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Highlights

Phytosterol-Enriched Functional Foods: New Tools in Cardiovascular Prevention

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Phytosterol-Enriched Functional Foods: New Tools in Cardiovascular Prevention

Symposium

This publication was based on the lectures presented by international experts during a Symposium entitled *Phytosterol-Enriched Functional Foods: New Tools in Cardiovascular Prevention* held during the *XIV International Symposium on Atherosclerosis* (Rome, June 18-22, 2006). The following invited speakers took part in the session:

Rodolfo Paoletti
School of Pharmacy, University of Milan, Milan, Italy.
Introduction

Peter J. Jones
Richardson Center for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Canada.
Phytosterols: Physiology and Mechanism of Action

Elke A. Trautwein
Unilever Food and Health Research Institute, Vlaardingen, The Netherlands.
Phytosterols and Cholesterol-Lowering Efficacy in Different Food Formats

Leiv Ose
Lipid Clinic, Medical Department, Rikshospitalet-Radium-hospitalet, Oslo, Norway.
Phytosterol Intake and Dietary Recommendations

Eberhard Windler
University Hospital, Hamburg, Germany.
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Eric Bruckert
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Closing Remarks

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Preface

The use of plant sterols (also called phytosterols) in the management of blood cholesterol and the prevention of cardiovascular disease has been investigated by the scientific community since the first half of the past century. The therapeutic potential of plant sterols was highlighted in 1961, in the "1st International Symposium on Drugs Affecting Lipid Metabolism", held in Milan, Italy.

Plant sterols are now once more under the spotlight, after a period of 'neglect' that coincided with the discovery of highly effective cholesterol-controlling drugs. Several products enriched with plant sterols have been launched and their market position has gone beyond the more general nutritional guidelines for the overall population, as they represent an additional dietary option in the prevention and treatment of coronary heart diseases.

The development of plant sterol-enriched foods has been accompanied by the normative evolution of the concept of *novel food* in the European Union. This concept will likely infuse new energy into research in the integrated field of nutrition and health in the coming years.

The range of plant sterol-enriched foods is rather extensive in Europe. Their commercial success confirms the high level of interest from the lay public into nutritional interventions in the control of blood cholesterol and in non-pharmacological prevention of cardiovascular risk.

It is foreseeable that interest in *cholesterol-lowering functional foods* will be further increased in the near future. A better understanding of the biochemical mechanisms of these foods, and of their synergy with other dietetic measures, lifestyle and pharmacological approaches, will strengthen the rationale for their use in the prevention of cardiovascular disease.

Phytosterols: Physiology and Mechanism of Action

Peter J. Jones, *Richardson Center for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Canada.*

Plant sterols are naturally present in plants and are structurally very similar to cholesterol.

Phytosterols, also known as plant sterols, are molecules that are structurally very similar to cholesterol and are naturally present in plants. There are different types of plant sterols; they differ from each other by the presence of ethyl or methyl groups in the lateral chain of the parent molecule (Figure 1). In the plant cell, their role is the same as that of cholesterol in animal cells, which is to provide stability to cell membranes and to some intracellular organelles.

The main food sources of plant sterols are vegetable oils, nuts, seeds, grain products, fruits and vegetables. On average, the dietary intake of plant sterols is between 160 and 400 mg/day.

Knowledge of the absorption of cholesterol and plant sterols, as well as the effects of plant sterols on cholesterol absorption, has significantly increased in the past few years. It is currently known that compounds are incorporated into the mixed micelle, pass through the brush border of cell membranes and are then internalised by intestinal cells. A protein called Niemann-Pick C1 Like1 (NPC1L1) is crucial to this process, such that animals deprived of this protein are unable to adequately absorb cholesterol and plant sterols from food. After they have entered intestinal cells, most of the plant sterols are immediately re-excreted into the intestinal lumen, through the action of specific protein transporters belonging to the ABC (ATP-binding cassette) family, namely ABCG5 and ABCG8. The net result of this re-excretion process is that, on average, less than 5% of the ingested plant sterols are absorbed. This percentage is much lower than that of cholesterol (about 50%, Figure 2). In turn, only minute quantities of these molecules are incorporated into chylomicrons and subsequently reach the liver. From the liver, small quantities of absorbed plant sterols enter the circulation, associated with very low density lipoproteins; traces of these molecules can be found in every lipoprotein class and in peripheral cells, both in the membrane and in intracellular organelles.

Most plant sterols that have entered intestinal cells are immediately re-excreted into the intestinal lumen, through the action of specific protein transporters.

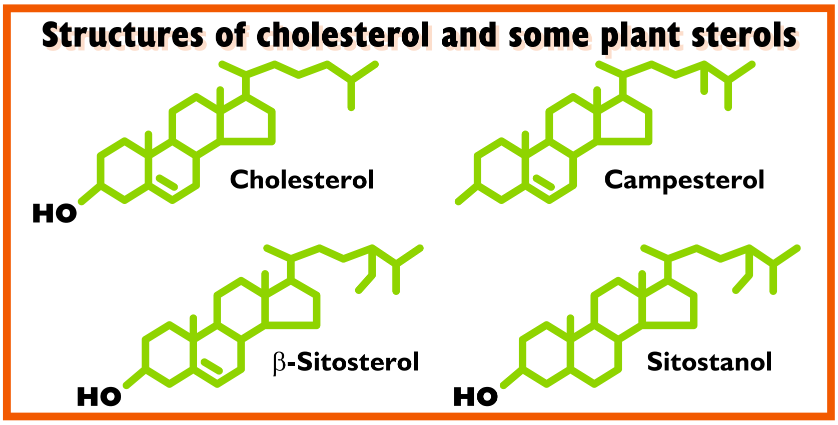


Figure 1

Phytosterols: Physiology and Mechanism of Action

If consumed regularly, amounts of plant sterols five to 10 times higher than those ingested as part of the usual diet are able to reduce total and LDL cholesterol.

It has been known for several years that plant sterols significantly lower blood concentrations of total and low-density lipoprotein (LDL) cholesterol, when consumed regularly at five to 10 times more than the amount usually ingested in the daily diet. There are many potential mechanisms through which plant sterols can reduce blood cholesterol concentrations, including the reduction of cholesterol intestinal absorption. Plant sterols compete with cholesterol, of both dietary and biliary origins, for solubilization and subsequent incorporation into mixed micelle a prerequisite for the absorption of cholesterol itself. In the intestinal lumen, they form with cholesterol insoluble complexes that precipitate and are excreted in the faeces. The highly specific trans-membrane transport is also affected: plant sterols compete with cholesterol for the activity of the above-mentioned protein NPC1L1. Additionally, they facilitate the re-excretion of cholesterol through the transporters ABCG5 and ABCG8 (see above and Figure 3).

