POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
http://journal.pan.olsztyn.pl
e-mail: pjfns@pan.olsztyn.pl
Pol. J. Food Nutr. Sci.
2009, Vol. 59, No. 2, pp. 105-112

BIOLOGICAL ACTIVITIES OF PHYTOSTEROLS WITH PARTICULAR ATTENTION TO THEIR EFFECTS ON LIPID METABOLISM

Elżbieta Bartnikowska

Department of Dietetics, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences, Warsaw, Poland

Key words: plant sterols (phytosterols), hypercholesterolemia, atherosclerosis, phytosterolemia (sitosterolemia)

The hypocholesterolemic effect of plant sterols (phytosterols) that relies on lowering the intestinal absorption of both dietary and endogenous cholesterol has been known since the middle of the twentieth century. Due to this fact, many food products, mostly margarines, have been enriched with phytosterols and used in management of moderate hypercholesterolemia. Phytosterol-enriched margarines enable effective therapy of moderate hypercholesterolemia with dosage of around 3 g phytosterols per day. A higher dose is not recommended due to the possibility of interference of phytosterols with the absorption of fat-soluble vitamins.

The characteristic feature of phytosterolemia (sitosterolemia) is enhanced absorption of phytosterols which in consequence leads to premature atherosclerosis.

The objective of this review is to discuss the results of available studies concerning metabolic effects of phytosterols with a closer look at their impact on lipid metabolism in humans.

INTRODUCTION

One of the common features of sterols is cyclopentanophenanthrene ring system with a side chain at carbon C17. Higher sterols are universally present in large amounts in eukaryotic plasma membranes. Cholesterol(cholest-5α-en-3β-ol) is an integral compound of animal and human cell membranes.

Sterols that can be found in plants are called phytosterols (*phyton* – plant). Plant cell membranes are usually built of β-sitosterol (24α-ethylocholesterol), campesterol (24α-methylocholesterol) and stigmasterol (Δ^{22}-24α--ethylocholesterol) and their unsaturated equivalents – stanols.

Phytosterols, both saturated and unsaturated ones, are usually found in a form of either free sterols, glycolipids or esterified sterols. The latter form is mostly esterified with fatty acids and less commonly with sulfuric acid or with coumaric acid [Moreau *et al.*, 2002]. Phytosterol molecules are composed of 28 or 29 carbon atoms. The unsaturated phytosterols have a double bound between the carbons C5 and C6 (Δ^5-sterols) or C7 and C8 (Δ^7-sterols) [Lagarda *et al*., 2006; Ostlund, 2002].

FOOD SOURCES OF PHYTOSTEROLS

Phytosterols found in plant foods are mostly unsaturated [Patel & Thompson, 2006]. Esterification makes phytosterols more fat-soluble. Therefore, in vegetable oils there is a noticeable preference to esterified forms of phytosterols, *e.g.* in soy bean oil the most common forms of esterified phytosterols are β-sitosterol and campesterol much less frequently Δ^5-avenasterol. In the process of phytosterols esterification more unsaturated fatty acids and less saturated fatty acids are used.

The concentration of phytosterols in food depends on the type of plant as well as the kind of product, for example, in corn seeds the concentration of phytosterols is usually at the level of around 70 mg/100 g, and in corn oil – around 950 mg/100 g [Ostlund, 2002]. The unrefined plant oils are considered to be the best source of phytosterols.

Considerably high amount of phytosterols can be found in wheat germ, nuts, pulses and grain products, but is worth underlying that their content may vary depending on the variety, for example *Triticum aestinum* wheat seeds contain twice as much phytosterols than *Triticum pelta* wheat seeds [Ruibal--Mendieta *et al*., 2004]. Pulses usually contain 124-135 mg phytosterols/100 g; whereas 108-207 mg phytosterols can be found in 100 g of nuts. A lower content content of phytosterols has been reported in vegetables (10-25 mg/100 g) as well as in fruits (8-24 mg/100 g) [Anon – Phytosterol content in food, 2007; Higdon, 2005].

The intake of phytosterols depends on the amount of plant products consumed. Therefore, diets of our ancestors were rich in phytosterols, likely providing around 1000 mg of phytosterols per day [Jenkins *et al*., 2003]. At present, the consumption of phytosterols has been estimated to range from 150 to 450 mg per day, and in the Western diet to be at the level of 160-360 mg/day. The consumption of phytosterols from

Author's address for correspondence: Prof. Elżbieta Bartnikowska, Department of Dietetics, Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences, ul. Nowoursynowska 159c, 02-787 Warsaw, Poland; e-mail: elzbieta_bartnikowska@sggw.pl

Western diet is two times lower in comparison with the vegetarian diet [Higdon, 2005; Michajlik & Bartnikowska, 1999; Nair *et al*., 1984; Ostlund, 2002].

ABSORPTION AND METABOLISM OF CHOLESTEROL AND PHYTOSTEROLS

Intestinal absorption of cholesterol can be divided into three stages: (i) transition from lipid phase to micellar phase, (ii) transfer from micellar phase to intestinal mucosal cells, and (iii) esterification and incorporation of cholesterol into chylomicrons and VLDLs synthesized in intestinal wall.

Intestinal absorption of dietary cholesterol from the Western diet usually reaches the level of 50-60%. This process begins in the duodenum and continues in the upper part of jejunum. Cholesterol is actively synthesized in villi. The villi cells are also a source of endogenous cholesterol in intestinal lumen because those cells are submitted to desquamative process [Michajlik & Bartnikowska, 1999].

The precise molecular mechanisms of sterols absorption are not well defined but both cholesterol and phytosterol absorption requires the Niemann-Pick C1 Like 1 Protein (NPC1L1). The essential role of NPC1L1 has been proven by the reduction of cholesterol absorption in transgenic mice deficient in this protein. Moreover NPC1L1 deficient transgenic mice have practically an undetectable level of phytosterols in blood serum [Altmann *et al*., 2004].

