than in type 1 diabetics, whereas there was no difference in Lp(a). In the whole study population there was an inverse correlation between n-3 PUFA and apoB (Spearman's rho -0.14; $p = 0.043$). In patients with type 2 diabetes there was an inverse correlation between the content of n-3 PUFA in platelets and hsCRP (Spearman's rho -0.10; $p = 0.012$). **Conclusion:** A high cellular content of n-3 PUFA appear to be associated with low apoB levels (both type 1 and 2 diabetes) and low hsCRP levels in patients with type 2 diabetes.

Funding: None

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DIETARY HABITS AND INTAKE OF OMEGA-3 FATTY ACIDS IN ADOLESCENCE: RESULTS FROM THE LIPID STUDY LEIPZIG (LSL)

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Objectives: A population-based lipid screening study – Lipid Study Leipzig (LSL) – was initiated in the city of Leipzig, Germany. The objectives were to evaluate the cardiovascular risk factor profile and its dependence on age, dietary and lifestyle factors. With the aim of identifying types of effective prevention strategies adolescents were included in LSL. **Methods:** Data included both the measurement of cardiovascular risk factors and the evaluation of dietary and lifestyle factors of 897 adolescents between 14 and 18 years of age at secondary and high schools. Seven-day diet diaries were used to assess the dietary intakes and were analysed with the computer program PRODI 4.5 expert. **Results:** Even in adolescence significant relationships between cardiovascular risk factors are existing. The mean total supply of fatty acids nearly corresponds to guiding values; however, their composition is still improvable. On the average, the ratio of saturated fatty acids: monounsaturated fatty acids: polyunsaturated fatty acids in the daily diet is 1: 0.83: 0.35. The mean ratio of omega-6 fatty acids and omega-3 fatty acids is above 5:1. The supply of eicosapentaenoic acid and docosahexaenoic acid is low. **Conclusions:** In adolescence the dietary intake of omega-3 fatty acids is inadequate. The public health challenge is achieving adoption of beneficial dietary fatty acid composition in the setting of influences that promote unhealthy lifestyles in adolescence.

Funding: Working Group Omega-3 Frankfurt/ Main, and Techniker Health Insurance Company Hamburg/Germany

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FLAXSEED OIL SUPPLEMENTATION INCREASES PLASMA AND TISSUES CONCENTRATIONS OF LINOLENIC ACID IN RATS

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We studied the bioavailability of acute supplementation of scalar doses of flaxseed oil by analysing the level of Linolenic acid (ALA, w-3) and Linoleic acid (w-6) in serum and tissues of rats tested at 2-4-8-16 h after the administration. The amount of flaxseed oil administered by oral rate was 1.9, 4.7, 9.5 mL/kg corresponding to 1, 2.5, 5 g ALA/kg. Two techniques of lipid extraction were investigated to achieve maximal free fatty acids recovery in a reasonably short time. The corresponding fatty acid methyl esters obtained with direct methylation with MeOH/HCl, were quantified by GC/MS technique. GC-MS analyses were performed on a Gas-Chromatograph Varian 3400 on a HP-INNOWAX column (30 m x 0.25 mm; 0.25 mm film thickness). Mass spectra were acquired on a Finnigan MAT SSQ 710A mass spectrometer in the electron impact (EI) mode. Serum ALA levels at 1 g/kg after 2h in the flaxseed oil group increased by 70% from 0.067 ± 0.007 to 0.096 ± 0.008 mg/mL ($P<0.001$ Anova) whereas no significant increase occurred in the flaxseed oil group at 2.5 g/kg (0.142 ± 0.009) or at 5 g/kg after 2 h (0.140 ± 0.008) when compared with the value obtained after 1 g/kg. A statistically significant increase of ALA was found in adipose tissue and in liver 4 h after the administration of 1 g/kg of ALA whereas higher doses did not produce any significant changes.