A daily intake of 2 g plant sterols (or their reduced forms, stanols) is associated with a consistent and reproducible reduction in LDL-cholesterol concentrations of 10%.

A daily intake of around 2 g of plant sterols (or of their reduced form, stanols) is associated with a consistent and reproducible 10% reduction of LDL-cholesterol concentrations. This reduction is mediated by a decrease in the intestinal rate of cholesterol absorption in the order of 30-40%. In addition with a reduction in cholesterol absorption, consumption of foods enriched with plant sterols results an increase in the hepatic rate of cholesterol synthesis. This is a compensatory mechanism by which the availability of cholesterol in the hepatocytes is maintained, which, from a therapeutic point of view, presents an opportunity for additional cholesterol-lowering by combining plant sterols with inhibitors of cholesterol synthesis (statins) (see below). The cholesterol-lowering effect of regularly consumed plant sterols is constant through time. In a recent 12-month study, LDL-cholesterol lowering, which was around 12%, was unaltered throughout the whole study period. Obviously, blood cholesterol

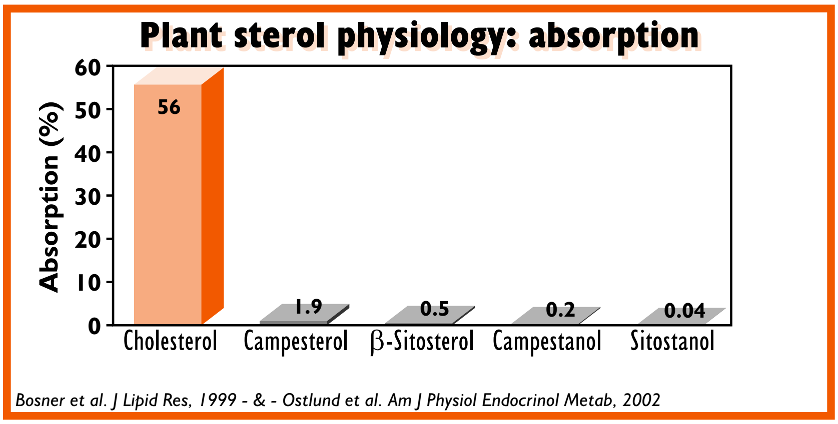


Figure 2

Phytosterols: Physiology and Mechanism of Action

concentration returns to basal level upon interruption of treatment.

The cholesterol-lowering property of plant sterols has now been demonstrated in more than 70 controlled clinical trials and two meta-analyses, the second of which stemmed from the works presented at the meeting *Plant-Based Products for Inhibition of Cholesterol Absorption: Efficacy, Safety and Future Research* held in 2001 by the Nutrition Foundation of Italy in Stresa, Italy. This meta-analysis by Katan et al. indicates the dose-dependency of the cholesterol-lowering properties of plant sterols. On average, a daily intake of 2 g of plant sterols reduces blood cholesterol concentrations by 10%. This meta-analysis also confirms that sterols and stanols have a similar cholesterol-lowering efficacy.

Several studies have confirmed the cholesterol-lowering property of plant sterols in subjects with different characteristics and pathologies.

Several studies confirmed the cholesterol-lowering properties of plant sterols in subjects with different characteristics and pathologies. Subsequently, these compounds have been incorporated into different food matrices (e.g. margarine, milk, yoghurt, and their derivatives) to modulate the human plasma lipid profile. In several countries, specific claims concerning the efficacy of plant sterols are permitted, thus informing the consumer of their cholesterol-lowering potential.

Research is currently focusing on the possibility of combining plant sterols with other compounds (or lifestyle modifications) that might result in additional lipid-lowering effects.

Currently, research is focusing on the possibility of combining plant sterols with other compounds (or lifestyles) that might result in additional lipid-lowering effects.

One such possibility concerns medium-chain triglycerides (MCT). These compounds represent an interesting source of energy and have recently been found in our laboratories to improve blood lipid profile significantly following their co-administration with plant sterols.

A second, interesting therapeutic opportunity is the combination of plant sterols with different types of dietary fibre. In a recently published study conducted by our group, the effect of glucomannan (a semi-digestible fibre) and plant sterols, either

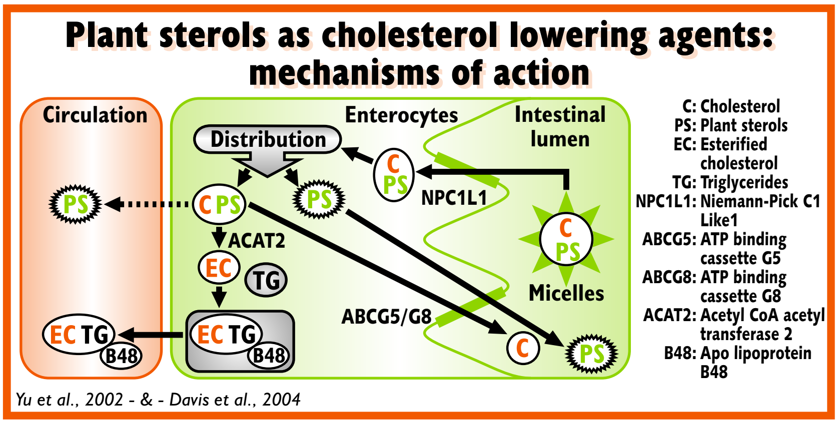


Figure 3

alone or in combination, on lipid profile was evaluated. Either molecule alone improved lipid profile, and this improvement was much amplified by the combination of the two molecules (up to 20% reduction in blood LDL-cholesterol concentration, or 17% more compared with the non-supplemented control group).

Plant sterols can also be added to an intervention programme of increased physical activity. In another recent study carried out by our group, the extent to which the combination of plant sterols with physical activity increases the high-density lipoprotein/LDL ratio was determined. This ratio is an important determinant of cardiovascular risk.

Another possibility to achieve greater lipid-lowering effects is by combining plant sterols with statins, which are inhibitors of cholesterol synthesis.

Available evidence indicates that plant sterols have a good safety profile.

Another possibility to further lower circulating cholesterol concentrations is to combine plant sterols with inhibitors of cholesterol synthesis (statins). The findings from several studies have shown that plant sterols have a similar LDL-cholesterol lowering efficacy (approx 10%) when they are administered in combination with a statin or as monotherapy, and that their effects are additive to those of statins. Hence, the statin-plant sterol combination results in larger reductions in blood cholesterol and allows the desired LDL reduction target to be achieved with a lower dose of statin, which represents obvious advantages for the patient. Available evidence indicates that plant sterols have a good safety profile, and are classified as GRAS (Generally Regarded As Safe) in the USA. No biochemical alteration, red blood cell deformability or hormonal synthesis alterations in either male or female subjects were reported in studies where plant sterols have been administered for up to 12 consecutive months.