Within the enterocyte most of the cholesterol is esterified with long–chain fatty acids by microsomal cholesterol acyltransferase (ACAT) then packaged with apo B48, triacylogliceroles, phospholipids, fat-soluble vitamins into chylomicrons and intestinal VLDLs. The latter are probably more important in the transport of cholesterol through the intestinal wall. Absorbed cholesterol from enterocytes is secreted into the lymphatic system and then into the circulatory system.

The pathway of the absorption of phytosterols is very similar. The only difference is in the amount absorbed. As previously mentioned, humans absorb around 50-60% of dietary cholesterol, whereas the absorption of plant sterols is less than 5% [Michajlik & Bartnikowska, 1999; Patel & Thompson, 2006]. Unesterified phytosterols form crystals that are difficult to incorporate into micelles formed in the intestine. However, most of the unesterified cholesterol and phytosterols that are able to enter enterocytes are transported back into intestinal lumen by specific pump called the "sterolin pump" containing proteins from ABC group, like ATP binding cassette proteins: ABCG5 and ABCG8 [Yu *et al*., 2002; Sudhop *et al*., 2005]. Phytosterols are secreted back into intestine by ABCG5 and ABCG8 proteins at a much greater rate than cholesterol. Phytosterols which are incorporated into chylomicrons and intestinal VLDLs from circulation are taken up by the liver. Inside the liver, phytosterols are secreted into bile by hepatic ABCG5 and ABCG8 transporters. It is worth emphasizing that phytosterols are secreted into bile to greater extent than cholesterol [Sudhop *et al*., 2002a].

Unabsorbed or transported back to intestinal lumen cholesterol and phytosterols are metabolized in colon by the intestinal microflora enzymes to coprosterol or coprostanol and excreted with faeces [Michajlik & Bartnikowska, 1999].

The absorption of phytosterols depends on the species; they are practically unabsorbed in rabbits, rats absorb less than 4% whereas, humans absorb around 6% of the dosage provided *via* the alimentary tract [Kritchevsky & Chen, 2005].

The level of absorption is also dependent on the chemical structure of phytosterols, more specifically on the existence of double bounds in the cyclopentanophenenthrene ring and the nature of additional C24 side chain. The absorption from intestine of unsaturated phytosterols is lower than of the saturated ones, *e.g.* it has been calculated that the absorption of β-sitosterol is around 5% of consumed amount whereas absorption of β-sitostanol is 0.1% [Kozłowska-Wojciechowska, 2002; Nguyen, 1999]. According to Ostlund *et al*. [2002], the reduction of the double bound at C5 decreased absorption by 90%. The more complex side chain at C24 diminishes the absorption [Heiemann *et al*., 1993]. Therefore, the efficiency of phytosterol absorption depends on the structure of both sterol nucleus and side chain.

Phytosterols inhibit the absorption of dietary cholesterol. Due to structural similarity, plant sterols take the place of both dietary and endogenous cholesterol in micelles produced in intestinal lumen. Therefore, less cholesterol is delivered to enterocytes and is incorporated into chylomicrons and VLDLs synthesized in the intestine. Furthermore, a high intake of phytosterols competitively blocks the absorption of cholesterol, and at the same time phytosterols are not absorbed [Michajlik & Bartnikowska, 1999].

INFLUENCE OF PHYTOSTEROLS ON LIPID METABOLISM IN EXPERIMENTAL ANIMALS AND HUMANS AS ESTIMATED IN THE MIDDLE OF THE XXTH CENTURY

According to Pollak [1953a], on the turn of the XIXth and XXth century it was stated that phytosterols are not absorbed from the animal alimentary tract. First studies on the influence of phytosterols on total cholesterol level in blood were conducted in the middle of the XXth century. Their results clearly showed that phytosterols decrease the development of hypercholesterolemia and accumulation of cholesterol in liver as well as inhibit the development of atherosclerosis evoked *via* alimentary tract in experimental animals and birds [Peterson *et al*., 1953; Pollak, 1953a].

Positive results of studies on animals motivated scientists to conduct clinical trials, the main aim of which was to assess the influence of different doses of phytosterols on total cholesterol level in serum. In the research carried out half a century ago large doses (5, 7 or 10 g/day) of phytosterols were used. That resulted in the decrease of blood total cholesterol after 7-14 days regardless of the dose. The continuation of the diet resulted in limited reduction of total cholesterol level. The effect of phytosterols was dependent on initial serum cholesterol level in patients and was more noticeable in the persons with hypercholesterolemia. In persons without hypercholesterolemia (total serum cholesterol level lower than 200 mg/dL) phytosterols had no significant effect on the concentration of total cholesterol in serum. After discontinuance of the administration of phytosterols the rebound effect was

not observed and the blood cholesterol level returned to pre-study level after 14 to 40 days [Pollak, 1953b].

FOOD PRODUCTS ENRICHED WITH PHYTOSTEROLS

The evidence of the positive impact of some food components on human health become the basis for the development of "functional foods" – a new generation of food products, mostly with the addition of bioactive components that display a therapeutic effect. Works in the field of functional food were resumed to use the hypocholesterolemic properties of phytosterols.

Extraction of phytosterols from vegetable oils is quite expensive. The development of the extraction of phytosterols, mostly stanols, from the pine pulp significantly decreased the costs [Hollingsworth, 2001]. Due to the fact that phytosterols, like cholesterol, are not water soluble, fat vehicles were suggested. The first vehicle considered was mayonnaise; but this product has a limited application in an everyday diet [Miettinen *et al*., 1995]. This has pushed specialists from Raisio group, Lipton and Uniliver to develop the production of margarines enriched with phytosterols [Hollingsworth, 2001]. Even though these products are more expensive than regular margarines they can be commonly used in the treatment of moderate hypercholesterolemia. The researchers noted that phytosterols dissolved in fat were characterised by better efficacy in lowering the concentration of total cholesterol in blood in comparison with crystal products containing phytosterols [Hollingsworth, 2001].