Funding: The research has been supported by CINSPAN

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PREVENTION OF ATRIAL FIBRILLATION RECURRENCE WITH AN α-LINOLENIC ACID ENRICHED DIET: A RANDOMIZED STUDY

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Context: In the Lyon-Diet-Heart Study mortality was reduced by α-linolenic acid (ALA) possibly by reducing cardiac arrhythmias. **Objective:** We have therefore assessed ALA in atrial fibrillation as a cardiac arrhythmia. **Design:** Randomized parallel design efficacy study. **Setting:** Three university hospital centers Bordeaux, France. **Patients:** 98 successive patients successfully underwent electro cardioversion of whom 75 completed the study without major deviations. Intervention: A canola margarine and oil (ALA ω-3, 1.4 g/d) versus a conventional diet (control), with a one year follow-up. Main outcome measure: Length of time to first atrial fibrillation recurrence. **Results:** In the 75 patients, at 2 months, atrial fibrillation recurred in 40% (14/35) of the control subjects versus 18% (7/40) of the ALA subjects (p=0.036), the respective figures at 6 months were 48.6% (17/35) versus 22.5% (9/40) (p = 0.018) and at 12 months, 54.3% (19/35) control versus 32.5% (13/40) (p=0.057). Kaplan-Meier Survival curves demonstrated a significant advantage of ALA over control at one year (p=0.037). **Conclusion:** ALA appears to be antiarrhythmic and this action may explain its cardioprotective effect in clinical trials and cohort studies. Trial registration : NCT00410020.

Funding: Onidol (France), Lesieur (France), Vandemoortele (Belgium), Danone Vitapole (France)

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MICRODISPERSED OXIDISED CELLULOSE, A NOVEL POTENTIAL SOLUBLE FIBER LIKE HYPOLIPIDEMIC SUBSTANCE

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Microdispersed Oxidised Cellulose (MDOC™) is a random copolymer of polyanhydroglucose and polyanhydroglucuronic acid. In this study we wanted to elucidate hypolipidemic mechanism of action of MDOC™ and its effects on cholesterol levels, and inflammatory markers in apoE-deficient mice. Male apoE$^{-/-}$ mice consumed an atherogenic diet for 4 weeks (control group). Mice in MDOC™ group mice consumed the same diet supplemented with MDOC™ (50 mg/kg/day) for 4 weeks. Biochemical analyses of blood cholesterol fractions, determination of cholesterol absorption, bile acid excretion, the fermentation both *in vivo* and *in vitro*, ELISA analysis of IL-6 and VCAM-1 levels in blood and in aortic sinus were performed. MDOC™ treatment significantly decreased LDL cholesterol and significantly increased HDL cholesterol. ELISA analysis revealed significant decrease of IL-6 levels after MDOC™ treatment. Moreover, MDOC™ was significantly fermented both *in vivo* and *in vitro* which resulted in a significant reduction of cholesterol content in liver. This study demonstrates that MDOC™ acting probably as a soluble fiber has hypolipidemic effects in apoE-deficient mice.

Funding: This work was supported in part by grants from Alltracel Pharma Ltd., Ministry of Education of the Czech Republic (MZO 00179906), and The Grant Agency of MSMT (FRVS 51/75105)

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CARDIOVASCULAR RISK FACTORS IN PARENTS AND CHILDREN: THE PEP FAMILY HEART STUDY

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Objectives: To compare prevalence of CVD risk factors (RF) among PEP-Families. **Subjects and Methods:** 1123 families were elected. In 1948 parents (37.1± 4.9 y) and 1559 children (6.9± 1.7 y) overweight, dyslipidemia and hypertension were assessed. Age- and gender- specific values were obtained by LMS Chart Maker, statistical analyses with SPSS 14.0. **Results:** Mean values for lipids, BMI, waist circumference (WC) and blood pressure (BP) were higher ($p<0,01$) in men than in women. 10% of 860 men and 9% of 1088 women were obese (BMI>30kg/m²), 42% respectively 21% overweight (BMI >25-<30 kg/m²). Prevalence of RF was in normal-overweight-obese men for LDL-C>130 mg/dl 49-56-61%, for HDL-C <40 mg/dl 51-66-76%, for TG >150 mg/dl 26-49-55% and for BP >130/85 mm Hg 42-64-81%. In normal-overweight-obese women prevalence for high LDL-C was 26-32-36%, for HDL-C <50 mg/dl 13-46-69%, for high TG 8-22-32% and for high BP 15-28-60 %. 54% of normal weight men had >2 RF increasing to 77% in overweight and 87% obese men, corresponding to 11% in normal, 32% in overweight and 59% in obese women. Lipids in the 781 girls were higher than in the 778 boys, who had higher ($p<0.01$) WC values. Prevalence of overweight at the >90th p.c. was for BMI 5 % in both boys and girls, for WC 9 respectively 4%. Girls had hypertension (>95th p.c.) in 6%, TG > 110 mg/dl 8%, HDL-C (£ 50 mg/dl) 11%, LDL-C (>130 mg/dl) 18% corresponding to 3, 4, 6 (<40mg/dl), 13% in boys. 84% of overweight boys and 18% girls had > 2 RF compared to 5 and 9% in normal weight children. **Conclusions:** Overweight was 6 times higher in parents than in children. The RF profile was worst in fathers and best (by 4-14 times for single RF) in sons. Dyslipdemia in daughters and mothers was similar.

