The importance of sitosterol and sitosterolin in human and animal nutrition

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Sitosterol, the principal phytosterol in most higher plants and hence in plant-derived food poducts, is found in the serum and tissues of healthy humans in concentrations 800-1000 times less than the endogenous cholesterol. The glucoside of sitosterol (sitosterolin) is present in mammalian serum at even lower concentrations. In many animals, sitosterol and sitosterolin concentrations relative to cholesterol are considerably higher than in humans. Only plants can synthesise these compounds and humans and animals obtain them from their diet. Even though their absorption efficiency is low (-1/10 and -1/50 for sitosterol and sitosterolin, respectively, relative to cholesterol), their apparent synergystic stimulatory effect on the immune system and prophylactic effect on a variety of diseases of civilisation indicates their importance in human and animal nutrition. Since modern food processing tends to reduce their concentration in processed plant-food products, and eating habits also affect their consumption adversely, it is desirable to eat sufficient unrefined or unprocessed plant foods or resort to food supplements containing sitosterol and sitosterolin.

Epidemiological studies have consistently shown that diets rich in vegetables and fruit reduce the risk of developing various types of cancer,[1] cardiovascular disease,[2] diabetes[3] and other common ailments of civilisation.[4] Ubiquitous components of such diets identifed as disease preventing agents are vitamin E (tocopherols), ß-carotene (carotenes), vitamin C (ascorbic acid), Havonoids (vitamin p, rutin, naringen, etc.) and various phyto-oestrogens (genistein, daidzein, biochanin A, formonetion, coumestrol, etc.). However, none of these substances, except for vitamin C and vitamin E, has shown any great improvement in health when given individually in its pure form and in certain instances caution may even be desirable when some of them are taken over protracted periods (e.g. ß-carotene and phyto-oestrogens[6]).Many of these studies also report that plant constituents other than those mentioned above, for example sitosterol, may contribute to or be responsible for the observed health promoting effect of plant diets.[4,7]

Animals synthesise only cholesterol, but plants have the biosynthetic ability to produce, besides small amounts of cholesterol; a number of plant sterols of which the most common are campesterol, sitosterol and stigmasterol, and in some plant families the Δ^7-sterol analogues, of which Δ^7-stigmastenol and spinasterol are the most abundant.[8-10] These plant sterols contain an extra alkyl group at C-24 in the side chain, providing more bulk (volume) to the molecule in comparison to cholesterol. One of the most important functions of cholesterol in animals and phytosterols in plants is their presence as components in the bilamellar (endofacial and exofacial) cell membranes.[11] In addition, cholesterol is converted in animals and plants to vitamin $D_3$2 and pregnenolone which, particularly in animals, then gives rise to various sex steroids and corticosteroids and in plants (and perhaps animals also)[13,14] the cardenolides and bufadienolides[8,9,15] Plants also use cholesterol to produce spiroketal steroids (diosgenin, solasodine, etc.).[15] Sitosterol can be degraded to pregnenolone in animals[16,17] and plants[15] and hence to all the steroid hormones derived from pregnenolone and its C_{21}, analogues, but plants do not seem effectively to utilise their 24-alkylsterols for vitamin D or spiroketal steroid synthesis.[8,9,15]

In animals cholesterol occurs either in its free form or esterified with fatty acids. In its free form it acts essentially as a membrane component or to a much smaller extent as a sex hormone or corticosteroid hormone precursor via pregnenolone. The cholesterol esters serve primarily as cell membrane components, and storage and fatty acid transport agents. In all higher plants cholesterol and the C-24 alkyi plant sterols occur free (S), as esters (SE), as ß-D-glucosides also known as sterolins (SG), and their *6-O'*-esters (ASG) in small but readily identifiable amounts as primary essential biosynthetic products.[8] As in animals, S and SE are essential cell membrane components and SE seems to serve a similar transport or storage function.[8] Evidence exists that in the bilamellar cell membranes the bulkier sitosterol and stigmasterol preferentially occupy the exofacial leaflet whereas cholesterol partitions readily into the cytofacial leafet. This has important conformational and permeability (fluidity) implications with respect to the overall cell membrane structure in both plants and animals.[10,18,19] The essential purpose of SG and ASG is as yet unknown but suspected to be of importance in cell membrane structures, particularly with respect to ASG, which is derived from SG in plants[8,10,20] and in mammals from absorbed SG.

