



Pilot Study Conducted at the University of Guelph, by the Human Nutraceutical Research Unit On The Supplement Sterol 117™

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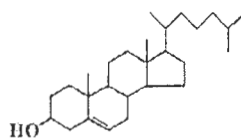
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A randomized, double blind, placebo-controlled clinical trial to determine the effects of Sterol 117™ supplement, containing plant sterols, pine bark antioxidants and essential fatty acid complex, on specific immune parameters and cardiovascular indices in both men and women with non-food allergies.

1.0 INTRODUCTION

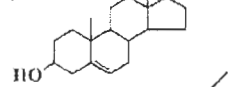
Phytosterols are fats that are present in all plants, including fruits and vegetables. Although structurally similar to cholesterol, the sterols synthesized by animals and plants differ in the nature of their side chain (see Figure 1) (Allayee et al. 2000).

Cholesterol

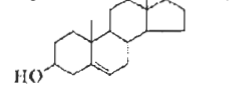


Phytosterols

β-Sitosterol



Stigmasterol



Campesterol

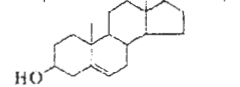


Figure 1: Structures of cholesterol and phytosterols

It has been proposed that phytosterols inhibit the uptake of dietary and endogenously produced cholesterol from the gut, causing a decrease in serum cholesterol levels (Nguyen 1999). One theory suggests that cholesterol in the intestine, already marginally soluble, is precipitated into a non-absorbable state by the presence of added phytosterols (Hicks and Moreau 2001). A second theory proposes that cholesterol must enter bile salt and phospholipid containing "mixed micelles" to be absorbed into the bloodstream. Cholesterol is only marginally soluble in these micelles and is displaced by phytosterols, preventing its absorption (Hicks and Moreau 2001). Due to the limited capacity in the micelles for carrying cholesterol, compounds with similar structures, such as plant sterols, can compete with cholesterol for this space. Therefore, increasing the amounts of plant sterols may result in less cholesterol in mixed micelles and hence, decreased absorption of cholesterol from the gut (Institute of Food Science and Technology 2003).

Enzogenol™:

While no specific information is available on the pharmacology of Enzogenol, data is available on the pharmacokinetics of flavonoids. Orally ingested flavonoids are largely present as aglycons in the intestine and become absorbed with micelles of bile acids into the epithelium and then into the blood. Through the portal vein, the major part of the flavonoids would be delivered to the liver, which decomposes them (Havsteen 1983).

Cellasate™:

Both EPA and DHA have been found to alter plasma membrane composition, cell-signaling mechanisms, eicosanoid responses, cytokine release, and immune cell responses (Arslan et al. 2002). One mechanism for these effects may be that EPA and DHA are incorporated into membrane phospholipids by replacing arachidonic acid (AA, n-6 fatty acid). AA is the substrate for the synthesis of eicosanoids, such as thromboxane A₂ and prostaglandin E₂ (O'Morain et al. 1990). N-3 fatty acids have a greater affinity for the cyclo- and lipoxygenase enzymes than n-6 fatty acids and they competitively inhibit the formation of prostaglandins and leukotrienes (Drevon 1992). Therefore, increased dietary intake of n-3 fatty acids may shift the balance of the eicosanoid production to a less inflammatory profile (Arslan et al. 2002). Another mechanism for the effect of n-3 fatty acids is associated with the change in fluidity by incorporation of fatty acids in the cell membranes and an influence on the activities of membrane-associated enzymes or receptors (Vognild et al. 1998).

3.0 PRE-CLINICAL STUDIES

Studies specifically involving Sterol 117™ have not been conducted to date. However, several toxicity studies have been conducted using the ingredients found in Sterol 117™. These studies are summarized below.

Sub-chronic toxicity

Phytosterols:

Studies conducted on phytosterols have consistently demonstrated a lack of toxicity in animals and humans, except for individuals with an extremely rare genetic condition, sitosterolaemia (Hicks and Moreau 2001). In sitosterolaemia, plasma plant sterol concentrations are elevated due to enhanced intestinal absorption and diminished removal (Salen et al. 1996).