Funding: Foundation for the Prevention of Arteriosclerosis

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ANTHROPOMETRIC EFFECT OF LIFESTYLE INTERVENTION

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Objective: To evaluate the anthropometric effect of a lifestyle intervention program given to healthy, moderately obese, employees at a Danish Hospital. **Methods:** In total 120 men and women aged 25-66 years with BMI between 25 and 30 were randomized to either group intervention or individual intervention. The intervention included 5 sessions with diet and activity counseling. BMI (kg/m^2), waist circumference (cm) and body fat % were measured at baseline, and after 6 and 12 months follow up. **Results:** The two groups were comparable at recruitment. Median BMI was 27.6 and 27.2; waist circumference 88.5 and 86.5; body fat % 35.8 and 36.9 among participants randomized to group and individual intervention, respectively. After 6 months all anthropometric measurements were reduced in both groups. For both waist circumference and body fat the reductions were largest after individual counseling ($p=0.0001$ and $p=0.004$). After 12 months all anthropometric measurements remained reduced, but slightly attenuated. **Conclusions**: The lifestyle intervention showed a modest effect on BMI, waist circumference and body fat % and the changes were maintained over time. Subjects who had the individual counseling seemed to benefit slightly more than subjects given group intervention.

Funding: The study was partly supported from the local Spar Nord Foundation

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OBESITY

***Liljana Sokolova Đokic**, Sandra Martinović, Milan Bosnjak, Ivan Ponjević. Institute of Public Health, Sombor, Yugoslavia; Heath Center, Sombor, Yugoslavia.*

Diabetes is a chronically life long disease and it is of a great importance as a consequence of the obesity. In a Preventive Service Center, Health Center Sombor, there has been done a research over 101 patients about their obesity and diabetes. There was 24 patients younger than 50 and 77 patients older than 50. 41% from both group had a diabetes history in their families. 29% of younger and 38% older had an irregular diet and more than 50% from both groups was physically inactive.46% of younger and 69% of older had an increased level of blood cholesterol; 54% of younger and 88% of older had an increased level of blood tryglycerids. 41% of younger and 81% of older had a Body Mass Index over 25. As a consequence, 37% of younger and a 76% of older had an increased level of a blood sugar. Obesity and increased blood fats are conected with a high level of a blood sugar, and the frequency of a diabetes is higher as we are getting older.

Funding: Health Center, Center of Prevention, Sombor, Serbia

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THERE ARE NO ADDITIONAL CHANGES ON LIPID PROFILE INDUCED BY METFORMIN IN OBESE PATIENTS ON CLASSICAL WEIGHT LOSS PROGRAMS

***Jose Silva Nunes**, Leone Duarte, Fernando Malheiro. Curry Cabral Hospital, Lisbon, Portugal.*

Objective: To study an additional effect on lipid profile (LP) of adding metformin to a classical weight loss program for obese patients, independently of anthropometric variation. **Methods**: We selected 93 files of obese patients (82 females) who were on any drug (other than metformin) during the first 6 months: 48 patients on diet and physical exercise and 45 on similar plans plus metformin (2.5-3 g/day). They were characterised for age, gender, anthropometrics, glucose, insulin, HOMA-IR and LP. Glycaemic status was determined by an oGTT. Parameters were reassessed 6 months later and its variation was compared between groups. We looked for correlations among variation in anthropometrics, insulin resistance (IR) and LP. **Results**: Patients were characterised by mean age=38±13 yrs, BMI=37.5±6.6 kg/m^2, waist (Wc)=106.7±13.7 cm, cholesterol=198.6±33.7 mg/dl, HDL-c=52.3±16 mg/dl, LDL-c=123.5±31.9 mg/dl, triglycerides=119.2±76.1 mg/dl, glucose=88.7±13.8 mg/dl, insulin=17±11.7 µU/l and HOMA-IR=3.85±2.9. There was no significant difference, between groups, in the distribution by gender, glycaemic status or presence of dyslipidaemia. Six months later, there was a significant decrease in BMI (p=0.000), Wc (p=0.000), glucose (p=0.005), insulin (p=0.000), HOMA-IR (p=0.000) and triglycerides (p=0.039). No difference in the variation of LP was perceived between groups. No correlation was observed among variation in LP, in anthropometrics and in IR. **Conclusions**: Except for triglycerides, no change in LP is observed after 6 months on a weight reduction program; its decrease is not dependent on the variation in anthropometrics or IR. There is no additional benefit on lipid profile of adding metformin to a classical weight loss program.

