

Plant sterols as dietary adjuvants in the reduction of cardiovascular risk: theory and evidence

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Abstract: Plant sterol-enriched foods are an effective dietary adjuvant in reducing cardiovascular risk by lowering total cholesterol and low density lipoprotein-cholesterol (LDL-C) in serum by up to ~15%. The mechanism of action of plant sterols is different from those of 3-hydroxy-3-methylglutaryl coenzyme A inhibitors (statins) and thus their effect is additive. Combining plant sterols with other dietary components known to reduce cholesterol in a portfolio approach has proven to be most effective for reduction of hypercholesterolemia and provide an alternative treatment option for clinicians. Plant sterol-enriched foods provides clinicians with a relatively cheap, safe, and effective way to help patients manage their cardiovascular risk.

Keywords: plant sterols, plant stanols, cardiovascular risk, cholesterol, LDL-cholesterol

The problem of cardiovascular disease

Cardiovascular diseases (CVD), including stroke, coronary occlusion, ischemic heart disease (IHD), atherosclerotic heart disease, vascular and coronary thrombosis, is the leading cause of death in most Western societies. This has wide public health implications. In 2002, CVD made up 16.7 million, or 29.2% of total global deaths (WHO 2005). It is predicted that CVD will be the leading cause of death in developing countries by 2010 (WHO 2005). Approximately 80% of CVD are prevalent in low and middle-income countries (WHO 2005). In Australia, CVD are the most costly diseases for the health system and in 1993–94, CVD accounted for \$AU3719 million or 12% of total direct health system costs in that year (AIHW 2004).

No authentic cause of atherosclerosis has been isolated, but is believed to be multifactorial (Buja 1996). To date, etiological research has only established risk factors which provide the basis for some efficient but incomplete means for prevention. Most of this evidence relating to risk factors is epidemiologic and therefore consists of statistical evidence for probability of evidence (Buja 1996). The three factors most consistently associated with an increase in the incidence of CVD include hypertension, hypercholesterolemia, and cigarette smoking. Other modifiable risk factors include obesity, lack of exercise, hypertriglyceridemia and low high-density lipoprotein-cholesterol (HDL-C) levels. There are also a number of nonmodifiable risk factors such as male gender, diabetes mellitus, family history of premature CVD, and the presence of definitive atherosclerosis.

There is considerable evidence that dietary saturated (and trans) fatty acids lead to increases in blood levels of cholesterol, and ultimately to atherosclerosis. In the past this has been the theoretical underpinning to many health-promoting and dietary prevention strategies (Kwiterovich 1997). Initially, expert committees

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from major scientific associations such as the National Heart, Lung and Blood Institute, and the American Heart Association initiated clinical trials with the aim of decreasing plasma cholesterol levels in an attempt to decrease the risk of atherosclerotic disease (the diet–heart hypothesis) (Haskell 2003).

Risk factors of cardiovascular disease

The clinical manifestations of CVD are chronic arterial obstructions or acute arterial occlusions in various territories, which ultimately lead to the condition where there is an inadequate supply of blood to the heart muscle, brain, or visceral limbs leading to ischemia and in severe cases, myocardial infarction (Ulbricht and Southgate 1991). Atherosclerosis is a disease of the tunica intima (arterial inner lining of the lumen) of the large and medium sized arteries, characterized by the development of fibrous, fatty deposits called plaques or atheromas (Zeman 1991). These atheromas eventually become calcified, rendering them rigid and narrow.