Besides margarines enriched with phytosterols on the market of developed countries, a variety of foods containing added phytosterols or phytostanols, including mayonnaises, oils, salad dressings, yogurt, milk, soy milk, orange juice, snak bars and meats, are available as well [Hollingsworth, 2001; Berger *et al*., 2004].

EFFICACY OF THE HYPOCHOLESTEROLEMIC EFFECT OF PHYTOSTEROLS

The extent of the hypocholesterolemic effect of phytosterols depends on various factors of which the most important are: (i) phytosterols origin determining their profile, (ii) the dose and manner of dosage (as one dose or divided into portions), (iii) duration of treatment, and (iv) age of the patient as well as the type and intensity of lipid metabolism disorders.

The studies examining the effect of plant sterols with the usage of margarines proved that phytosterols have a positive effect solely on cholesterol metabolism. However, it is not recommended to include the phytosterol therapy in treatment of hypertriglicerydemia.

The hypocholesterolemic effect of margarines with the addition of phytosterols depends on the amount of sterols and stanols used, the value of the proportion of free to esterified phytosterols and the composition of fatty acids pool in margarine [Jones *et al*., 2000; Weststrate & Meijer, 1998].

The efficacy of the hypocholesterolemic effect of the phytosterols differs in respect to their profile – their origins. Hallikainen & Uusituta [1999] stated that inclusion of margarines enriched with stanol esters derived from pine pulp decreased total cholesterol and LDL cholesterol in serum more efficiently than margarines enriched with stanol esters from vegetable oils. The differences in the hypocholesterolemic effect of stanols derived from pine pulp and vegetable oils are probably caused by the differences in the stanol profile. As previously mentioned, the absorption of phytosterols depends on complexity of C24 side chain. Phytosterols with a less complex side chain at carbon C24 are characterised by higher absorption and therefore exert a lesser hypocholesterolemic effect.

Miettinen & Vanhanen [1994] demonstrated that esterified β-sitostanol was more efficient than free β-sitosterol, free β-sitostanol or rapeseed-based margarine alone in lowering serum cholesterol in patients with hypercholesterolemia. More recent study indicated however that soybean sterol esters had a similar serum cholesterol-lowering effect as the β-sitostanol ester [Westsrate & Meijer, 1998].

In a cross-sectional study Andersson *et al*. [2004] indicated that dietary phytosterol intakes were inversely related to total cholesterol and LDL-cholesterol concentrations in serum even after adjusting for saturated fat and fiber intake. Law [2000] subjected the results of 18 controlled clinical trials to meta-analysis and found that the consumption of spreads providing an average of 2 grams per day of phytosterols or phytostanols lowered the concentration of LDL-cholesterol in serum by 9-14%. In a more recent meta-analysis that combines the results of 23 controlled clinical trials Chen *et al*. [2005] found that the consumption of phytosterols or phytostanols in an average dose of 3.4 grams per day decreased the concentration of LDL-cholesterol in serum by about 11%. Reduction of fat intake combined with a phytosterol therapy resulted in much greater effects. For example, Jones *et al*. [1999] demonstrated that substituting monounsaturated and polyunsaturated fats for saturated fat resulted in 9% reduction of LDL-cholesterol concentration in the serum after 30 days, but the addition of 1.7 grams per day of phytosterols or phytostanols to the same diet resulted in a 24% reduction. The results indicating the hypocholesterolemic effect of phytosterols have been reported in many reviews [Berger *et al*., 2004; Kozłowska-Wojciechowska, 2002; Patel & Thompson, 2006].

The differences in dosage of phytosterols influence their efficacy. A small phytosterol dose of 0.8 g per day or less has no significant lowering effect on both total and LDL cholesterol concentration in serum [Hendriks *et al*., 1999; Miettinen & Vanhanen, 1994; Volpe *et al*., 2001; Lichtenstein & Deckelbaum, 2001]. The therapeutic phytosterol dose of 2-3 g per day is recommended [FDA, 2000; Lichtenstein & Deckelbaum, 2001]. The dose above 3 g/day is not advised for a lack of considerably increased hypocholesterolemic effect and the threat of side effects as a result of interfering with the absorption of fat-soluble vitamins, mostly β-carotene. THE EU Scientific Committee on Foods also concluded that plant sterols and stanols are safe for human use; however, the Committee recommended that intakes of plant sterols and stanols should not exceed 3 grams per day [Scientific Committee on Food of the EU, 2003].

The results of most of the studies indicate that the hypocholesterolemic effect is greater when the dose of phytosterols is divided into 3 portions a day than whey they are administrated in a single dose. The Food and Drug Administration specialists (USA) as well as the EU experts claim that for better hypocholesterolemic effect the dose of phytosterols should be divided into 3 portions a day [FDA, 2000; Scientific Committee on Food of the EU, 2003].

Long-term studies (12 months) showed that the highest decline in cholesterol concentration in patients with moderate hypercholesterolemia occurred during the first three months of the phytosterol therapy [Miettinen *et al*., 1995]. The continuation of the trial had less apparent hypocholesterolemic effect. Also the age of the patient could be accounted for the level of the effect of phytosterols. The hypocholesterolemic effects of phytosterols are greater in elderly persons than in younger persons with moderate hypercholesterolemia [Law, 2000].