A topic that attracts much interest concerns the effects of plant sterols on the absorption of carotenoids and lipid-soluble vitamins. The findings from the meta-analysis by Katan et al. mentioned earlier showed that the serum concentrations of lycopene, carotenoids and alpha-tocopherol were significantly

The effect of plant sterols on the absorption of carotenoids may be neutralized by a higher consumption of yellow, orange, and red-coloured fruit and vegetables.

reduced with doses of 1.5 g plant sterols/day or greater. This is because these lipid-soluble molecules are transported by LDL. However, after correction for the decreases in cholesterol levels, the reduction of serum vitamins was found to be only significant for beta-carotene (Figure 4). This effect may be neutralized by a higher consumption of yellow, orange and red-coloured fruit and vegetables.

Based on available evidence, it can be concluded that plant sterols compete with cholesterol (of both dietary and biliary origins) for its intestinal absorption, reducing this absorption by approximately 30-40%. The reduction in cholesterol intestinal absorption translates into a reduction in blood LDL-cholesterol concentration of around 10% for a dose of 2-2.5 g plant sterols/day. Greater effects on blood lipid profile can be obtained by combining plant sterols with lifestyle modifications or other nutritional approaches. Plant sterols appear to have good safety profile. A moderate reduction in the intestinal absorption and plasma concentrations of carotenoids is possible but can be reversed by an adequate consumption of coloured fruit and vegetables.

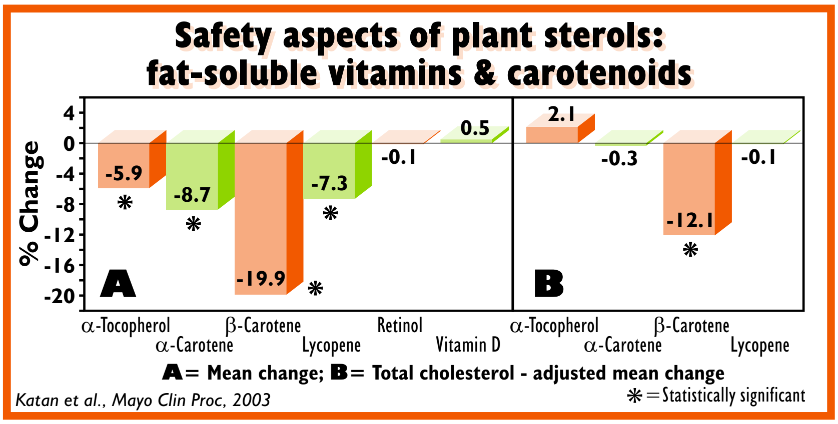


Figure 4

Phytosterols and Cholesterol-Lowering Efficacy in Different Food Formats

Elke A. Trautwein, *Unilever Food and Health Research Institute, Vlaardingen, The Netherlands.*

Although initial data on the cholesterol-lowering efficacy of plant sterols were available in the 1950s, additional information only began to accumulate markedly in the mid-1990s with the launch of the first foods enriched with plant sterols.

The first studies on foods enriched with plant sterols were carried out with these molecules incorporated into lipid matrices (mostly spreads, but also salad dressings). However, several recent studies have shown that these compounds maintain their significant cholesterol-lowering efficacy if they are incorporated into non-lipid matrices (e.g. fruit juices).

The correlation between the dose of administered plant sterols and their cholesterol-lowering effects has been elucidated in the meta-analysis by Katan et al. mentioned in the previous presentation (Figure 1). The results from 41 studies, in which very different daily doses (from 0.5 to 3.5 g/day) of plant sterols were used, show a non-linear dose-response relationship between the plant sterol dose and the reduction in blood cholesterol concentration.

We know that plant sterol consumption of 2-2.5 g/day reduces blood LDL-cholesterol concentrations by approximately 10%.

Several studies have demonstrated the cholesterol-lowering efficacy of plant sterols incorporated into fatty and non-fatty food matrices.

It is important to note that a 10% decrease in blood LDL-cholesterol concentration was obtained following a daily consumption of 2-2.5 g plant sterols with further increases in the dose of plant sterols adding little additional benefit.

Several studies have demonstrated the efficacy of plant sterols incorporated into fatty food matrices. For example, in the study by Cleghorn, shifting from a daily consumption of 20 g saturated fat (such as butter) to margarine rich in polyunsaturated fat led to a decrease by approximately 5% in blood concentrations of both total and LDL-cholesterol. Enriching the same margarine with 2 g plant sterols resulted in a decrease in blood LDL-cholesterol concentration of 12%.

The cholesterol-lowering effect of plant sterols has been reported to be maintained over time. In a study by Hendriks published in

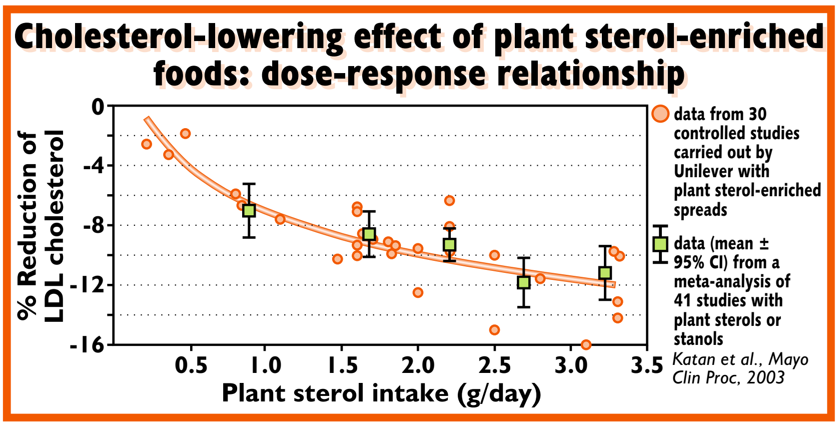


Figure 1

Phytosterols and Cholesterol-Lowering Efficacy in Different Food Formats

2003, a daily dose of 1.6 g plant sterols administered for 12 months significantly lowered blood cholesterol concentrations compared with the control treatment throughout the study period (Figure 2).

Several factors can affect the cholesterol-lowering property of plant sterols. Among these are the composition (namely, the fat content), the consistency (solid or liquid) of the food to which plant sterols are added, and the temporal relationship between the food and the meal (i.e. how much time is needed between a meal and the consumption of the plant sterol-enriched food to ensure maximum efficacy).

To investigate if there are differences in the cholesterol-lowering efficacies of plant sterols incorporated in a solid matrix and those incorporated in a liquid matrix, a study was conducted with a margarine and a milk enriched with plant sterols. The results clearly demonstrated that the ability of the two products to reduce LDL blood cholesterol concentration was comparable (8-10%).

Although the efficacy of plant sterols added to margarine appeared to be slightly greater, the difference between the two products was not statistically significant.