As yet, the pathogenesis is only partially understood. The development of an atheroma (or plaque) in the intima of the major arteries marks the pathogenesis of atherosclerosis. Arterial wall abnormalities, blood composition abnormalities, and hemodynamic alterations are generally accepted to be causative (Virchow's triad) (von Baeyer et al 2003). There is strong support for a causative relationship between hypercholesterolemia and atherosclerosis originally evidenced by the premature development of CVD in younger individuals with familial hypercholesterolemia (Ross 1986). There is also direct evidence from animal models of the effect of elevated low-density lipoprotein-cholesterol (LDL-C) on atherogenesis (Ross 1986) initiating the cascade of events eventually leading to atherosclerosis (Steinberg et al 1989). Shear-stress induced micro injuries of the endothelium in hemodynamically compromised regions together with local coagulation activation associated with microinflammation of the plaque are currently thought to cause plaque rupture (von Baeyer et al 2003). The resultant local clot formation is the ultimate reason for heart failure. This has been confirmed in a number of large trials showing a combined reduction of cardiac deaths and non-fatal myocardial incidents in post infarction patients by approximately 35% through the use of cholesterol-lowering medications (Anonymous 1994; Sacks et al 1996).

Current dietary guidelines – the use of plant sterols

The Adult Treatment Panel (ATPIII) of the US National Cholesterol Education Program (NCEP) recommend as part of their therapeutic lifestyle changes for the reduction of CVD risk, a diet which contains 25%–35% of total calories from fat (<7% saturated fatty acids (SFA), up to 10% polyunsaturated fatty acids (PUFA), and up to 20% monounsaturated fatty acids (MUFA), 50%–60% of total calories from carbohydrates (CHO), ~15% of total energy from protein (PRO), <200 mg cholesterol/day, dietary fibre 20–30 g/day and total energy to be balanced with energy expenditure to maintain desirable body weight or prevent weight gain (NCEP 2001). More recently there is a recommendation to include 2 g per day of plant sterols into the diet for those with elevated serum LDL-C (NCEP 2001) in response to the growing amount of evidence showing significant cholesterol-lowering from plant sterol/stanol-enriched margarines. The use of plant sterol-enriched products is also recommended by the International Atherosclerosis Society (IAS 2003) and the National Heart Foundation of Australia (NHF 2001).

The most common are unsaturated plant sterols β -sitosterol, campesterol, and stigmasterol and the saturated sitostanol and campestanol (Kochhar 1983). Research over the past decade has focused on the esterified form of plant sterols which is added to food products such as margarine for reasons of greater product stabilization, although free-forms of plant sterols have been studied in yoghurt (Thomsen et al 2004). Plant sterols reduce the absorption of both dietary and biliary cholesterol from the intestinal tract by 30%–50% (Jones et al 2000). The exact mechanism is yet to be elucidated; it is generally assumed that the presence of increased quantities of plant sterols in the gut lowers the micellar solubility of cholesterol, therefore lowering the amount of cholesterol available for absorption (Jones et al 2000). Recent advancements in food technology have seen the emergence of food products such as margarine, milk, yoghurt, and cereal products being enriched with plant sterols/stanols and promoted as a food which can help lower serum cholesterol.

Efficacy of plant sterol-enriched products

The results of dietary interventions aimed at reducing total serum cholesterol have been largely ineffectual. It has been suggested that dietary advice to individuals produced small

reductions in total cholesterol concentration (less than 4%), rendering it of little value in clinical management (Ramsay et al 1991). Tang et al (1998) published a systematic review of 19 randomized, controlled dietary intervention trials under free-living conditions aimed at lowering total cholesterol. The overall weighted mean reduction in blood cholesterol across all dietary comparisons was only 5.3%, using trials of at least six months duration. Although this may translate into a 5%–10% reduction of CVD in a 10-year period, compared with the effectiveness of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) inhibitors (statins: the most commonly used drugs for lowering serum cholesterol), dietary interventions risked being undervalued as a front line therapy for the management of serum cholesterol if it wasn't for the emergence of plant stanol/sterol-enriched functional foods.