Malini and Vanithakumari investigated beta-sitosterol using rat toxicity studies. No mortality was observed in any of the groups tested with beta-sitosterol. The response of the liver and kidney to beta-sitosterol treatment was equivocal. Inspection of these organs did not reveal any visual lesions attributable to the sterol treatment. Histological studies also did not find any dramatic adverse changes (Malini and Vanithakumari 1990). These findings are in agreement with previous reports that the liver and kidneys did not have any adverse effects of long-term exposure to oral administration of beta-sitosterol in rat, rabbit, and dog models (Swell et al. 1956)

A study conducted by Hepburn et al. investigated the effects on oral toxicity using phytosterols. Diets containing phytosterols did not produce any general organ or systemic toxicity when fed to male and female rats at doses as high as 8.1% of the diet for a period of 90 days. There were no organ weight changes, no macroscopic observations of necropsy, and no histological changes associated with treatment (Hepburn et al. 1999). These results are similar to previous studies that found no evidence of toxicity in rats, rabbits or dogs, when phytosterols were fed at high levels for periods of up to two years (Shipley et al. 1958).

Enzogenol™:

The toxicity of flavonoids is very low in animals. For rats, the LD₅₀ is 2-10 g per animal for most flavonoids. Similar doses in humans are unrealistic, however, as a precaution, doses less than 1 g per adult per day has been recommended (Havsteen 1983).

Phytosterols:

Studies have reported that some phytosterols may elicit an estrogenic response *in vivo*, although these have mostly been observed following the subcutaneous route of administration (Malini and Vanithakumari 1991, 1993). However, this is not appropriate for assessing the hazard of materials intended for oral consumption as the kinetics of subcutaneously administered phytosterols differs from that following oral administration. Baker et al. investigated the estrogenicity of phytosterols using *in vivo* and *in vitro* assays. The *in vitro* studies found that phytosterols were unable to bind to the estrogen receptor. Therefore, phytosterols were not estrogenic via the oral route of administration (Baker et al. 1999).

Enzogenol™:

A literature search did not reveal any published data on the hormonal effects of flavonoids. Therefore, this suggests that there is little to no hormonal activity associated with the use of flavonoids.

Cellasate™:

There does not appear to be any estrogenic or testosterone activity with the use of n-3 fatty acids. A study of the current literature available did not produce any information with regard to n-3 fatty acids and hormonal functions. Therefore, this suggests that there is little to no hormonal activity associated with the use of n-3 fatty acids.

4.0 CLINICAL STUDIES

Several human trials using phytosterols, Enzogenol™, or components of Celasate™ have been conducted for cardiovascular disease. These trials are summarized below.

Phytosterols

Effect of plant sterols from rice bran oil and triterpene alcohols from sheanut oil on serum lipoprotein concentrations Am J Clin Nutr 2000

This nine-week double blind study with multiple crossovers was investigated in 60 male and female subjects. Subjects consumed 29 g/day of three margarines

for three-weeks each. The margarines consisted of a commercially available diet margarine and margarines containing plant sterols from rice bran oil or triterpene alcohols from sheanut oil. The mean intake of total plant sterols was 0.06 g/day from the control margarine, 2.1 g/day from the rice bran oil margarine, and 2.6 g/day from the sheanut oil margarine. Fasting venous blood samples were collected at the end of each three-week period, one on day 18 and another on day 21. Samples were analyzed for total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triacylglycerol concentrations. The results showed that 2.1 g/day of plant sterols from rice bran oil lowered TC by 5% and LDL cholesterol by 9% whereas triterpene alcohols from sheanut oil had little to no effect on these indices.

Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolemic and mildly hypercholesterolemic subjects Eur J Clin Nutr 1999

In this study, 100 male and female volunteers consumed 25 g/day of spreads over a 14-week period. The study had a double blind, placebo-controlled, balanced incomplete Latin square design using five spreads and four time periods. The five spreads included butter, a commercially available spread (control), and three test spreads fortified with plant sterols (0.85, 1.62, and 3.26 g/day). Fasting venous blood samples were collected and analyzed for TC, HDL, LDL, and triglycerides. TC and LDL cholesterol significantly decreased by 5-7% and 7-10% respectively, with plant sterol consumption, while triglyceride concentration was not affected. Furthermore, HDL cholesterol was not affected after consumption of the plant sterol enriched spreads.

Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolemic and mildly hypercholesterolemic subjects Eur J Clin Nutr 1998

This study consisted of 100 male and female subjects who consumed 30 g/day of margarine over a 14-week period. The study had a randomized, double blind, placebo-controlled balanced incomplete Latin square design with five treatments and four time periods. The five treatments included "Benecol", which is fortified mainly with sitostanol-ester, a commercially available spread, and three phytosterol treatments from either soybean oil, rice bran oil, or sheanut oil concentrates. Fasting venous blood samples were collected after 2.5 and 3.5 weeks and analyzed for TC, HDL, LDL, and triacylglycerol concentration. Margarine enriched with

Table 2. Biochemical and hematological safety parameters. Mean [SD]

Index	Baseline		Week 6		Week 12	
Glycemic control						
Plasma glucose [mmol/L]	5.2	[0.6]	5.1	[0.8]	5.0	[0.6]
Renal function						
Plasma creatinine [mmol/L]	0.07	[0.01]	0.07	[0.01]	0.07	[0.01]
Urine albumin creatinine ratio	1.2	[1.4]	1.0	[0.7]	1.1	[0.8]
Liver function						
Plasma bilirubin [mmol/L]	13.7	[5.3]	12.6	[4.1]	11.4	[3.8] *
Plasma alkaline phosphatase [mmol/L]	81.4	[20.5]	83.0	[22.8]	78.7	[21.7]
Plasma AST [mmol/L]	21.4	[6.6]	21.6	[5.8]	21.8	[5.8]
Plasma ALT [mmol/L]	22.5	[10.6]	21.3	[10.9]	21.3	[9.6]
Plasma GGT [mmol/L]	27.3	[16.1]	28.6	[19.8]	28.9	[21.4]
Lipid profile						
Plasma total cholesterol [mmol/L]	5.7	[1.0]	5.8	[1.0]	5.6	[1.2]
Plasma HDL-cholesterol [mmol/L]	1.60	[0.51]	1.62	[0.53]	1.65	[0.47]
Plasma LDL-cholesterol [mmol/L]	3.4	[0.9]	3.5	[0.8]	3.3	[0.8]
Plasma triglyceride [mmol/L]	1.57	[0.74]	1.47	[0.72]	1.43	[1.12]
Total cholesterol : HDL-cholesterol ratio	3.9	[1.3]	3.8	[1.2]	3.6	[1.2]
Plasma apolipoprotein B [mmol/L]	1.09	[0.25]	1.03	[0.26]	1.09	[0.30]
Hematology						
Hemoglobin [g/L]	139	[11]	139	[9]	137	[10]
RBC count [...10 ¹² /L]	4.5	[0.4]	4.5	[0.4]	4.4	[0.4]
RBC mean cell volume [fL]	90.5	[4.0]	90.9	[4.0]	91.0	[4.0]
MCHC [g/L]	342	[4]	340	[5]	340	[8]
WBC count [...10 ⁹ /L]	5.3	[1.3]	5.5	[1.8]	5.6	[1.6]
Platelet count [...10 ⁹ /L]	235	[43]	233	[47]	234	[50]
Platelet mean cell volume [fL]	7.9	[0.7]	8.0	[0.7]	7.9	[0.6]

p value of comparison with baseline. * =0.05

Other related studies of interest:

Kell SO, et al. Dietary flavonoids, antioxidant vitamins and incidence of stroke: the Zutphen study. *Arch Intern Med* 1996; 156:637-642.

Knekt P, et al. Flavonoid intake and coronary mortality in Finland: a cohort study. *BMJ* 1996; 312:478-481.

Cellasate™

Effect of Marine Oils Supplementation on Coagulation and Cellular Activation in Whole Blood Lipids 1995

This study investigated the effects of various marine oils in 134 healthy male and female volunteers for ten weeks. The subjects consumed 15 mL/day of oil and were randomly divided into the following groups: seal, cod liver, seal/cod liver, blubber of Minke whale, or no oil. Fasting venous blood samples were taken and analyzed for TC, HDL, and TG. TC remained unchanged in all of the oil groups, whereas HDL cholesterol sig-

nificantly increased by 7% in the seal/cod liver oil group. TG was significantly reduced in the cod liver oil group only. The study concluded that supplementation of a regular diet with a combination of seal and cod liver oil seems to have some beneficial effects on cardiovascular disease risk factors.

Other related studies of interest:

Vognild E, et al. Effects of dietary marine oils and olive oil on fatty acid composition, platelet membrane fluidity, platelet responses, and serum lipids in healthy humans. *Lipids* 1998; 33:427-436

Reported Adverse Effects

Phytosterols:

No significant side effects, including gastrointestinal side effects, have been observed with consumption of plant sterol esters (Nguyen 1999). It has been suggested that consumption of sterol esters slightly reduce the

Exclusion Criteria:

- Pregnancy/breast feeding
- History of clinical significant and unstable cardiovascular, pulmonary, renal, neurological, dermatological, hepatic or endocrine disease in the past 6 months
- Change in medication 4 weeks before entry into study
- History of frequent respiratory infections
- Diabetes or immune disorder such as lupus erythematosus or HIV/ AIDS
- Known allergy to Sterol 117™ or any of its components
- History of drug, alcohol or substance abuse in the past 6 months
- Participation in any clinical trial within 6 weeks preceding day 1 of the study

All participants were recruited from the University of Guelph or the City of Guelph. At the study screening, participants were given a letter of information and the consent form to sign. Respondents received a verbal briefing of the study protocol and the same information in writing. They signed an informed consent and completed a questionnaire to ensure compliance with the inclusion/exclusion criteria.