In several studies, plant sterols were added to milk, which was consumed in quantities ranging from 250 to 500 mL/day in a single or multiple portions throughout the day. LDL-cholesterol concentration was reduced by 7% to 15%, with a high inter-study variability, but with a constantly significant effect. Similar results were obtained using yoghurt as the food matrix. Different dosages were used in the numerous published studies, but a consistent decrease in LDL-cholesterol concentration by 5% to 13% has been reported.

The use of cereal-based matrices also yielded a comparable outcome. A significant reduction of LDL-cholesterol

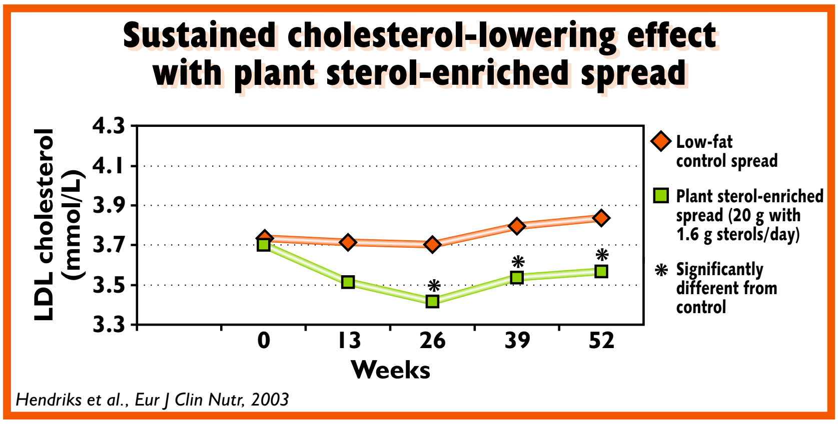


Figure 2

Phytosterols and Cholesterol-Lowering Efficacy in Different Food Formats

Supplementation with plant sterols appears to be efficacious regardless of the food matrix.

The mode of administration of plant sterols (once daily or in divided doses throughout the day) does not exert much influence on their cholesterol-lowering activity.

concentration was seen in all the studies where plant sterols were administered in bread, cereals, pasta or tortilla chips.

The cholesterol-lowering efficacy of fat-free liquid matrices, such as fruit juices, has also been studied. With both orange juice and lemonade a reduction in LDL-cholesterol of 12-14% was observed, which was comparable to that obtained with any other food matrix. Overall, these studies suggest that the nature of the food matrix used to deliver plant sterols is less important than initially thought. Supplementation with plant sterols appears to be efficacious regardless of the food matrix, at least within the wide range of food items studied so far.

From a practical point of view, another important aspect that needs to be considered is the extent to which the mode of administration of plant sterol-enriched foods (i.e. once daily or in divided doses throughout the day) affects their efficacy in lowering cholesterol.

A study by Plat et al. was specifically conducted to compare the intake of 2.5 g plant sterols in a single daily dose or in three separate meals (breakfast, 0.42 g; lunch, 0.84 g; dinner, 1.25 g) (Figure 3).

No significant difference in the ability of plant sterols to lower LDL-cholesterol was observed between the two regimens, which was in the order of 10% in both cases. In a study conducted in the USA, the consumption of hamburgers supplemented with 2.7 g plant sterols once a day led to a similar decrease in blood LDL-cholesterol concentration. Similar results were obtained with yoghurt administered in a single daily dose ranging from 65 g to 150 g.

A third aspect, which is of obvious practical relevance, is whether consumption of food items enriched with plant sterols should take place close to a meal or at any time during the day.

Doornbos et al. recently examined the effect of consuming 2.8 g

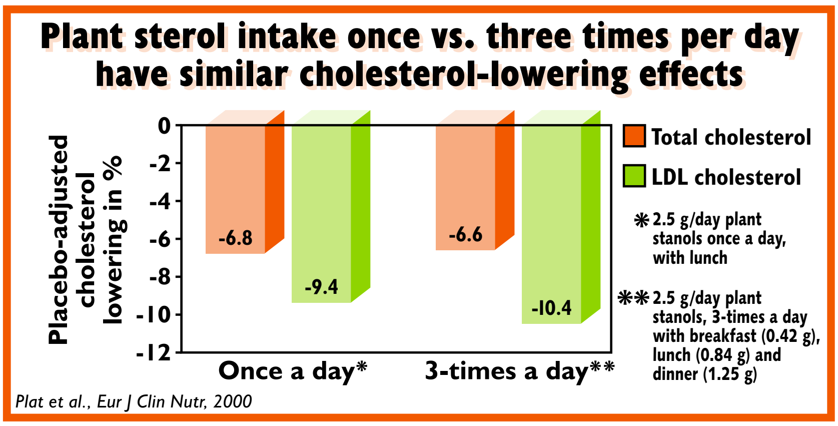


Figure 3

Phytosterols and Cholesterol-Lowering Efficacy in Different Food Formats

plant sterols after a usual overnight fast (half an hour before breakfast), or during or at the end of lunch. In both situations, consumption of plant sterols reduced blood cholesterol concentration, but the reduction in LDL was significantly more pronounced when plant sterols were ingested with lunch rather than on an empty stomach (-9.4% vs -6.8%, respectively, $p < 0.05$) (Figure 4).

A more pronounced cholesterol-lowering effect is obtained with a consumption of plant sterols with the main meal rather than after a usual overnight fast.

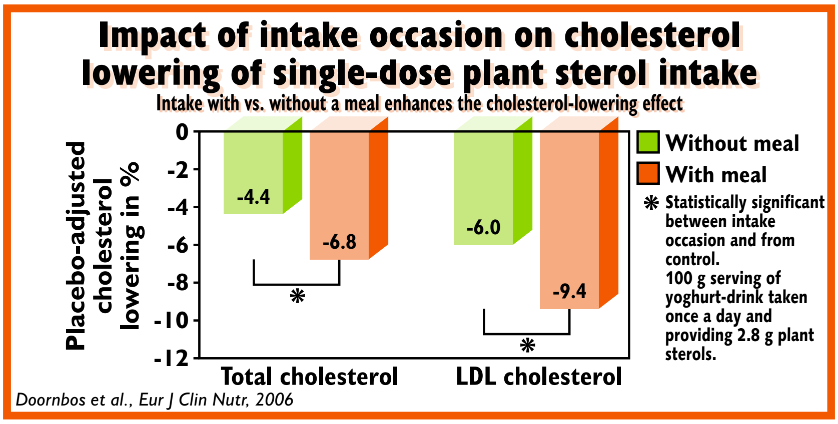
Consumption of plant sterols with the main meal leads to a more pronounced cholesterol-lowering effect, compared with consumption after a usual overnight fast. This is an important functional aspect to consider when plant sterols are added in liquid matrices. The gastric and intestinal transit times for such matrices are very different (usually much shorter) from those of solid matrices.