Dietary sterols are absorbed by all animals and most of those investigated (including dog, pig, mouse, rat and sheep) contain about 10-20 times more sitosterol in their serum and tissues than humans (~5 μM).[22,27] In healthy humans the sitosterol to cholesterol ratio on a molar basis is about 1 to 800-1000.[28-32] In humans the absorption of cholesterol is about 50% while that of sitosterol is about 5%.[17,33] Campesterol (240-methylcholesterol), with its smaller C-24 alkyl group, is absorbed more readily (~20%) than sitosterol (24α-ethytcholesterol) but less efficiently than cholesterol.[17,29] A small percentage of humans are hyperabsorbers of plant sterols (called phytosterolaemics, with 5-10 times the normal serum sitosterol and higher campesterol levels) and there are a few individuals in whom sitosterol exceeds the absorption of campesterol (sitosterolaemics, with 10-100 times the normal serum sitosterol and lower campesterol levels), reaching sitosterol serum levels found in many animals but accompanied in humans by serious health consequences.[17,34,35] It is known, however, that sitosterol and sitosterolin on oral administration are not toxic.[36-38] Moreover, sitosterol acts as a plasminogen activator[39,40] and promotes the formation of polyunsaturated fatty acids from linoleic acid,[41] whereas high serum cholesterol levels seem to reduce tissue plasminogen activator activity[39,42] and the ability to convert linoleic acid to the essential polyunsaturated fatty acids[43] needed for prostaglandin and leukotriene synthesis important in cell-mediated immune functions.[44]

Little is known about the absorption of SG in animals and humans, but it has been reported to be about 1-2.5% in rats followed by conversion to S, SE and ASG. Its presence has been identified in cows' milk and in the serum of humans, dogs, rabbits, rats and mice.[21,45] Here it is of interest to note that the 6´-*O*-oleate ester of cholesteryl ß-D-glucoside is synthesised by birds[46] and snakes,[47] indicating that these compounds are indeed of some biological importance in the animal kingdom. Of particular interest in this connection is the recent finding that the 6´-*O*-oleate ester of sisterol ß-D-glucoside has an ED_{50} of 0.069µM in the HL-60 cell differentiation test in which 1α,25-dihydroxyvitamin D_3 was only five times more effective on a molar basis.[48] In contrast, sitosterol (plant sterols) itself has been shown to be effective in inhibiting HT-29 human colon cancer cell growth[49] and epithelial cell proliferation[50.51] as well as chemically-induced colon tumours in rats,[52-54] mammary lesions in organ culture,[55] and as an antimutagenic agent.[55-56] Cholesterol, on the other hand, promotes chemically induced colon carcinogenesis in rats[57-58] and inflammation,[59] but this does not necessarily apply to its glucoside, cholesterolin.[38] Sitosteryl glucoside has a protective effect on saponin-induced haemolysis at a dose (PD_{50}) of 1.7 $mg l^{-1}$ (3.0µM), whereas sitosterol showed no such effect.[60] Intravenous administration of SG at 50µg kg^{-1} protects against histamine-induced vascular permeability (up to 50%) in rats[21] and in guinea pigs[61] and it increases haemostatic activity at 25µg kg^{-1} by about 18% in mice; this latter effect is slightly reduced (-10%) on oral administration (2mg kg^{-1}).[21] It has also been shown that, individually, sitosterol and its glucoside have a proliferating effect on T-cell production *in vitro*, still noticeable at the remarkable low level of 10 pg l^{-1} and 1 pg l^{-1} (24fM and 1.7 fM), respectively, with a synergistic enhancement when both are given together. Also, when these two compounds were given to human volunteers on a normal diet, an enhanced T-cell proliferative response was observed after 4 weeks of daily oral supplementation with 60 mg sitosterol and 0.6 mg of its glucoside.[62] Interestingly, cholesterol showed rather a T-cell suppression response in the *in vitro* test, even at 1µg l^{-1}.[63] This strongly suggests that the plant sterols and sterolins, and particularly sitosterol and sitosterolin at surprisingly low concentrations, have a beneficial effect on the immune system and that their low absorption rate is of little consequence in relative terms. The important factor is an adequate maintenance of their body pools via a sufficient and constant dietary supply in view of the fact that body pools of both sitosterol and sitosterolin are rapidly diminished on a diet devoid of either compound.[21,33,62] Reports exist about the anti-inflammatory,[64-69] anti-ulcer,[70-74] anti-diabetic[75-77] and anticancer activity[49-56,65,67,78-82] of both sitosterol and sitosterolin.

