The role of phytosterols and phytosterolins in immune modulation: a review of the past 10 years

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Although plant sterols (phytosterols) were chemically described in 1922, their biological role in human and animal health has been underestimated. Their ability to control cholesterol plasma levels in hypercholesterolimic patients was first described in 1983 when the structure of phytosterols implied that they could, by steric hindrance, inhibit the absorption of cholesterol from our diets. This has led to the development of functional foods containing high contents of these plant molecules or their esters as cholesterol controlling foods. Over the last 15 years, however, several reports have appeared in the literature indicating that phytosterols have some immunological activity as highlighted in animal models of inflammation or even in *in-vitro* and *in-vivo* models of cancer (colorectal and breast cancer). These findings were paralleled by epidemiological studies correlating the reduced risk of numerous diseases and the dietary intake of phytosterols. It is only in the last 10 years, however, that their direct immune modulatory activity on human lymphocytes has been proven and the mechanism of action in cancer cells has been elucidated. The use of phytosterols as supportive therapies in certain chronic conditions has been tested under clinical trial conditions. This review presents a summary of the *in-vitro* and *in-vivo* studies published to date. Curr Opin Clin Nutr Metab Care 4:471–475.

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Abbreviations

BSS	β-sitosterol
BSSG	β-sitosterol glucoside
HIV	human immunodeficiency virus

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Introduction

β-Sitosterol (BSS) is the major phytosterol of higher plants and is found in the tissues and plasma of healthy individuals at concentrations 800–1000 times lower than that of endogenous cholesterol. Its glucoside, β-sitosterol glucoside (BSSG), is present in even lower concentrations [1]. Many epidemiological studies have indicated a reduced incidence of various types of cancer, cardiovascular disease, diabetes and other chronic conditions in populations consuming diets rich in vegetables and fruits [2]. Although many such studies have concentrated on the protective effects of minerals, trace elements and vitamins, it is only in recent years that the phytosterol content of the foods has been taken into account and yielded positive correlations [3].

In order to understand the possible impact of one of the major phytosterols, namely BSS, and its glucoside BSSG on human health, it is necessary to consider the *in-vitro* data and how these could translate into *in-vivo* benefits. Clinical studies examining the immunological impact are included.

β-Sitosterol and β-sitosterol glucoside as immune modulators *in vitro*

Initial observations made in 1996 showed that the addition of BSS and BSSG mixture to mitogen-activated human peripheral blood lymphocytes could increase the proliferative response several fold. This was confirmed in the same study by a small pilot investigation in volunteers who ingested the mixture orally, followed by ex-vivo testing of the proliferative responses of their lymphocytes. In parallel, the lytic/cytotoxic activity of the natural killer cells *vis-à-vis* a cancer cell line was greatly enhanced when pre-incubated with the BSS/BSSG mixture. This enhanced killing ability was possibly due to the enhanced secretion of interleukin 2 and gamma interferon, both of which are known to promote the cellular activities of natural killer cells [4].

When the profile of cytokine secretion by activated T lymphocytes was measured, it was found that the phytosterol mixture seemed selective in the above-mentioned activities. Lymphokines belonging to the T_{H1} type helper cells were increased whereas those belonging to the T_{H2} helper cells remained relatively unchanged. Indeed, the secretion of interleukin 2 and gamma interferon was enhanced whereas the release of the T_{H2} cytokine interleukin 4 remained unchanged. This specificity implied that the phytosterol mixture has

important regulatory and modulatory properties in diseases where priming of the T_{H1} helper cells is warranted (e.g. effective clearance of particular pathogens) or under other conditions where the overt T_{H2} responses need balancing. It is possible that the T_{H1}:T_{H2} balance is reinstated by using the BSS/BSSG mixture [5]. This was further substantiated by an *in-vitro* study showing the BSS/BSSG mixture to have anti-glucocorticoid activity: the mixture was able to abrogate the response induced by dexamethasone [6].

Further investigation of the BSS and glucoside mixture showed it to have anti-inflammatory properties: the secretion of the monokines interleukin 6 and tumor necrosis factor alpha by endotoxin activated human monocytes was significantly inhibited. This led the authors to suggest that the BSS and glucoside mixture would have a role to play in the control of chronic inflammatory conditions.

Clinical studies investigating immune modulation and phytosterols

Due to the fact that the BSS and BSSG mixture showed profound effects *in vitro* and due to the safety profile of these molecules, it seemed appropriate to investigate the effects under clinical trial situations. The clinical studies undertaken concentrated on the anti-inflammatory properties of BSS and BSSG and the diseases targeted included those in which the fine balance between the activities of T_{H1} and T_{H2} CD4 helper cells needed to be modulated. Such diseases include those described in the literature with overt imbalances between the regulatory T cells, as described below.