All participants were questioned on the nature of their allergies. Eligible subjects were required to have non-food allergies, but were required to have allergies to dust, animal dander, animal saliva, molds, etc. In addition, the study required that all subjects had received an allergy test by their physician to verify allergen response, or had been prescribed medication in the past to treat their allergies.

Experimental Design

This study was conducted as a double blind, placebo-controlled clinical trial. Subjects in the treatment group consumed two capsules of the Sterol 117™ supplement for the first seven days in order to reach phytosterol

threshold levels, followed by a maintenance dosage of 1 capsule per day. Each capsule contains 300mg of phytosterols (117mg of b-Sitosterol) derived from soy, 20mg of Enzogenol™ (antioxidants) extracted from pine bark, and 50mg of Cellasate™ (a mixture of proteins and essential fatty acids) from seeds and fish oils. The placebo consisted of a rice flour mixture, identical in appearance to the supplement, and the consumption pattern for the placebo was the same for the placebo group as for the treatment group. The Sterol 117™ and placebo supplementation phase lasted 28 days.

Subjects were required to visit the HNRU human clinical testing unit on three separate occasions. Subjects came in for baseline testing (day 0) and returned on day 7 of the study and again on day 28, the last day of the study. On the initial visit, height, weight and a blood sample were taken. Blood was analyzed for complete blood cell count (CBC), plasma DHEA, Cortisol, total HDL and LDL-cholesterol concentrations, and triglycerides. A Quantikine high sensitivity Elisa kit was used in order to measure human IL-6 in the plasma collected. Throughout the study, subjects were required to complete a daily journal listing specific allergy symptoms, non-allergy symptoms and any medications taken during the supplementation phase.

RESULTS

Immune Parameters

Basophils

The effects of the supplement on immune parameters are presented in **Table 1**. A number of studies support the belief that human basophils play an important role in allergic inflammation. Mast cells and basophils express the high affinity receptor for IgE (FcεRI) and play a central role for IgE-associated immediate hypersensitivity reactions and allergic disorders. During allergic reactions, basophils migrate from the blood compartment to inflammatory sites, where they act as effector cells in concert with eosinophils. Basophils release histamine during inflammation and allergic reactions.

body's inappropriate response to prolonged stress. The normal reaction of the body to stress is to produce greater quantities of both cortisol and DHEA. When the stress is gone, the body reduces its output of cortisol and DHEA to resting levels and everything is fine. This is what happens with short episodes of stress. However, when the stress is prolonged, the body prefers to make increasingly greater amounts of cortisol and less DHEA. How long does it take for this to occur?

One study showed that after just 28 days of continuous stress cortisol levels had climbed to 240 percent of starting values and DHEA had dropped to 15 percent of initial levels! What's even worse is that even after the stress is removed, the body sometimes does not recover and bring these hormones back to normal levels, but instead, remains in the stress response mode with high cortisol and low DHEA output. The consequences of elevated cortisol and reduced DHEA levels are devastating: The immune system is compromised with increased risk to infections, certain cancers, allergies and autoimmune diseases.

A tremendous body of research has shown that when cortisol goes up, DHEA drops and when DHEA is normal, cortisol also normalizes. Low DHEA levels are seen in those that are immune compromised, have arteriosclerosis (hardening of the arteries), diabetes and lupus.

Cortisol helps the body maintain homeostasis in the face of stressors counteracts inflammatory and allergic reactions and controls the metabolism of protein and carbohydrates. Cortisol is a very misunderstood hormone. Balance is the key. In naturally low doses it stimulates the immune system and in high doses, as prescribed in synthetic drug form, it can be immune suppressing. Remember that cortisol plays a role in counteracting inflammatory responses in the immune system and when cortisol is not available because the adrenal glands have become exhausted from too much stress, inflammation is allowed to continue unchecked. Conversely,

too much cortisol and you have immune suppression.