Because sitosterol is absorbed less efficiently (1/10) than cholesterol, it has unsurprisingly been found to lower the absorption rate of cholesterol when given at relatively high oral doses (3-30 g),[17] and even the normal daily dietary supply of plant sterols at 200-240 mg (-130-160 mg sitosterol) seems to have an effect on cholesterol serum levels.[83,84] For this reason sitosterol was and still is used in some countries for the treatment of of mild cases of hypercholesterolaemia.[17,36,37,85] The side effects of these high doses (other than occasional mild constipation[85] or diarrhoea[36]) have never been reported nor have any health promoting effects been observed or particularly looks for other than an improvement in serum cholesterol status.[36] Since 1974, sitosterol in combination with its glucoside (S:SG in the ratio 10:0.1 mg per capsule, Harzol®) has been used in Germany for the treatment of benign prostate hypertrophy (BPH), usually at a daily dose of 3 x 2 capsules or 60_1 mg plant sterols per day (-44 mg sitosterol plus campesterol, campestanol, dihydrosistosterol and stigmasterol).[86-89] BPH patient response is in general positive, although other health benefits have not been reported.[86,89] A similar product used to be available in Germany for the treatment of soft-tissue rheumatism (Flemun®, 10 mg sitosterol with 0.1 mg glucoside) with moderate success,[90] but it is no longer marketed. Sitosterol and its glucoside, either singly or in combination, are found in BPH remedies derived from *Serenoa repens* (*Sabal serulata*),[91,92] *Pygeum africanum* (*Prunus africana*)[92,93] and as the Δ^7 analogues in pumpkin seed.[92,94] Sitosteryl glucoside and hence sitosterol are also present in many popular plant remedies such as *Harpagophytum procumbens* (devils claw), commonly used for the treatment of rheumatic complaints,[95,96] *Silybum marianum* (milk thistle) extracts used for the treatment of liver complaints,[97,98] *Gingko biloba* extracts for the treatment of cardiovascular illnesses,[99,100] *Panax ginseng* popular as a universal tonic,[96,101] and *Eleutherococcus senticosus* (Siberian ginseng) also used as a general tonic, in which sitosteryl glucoside has been identified as one of the 'adaptogens' (eleutheroside A).[96,102,103] Adaptogens are a group of natural plant products which promote overall health without the rapid response normally elicited by a drug and without the side effects associated with any drug used.[102-104]

Plant sterols are obligatory metabolites in all higher plants, ferns and many algae together with their glucosides. Since sitosterol is in most instances the major plant sterol it is not surprising that this compound, often together with its glucoside, has been isolated from or identified in many plants and its occasionally mentioned in epidemiological reports as a possible contribution to the health promoting effect of vegetable and fruit diets.[4,105,106] Indeed, sitosterol and its glucoside have been evaluated from a variety of biological activities.[21,36-42,45,48-56,60-90,107,108] However, it has been stated that 'the ubiquitous occurrence of sitosterol, plant sterols in general, and their glucosides in all vegetables makes it highly unlikely that they have any drug related properties and many reports on their medicinal properties are based on *in vitro* or unrealistically high *in vivo* doses which make a therapeutic application of these compounds highly unlikely'.[76,77]