The results of the studies revealed that diet therapy allows normalizing cholesterol concentration in patients with moderate hypercholesterolemia. Patients with intensive hypercholesterolemia require appropriately planned drug therapy which depends on the type of lipid metabolism disorders. Both phytosterols and phytostanols augment the effectiveness of treatment of lipid metabolism disorders with HMG-CoA inhibitors (statins) and fibrates [Becker *et al*., 1992; Patel & Tompson, 2006; Vuorio *et al*., 2000]. Results of controlled clinical studies demonstrated that consumption of 2-3 grams per day of phytosterols or phytostanols by patients on statin theraphy resulted in an additional 7-11% reduction of LDL cholesterol concentration in serum [Blair *et al*., 2000; O'Neill *et al.*, 2004; Simons, 2002]. Therefore, it seems prudent to decrease the dose of medicines at the same time reducing the risk of occurrence of side effects caused by long-term application of drug therapy.

Phytosterols have no significant effect on the concentration of HDL cholesterol in serum. However, the plant sterol therapy increases the value of proportion of HDL/total cholesterol and HDL-cholesterol/LDL-cholesterol. Therefore, during the implementation of a diet therapy of moderate hypercholesterolemia the partial substitution of animal fats with margarines enriched in phytosterols is recommended [Gylling *et al*., 1995].

Apo E plays an essential role in the metabolism of fats, hence apo E polymorphism plays a crucial role in the development of hypercholesterolemia and atherosclerosis. Phenotype of the Epo E can be accounted for the individual variability in lipemic response to diet modifications.

Three isoforms of the apo E can be found, namely: E2 (7% of the population), E3 (78% of the population) and E4 (15% of the population). Everybody inherits one isoform of apo E from each parent. Due to predominance of E3 isoform, the phenotype E3/E3 is most common. Other combinations are associated with the existence of a rare gene contributing to changes in the postprandial fat metabolism as well as apo E affinity for receptors which in turn respond to the development of hypercholesterolemia and an increasing the risk of atherosclerosis [Eischner *et al*., 2002; Nowicka, 2004; Superko, 2007].

The results of many studies showed that the most sufficient serum cholesterol reducing effect of phytosterols was noted in patients with apo E4 isoform [Patel & Tompson, 2006]. Vanhanen *et al*. [1993] demonstrated higher efficiency of phytosterol in reducing the concentration of cholesterol in patients with apoE4 in comparison with apo E3, respectively 11.8% and 6.0%. These results proved the individual variability of the level of reduction in cholesterol concentration in response to the phytosterol therapy.

POTENTIAL THREATS ASSOCIATED WITH AN ELEVATED INTAKE OF PHYTOSTEROLS

In the US, plant sterols and stanols added to food products are recognized as safe, getting the GRAS status [FDA, 2003]. Patients who consumed a plant sterol enriched spread providing 1.8-2.6 grams per day for up to one year did not report any adverse effects [Miettinen *et al*., 1995]. However, other authors described that occasionally nausea, indigestion, diarrhea and constipation have been reported [Berges *et al*., 1995; Klippel *et al*., 1997].

Even though there is a shortage in firm evidence for the existence of negative side effects of phytosterols consumption, they are not recommended for children under 5 years of age with the normal concentration of serum cholesterol. This recommendation is based on the assumption that phytosterols may disrupt absorption of fat-soluble vitamins. Furthermore, the cholesterol requirements for rapidly growing children are great and according to *Cholesterol Imprinting Hypothesis* insufficient intake of cholesterol in infancy and early childhood may increase the risk of lipid metabolism disorders and cardiometabolic diseases in the later stages of life [Berger *et al*., 2004; Fall *et al*., 1992].

The studies on children with cow's milk, eggs and fish allergy remaining on elimination diet, low in cholesterol and high in vegetable fats, showed that increased consumption of products rich in phytosterols had no connection with lipid metabolism disorders [Joki *et al.*, 2003].

It is believed that the results of phytosterol consumption have the same effect on both women and men however, high doses may cause hormonal disorders in females. The studies conducted on animals showed that phytosterols were responsible for the growth of testicle mass and the volume of semen in male rats and for an increase in the mass of uterus in female rats [Patel & Tompson, 2006]. If or in what dosage the phytosterols may cause similar effects in human organism is still unknown.

The negative effects of phytosterols on vegetarian females that consume more than 500 mg of phytosterols per day are unknown. Even though there is a visible lack of results of adequately planned studies, pregnant and lactating woman should refrain from diet supplementation with phytosterols [Berger *et al*., 2004].

Potentially, the most dangerous aspect of increased consumption of phytosterols is connected with their negative effect on the absorption of fat-soluble vitamins. There is still not enough studies considering the effects of phytosterols consumption on the concentration of tocopherols, carotenoids and retinoids in serum. Hallikainen *et al*. [2000] concluded

that a dose of 0.8 grams per day to 3.2 grams per day of phytosterol significantly decreased the concentration of both α and γ-tocopherol in serum. The concentration of alpha tocopherol in serum declined proportionally to the dose of phytosterol used. It was also noted that only 2.4 and 3.2 grams per day of phytosterols significantly decreased the concentration of γ-tocopherol. Law [2000] claims that phytosterols consumption results in 25% decline in β-carotene concentration. According to Hicks & Moreau [2001], however, a dose of 1.6 grams of phytosterols/day has no significant effect on both vitamin E nor carotenoids concentration in plasma.

Margarines enriched with phytosterols are also fortified with fat-soluble vitamins to decrease the possibility of inadequate utilization of these vitamins. However, according to the opinion of the EU Scientific Committee for Nutrition it is prudent to avoid the consumptions of sterols above the dose of 3.0 grams per day [UE Legislation, 2000]. According to the decision of the Commission 200/500/UE (established on the 24th of July 2000) it is advisable to label a food product with sterol content.

PHYTOSTEROLEMIA (SITOSTEROLEMIA)

Phytosterolemia is a rare genetic disorder inherited as an autosomal recessive feature. It is induced by defects in one of the ABC protein coding genes, either ABCG5 or ABCG8. These proteins are a part of a transporter that is responsible for the preferential excretion of free cholesterol and phytosterols from enterocytes back to intestine's lumen and from hepatocytes to the biliary tract. Mutation of ABCG8 or ABCG5 genes is responsible for disturbed secretion of sterols into the intestinal tract and therefore decreases sterol excretion [Patel & Thompson, 2006; Yu *et al*., 2002].