In the conventional standard of care, any cortisol level within a very broad range is considered normal, and anything outside that range indicates disease. Cortisol production has an ACTH dependant circadian rhythm with peak levels in the early morning and an nadir at night (**salivary cortisol ranges can vary from 8.0 to 1.0 in the morning and 1.0 to 0.1 in the evening**) The factor controlling this rhyme is not completely defined and can be disrupted by a number of physical and psychological conditions. ACTH and cortisol are secreted independent of circadian rhythm in response to physical and psychological stress.

In the early stages of adrenal stress, cortisol levels will be too high during the day and continue rising in the evening. This is called "hyperadrenia". In the middle stages, cortisol may rise and fall unevenly as the body struggles to balance itself despite the disruptions of caffeine, carbs and other factors, but levels are not normal and are typically too high at night. In advanced stages, when the adrenals are exhausted from overwork, cortisol will never reach normal levels ("hypoadrenia").

None of the participants in the trial were known to have any of the auto immune diseases that are associated with elevated cortisol levels. The change in cortisol levels noted in the pilot trial appear to be in the normal range, and neither DHEA nor cortisol levels, nor the ratio of these two parameters, showed significant changes at the $p < 0.05$ level. Any future trials to detect the impact on cortisol levels would have to include participants who have been clinically diagnosed with autoimmune diseases associated with abnormal cortisol levels.

Cardiovascular Parameters

The effects of the supplement on lipid and lipoprotein parameters are illustrated in **Table 2** and **Table 3**.

Table 2. The effects of Sterol 117™ on blood lipid parameters in experimental and placebo groups from day 0 to day 28.

Blood Lipid Parameters (mmol/L)	Sterol 117 Day 0	Sterol 117 Day 28	Sterol 117 Difference Day 28 - Day 0	Control Day 0	Control Day 28	Control % Difference Day 28 - Day 0
Total Cholesterol	4.36	4.13	-5.3%	4.87	4.91	8.2%
LDL	2.27	1.93	-15.0%**	2.85	2.87	0.7%
HDL	1.63	1.70	4.3%	1.48	1.41	-4.7%
TG	1.00	1.09	9.0%	1.18	1.38	16.9%

** statistically significant, $p < 0.05$

level of LDL cholesterol, a high level of HDL cholesterol, and a moderate total of both.

The specific objective of this portion of the trial was to determine the effects of the supplement Sterol 117™ on blood lipid parameters. Significant reduction was noted in the overall LDL levels of the treatment group from day 0 to day 28. Perhaps what is more interesting is the increase in HDL levels, compared with a relative decrease in the placebo group.

However it is the ratios of various lipids and lipid proteins rather than the absolute values that are important in assessing cardiovascular risk, and consequently these ratios were calculated and tabulated.

A significant decrease in the ratio of TC/HDL, and in the ratio of LDL/HDL cholesterol, in the Sterol 117™ group, was noted. A decrease in these ratios corresponds to an associated decrease in the risk of cardiovascular disease (CVD). These ratios are markers for a reduction in the risk of developing atherosclerosis. **Consequently it is our opinion that these results indicate that Sterol 117™ could be very beneficial to the health of hypercholesterolemic individuals at risk of developing CVD.**

Conclusions and Suggestions For Future Research

Sterol 117™ and its components appear to have an effect on immune parameters and, in particular, in basophil and possibly IL-6 levels. Given these changes, Sterol 117™ would appear to have the potential to substantially alleviate allergic responses.

Sterol 117™ could also have an effect in auto-immune diseases such as Crohn's disease or rheumatoid arthritis, or in the ability of subjects to resist the common cold virus, although studies on these particular populations would be required to verify possible beneficial effects.

This study verified that Sterol 117™ supplement is effective in reducing circulating levels of LDL-cholesterol and increasing circulating levels of HDL cholesterol. It is of interest to note that there was a significant decrease in the ratio of TC/HDL, and in the ratio of LDL/HDL cholesterol, in the Sterol 117™ group. A decrease in these ratios corresponds to an associated decrease in cardiovascular disease (CVD) risk, because these ratios are markers for a reduction in the risk of developing atherosclerosis. Consequently, these results would be of considerable benefit to the health of hyper-

cholesterolemic individuals at risk of developing CVD.

Although pre-clinical study data for Sterol 117™ is not available, clinical studies of the components of Sterol 117™ indicate that there are few adverse effects. Furthermore, the components of Sterol 117™ do not appear to be associated with any mutagenic or genotoxic activity.

Appropriately designed research might help to clarify the role of Sterol 117™ in immune function.

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