Pulmonary tuberculosis

Since the original published *in-vitro* study showing the immune modulatory activity of the phytosterol/glucoside mixture, several clinical studies have been initiated. The first of these examined the adjuvant property of the sterol/sterolin mixture in the treatment of patients with sputum proven pulmonary tuberculosis [7]. This double blind, randomized, placebo-controlled trial showed significant differences between the groups. The placebo group had higher erythrocyte sedimentation rates, lower lymphocyte counts and other hematological parameters, as well as differences in weight gain over the 6-month follow-up period. In general, the phytosterol/glucoside mixture-treated group demonstrated a faster clinical recovery.

Feline immunodeficiency virus – a model of human immunodeficiency virus

Domestic cats infected with feline immunodeficiency virus exhibit the same immunopathogenic processes as those revealed in humans infected with human immunodeficiency virus (HIV). Infected cats typically succumb to the disease due to immune suppression. A group of 33 infected cats randomized into two groups and either treated with the phytosterol mixture or placebo capsules exhibited major differences in their median CD4 cell numbers over an extended period of time. Those cats treated with the active capsules maintained stable CD4 cell counts whereas in those treated with placebo capsules the counts of the immune cells decreased and finally they died of the disease [8]. The mortality of the two groups was significantly different [9]. These findings have been published and presented at international congresses.

Use of the β-sitosterol and β-sitosterol glucoside mixture for management of human immunodeficiency virus infected patients

An open labeled study in HIV infected patients in which no retroviral drugs were used for control of viral replication was initiated in South Africa, where access to registered drugs is denied to most patients due to the cost of such medication [10]. Those enrolled in the study with relatively intact immunities (more than 500 CD4 cells/μl blood) showed the most promising results: their baseline CD4 cell numbers were maintained and their plasma viral loads were significantly decreased. In fact, 15% of the individuals in this sub-group became viral load undetectable (less than 200 copies per ml plasma) within 12 months of starting the study [11•]. Unfortunately, in those whose immune cells were low at the start (<200 CD4 cells/μl blood), disease progression was still evident. Analysis of the CD4 cell type (T_{H1} versus T_{H2} type) showed that those ingesting the BSS/BSSG mixture maintained a favorable T_{H1} response [12••], which implies that their cell mediated immunity was possibly responsible for the viral control and stable CD4 cell counts maintained over 40 months in the absence of any drugs.

Phytosterol and its glucoside in preventing stress induced immune suppression

Marathon running or any endurance sport leads to transient immune suppression, possibly due to hormone-induced redistribution of immune cells as well as a decline in the functionality of the cells. Individuals competing in such activities are therefore prone to bacterial and viral infections, especially affecting the upper respiratory tract. A model of immune stress was used to test the potential of the BSS/BSSG mixture in preventing these physiological changes in a group of marathon runners [13]. The study found the hematological changes that accompany endurance exercise were more pronounced in individuals who received placebo capsules compared with those who received the active compounds. The primary endpoints of the study were the presence of neutrophilia, lymphopaenia, inversion of the CD4 to CD8 cells in the peripheral blood, increases

in the adrenal hormone cortisol and the resulting decline in the antagonist DHEAs (dihydroepiandrosterone sulphate), as well as increases in the pro-inflammatory cytokine, interleukin 6. All of these parameters were present in the placebo group but were partially reversed or totally abrogated in the treatment group. This study therefore showed that the phytosterol mixture appears to have anti-inflammatory properties *in vivo* and that it could be used to prevent infectious episodes in endurance sport athletes.

More recently, in a murine model of inflammation, Navarro *et al.* [14] showed a mixture of phytosterols (made up of 61% BSS) could inhibit the inflammatory response in the carrageenan paw edema experiments when the mice were fed the mixture.

Phytosterol mixture and rheumatoid arthritis

A double blind, placebo-controlled study conducted in patients with active rheumatoid arthritis was recently completed and showed that the attenuation of disease activity (criteria according to the American College of Rheumatology: scores changing >20% relative to baseline) in the treated group was achievable with the use of the BSS/BSSG mixture. The rationale of this study was that due to the potent anti-inflammatory properties of the phytosterol mixture, patients diagnosed with this chronic inflammatory condition should benefit from such an intervention. Markers of activity included erythrocyte sedimentation rates, tender joint counts, swollen joint counts, clinician's assessment of disease activity, and so on. Although unpublished at present, this study opens up new avenues as far as the chronic inflammatory conditions are concerned and we await further studies in this field.

Use of the phytosterol mixture in allergic rhinitis/sinusitis patients

As the phytosterol mixture changes the cytokine profile of T cells to a predominant T_{H1} type, it would seem applicable to test this mixture in allergic conditions where it is known that a predominant T_{H2} profile prevails (increased interleukin 4 release and abnormal gamma interferon secretion). This ultimately leads to increased IgE antibody synthesis and allergic symptoms, the hallmarks of allergies. A group of 24 atopic individuals (mainly pollen sensitivity) were included in a small pilot study and several laboratory and clinical markers of activity were measured over a 12-week period. Statistically significant changes occurred including less rhinorea, less turbinate hypertrophy, less post nasal drip symptoms, lower IgE plasma levels and higher T_{H1}-producing cells. Subjective improvements were reported by the patients themselves when an international questionnaire was used to record patient symptoms. This study has been submitted for publication. Some limited ex-vivo data had previously shown immunological changes occurring in the cytokine profiles of lymphocytes of allergic individuals ingesting capsules of the phytosterol mixture [15].