In phytosterolemia the concentration of sterol is elevated dozen times in comparison with sterol concentration in persons without this disorder. In healthy individuals, net absorption of phytosterols is usually below 5-6% of the dose consumed. Patients with phytosterolemia absorb dietary plant sterols in 16-63% [Patel & Thompson, 2006]. In consequence, phytosterols concentration in serum is about 0.3-1.0 mg/100 mL in healthy individuals and about 10-65 mg/100 mL – in patients with phytosterolemia [Steiner, 2005].

Phytosterolemia was first described in 1974 by Bhattacharyya & Connor [1974]. The main symptoms of this disorder are elevated serum phytosterol concentration and tendon xantoma – the disposition of cholesterol in skin, mostly in tendons, knees, elbows, buttocks, hands, and eyelids, occurring predominantly in childhood and adolescents. As a result of untreated phytosterolemia atheroscelrosis and coronary heart disease may occur [Steiner, 2005; Sudhop *et al*., 2002b]. The phytosterolemia may be manifested with lower than expected decline of serum total cholesterol in response to HMG CoA reductase inhibitor treatment. In such cases, it seems necessary to evaluate serum sterol profile using chromatographic techniques.

Phytosterolemia occurs in 1 per 1 million people [Patel & Thompson, 2006]. Many phytosterolemia cases are undiagnosed or diagnosed as hyperlipidemia because routine tests assessing the concentration of serum cholesterol using enzymatic and colorimetric methods do not distinguish phytosterols and cholesterol. In most of the cases it not important as phytosterols account for less than 1% of total serum sterols. In order to properly differentiate the concentration of each sterol either gas liquid chromatography (GLC) or high performance liquid chromatography (HPLC) should be applied.

The treatment of phytosterolemia consists in restrictions of phytosterols food sources in the diet (mostly vegetable fats, nuts, grain products, chocolate), as well as pharmaceutical treatment with bile sequestrant resins and sterol absorption inhibitors and/or chirurgic treatment – ileal bypass surgery. The phytosterolemia-coexisting diseases, such as anaemia, should be treated as well [Patel & Thompson, 2006; Steiner, 2005].

OTHER METABOLIC ACTIVITIES OF PLANT STEROLS

Experiments in animals and cell cultures indicated that plant sterols may exert biological activities unrelated to the hypocholesterolemic effect. However, their significance in humans is not yet explained. As mentioned earlier, cholesterol is a structural component of animal and human cell membranes. In experiments *in vitro* the substitution of cholesterol with phytosterols altered the physical properties of cell membranes, which could affect the activity of membrane-bound enzymes or signal transduction [Halling & Slotte, 2004; Awad *et al*., 1996]. These activities of plant sterols are not, however, defined in humans.

In contrast to normal cells, cancerous cells lose their ability to respond to a death signal by undergoing apoptosis. In experiments *in vitro* the addition of sitosterol resulted in inducing apoptosis of the cultured cancer cells of prostate [von Holtz *et al*., 1998], breast [Awad *et al*., 2003] and colon [Choi *et al*., 2003]. Results of some studies, particularly from Uruguay, demonstrated that the intake of phytosterols was inversely associated with esophageal [De Stefani *et al*., 2000a], stomach [De Stefani *et al*., 2000b] and breast [Ronco *et al*., 1999] cancers. On the other hand, in a prospective epidemiological study, a high intake of plant sterols was not associated with a reduced risk of colon and rectal cancers [Normén *et al*., 2001]. Strom *et al*. [1999] similarily reported that total phytosterol consumption was not associated with prostate cancer risk, however, they found that men with prostate cancer had higher dietary campesterol intake than the healthy individuals.

The anticancer activity of plant sterols has been reviewed by Awad & Fink [2000]. Limited data from cell culture [Awad *et al*., 2004] and animal [Navarro *et al*., 2001] studies indicate that plant sterols may attennuate the inflammatory activity of immune cells. Bouic [2001] has reviewied the potential role of phytosterols both in the etiology as well as in the prevention of immunological diseases. The ati-inflammatory activity of phytosterols most likely relies on the inhibition of secretion of inflammatory mediators, such as Il-6 and TNFα [Bouic, 2001].

Plant sterols may also possess antioxidative properties [Wang *et al*., 2002]. The observation that *in vitro* phytosterols decrease lipid peroxidation of platelet membranes in the presence of iron [van Rensburg *et al*., 2000] may confirm this assumption.

Plants usually contain traceable amounts of oxidized derivatives of phytosterols, *e.g.* rape seeds contain approximately 10-15 mcg/g of oxyphytosterols. These compounds are formed during enzymatic reactions proceeding in the ripening process. The most dominant oxidative derivatives are 7 hydroxy- and epoxy-phytosterols [Rudzińska *et al*., 2005].

The oxidation of phytosterols in food, likewise that of cholesterol, is enhanced by food processing with an air access and temperature usage. The concentration of oxyphytosterols in food products increases also with the time of storage [Rudzińska *et al*., 2005].

Oxyphytosterols can be absorbed from the alimentary tract of animals [Tomoyori *et al*., 2004]. Homma *et al*. [2003] suggested that stanol esters in healthy men might protect particles of low density lipoproteins against oxidation. On the other hand, oxyphytosterols are detectable in atheromatous plaque, which may indicate their involvement in the development of atherosclerosis [Patel & Tompson, 2006]. Oxyphytosterols similarly as oxycholesterols may affect many metabolic processes. Unfortunately, the number of studies conducted so far is not sufficient to determine the exact metabolic effects of oxyphytosterols.