In a way this statement is correct, since sitosterol and sitosterolin are not drugs in the accepted sense, but rather slow acting essential micronutrients or adaptogens better considered as minor but nevertheless important cell membrane constituents. Nevertheless, popular micronutrients such as vitamin E, vitamin C and ß-carotene belong to this category and have found wide acceptance associated with a considerable volume of research and promotional literature publicising their use and health advantages. These three compounds (vitamin E, vitamin C and ß-carotene), like plant sterols and sterolins, are primary plant metabolites and , in the course of evolution, animals and humans have become adapted to their dietary availability and integrated them more or less with their own metabolic needs.[109] Animals cannot synthesise carotenoids, but they need a small steady supply of ß-carotene to produce vitamin A (retinol). Animals make their own vitamin E analogue, ubiquinone/ubiquinol,[110] which is more hydrophilic (water soluble) than vitamin E, and which provides antioxidant protection in fatty tissues/environments. This is perhaps not so important in a lean healthy population, but may be of vital consequence in an affluent adipose society. Vitamin C can be synthesised by most animals, but in humans this ability has been lost probably because the larger proportion of their evolutionary diet was based on unprocessed plant food, as it still is in primitive societies. Any prolonged reduction in serum and tissue levels of vitamin C will result in serious health problems. Vitamin C supplementation of the diet then becomes a necessity. This may also apply to the plant sterols and their glucosides (sterolins).[36,37,107,108] A good quality diet providing mainly unprocessed plant foods will readily the supply a daily amount of 200-300 mg plant sterols (containing -65% sitosterol, 130-195 mg)[27,88,111-113] and roughly 10% that value of their glucosides.[20] However, the actual intake of plant sterols in different countries and among population groups may differ considerably and range from 40-400 mg per day.[17,27,32,37,51,88,111-118] Surprisingly, some vegetarians have daily intakes of below 100 mg (-65 mg sitosterol).[51]

Vegetables contain from 5-40 mg of plant sterols, on average 20 mg,[119] per 100 g and this can greatly increase to 100 mg on drying since they contain about 80% water.[120] Fruit contains from 2-30 mg plant sterols, on average 15 mg, per 100 g. All seeds are rich in plant sterols when mature, ranging from 22-714 mg per 100 g – an average value is about 120 mg 100 g^{-1},[119] – but it can be considerably less when seeds are immature.[121] This includes all edible seed and culinary products produced from them such as nuts, cereals, beans and seed-derived spices. The plant sterol content in vegetables, fruit and seeds embraces the four classes of sterol compounds (S, SE, SG and ASG), of which the glucoside portion, SG and ASG, is usually one tenth[20,122] but can occasionally be surprisingly high as in potatoes, which contain per 100 g about 40 mg plant sterols, of which 16 mg is sitosterol and 28 mg is in the form of SG and ASG.[123] The sitosterol content of these plant sterols varies between 40-80% and a good average can be taken as 65%.[27,88,111-113] Cooking does not destroy these compounds, but boiling water may remove some of the sterolins (SG and ASG) if the water is discarded, since sterolins have a water solubility of 10 mg 1^{-1} at room temperature,[87,122] which is considerably higher at 100°C. Sterols (S and SE) are practically insoluble even in boiling water.[124] Slicing, grating, macerating and juicing of fresh plant material may reduce the SG and ASG content through enzymatic hydrolysis to S, but will not affect the overall S content; the same applies when fruit and vegetables age.[121,123,125] The sterol content of processed and refined plants can alter greatly. Thus while wheat grains contain per 100 g about 4200 mg of plant sterols (S, SE, SG and ASG), of which 1900 mg is in the glucoside fraction, its flour contains per 100 g only about 52 mg of total plant sterols, of which 15 mg is in the glucoside fraction. The removed bran, however, contains about 4500 mg of total plant sterols and the unrefined oil about 2600 mg, of which 1740 mg is sitosterol per 100 g.[126-128] The same applies to other cereal products like rice, corn (maize) and rye. Crude plant oils are thus a relatively rich source of phytosterols and their glucosides, but a large proportion, and especially glucosides, are removed during the refining process.[36,119,126] Thus, while soya beans contain per 100 g about 160 mg of total plant sterols, of which 90 mg is sitosterol and 50 mg is in the glucoside fraction, the crude oil contains about 350 mg of total sterols, which is reduced to 220 gm on refining, completely removing all glucoside fraction; on hydrogenation the sterol content is further reduced to 130 mg per 100 g.[36,119,129,130] This applies to all plant oils, which even in the refined form are a rich source of plant sterols, but then lack sterolins. Plant sterols, but not their glucosides, are therefore also present in margarines,[36,131] but only traces are found in butter obtained from free range cows.[132]