In 57 trials, the efficacy of plant sterol esters and plant stanol esters in reducing mean total cholesterol (TC) and LDL-C was studied in normocholesterolemic, hypercholesterolemic, or diabetic individuals (n=3609) (Normén et al 2005). When plant sterols/stanols are added to foods such as margarine with an average dose of 2.4 g/day (standard deviation [SD] \pm 1.3 g/day), lowering was 9.9% (SD \pm 3.9%) for LDL-C (Normén et al 2005). It has been shown that there is no significant difference in the efficacy between plant sterols and plant stanols when they are esterified, which is the form added to foods (Hallikainen et al 2000). Most of these studies used esterified plant sterols in margarines. Further studies have investigated the cholesterol-lowering effects in different food vehicles such as milk, yoghurt, bread, and cereal. Clifton and colleagues (Clifton, Noakes, Sullivan, et al 2004) have concluded that serum total and LDL-C were significantly lowered when plant sterols were added to: milk (8.7% and 15.9%) and yoghurt (5.6% and 8.6%), but significantly less when added to bread (6.5%) and cereal (5.4%), while other investigators have made similar observations using yoghurt (Volpe et al 2001; Mensink et al 2002), and milk (Thomsen et al 2004).

Effectiveness of plant sterol-enriched products

Most studies to date have been in controlled settings using willing volunteers and providing subjects with plant sterol- or stanol-enriched products. It is important to evaluate the effectiveness of this treatment in a clinical or 'free-living' context. Effectiveness answers the question of compliance, ie, do subjects comply with the therapy to ensure that the

effect is still observed. Whilst it may be correct to assume that efficacy in controlled settings often equates to effectiveness with therapeutic drugs, with functional foods, issues relating to the belief in the benefit of use, taste, preference, and price may affect consumption and ultimately compliance (Anderson et al 1998; Povey et al 2001). Given the reported inconsistency in consumption of these products (Simojoki et al 2004), there have been recommendations to replicate these results in subjects who assemble the diets for themselves on a routine basis, thus determining the true effectiveness of this strategy in the clinical setting (Jenkins et al 2003). In addition, the net effect on the whole diet needs to be considered in those who select functional foods, given the importance of other dietary aspects such as restricting saturated fat and cholesterol and increasing n-3 fatty acids in reducing CVD risk (Schaefer 2002). In a clinical trial with hospital outpatients, using the number-needed-to-treat index, it was demonstrated, that for each 2.8 patients counseled with routine prescription of plant stanol/sterols, one additional patient would obtain a reduction in cholesterol by \geq 15% compared with conventional treatment (Patch et al 2005). What was demonstrated in a controlled environment can now be safely extrapolated in a free-living context.

Safety

There have been concerns raised over the reduced absorption of some fat soluble vitamins from intake of plant sterols. Plant sterols and stanols have been shown to reduce β -carotene, α -carotene, and vitamin E levels by around 25%, 10%, and 8% respectively (Gylling and Miettinen 1999; Hallikainen et al 1999; Hendriks et al 1999). This appears to be more pronounced at higher levels of intake (6.6 g/day), however this does not provide any additional cholesterol-lowering benefits beyond what would be obtained from an intake of around 3 g/day (Clifton, Noakes, Ross, et al 2004). In addition, Noakes et al (2002) found that a reduced absorption can be abated by an increased consumption of carotene-containing vegetables within current dietary recommendations.

There have been concerns regarding the possible atherogenic effects of plant sterols in certain individuals. Most recently, Miettinen et al (2005) concluded that statin treatment was associated with an increase in plant sterols absorption and also arterial plaque. In contrast, recent experimental models that have tested whether elevated plasma levels of plant sterols were associated with

atherosclerosis in genetically modified mice and in middle-aged men and women found no association (Wilund et al 2004). In mice with inactivation of adenosine 5'-triphosphate (ATP)-binding cassette leading to >20-fold higher plasma levels of plant sterols, no significant differences in aortic lesion area compared with controls were found (Wilund et al 2004). Furthermore, in a study consisting of 2542 men and women to determine the relationship between plasma plant sterols and atherosclerosis, no association was found (Wilund et al 2004). The role of dietary plant sterols in the development of atherosclerotic plaque is unclear and further research is warranted to determine its safety in subgroups of the population.