CONCLUDING REMARKS

Studies concerning the lipid metabolic disorders, especially their response to dietary changes are very intensive. Their main purpose is to explain the impact of food components and their interactions in both progression and inhibition of atherogenesis and other metabolic disorders. The bioactive substances found in plant food inlcude phytosterols. These compounds may affect many metabolic processes. Until now, their hypocholesterolemic properties have been firmly confirmed and they are recommended for the use in the treatment of moderate hypercholesterolemia. Hopefully, in the near future those studies will help in better understanding of the metabolic role of phytosterols, which in turn will enable better composition preventive diets.

REFERENCES

1. Altmann S.W., Davis Jr H.R., Zhu L.J., Yao X., Hoos L.M., Tetzloff G., Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science, 2004, 303, 1201–1204.
2. Andersson S.W., Skinner J., Ellegard L., Welch A.A., Bingham S., Mullingan A., Anderson H., Khaw K.T., Intake of dietary plant sterols is inversely related to serum cholesterol concentration in men and women in the EPIC Norfolk population: a cross-sectional study. Eur. J. Clin. Nutr., 2004, 58, 1378–1385.
3. Anon. Phytosterol content of food, [http://www.dietaryfiberfood.com/cholesterol-low.php] (accessed on the 5th January 2007).
4. Awad A.B, Fink C.S,. Phytosterols as anticancer dietary components: evidence and mechanism of action. J. Nutr., 2000, 130, 2127–2130.
5. Awad A.B, Roy R., Fink C.S., Beta-sitosterol, a plant sterol, induces apoptosis and activates key caspases in MDA-MB-231 human breast cancer cells. Oncol. Rep., 2003, 10, 497–500.
6. Awad A.B., Chen Y.C., Fink C.S., Hennessey T., Beta-sitosterol inhibits HT-29 human colon cancer cell growth and alters membrane lipids. Anticancer Res., 1996, 16 (5A), 2797–2804.
7. Awad A.B., Toczek J., Fink C.S., Phytosterols decrease prostaglandin release in cultured P388D1/MAB macrophages. Prostaglandins Leukot Essent Fatty Acids., 2004, 70, 511–520.
8. Becker M., Staab D., von Bergmann K., Long-term treatment of severe familial hypercholesterolemia in children: effect of sitosterol and Bezafibrat. Pediatrics, 1992, 89, 138–142.
9. Berger A., Jones P.J., Abumweis S.S., Plant sterols: factors affecting their efficacy and safety as functional food ingredients. Lipids Health Dis., 2004, 3, 5–7.
10. Berges R.R., Windeler J., Trampisch H.J., Senge T., Randomised, placebo-controlled, double-blind clinical trial of beta-sitosterol in patients with benign prostatic hyperplasia. Beta-sitosterol Study Group. Lancet, 1995, 345, 1529–1532.
11. Bhattacharyya A.K., Connor W.E., Beta-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. J. Clin. Invest., 1974, 53, 1033–1043.
12. Blair S.N., Capuzzi D.M., Gottlieb S.O., Nguyen T., Morgan J.M., Cater N.B., Incremental reduction of serum total cholesterol and low-density lipoprotein cholesterol with the addition of plant stanol ester-containing spread to statin therapy. Am. J. Cardiol., 2000, 86, 46–52.
13. Bouic P.J., The role of phytosterols and phytosterolins in immune modulation: a review of the past 10 years. Curr. Opin. Clin. Nutr. Metab. Care, 2001, 4, 471–475.
14. Chen J.T., Wesley R., Shamburek R.D., Pucino F., Csako G., Meta-analysis of natural therapies for hyperlipidemia: plant sterols and stanols versus policosanol. Pharmacotherapy, 2005, 25, 171–183.
15. Choi Y.H., Kong K.R., Kim Y.A., Jung K.O., Kil J.H., Rhee S.H., Park K.Y., Induction of Bax and activation of caspases during beta-sitosterol-mediated apoptosis in human colon cancer cells. Int. J. Oncol., 2003, 23, 1657–1662.
16. De Stefani E., Boffetta P., Ronco A.L., Brennan P., Deneo-Pellegrini H., Carzoglio J.C., Mendilaharsu M., Plant sterols and risk of stomach cancer: a case-control study in Uruguay. Nutr. Cancer, 2000b, 37, 140–144.
17. De Stefani E., Brennan P., Boffetta P., Ronco A.L., Mendilaharsu M., Deneo-Pellegrini H., Vegetables, fruits, related dietary antioxidants, and risk of squamous cell carcinoma of the esophagus: a case-control study in Uruguay. Nutr. Cancer, 2000a, 38, 23–29.
18. Eischner J.E., Dunn S.T., Perveen G., Thompson D.M., Stewart K.E., Stoehla B.C., Apolipoprotein E polymorphism and cardiovascular disease: A Huge review. Am. J. Epidemiol., 2002, 155, 487–495.
19. Fall C.H., Barker D.J., Osmond C., Winter P.D., Clark P.M., Hales C.N., Relation of infant feeding to adult serum cholesterol concentration and death from ischaemic heart disease. Brit. Med. J., 1992, 304, 801–805.
20. FDA, Food and Drug Administration. Food labeling: Health claims; plant sterol/stanol esters and coronary heart disease. Food and Drug Administration Fed. Reg., 2000, 65, 54685––54739.
21. FDA, Food and Drug Administration. GRAS Notice No. GRN 000112. 2003. Available at: [http://www.cfsan.fda.gov/~rdb/opa-g112.html] (accessed on the 1st of February 2007).
22. Gylling H., Siimes M.A., Miettinen T.A., Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia. J. Lipid Res., 1995, 36, 1807–1811.