Funding: None

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THE EFFECT OF A COMBINATION OF PLANT STEROL-ENRICHED FOODS ON PLASMA LIPIDS AND LIPOPROTEINS

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The purpose of this clinical trial was to evaluate the effect of combining low-fat margarine and milk enriched with plant sterols as part of a National Cholesterol Education Program (NCEP) step I diet on serum lipids and lipoproteins. This study was a randomised, double-blind, placebo-controlled, cross-over design with a run-in period and 2 intervention periods, all lasting 4 weeks. The subjects were provided dietary advice by an experienced clinical dietician before the start of the run-in period in order to meet the guidelines of the NCEP Step 1 diet. Fifty mildly hypercholesterolemic (LDL 3.0 – 5.5 mmol/L) subjects were recruited from the outpatient Lipid Clinic, Aalborg Hospital and the general community. Study products consisted of 20 g low-fat margarine (37% fat) containing 1.6 g plant sterols and 250 ml low-fat milk (0.6% fat) containing 0.7 g plant sterols, for a total daily intake of 2.3 g plant sterols. Placebo products were identical but without sterols added. Forty-six volunteers completed the study. Serum total and LDL cholesterol decreased significantly by 5.5% ($p<0.001$, 95% CI: 2.5; 8.3) and 7.7% ($p=0.001$, 95% CI: 3.4; 11.9), respectively, after consumption of plant sterol-enriched products compared to placebo. Serum apolipoprotein B was significantly reduced by 4.6% ($p<0.05$, 95% CI: 1.7; 7.5), and apolipoprotein A-1 was lowered by 1.3% ($p>0.05$, CI: +0.8; -3.2) after plant sterol intake compared to placebo. A combination of low-fat margarine and milk enriched with plant sterols significantly reduced total, LDL cholesterol and apolipoprotein B. Low fat products enriched with plant sterols may therefore lower CHD risk in hypercholesterolemic individuals.

Funding: The study was partially funded by Unilever Denmark A/S

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ASSESSING THE CONTRIBUTION OF PLANT STEROLS TO CHOLESTEROL REDUCTION IN THE DIETARY PORTFOLIO

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Objective: To determine the effect of removing plant sterols from the dietary portfolio on blood lipid reductions, since plant sterols (2g/d), consumed alone, have been shown to decrease low density lipoprotein-cholesterol (LDL-C) by approximately 9-14%. **Methods:** Diets high in soy protein (22.5g/1000 kcal), viscous fibers (10g/1000 kcal), plant sterols (1.0g/1000kcal) and almonds (23g/1000 kcal) were prescribed to 42 hypercholesterolemic subjects for 80 weeks. For 10 of these weeks (weeks 52-62), plant sterols were excluded from the diet. **Results:** The mean LDL-C reduction from baseline was 15.4±1.6% ($P<0.001$) when subjects were consuming the full dietary portfolio, including plant sterols. After elimination of sterols, LDL-C reduction was 9.0±1.5% ($P<0.001$). Results from 18 subjects with complete data demonstrated comparable LDL-C reductions: on-sterols: 16.7±3.1% ($P<0.001$); off-sterols: 10.3±2.6% ($P<0.001$). This attributed a reduction of 6.3±2.0% ($P=0.005$) to plant sterols. Compliance in the group of 18 subjects was 67.0±5.9% for plant sterols and 61.9±4.8% for the other 3 components. When 100% compliance was assumed, LDL-C reduction would be estimated at 9.4% for the plant sterols (2g/d) and 17.2% for the other 3 components. **Conclusion:** In the context of a low saturated fat diet, plant sterols, combined with other cholesterol-lowering foods, decreased LDL-C to the same extent as in trials in which plant sterols were the only cholesterol-lowering agent.