In order to consume 100 mg of plant sterols, a person would have to eat about 500-700 g of fresh vegetables and fruit, about 200 g of flour products without additives or 250 g of potatoes, and this amount has to be doubled to reach 200 mg, which is acceptable for a normal dietary supply. Even an apparently quality diet may be inadvertently selected in such a manner that the total plant sterol and sterolin intake is just not adequate to maintain a proper serum level of sitosterol[51,133] and its glucoside relative to serum cholesterol levels apparently necessary for an efficiently functioning immune system.[62] This situation is made worse when daily consumption relies on processed foods[3] or a reduced food intake. The first occurs frequently under conditions of stress, while the latter commonly arises during slimming routines, ill health and old age.[116] Especially in these cases dietary plant sterol and sterolin supplementation, either by judicious selection of plant food or products or by plant sterol and sterolin supplements, is indicated. Since many animals have considerably higher sitosterol concentration levels in their serum when living in their natural environment, any artificial feeding may result in plant sterol[33,132] and sterolin[21] deficiencies with serious long-term consequences[62] which as yet have never been investigated nor even suggested.

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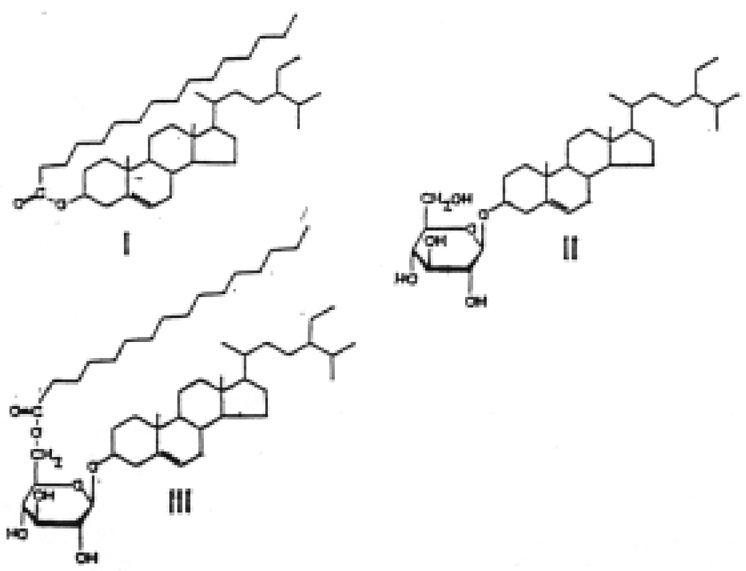
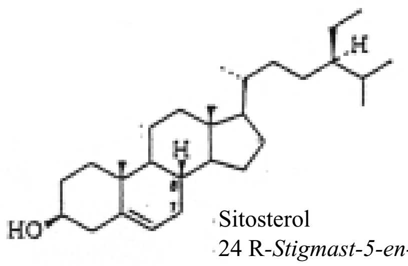
This article was written to promote awareness of the nutritional importance of sitosterol and its glucoside sitosterolin and their effect on maintaining or improving immuno competence. The accumulated literature about the biological behaviour of sitosterol is vast and dominated by opinions about its non-absorbability and cholesterol absorption reducing effects. It is however recognised by many investigators that sitosterol is absorbed to a limited extent and joins cholesterol in all cellular systems where it must exert an effect different from cholesterol. Since sitosterol is an ubiquitous micro-nutrient evolutionary forces should have provided a beneficial niche for this plant sterol and any reduction in its dietary availability could therefore disturb an established balance. The synergistic effect with its glucoside is surprising,[62] but highlights the nutritional importance of sitosterol and sitosterolin. Modern nutritional practices tend to reduce the availability of both compounds for many individuals. The often quoted dietary availability of 250 ± 50 mg sitosterol per day is probably true for well managed family households and "balanced diets", but in my opinion does not apply to the chaotic dietary habits of many present-day individuals.

However, while this article attempts to highlight the importance of sitosterol and sitosterolin it must be kept in mind that other essential micro-nutrients are equally important. Without an adequate average daily supply of ascorbic acid, magnesium and other vitamins and essential micronutrients, no real benefit can be obtained from even an over supply of sterol and sterolin. On the other hand, any dietary or medical procedures that reduce sitosterol absorption may have adverse health effects.





Sitosterol
24 R-*Stigmast-5-en-3ß-ol*

Fig. The most typical phytosterol conjugates: Sitosteryl palmitate (I), Sitosteryl ß-D-glucoside (II), Sitosteryl (6´-O-palmitoyl) -ß-D-glucoside (III)