23. Hallikainen M.A., Sarkkinen E.S., Uusitupa M.I.J., Plant stanol esters affect serum cholesterol concentrations of hypercholesterolemic men and women in a dose-dependent manner. J. Nutr., 2000, 130, 767–776.
24. Hallikainen M.A., Uusitupa M.I.J., Effects of 2 low-fat stanol ester–containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolemic subjects. Am. J. Clin. Nutr., 1999, 69, 403–410.
25. Halling K.K., Slotte J.P., Membrane properties of plant sterols in phospholipid bilayers as determined by differential scanning calorimetry, resonance energy transfer and detergent-induced solubilization. Biochim. Biophys. Acta, 2004, 1664, 161–171.
26. Heinemann T., Axtmann G., von Bergmann K., Comparison of intestinal absorption of cholesterol with different plant sterols in man. Eur. J. Clin. Invest., 1993, 23, 827–831.
27. Hendriks H.F., Weststrate J.A., van Vliet T., Meijer G.W., 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., 1999, 53, 319–327.
28. Hicks K.B., Moreau R.A., Phytosterols and phytostanols: Functional food cholesterol busters. Food Technol., 2001, 55, 63–69.
29. Higdon J., Phytosterols. 2005 [lpi.oregonstate.edu/infocenter/phyto chemicals/sterols/index.html] (accessed on the 27th October 2006)..
30. Hollingsworth P., Margarine: The over-the top functional food. Food Technol., 2001, 55, 59–63.
31. Homma Y., Ikeda I., Ishikawa T., Tateno M., Sugano M., Nakamura H., A randomized, placebo-controlled trial: Decrease in plasma low-density lipoprotein cholesterol, apolipoprotein B, cholesteryl ester transfer protein, and oxidized low-density lipoprotein by plant stanol ester-containing spread. Nutrition, 2003, 19, 369–374.
32. Jenkins D.J., Kendall C.W., Marchie A., Jenkins A.L., Connelly P.W., Jones P.J.H., Vuksam V., The Garden of Eden-plant based diets, the genetic drive to conserve cholesterol and its implications for heart disease in the 21st century. Comp. Biochem. Physiol. Mol. Integr. Physiol., 2003, 136, 141–151.
33. Joki P., Suomalainen H., Jarvinen K.M., Juntunen-Backman K., Gylling H., Miettinen T.A., Antikainen M., Cholesterol precursors and plant sterols in children with food allergy. Am. J. Clin. Nutr., 2003, 77, 51–55.
34. Jones P.J, Raeini-Sarjaz M., Ntanios F.Y., Vanstone C.A., Feng J.Y., Parsons W.E., Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. J. Lipid Res., 2000, 41, 697–705.
35. Jones P.J., Ntanios F.Y., Raeini-Sarjaz M., Vanstone C.A., Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. Am. J. Clin. Nutr., 1999, 69, 1144–1150.
36. Klippel K.F., Hiltl D.M, Schipp B., A multicentric, placebo-controlled, double-blind clinical trial of beta-sitosterol (phytosterol) for the treatment of benign prostatic hyperplasia. German BPH-Phyto Study group. Br. J. Urol., 1997, 80, 427–432.
37. Kozłowska-Wojciechowska M., Phytoterols and phytostanol a new chance in prophylactics of atherosclerosis. Czynniki Ryzyka, 2002, 1, 5–12 (in Polish).
38. Kritchevsky D., Chen S.C., Phytosterols – Health benefis and potential concers: a review. Nutr. Rev., 2005, 25, 413–428.
39. Lagarda M.J., Garcia-Llatas G., Farre R., Analysis of phytosterols in food. J. Pharmaceut. Biomed., 2006, 41, 1486–1496.
40. Law M., Plant sterol and stanol margarines and health. Br. Med. J., 2000, 320, 861–864.
41. Lichtenstein A.H., Deckelbaum R.J., Stanol/sterol ester–containing foods and blood cholesterol levels. A statement for healthcare professionals from the Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. Circulation, 2001, 103, 1177–1179.
42. Michajlik A., Bartnikowska E., Lipidy i lipoproteiny osocza. 1999, 1st ed., Wydawnictwo Lekarskie PZWL, Warszawa (in Polish).
43. Miettinen T. A., Puska P., Gylling H., Vanhanen H., Vartiainen E., Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N. Engl. J. Med., 1995, 333, 1308–1312.
44. Miettinen T.A., Vanhanen H., Dietary βsitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. Atherosclerosis, 1994, 105, 217––226.
45. Moreau R.A., Whitaker B.D., Hicks K.B., Phytosterols, phytostanols and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Prog. Lipid Res., 2002, 41, 457–500.
46. Nair P.P., Turjman N., Kessie G., Diet, nutrition intake, and metabolism in populations at high and low risk for colon cancer. Dietary cholesterol, beta-sitosterol, and stigmasterol. Am. J. Clin. Nutr., 1984, 40 (4 Suppl.), 927–930.
47. Navarro A., De las Heras B., Villar A., Anti-inflammatory and immunomodulating properties of a sterol fraction from *Sideritis foetens* Clem. Biol. Pharm. Bull., 2001, 24, 470–473.
48. Nguyen T.T., The cholesterol-lowering action of plant stanol esters. J. Nutr., 1999, 129, 2109–2112.
49. Normén A.L., Brants H.A., Voorrips L.E., Andersson H.A., van den Brandt P.A., Goldbohm R.A., Plant sterol intakes and colorectal cancer risk in the Netherlands Cohort Study on Diet and Cancer. Am. J. Clin. Nutr., 2001, 74, 141–148.
50. Nowicka G., Polimorfizm genów a odpowiedź metaboliczna na składniki diety. (Gene polymorphisms and dietary response. 2004, *in:* Fizjologiczne uwarunkowania postępowania dietetycznego. Międzynarodowa Konferencja Naukowa, Listopad, 2004 Część 1. Wyd. SGGW, Warszawa, 2004, 87–96 (in Polish).
51. O'Neill F.H., Brynes A., Mandeno R., Comparison of the effects of dietary plant sterol and stanol esters on lipid metabolism. Nutr. Metab. Cardiovasc. Dis., 2004, 14, 133–142.
52. Ostlund R.E, Jr., Phytosterols in human nutrition. Annu. Rev. Nutr., 2002, 22, 533–549.
53. Patel M.D, Thompson P.D., Review. Phytosterols and vascular disease. Atherosclerosis, 2006, 186, 12–19.
54. Peterson D.W., Shneour E.A., Peek N.F., Dietary constituents affecting plasma and liver cholesterol in cholesterol-fed chicks. J. Nutr., 1953, 50, 191–201.
55. Pollak O. J., Reduction of blood cholesterol in man. Circulation, 1953b, 7, 702–706.
56. Pollak O.J., Successful prevention of experimental hypercholesterolemia and cholesterol atherosclerosis in the rabbit. Circulation, 1953a, 7, 696–701.
57. Ronco A., De Stefani E., Boffetta P., Deneo-Pellegrini H., Mendilaharsu M., Leborgne F., Vegetables, fruits, and related nutrients