It may be hypothesized that the stimulation of bile flow, which is associated with the presence of food in the upper part of the gut, significantly influences the cholesterol-lowering effect of plant sterol-enriched foods. Indeed, the presence of bile is important because it contributes to the formation of mixed micelle, which are crucial to the process of cholesterol absorption. In addition, bile contains significant amounts of cholesterol, which are less effectively re-absorbed in the presence of plant sterols and are therefore excreted in faeces.

Available research evidence indicates that foods enriched with plant sterols are able to lower cholesterol significantly.

In conclusion, available research evidence indicates that food items enriched with plant sterols can have significant cholesterol-lowering effects. The findings from over 40 studies conducted in more than 2,000 subjects strongly support that the intake of 2-2.5 g/day plant sterols reduces blood LDL-cholesterol concentration by 10% on average. No greater benefit was obtained with higher doses of plant sterols.

The cholesterol-lowering effect is maintained over time and does not markedly depend upon the food matrix used to deliver plant



Doornbos et al., Eur J Clin Nutr, 2006

Figure 4

sterols. Furthermore, the mode of administration of plant sterols (once daily or in divided doses throughout the day) does not exert much influence on their activity. However, the consumption of plant sterols close to a meal significantly increases the activity of plant sterols, particularly if they are added to liquid matrices.

Phytosterol Intake and Dietary Recommendations

Leiv Ose, *Lipid Clinic, Medical Department, Rikshospitalet-Radium-hospitalet, Oslo, Norway.*

A Dutch study has found that the mean daily intake of plant sterols was approximately 300 mg for men and 250 mg for women.

Subjects in the highest plant sterol consumption quintile had a lower blood cholesterol concentration compared with those in the lowest quintile.

Several observational studies conducted to evaluate the correlation between diet and degenerative diseases have allowed an estimation of the mean dietary intake of plant sterols in different populations (Figure 1).

In a Dutch study conducted in more than 120,000 male and female subjects, the mean intake of plant sterols was found to be approximately 300 mg/day for men and 250 mg/day for women. The study also assessed the intake of plant stanols. It was found that this intake was much lower (i.e., no more than 5%) than that of sterols. The main dietary sources of plant sterols were cereals and vegetable oil (36% and 26% of total intake, respectively), while vegetables and fruit contributed 20% of the total.

In the British arm of the European Prospective Investigation into Cancer and Nutrition (EPIC) study conducted between 1993 and 1997 in Norfolk, UK to evaluate the correlation between diet and cancer risk , dietary intake of plant sterols in men was the same as that found in the Dutch study. However, plant sterol intake in women was slightly higher than that in the Dutch study and was found to be similar to that in men (approximately 300 mg/day). The British EPIC was the first study that investigated the correlation between dietary intake of plant sterols and blood cholesterol concentration in a large cohort of more than 20,000 people. Subjects in the highest plant sterol consumption quintile (> 450 mg/day) had a lower total blood cholesterol concentration than those in the lowest quintile (178 mg/day). These differences were wider in men (approximately 10 mg/dL for total blood cholesterol concentration, and 7 mg/dL for blood LDL-cholesterol concentration), and narrower in women (approximately 7 mg/dL for total and blood LDL-cholesterol concentrations).

Similar findings to those of the Dutch study on plant sterol intake in men (300 mg/day) were obtained from a national survey on dietary habits of the Finnish population (FINDIET) conducted in

Plant sterols intakes in various populations

Data from the "Netherlands Cohort Study"

A *Total plant sterol intake:* ♂ **307** mg/day ♀ **263** mg/day

Normen et al., Am J Clin Nutr, 2001

Data from the "EPIC Norfolk population"

B *Total plant sterols intake:* ♂ **310** mg/day ♀ **303** mg/day

Andersson et al., Eur J Clin Nutr, 2004

Data from the "national FINDIET survey"

C *Total plant sterols intake:* ♂ **305** mg/day ♀ **237** mg/day

Valsta et al., Br J Clin Nutr, 2004

Figure 1

Phytosterol Intake and Dietary Recommendations

In the Finnish population, plant sterol-enriched margarines are consumed by 5% of subjects between 35 and 85 years of age. This percentage tends to increase with age and is mostly related to subjects with higher education and of higher socio-economic status.

The report of the US National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) recognises the control of blood LDL-cholesterol concentration as a key factor in cardiovascular prevention. Accordingly, a sequential strategy to achieve target blood cholesterol has been suggested.

1997, whereas the figure in women was 237 mg/day on average. The FINDIET survey was conducted in 2,738 male and female subjects using a 24-hour dietary recall questionnaire. A positive correlation was found between total intake of plant sterols from natural sources and enriched foods, and education evaluated by the number of attended school years. The major dietary sources of plant sterols were cereal-based products, followed by fortified margarines. In women, but not in men, fruit (in particular, berries) were important sources of plant sterols. Data from the Public Health Institute in Finland showed that plant sterol-fortified margarines were consumed by 5% of subjects aged between 35 and 85 years. This percentage tended to increase with age and related mostly to subjects with higher education and of higher socio-economic status, "white collar" professionals, and married individuals.

A post-marketing surveillance performed by Unilever after the launch of plant sterol-enriched margarines has documented that the consumption of these products is overall in line with the on-label indications. In every country where the poll was conducted, median consumption of the products, was between 15 and 20 g/day (suggested amount 25 g/day), while the 95th percentile of consumption fell between 25 g/day in France and 45 g/day in The Netherlands (highest recorded value).

Many guidelines have been issued by the international health organizations on the use of plant sterol-enriched foods. A comprehensive evaluation of these guidelines reveals that they support the efficacy and safety of sterols and stanols for the treatment of people with hypercholesterolaemia.

The US National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) report issued in 2001 recognises the control of blood LDL-cholesterol concentration as a key factor in cardiovascular prevention and suggests a sequential lipid-lowering strategy. This strategy includes the initiation of a lipid-lowering diet low in saturated and *trans*-fatty acids, following which the so-called "Therapeutic Lifestyle Changes" should be adopted if blood cholesterol concentration does not reach the target value (Figure 2). This "augmented" lipid-lowering approach includes weight loss, increased physical activity, further reduction of total and saturated fat intake and, as an additional "therapeutic option," an increased intake (10-25 g/day) of soluble fibre and the consumption of 2 g/day of plant sterols from enriched foods. It is worthy to note that the overall effect of this dietetic adjustment may reach a 25-30% reduction in blood LDL-cholesterol concentration even though its applicability to real-life situations and, in particular, its long-term compliance, have not been adequately studied.

The ATP III document stipulates that the reduction of coronary heart disease risk achieved by a daily intake of 2 g plant sterols should be twice as much as that achieved by reducing dietary saturated fatty acids and cholesterol.