The daily supply of plant sterols in a typical 'Western diet' amounts to an average of 150–400 mg per person (SCF 2002). Human and animal studies have shown that plant sterol and stanol esters are non-toxic (Ayesh et al 1999; Baker et al 1999; Hepburn et al 1999; Waalkens-Berendsen et al 1999; Westrate et al 1999). Since 1997, when the first plant sterol containing margarine was introduced to the European market, many more applications have been lodged to add plant sterols to dairy products, cheeses, bakery products, sausages, plant oils, and other products (Kuhlmann et al 2005). Concerns have been expressed regarding the potential excessive consumption of plant sterols as it cannot be excluded that high intakes might induce undesirable effects. A study in Germany, based on a dietary simulation method, demonstrated that if a range of products are available at an enrichment amount delivering 2 g of plant sterols per serve, it resulted in an intake maximum of 13 g/day (Kuhlmann et al 2005). A model designed to stimulate such a scenario in the Finnish populations also revealed that an intake of 4–9 g could be achieved when alternative plant sterol containing products to margarine were added to the food supply (Raulio et al 2001). However, a cohort of 29 772 plant stanol ester margarine users based on a compilation of 15 surveys conducted by the National Public Health Institute in Finland between 1996–2000 concluded that sterol-enriched margarine was used by persons for whom they were designed and in the way it was intended (Simojoki et al 2005).

Plant sterols in combination with medications and other functional foods

There is growing support for plant sterol use as an adjuvant to current pharmaceutical interventions with the

aim of preventing CVD. Statins are most effective in ameliorating intrinsic high cholesterol synthesis (Thompson et al 2002), whereas plant sterols are most effective in individuals with high intrinsic cholesterol absorption from the gastrointestinal tract (Jandacek et al 1977). Statins differ in their lipid-lowering potency, with cholesterol reduction ranging from 32% and 41% for TC and LDL-C respectively on a low dose statin (10 mg/day of Rosuvastatin) to 42% and 55% respectively for a high dose statin (40 mg/day Rosuvastatin) (Jones et al 2003). There have been five randomized, controlled trials combining the use of plant sterols and statin medications (Vanhanen 1994; Gylling et al 1997; Miettinen et al 2000; Neil et al 2001; Simons 2002) which show that in combination plant sterols have an additive effect of $4.5\% \pm 2.4\%$ per g sterols. This translates to an additional risk reduction of cardiovascular events by ~9%–14% using risk modelling equations (Keys et al 1957) and there have been no reported nutrient–drug interactions. In a meta-analysis the predicted CVD risk reduction from including plant sterol in the diet was up to 20% in the longer term (Katan et al 2003). With the escalating cost associated with statin medications, plant sterol-enriched products provide a clinical and financially viable strategy. In what is a world first, a Dutch health insurer (VGZ) has begun reimbursing members (€40 worth of products each year, or about US\$52 annually) for buying heart-healthy foods such as margarine, yogurt, and milk enriched with cholesterol-lowering plant sterols (Harding 2004).

More recently, it has been suggested that newer dietary approaches combining a number of cholesterol-lowering foods may offer another option to routine medication prescription. There is now enough evidence for the US Food and Drug Administration (FDA) to permit health claims for coronary heart disease risk reduction and dietary plant sterols, oat β -glucan, psyllium, soy protein, and nuts (Jenkins et al 2005). Earlier last year, an important study was published that investigated the effectiveness of a combining all of these foods in a portfolio approach to dietary intervention. After 1 month's intervention, participants taking lovastatin (20 mg/day) had a mean (SE) decrease in LDL-C of 30.9% (3.6%) ($p < 0.001$) compared with 28.6% (3.2%) ($p < 0.001$) for the portfolio diet group, with no significant difference between the two groups (Jenkins et al 2005). This has practical significance as this is the first report showing that a 'whole of diet' approach incorporating plant sterols increases the effectiveness of the diet as a treatment for

hypercholesterolemia and is as effective as a low dose statin therapy.