and risk of breast cancer: a case-control study in Uruguay. Nutr. Cancer, 1999, 35, 111–119.
58. Rudzińska M., Uchman W., Wąsowicz E., Plant sterols in food technology. Acta Sci. Pol. Technol. Aliment., 2005, 4, 147–156.
59. Ruibal-Mendieta N.L., Rozenberg G., Delacroix D.L., Spelt (*Triticum spelta* L.) and winter wheat (*Triticum aestivum* L.) wholemeals have similar sterol profiles as determined by quantitative liquid chromatography and mass spectrometry analysis. J. Agric. Food Chem., 2004, 52, 4802–4807.
60. Scientific Committee on Food. Opinion on applications for approval of a variety of plant sterol-enriched foods. 2003. [http://europa.eu.int/comm/food/fs/sc /scf/out174_en.pdf] (accessed on the 19th May 2007).
61. Simons L.A., Additive effect of plant sterol-ester margarine and cerivastatin in lowering low-density lipoprotein cholesterol in primary hypercholesterolemia. Am. J. Cardiol., 2002, 90, 737–740.
62. Steiner R.D., Sitosterolemia. 2005, [http://www.emedicine.com/ped/topic2110.htm] (accessed on the 14th January2007).
63. Strom S.S., Yamamura Y., Duphorne C.M., Phytoestrogen intake and prostate cancer: a case-control study using a new database. Nutr. Cancer, 1999, 33, 20–25.
64. Sudhop T., Gottwald B.M., von Bergmann K., Serum plant sterols as a potential risk factor for coronary heart disease. Metabolism, 2002b, 51, 1519–1521.
65. Sudhop T., Lutjohann D., von Bergmann K., Sterol transporters: targets of natural sterols and new lipid lowering drugs. Pharmacol. Ther., 2005, 105, 333–341.
66. Sudhop T., Sahin Y., Lindenthal B., Hanh C., Luers H.K., von Bergmann K., Comparison of the hepatic clearances of campesterol, sitosterol, and cholesterol in healthy subjects suggests that efflux transporters controlling intestinal sterol absorption also regulate biliary secretion. Gut, 2002a, 51, 860–863.
67. Superko H.R., Inherited disorders contributing to coronary heart disease. Cholesterol, Genetics, and Heart Disease Institute [www.heartdisease.org/Traits.html] (accessed on the 14th January 2007).
68. Tomoyori H., Kawata Y., Higuchi T., Ichi I., Sato H., Sato M., Ikeda I., Imaizumi K., Phytosterol oxidation products are absorbed in the intestinal lymphatics in rats but do not accelerate atherosclerosis in apolipoprotein E–deficient mice. J. Nutr., 2004, 134, 1690–1696.
69. UE Legislation. [http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX: 32004R0608:PL.:NOT] (accessed on the 27th April 2007).
70. van Rensburg S.J., Daniels W.M., van Zyl J.M., Taljaard J.J., A comparative study of the effects of cholesterol, beta-sitosterol, beta-sitosterol glucoside, dehydroepiandrosterone sulphate and melatonin on in vitro lipid peroxidation. Metab. Brain Dis., 2000, 15, 257–265.
71. Vanhanen H.T., Blomqvist S., Ehnholm C., Hyvonen M., Jauhiainen M., Torstila I., Miettinen T.A., Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. J. Lipid Res., 1993, 34, 1535–1544.
72. Volpe R., Niittynen L., Korpela R., Effects of yoghurt enriched with plant sterols on serum lipids in patients with moderate hypercholesterolaemia. Br. J. Nutr., 2001, 86, 233–239.
73. von Holtz R.L., Fink C.S., Awad A.B., Beta-sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells. Nutr. Cancer, 1998, 32, 8–12.
74. Vuorio, A.F., Gylling, H., Turtola, H., Kontula, K., Ketonen, P., Miettinen, T.A, Stanol ester margarine alone and with simvastatin lowers serum cholesterol in families with familial hypercholesterolemia caused by the FH-North Karelia mutation. Arterioscler. Thromb. Vasc. Biol., 2000, 20, 500–506.
75. Wang T., Hicks K.B,, Moreau R., Antioxidant activity of phytosterols, oryzanol, and other phytosterol conjugates. J. Am. Oil Chem. Soc., 2002, 79, 1201–1206.
76. Weststrate J.A., Meijer G.W., Plant sterol-enriched margarines and reduction of plasma total and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur. J. Clin. Nutr., 1998, 52, 334–343.
77. Yu L., Hammer R.E., Li-Hawkins J., Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. Proc. Natl. Acad. Sci. USA, 2002, 99, 16237–16242.

Received June 2008. Revision received August and accepted September 2008.