Similar recommendations have been published by other health organizations worldwide, which advise the administration of 2 g/day of plant sterols to subjects who need an LDL-cholesterol reduction greater than that obtainable with modifications in dietary fat intake.

The ATP III document stipulates that the reduction of coronary heart disease risk achieved by a daily intake of 2 g plant sterols should be twice as much as that achieved by reducing dietary saturated fatty acids and cholesterol. Moreover, the document highlights the much larger LDL-cholesterol reduction observed when dietary changes, including plant sterol consumption, are combined to statins. Because of this additional effect, patients may not need to take cholesterol-lowering drugs or may be able to reduce their doses. Of particular note is the focus given by the ATP III document on the use of plant sterol-enriched food items in subjects at high cardiovascular risk, for whom the risk/benefit ratio is more favourable.

Similar recommendations have been published by the American Heart Association, which suggest the administration of 2 g/day of plant sterols to subjects who need an LDL-cholesterol reduction greater than that obtainable with modifications in dietary fat intake. The International Atherosclerosis Society recently introduced and publicized complete nutritional guidelines, which also recommend an intake of 2 g/day of plant sterols by hypercholesterolaemic subjects who cannot reach their

NCEP ATP III: guidelines 2001

Main features of the "Therapeutic Life Style Changes" as defined by NCEP ATP III:

1. *Weight reduction*
2. *Increase physical activity*
3. *Limit total fat intake to 25-30% of total calories*
4. *Reduce intakes of saturated fat and cholesterol*
   - *saturates <7% of total calories*
   - *cholesterol <200 mg/day*
5. *Additional therapeutic options for enhancing LDL lowering*
   - **plant sterols/stanols 2 g/day**
   - *increased soluble fibre intake to 10-25 g/day*

National Cholesterol Education Program Expert Panel, JAMA, 2001

Figure 2

Phytosterol Intake and Dietary Recommendations

therapeutic target. Similar guidelines have recently been compiled and distributed by the British Heart Foundation which underline how the intake of 2-3 g/day of plant sterols or stanols can decrease blood LDL-cholesterol concentration by 9-20% (Figure 3). Other international health organizations such as the National Heart Foundation of Australia, the Dutch Heart Foundation, the Finnish Medical Society, the Finnish Nutritionist Association, and the Spanish Atherosclerosis Society also acknowledge the cholesterol lowering efficacy of plant sterols.

It is important to note that several studies have confirmed the potential of daily intakes of 2 g plant sterols to reduce blood LDL-cholesterol concentration further than the effect of a low-fat diet. In a study conducted in New-Zealand, volunteers on a low-fat diet included in their menu 20 g/day of butter during the control phase. In the next phases, they replaced the butter by an equi-energetic amount of vegetable oil spread or by a vegetable oil spread enriched with plant sterols. Replacing butter by a vegetable oil spread lowered LDL-cholesterol by 6%, while using the plant sterol-enriched spread resulted in a 12% decrease in LDL-cholesterol. These results show that, as part of a low-fat diet, a plant sterol-enriched vegetable oil spread lowers cholesterol concentrations more than a regular vegetable oil spread.

In a study evaluating the efficacy of the daily intake of a margarine containing 1.6 g of plant sterols in 40 children with familial hypercholesterolaemia for 8 weeks, our research group observed a decrease of blood LDL-cholesterol concentration by approximately 10%. This effect was unchanged 26 weeks of open-label follow-up (Figure 4).

Most of the children's parents had the same disease and were being treated with statins. Within the same study protocol as their children, these subjects were administered plant sterol-enriched products in addition to statins for 8 weeks. As seen in other studies, a further reduction in LDL-cholesterol of approximately

International bodies that officially acknowledge the cholesterol lowering efficacy of plant sterols

- **International Atherosclerosis Society**
- **American Heart Association (AHA - NCEP ATP III Therapeutic Lifestyle Change Diet)**
- **British Heart Foundation**
- **Heart Foundation Australia**
- **Dutch Heart Foundation**
- **Finnish Medical Society, Finnish Nutritionist Association**
- **Spanish Atherosclerosis Society**

Figure 3

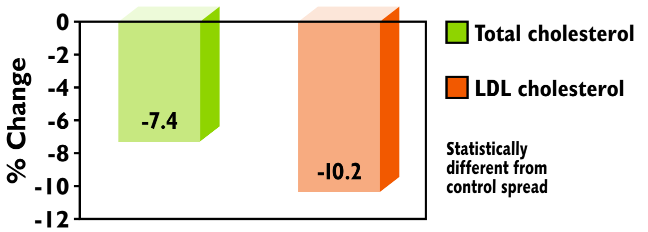
Phytosterol Intake and Dietary Recommendations

The effect of plant sterols is additive to that of other cholesterol-lowering foods: the Portfolio Diet, which has been developed by the Jenkins group and combines plant sterols with soy protein, viscous fibres, and almonds, leads to a reduction in LDL-cholesterol concentration of approximately 30%.

10% over statins was observed. The effects of plant sterols in subjects with familial hypercholesterolaemia have also been evaluated in a meta-analysis of six studies. This study confirmed that a reduction in blood LDL-cholesterol concentration by approximately 10% can be obtained by a daily intake of plant sterols starting from 1.6 g/day, and that no additional benefit was achieved with levels higher than 2.5 g.
The effect of plant sterols has been found to be additive to that of other cholesterol-lowering foods. The Portfolio Diet developed by the Jenkins group combines plant sterols with soy protein, viscous fibres and almonds. This diet leads to a reduction in LDL-cholesterol concentration of approximately 30%, similar to that obtained with a standard dosage of lovastatin.
In conclusion, available evidence clearly indicates that a low-fat diet provides a reduction in blood LDL-cholesterol concentration of approximately 4-5%. The addition of 2 g plant sterols to the daily diet, amplifies this reduction to approximately 15%, with an additional drop in blood LDL cholesterol concentration of 10%. The same additive effect can be observed with hypolipidaemic drugs such as the statins, allowing the target cholesterol concentrations to be reached in subjects who require a greater improvement of their blood lipid profile.

Cholesterol-lowering efficacy of plant sterols in children with familial hypercholesterolemia (FH)

18 g spread (enriched with 1.6 g/day plant sterols) vs. control spread.
38 children with FH, 8 week intervention.



Amundsen, Ose et al., Am J Clin Nutr, 2002

Figure 4

Plant Sterol Intake to Reduce Risk for Coronary Heart Disease

Eberhard Windler, *University Hospital, Hamburg, Germany.*

Despite a significant increase in total life expectancy, healthy life expectancy has only been modestly affected: many people at the age of 50 years and older are under medical care and on medication because they are affected by various diseases.

At about 50 years of age, 80% of human beings carry some atherosclerotic lesions (plaques).