Conclusions

The inclusion of plant sterols in a variety of foods provides consumers and clinicians with alternatives in the management of hypercholesterolemia. Used alone in the diet, or as an adjuvant to drug therapy, or in combination with other functional food components, plant sterol enriched products are effective at reducing serum total and LDL-C. There are no risks associated with their use and widespread acceptance has the potential to reduce the costs associated with an over-dependence on pharmacological approaches to cholesterol management.

References

- Anderson AS, Cox DN, McKellar S, et al. 1998. Take Five, a nutrition education intervention to increase fruit and vegetable intakes: impact on attitudes towards dietary change. *Brit J Nutr*, 80:133-40.
- Anonymous. 1994. Randomised trial of cholesterol-lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*, 344:1383-9.
- [AIHW] Australian Institute of Health and Welfare. 2004. National Cardiovascular Disease Database. Canberra. 2005.
- Ayesh R, Westrate JA, Drewitt PN, et al. 1999. Safety evaluation of phytosterol esters. Part 5. Faecal short-chain fatty acid and microflora content, faecal bacteria enzyme activity and serum female sex hormones in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester-enriched margarine. *Food Chem Toxicol*, 37:1127-38.
- Baker VA, Hepburn PA, Kennedy SJ, et al. 1999. Safety evaluation of phytosterol esters. Part 1. Assessment of oestrogenicity using a combination of in vivo and in vitro assays. *Food Chem Toxicol*, 37:13-22.
- Buja LM. 1996. Does atherosclerosis have an infectious etiology? *Circulation*, 94:872-3.
- Clifton PM, Noakes M, Ross D, et al. 2004. High dietary intake of phytosterol esters decreases carotenoids and increases plasma plant sterol levels with no additional cholesterol-lowering. *J Lipid Res*, 45:1493-9.
- Clifton PM, Noakes M, Sullivan D, et al. 2004. Cholesterol-lowering effects of plant sterol esters differ in milk, yoghurt, bread and cereal. *Eur J Clin Nutr*, 58:503-9.
- Gylling H, Miettinen TA. 1999. Cholesterol reduction by different plant stanol mixtures and with variable fat intake. *Metabolism*, 48:575-80.
- Gylling H, Radhakrishnan R, Miettinen TA. 1997. Reduction of serum cholesterol in post menopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine. *Circulation*, 98:4226-31.
- Hallikainen M, Sarkkinen ES, Uusitupa, MI. 1999. Effects of low-fat stanol ester enriched margarines on concentrations of serum carotenoids in subjects with elevated serum cholesterol concentrations. *Eur J Clin Nutr*, 53:966-9.
- Hallikainen MA, Sarkkinen ES, Gylling H, et al. 2000. Comparison of the effects of plant sterol ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolaemic subjects on a low fat diet. *Eur J Clin Nutr*, 54:715-25.
- Harding A. 2004. An insurance break for margarine. MSNBC, New York, NY, USA.
- Haskell WL. 2003. Cardiovascular disease prevention and lifestyle interventions: effectiveness and efficacy. *J Cardiovasc Nurs*, 18:245-55.
- Hendriks H, Westrate JA, van Vliet T, et al. 1999. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol-lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. *Eur J Clin Nutr*, 53:319-27.