Use of the β-sitosterol and β-sitosterol glucoside mixture in the protection against cancer

The role of the BSS/BSSG mixture in the protection against cancer is unexplored at present. It is generally accepted that the immune surveillance mechanisms are vital for protection against the development of solid tumors. Such mechanisms depend heavily on a well-balanced immune response favoring a cellular outcome (T_{H1} mediated) rather than a humoral response (T_{H2} type). The above-described immunological activities would suggest that the phytosterol mixture would play a pivotal role in the prevention of cancers. Such studies, however, are lacking at present. Nevertheless, the author feels that the elegant work showing the action of phytosterols (predominantly BSS) on cancer cells *in vitro* and in some limited *in-vivo* models should be brought to the attention of the reader. Hereunder such studies are described.

Phytosterols and effects on tumor cell lines *in vitro*

The initial interest in studying phytosterols was due to their efficacy in reducing the absorption of dietary cholesterol, thus offering protection from cardiovascular diseases. More recently, due to the abundant epidemiological data of protection afforded by phytosterol intake and cancer risks, several authors have embarked on studies to prove the direct effects of phytosterols on tumor cells *in vitro*. Such studies [16,17] have used established human colon cancer cell lines (e.g. HT-29), human breast cancer cell lines (e.g. MDA-MB-231) or prostate cancer cell lines (e.g. LNCaP) to show that cell growth was inhibited at doses of phytosterols which were within physiological ranges. Comparison with cholesterol showed no such activity, thereby suggesting that phytosterols could provide protection against the development of cancer.

Some further studies have actually provided mechanistic evidence of phytosterols and the inhibition of cell growth in these cell lines. In these studies it was shown that by adding phytosterols to the cell lines, programmed cell death (apoptosis) was initiated, probably by activating the protein phosphatase A2 pathway and the subsequent sphingomyelin cycle [18–20]. Cholesterol, the comparative compound, showed no equivalent activity, suggesting that the phytosterol was incorporated into the cell membranes and subsequently activated membrane bound enzymes. An excellent review regrouping the above studies was recently published [21••].

Lipid peroxidation is a process whereby cellular membranes are damaged due to the oxidative deterioration of polyunsaturated lipids and this may lead to cell death and some degenerative conditions as seen in old age. A single study reported that phytosterol was able to decrease the degree of membrane lipid peroxidation in platelet membranes [22]. Such findings would have implications in the understanding of cellular damage as observed in many neurological conditions.

Phytosterols and *in-vivo* models of cancer

The initial report of Raicht and co-workers [23], showing the protection afforded by phytosterols in a rat model of colorectal cancer, was possibly the impetus to many epidemiological studies trying to link the intake of phytosterols and the reduced risk of certain Western diet related cancers (breast, prostate and colon). Resulting from their rat model, Raicht *et al.* suggested that a diet supplemented with 0.2% phytosterols for 28 weeks could decrease the number of rats developing the tumors (induced by chemical means) by 39% and the number of tumors per rat by 60%. Other authors have confirmed these studies more recently: phytosterol feeding was found to dramatically reduce the growth and development of metastases of a human breast cancer cell line (MDA-MP-231) in immune deficient mice [24••]. The limitations of such studies, however, are that human clinical studies to prove or disprove such results are lacking. It would be encouraging to see oncology centers advise some dietary changes to include high phytosterol intake in patients diagnosed with cancer. Can such a dietary change have any impact on the outcomes of such patients? The animal models suggest that this would indeed be the case. The latest research into the role of phytosterols and cancer indicates the potential of these molecules in the management of oncology patients and we await such interventions.

Conclusions

Since the discovery of phytosterols in 1922 the world has seen a flurry of scientific studies concentrating on their cholesterol-lowering activities. It is only in the last 15 years that other biological properties have been ascribed to these plant compounds. These include their anti-inflammatory, anti-pyretic and anti-diabetic properties. There is now clear evidence that these molecules can be applied as supplements to combat life-threatening diseases where the immune system needs modulation. Their importance in cancer is still limited and lacks research, although epidemiological evidence exists for their importance in not only preventing the condition but also in its treatment. We hope to see further evidence in the forthcoming years.

Optimal immune health is vital for the prevention of many chronic conditions. Phytosterols are only part of the total puzzle and the role of other micronutrients and minerals must never be oversimplified. The role played by phytosterols in general health, however, has been underestimated for too long. It is time for us to reconsider these simple plant fats, those that are removed from our natural foods, to present to society more appealing products with longer shelf lives!

References and recommended reading

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