There has been a significant increase in total life expectancy in industrialized countries over the past decades. However, healthy life expectancy, or the number of years people can expect to live in good health, has only been modestly affected. Many people at the age of 50 years and older are under medical care and on medication because they are affected by various diseases.

One such condition is atherosclerosis, which accounts for more than 50% of deaths in developed countries. According to statistics, 50% of men and 33% of women will be affected by a cardiovascular event during their lifetime.

The pathogenic mechanisms of atherosclerosis start much earlier than its clinical manifestations. At about 50 years of age, 80% of human beings carry some atherosclerotic lesions (plaques) (Figure 1). The fact that these lesions will eventually undergo "cap" rupture, inducing a clinical event, or will remain stable and asymptomatic appears to be due to chance. Based on current knowledge, cardiovascular events originate from interactions between genetic factors and lifestyle, which trigger risk factors that are linked to cardiovascular disease. Lifestyle modifications can be adopted to reduce cardiovascular risk, but these need to be initiated early enough in life. Alternatively, pharmacological interventions can be targeted at specific risk factors, but this approach should only be used in the latter half of life and only in subjects with clearly altered risk factors.

The efficacy of pharmacological interventions has been extensively demonstrated. Several studies conducted with statins have confirmed a drastic reduction in coronary events induced by a reduction in blood cholesterol concentration. The magnitude of reduction in coronary events is proportional to that of the reduction in blood cholesterol, in particular LDL-cholesterol. The decrease in coronary events usually ranges between 20% and 40%, but never exceeds the upper limit even if LDL- cholesterol is

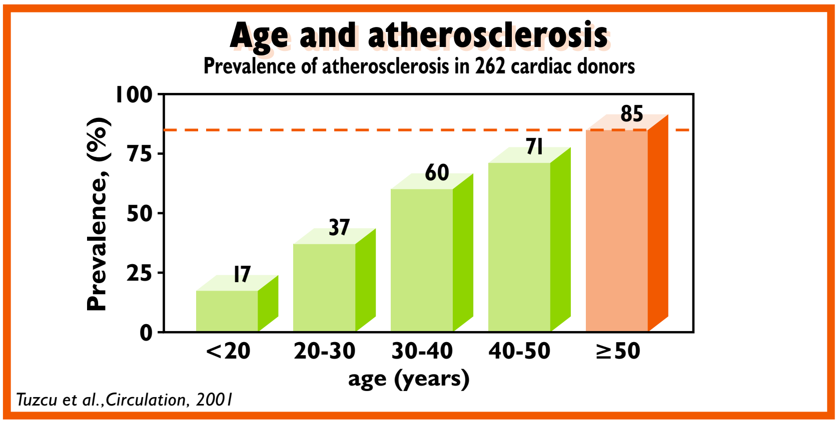


Figure 1

Plant Sterol Intake to Reduce Risk for Coronary Heart Disease

It has been shown in different populations that only moderate differences in blood cholesterol concentrations, maintained for prolonged periods, can translate into wide variations in cardiovascular risk.

LDL influences cardiovascular risk regardless of other risk factors.

largely reduced. This can be explained by the fact that 60-80% of coronary events are not influenced by statin treatment. It is therefore difficult to imagine that increasing the efficacy of the available drugs further can improve the situation, as treatment may have been provided too late owing to late diagnosis. On the other hand, it has been shown in different populations that only moderate differences in blood cholesterol concentrations, maintained for prolonged periods, can translate into wide variations in cardiovascular risk. In Japan where average blood cholesterol concentration is lower than that in Western countries, probably due to different dietary habits and a different genetic pattern, the incidence of coronary events is much lower than that in Western countries (Figure 2). Interesting findings from recent studies have shown that individuals who carry an antisense mutation in the protease PCSK9 gene (PCSK9 degrades the apo-B100 receptor) have LDL-cholesterol levels 15% lower than those of the non-carriers. This difference is associated with a reduction in coronary events in carriers of approximately 50% (Figure 3). A genetically-determined, and thus present early in life, 1% difference in blood LDL-cholesterol concentration is associated with a 3% reduction in cardiovascular risk, which is by far more interesting than the 1% reduction in risk for each 1% decrease in LDL-cholesterol observed in clinical trials of statins. Furthermore, this 1-3% relationship emphasises the importance of LDL in atherogenesis. It is worthy to note that LDL influences cardiovascular risk regardless of other risk factors, and that the protective effect of LDL reduction increases in absolute terms along with increased risk.
Reduction of LDL-cholesterol can be obtained by modifications of diet and lifestyle and if these are started early in life, they can contribute greatly to reducing cardiovascular risk over time. The use of functional foods, such as those fortified with plant sterols,

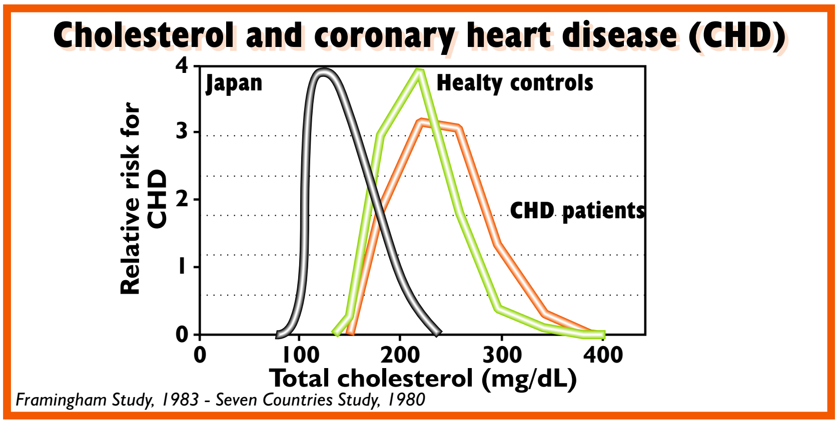


Figure 2

Plant Sterol Intake to Reduce Risk for Coronary Heart Disease

The use of functional foods, such as those enriched with plant sterols, can make it easier to reach therapeutic targets.

can make it easier to reach this target. For example, plant sterols can induce a reduction in blood LDL-cholesterol concentration in the same range as that induced by the protease PCSK9 mutation. It is thus conceivable that the use of plant sterols from the early years of life can bring about a reduction of cardiovascular risk comparable with that of carriers of the antisense mutation of PCSK9 protease (-50%).

It has been reported that the use of plant sterol-enriched foods increases plasma plant sterol concentrations. It is to be underlined that plant sterol concentrations in plasma are 100 times lower than those of cholesterol. In absolute terms, the increase in blood plant sterol concentrations that follow consumption of plant sterol-enriched foods is much smaller than the reduction in LDL concentrations induced.

The vast majority of studies conducted so far have concluded that modest increases in blood plant sterol concentrations do not exert any negative effect on cardiovascular risk.