- Hepburn PA, Horner SA, Smith M. 1999. Safety evaluation of phytosterol esters. Part 2. Sunchronic 90-day oral toxicity study on phytosterol esters – a novel functional food. *Food Chem Toxicol*, 37:521-32.
- [IAS] International Atherosclerosis Society. 2003. Harmonized clinical guidelines on the prevention of atherosclerotic vascular disease. International Atherosclerosis Society, Hamburg, Germany, p 1-28.
- Jandacek RJ, Webb MR, Mattson FH. 1977. Effect of an aqueous phase on the solubility of cholesterol in an oil phase. *J Lipid Res*, 18:203-10.
- Jenkins DJ, Kendall CW, Marchie A, et al. 2005. Direct comparison of a dietary portfolio of cholesterol-lowering foods with a statin in hypercholesterolemic participants. *Am J Clin Nutr*, 81:380-7.
- Jenkins DJA, Kendall CWC, Marchie A, et al. 2003. Effects of a dietary portfolio of cholesterol-lowering foods vs lovastatin on serum lipids and c-reactive protein. *JAMA*, 290:502-9.
- Jones PH, Davidson MH, Stein EA, et al. 2003. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR Trial). *Am J Cardiol*, 92:152-60.
- Jones PJ, Raeini-Sarjaz M, Ntanios F, et al. 2000. modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. *J Lipid Res*, 41:697-705.
- Katan MB, Grundy SM, Jones P, et al. 2003. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clin Proc*, 78:965-78.
- Keys A, Anderson JT, Grande F. 1957. Prediction of serum cholesterol responses of man to changes in fat in the diet. *Lancet*, ii:959-66.
- Kochhar S. 1983. Influence of processing on sterols of edible vegetable oils. *Prog Lipid Res*, 22:161-88.
- Kuhlmann K, Lindtner O, Bauch A, et al. 2005. Simulation of prospective phytosterol intake in Germany by novel functional foods. *Brit J Nutr*, 93:377-85.
- Kwiterovich PJ. 1997. The effect of dietary fat, antioxidants, and pro-oxidants on blood lipids, lipoproteins, and atherosclerosis. *J Am Diet Assoc*, 97:S31-41.
- Mensink RP, Ebbing S, Lindhout M, et al. 2002. Effects of plant stanol esters supplied in low-fat yoghurt on serum lipids and lipoproteins, non-cholesterol sterols and fat soluble antioxidant concentrations. *Atherosclerosis*, 160:205-13.
- Miettinen TA, Railo M, Lepantalo M, et al. 2005. Plant sterols in serum and in atherosclerotic plaques of patients undergoing carotid endarterectomy. *J Am Coll Cardiol*, 45:1794-801.
- Miettinen TA, Strandberg TE, Gylling H. 2000. Noncholesterol sterols and cholesterol-lowering by long-term simvastatin treatment in coronary patients: Relation to basal serum cholesterol. *Arterioscler Thromb Vasc Biol*, 20:1340-6.
- [NCEP] Expert panel on detection evaluation and treatment of high blood cholesterol in adults. 2001. Executive summary of the third report of the National Cholesterol Education Program (NCEP) (Adult Treatment Panel III). *JAMA*, 285:2486-97.
- Neil VA, Meijer GW, Roe L. 2001. Randomised controlled trial of use by hypercholesterolaemic patients of a vegetable oil sterol-enriched fat spread. *Atherosclerosis*, 156:329-37.
- [NHF] National Heart Foundation. 2001. Lipid management guidelines – 2001. *Med J Aust*, 175:S1-36.
- Noakes M, Clifton P, Ntanios F, et al. 2002. An increase in dietary carotenoids when consuming plant sterols or stanols is effective in maintaining plasma carotenoid concentrations. *Am J Clin Nutr*, 75:79-86.