Funding: Unilever Food and Health Research Institute, Unilever R&D Vlaardingen, The Netherlands

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PLANT STEROL-ENRICHED FERMENTED MILK DECREASES PLASMA CHOLESTEROL LEVEL IN HYPERCHOLESTEROLEMIC SUBJECTS

***C. Nicolle**, C. Cortese, R. Fellin, A. Mezzetti, S. Bertolini, M. Parillo, A. Pujia, G. Lupattelli, A. Notarbartolo, E. Mannarino. Danone Research, Palaiseau, France; Policlinico Universitario Tor Vergata, Rome, Italy; Azienda Ospedaliera, Ferrara, Italy; Centro Studi sull'Invecchiamento, Chieti, Italy; Ospedale San Martino, Genoa, Italy; Azienda Ospedaliera di Caserta, Caserta, Italy; Campus Germaneto V Livello, Catanzaro, Italy; Policlinico Universitario, Palermo, Italy; Ospedale Silvestrini, Perugia, Italy.*

We examined the effect of daily consumption of plant sterols-enriched low fat fermented milk (FM) on plasma lipid profile. Hypercholesterolemic subjects consumed a FM daily for 6 weeks. Subjects were randomized into two groups: either enriched with 1.6 g of PS ester/bottle (n=57), or control (n=53). Lipids, β-carotene, β-sitosterol, campesterol and hs-CRP plasma levels were determined. Plasma LDL-C levels were reduced by around 10% (95% CI [5.41 ; 13.59]), corresponding to a 0.42 mmol/L decrease after 6 weeks in subjects consuming plant sterol-FM compared to the controls ($P < 0.001$). The LDL-C lowering effect was already achieved with the same magnitude after 3 weeks of consumption. Similar pattern was observed for total cholesterol: a significant 6% reduction in average was achieved in the PS group as compared to controls, whereas plasma TG and HDL-C concentrations were not affected. The percentage of subjects decreasing LDL-C at the end of the trial was significantly higher ($P < 0.001$) in the plant sterol (91%) group compared to controls (64%). We will also assess changes in plasma β-carotene, β-sitosterol and campesterol concentrations. We will also investigate hs-CRP modification as inflammation biomarker. Our data suggest that the daily consumption of low fat plant sterol-dairy product favourably change lipid profile by reducing LDL-cholesterol by an average of 10%.

Funding: Danone Research

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In Grateful Appreciation

The Organizing Committee gratefully acknowledges the generous support in the form of unrestricted educational grants from the following:

Platinum

Abbott Laboratories

Gold

AstraZeneca
Merck & Co., Inc.
Merck/Schering-Plough Pharmaceuticals
Pfizer, Inc.

Silver

Reliant Pharmaceuticals, Inc.
sanofi-aventis
Takeda Pharmaceuticals North America, Inc.

Bronze

Aegerion Pharmaceuticals
Daiichi Sankyo Inc.
F. Hoffmann-LaRoche

National Lipid Association Educational Activities & Meetings Calendar

Name and Time of Activity		Contact and Registration Information
Online: November 2006–November 2007 **CME Newsletter: Lipid Management Today**	Sponsored – CME, CE Online activity	www.nlacme.com/lipidmanagementtoday
Online: January 2007–January 2008 **Webcast: Why Your Patients Are Not Getting to Goal – Steps You Can Use to Improve Dyslipidemia Treatment Outcomes** Sponsored by American Osteopathic Association	Endorsed – AOA, CME Online Activity	www.NLACME.com
Online: March 2007–March 2008 **Meeting Highlights: Presentations from the 2006 NLA Scientific Meetings**	Sponsored – CME, CE Online Activity	www.NLACME.com
October 11–13, 2007 **5th Annual World Congress on the Insulin Resistance Syndrome** Boston Marriott Newton, Boston, MA	Endorsed – CME Live Meeting	www.insilinresistance.US
October 12–13, 2007 **14th Annual Fall Symposium on Atherosclerosis Prevention** Chetola Resort, Blowing Rock, NC	Endorsed – CME Live Meeting	Email: sfrankli@wfubmc.edu
October 19–20, 2007 **2007 Comprehensive Cardiovascular Conference** The Westin Colonnade, Coral Gables, FL	Endorsed – CME Live Meeting	Email: meded@baptisthealth.net Ph: 786-596-2398
November 3, 2007 **NLA Masters Summit – Digestive Tract Lipid Modifying Therapies** Orlando, FL	Sponsored – CME, CE Live Meeting	www.lpid.org
November 4–7, 2007 **American Heart Association 2007 Scientific Sessions** Orlando, FL	Other	www.scientificsessions.org
December 2–4, 2007 **34th Annual Williamsburg Conference on Heart Disease** Williamsburg, VA	Endorsed – CME Live Meeting	Email: e.aiken@imedex.com