Moreover, the vast majority of studies conducted so far have concluded that modest increases in blood plant sterol concentrations do not exert any negative effect on cardiovascular risk. Similar findings were obtained from the Atherosclerosis Risk in Communities (ARIC) and Coronary Risk Factors for Atherosclerosis (CORA) studies. The latter study was a case-control study in which control subjects had, on average, higher plasma levels of campesterol, sitosterol and total plant sterols compared with patients with myocardial infarction (2.7 vs. 2.1 mg/dL; 1.7 vs. 1.3 mg/dL, and 4.3 vs. 4.1 mg/dL, respectively). Nevertheless, it is possible that the higher plasma levels of plant sterols observed in control subjects was related mostly to higher high-density lipoprotein (HDL)-cholesterol concentrations in these subjects. Indeed, plant sterols are not only transported by LDL; a great proportion of these sterols is also transported by HDL. In the CORA study, when HDL and LDL concentrations were taken into account, thus normalizing for the concentrations of plant sterol transporters, the correlation between plasma plant

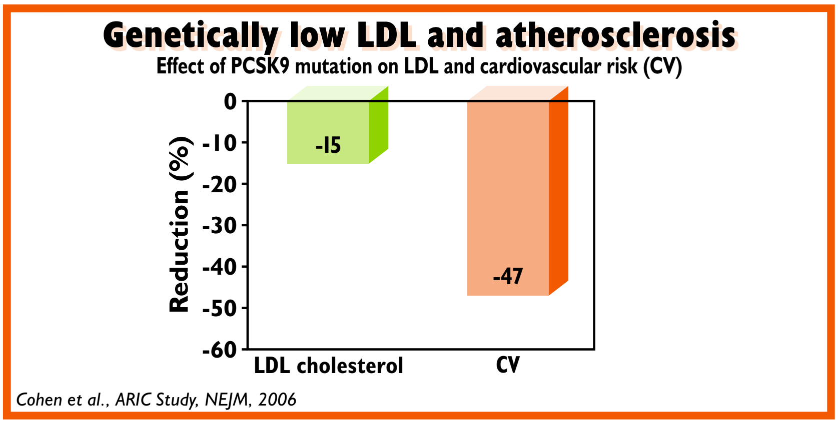


Figure 3

Plant Sterol Intake to Reduce Risk for Coronary Heart Disease

Based on data available to date, it is very unlikely that an increase in plasma plant sterols plays any detrimental role in cardiovascular risk.

sterol concentrations and myocardial infarction risk (expressed as odds ratio) was no longer statistically significant (Figure 4).
Based on data available to date, it is very unlikely that an increase in plasma plant sterols plays any detrimental role in cardiovascular risk.
Data from the CORA study also suggest that individual food items may have favourable or unfavourable effects on global risk, which are greater than those on individual cardiovascular risk parameters. This suggests that such effects may be directly correlated with the intake of individual food items. For example, consumption of meat and sausage is associated with an approximately 300% increase in cardiovascular risk for every 100 g of daily intake. Conversely, the consumption of 100 g fruit and vegetables is associated with a one-third reduction in risk. It is worthy to note that, even when the effects of such foods on cardiovascular risk factors are taken into account, most of the statistical association remains unaltered. As an example, the 50% increase in cardiovascular risk associated with meat and sausage consumption still exists after statistical adjustments for risk factor variations.
Likewise, the near-totality of the protective effect of vegetables is unaffected. It can thus be hypothesised that the effect of food items and, in general, of lifestyle on cardiovascular risk becomes only partially evident through their effects on classic risk factors; another portion acts directly on cardiovascular risk through mechanisms yet to be fully identified. Obviously, this hypothesis amplifies the preventive potential of lifestyle changes, especially if implemented early in life and maintained for prolonged periods.
From a practical point of view, it is likely that prevention of cardiovascular disease will be markedly modified in the future, by harmonizing a pharmacological approach (which is dominant

Correlation between plasma plant sterols and myocardial infarction risk

Total plant sterols adjusted for:	Odds ratio	95% CI
Age	0.929	0.857 - 0.998
HDL and LDL	0.977	0.906 - 1.054
Risk factors	1.014	0.916 - 1.152
Risk factors and nutrition	1.016	0.907 - 1.162

Figure 4

Plant Sterol Intake to Reduce Risk for Coronary Heart Disease

From a practical point of view, it is likely that prevention of cardiovascular disease will be markedly modified in the future, by harmonizing a pharmacological approach (which is dominant today) with life style changes, and will be optimized and implemented as early in life as possible.

today) with life style changes, and will be optimized and implemented as early in life as possible.
The information discussed above suggests that a lifestyle change adopted early enough in life may have a sufficient effect on reduction of cardiovascular risk, such that the use of pharmacological therapy may not be needed.
From this viewpoint, the availability of functional foods appears to be of great interest and it may be hypothesised that these provisions will, over time, further amplify the overall efficacy of cardiovascular risk control strategies.

Conclusions

The consumption of plant sterol-enriched foods has been shown to reduce intestinal absorption of cholesterol, of both dietary and biliary origins, by 30-40%. Plant sterols exert their effect by interfering with various physiological mechanisms involved in cholesterol absorption, such as the formation of mixed micelle and the activity of specific protein transporters (e.g. NPL1C1, ABCG5 and ABCG8 transporters).

The findings from over 40 studies conducted in more than 2,000 subjects have indicated that a daily intake of 2-2.5 g plant sterols reduces blood LDL-cholesterol concentrations by 10% on average. Higher daily intakes of plant sterols do not significantly increase this reduction further.

The cholesterol-lowering effect of plant sterols is maintained over time and does not depend on the food matrix in which they are incorporated. For example, plant sterols are equally effective when added to milk, yoghurt fruit juices, and vegetable oil based margarines.

Plant sterol-enriched foods are effective when consumed once daily or in divided doses throughout the day. For best cholesterol-lowering results, plant sterol-enriched foods should be consumed with a meal.

The international scientific bodies and associations that have examined the use of plant sterols recommend the consumption of 2 g/day of plant sterols to significantly lower blood cholesterol concentrations.

The cholesterol-lowering effect of plant sterols is additive to that of other cholesterol-lowering diet and lifestyle interventions. A combination of diet and lifestyle changes, including weight loss, physical activity, reducing total and saturated fat intake, and increasing soluble fibres and plant sterols can have a significant cholesterol lowering effect in the region of 30%.

The efficacy of plant sterols is additive to that of cholesterol-lowering drugs, such as statins. The combined effect results in approximately 10% additional reduction in blood LDL-cholesterol concentration than what is obtained with statins alone. Combining plant sterols and statins therefore allows the target cholesterol concentration to be achieved in individuals with more pronounced hypercholesterolaemia requiring more drastic cholesterol-lowering therapy.

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