- Normén L, Holmes D, Frohlich J. 2005. Plant sterols and their role in combined use with statins for lipid lowering. *Curr Opin Investig Drugs*, 6:307-16.
- Patch CS, Tapsell LC, Williams PG. 2005. Plant sterol/stanol prescription is an effective treatment strategy for managing hypercholesterolemia in outpatient clinical practice. *J Am Diet Assoc*, 105:46-53.
- Povey R, Wellens B, Conner M. 2001. Attitudes towards meat, vegetarian and vegan diets: an examination of the role of ambivalence. *Appetite*, 37:15-26.
- Ramsay LE, Yeo WW, Jackson PR. 1991. Dietary reduction of serum cholesterol concentration: time to think again. *BMJ*, 303:953-7.
- Raulio S, Nurtilla A, Mannonen L. 2001. Adding phytosterols and -stanols to food – modelling the amount received by Finnish adults. *Pub Nat Food Agency*, 10:3-22.
- Ross R. 1986. The pathogenesis of atherosclerosis. *N Eng J Med*, 314:488-94.
- Sacks FM, Pfeffer MA, Moye LA, et al. 1996. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N Eng J Med*, 335:1001-9.
- Schaefer EJ. 2002. Lipoproteins, nutrition, and heart disease. *Am J Clin Nutr*, 75:191-212.
- [SCF] Scientific Committee on Food. 2002. General view of the scientific committee on food on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources, with particular attention to the effects of β -carotene. Brussels, Belgium: Commission E.
- Simojoki M, Luoto R, Uutela A, et al. 2004. Consistency of use of plant stanol ester margarine in Finland. *Pub Health Nutr*, 7:63-8.
- Simojoki M, Luoto R, Uutela A, et al. 2005. Use of plant stanol ester margarine among persons with and without cardiovascular disease: Early phases of the adoption of a functional food in Finland. *Nutr J*, 4:1475-2891.
- Simons LA. 2002. Additive effect of plant sterol-ester margarine and cerivastatin in lowering low-density lipoprotein cholesterol in primary hypercholesterolemia. *Am J Cardiol*, 90:737-40.
- Steinberg D, Parthasarathy S, Carew TE, et al. 1989. Beyond cholesterol – modifications of low-density lipoprotein that increase its atherogenicity. *N Eng J Med*, 320:915-20.
- Tang JL, Armitage JM, Lancaster T, et al. 1998. Systematic review of dietary intervention trials to lower blood total cholesterol in free living subjects. *BMJ*, 316:1213-20.
- Thompson GR, O'Neill F, Seed M. 2002. Why some patients respond poorly to statins and how this might be remedied. *Eur Heart J*, 23:200-6.
- Thomsen AB, Hansen HB, Christiansen C, et al. 2004. Effect of free plant sterols in low-fat milk on serum lipid profile in hypercholesterolemic subjects. *Eur J Clin Nutr*, 58:860-70.
- Ulbricht TLV, Southgate DAT. 1991. Coronary heart disease: seven dietary factors. *Lancet*, 338:985-8.
- Vanhanen H. 1994. Cholesterol malabsorption caused by sitosterol ester feeding and meomycin in pravastatin-treated hypercholesterolaemic patients. *Eur J Pharmacol*, 47:169-76.
- Volpe R, Niittynen L, Korpela R, et al. 2001. Effects of yoghurt enriched with plant sterols on serum lipids in patients with moderate hypercholesterolaemia. *Brit J Nutr*, 86:233-9.
- von Baeyer H, Hopfenmuller W, Riedel E, et al. 2003. Atherosclerosis: current concepts of pathophysiology and pharmacological intervention based on trial outcomes. *Clin Nephrol*, 60:S31-48.
- Waalkens-Berendsen DH, Wolterbeek AP, Wijnands MV, et al. 1999. Safety evaluation of phytosterol esters. Part 3. Two-generation reproduction study in rats with phytosterol esters - a novel functional food. *Food Chem Toxicol*, 37:683-96.
- Westrate JA, Ayes R, Bauer-Plank C, et al. 1999. Safety evaluation of phytosterol esters. Part 4. Faecal concentrations of bile acids and neutral sterols in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester-enriched margarine. *Food Chem Toxicol*, 37:1063-71.
- Wilund KR, Yu L, Xu F, et al. 2004. No association between plasma levels of plant sterols and atherosclerosis in mice and men. *Arterioscler Thromb Vasc Biol*, 24:2326-32.
- [WHO] World Health Organization. 2005. Global strategy on diet, physical activity and health [online]. Accessed 1 November 2005. URL: <http://www.who.int/dietphysicalactivity/publications/facts/cvd/en/>.
- Zeman FJ. 1991. Clinical nutrition and dietetics. New York, USA: Macmillan.