



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

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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).

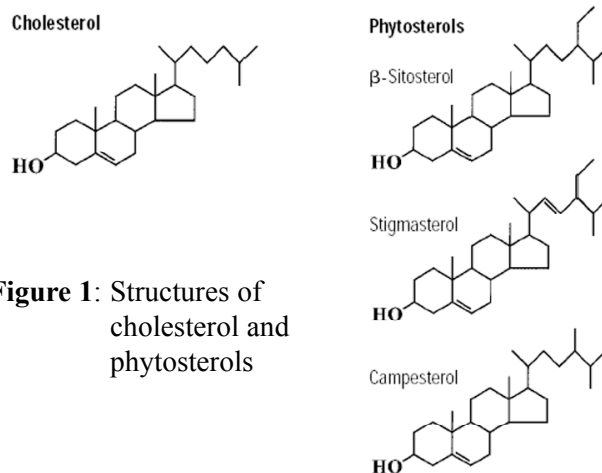


Figure 1: Structures of cholesterol and phytosterols

In animals, cholesterol is the most abundant sterol. In plants, more than 40 sterols have been identified, of which beta-sitosterol, stigmasterol, and campesterol are the most common (Hicks and Moreau 2001). These phytosterols occur in free form or are esterified to free fatty acids, sugar moieties or phenolic acids (Baker et al. 1999). Studies have demonstrated that beta-sitosterol possesses anti-inflammatory and immune modulating properties (Plant Sterol Monograph 2001). It has been estimated that consumption of 3 g/day of phytosterols could reduce the risk of heart disease by 15% to 40%, depending on age and other dietary factors (Hicks and Moreau 2001). However, the western diet typically contains 100-300mg of plant sterols (Nguyen 1999).

Enzogenol™ is a water soluble extract of monomeric and oligomeric proanthocyanidins, flavonoids, flavonoid glycosides, esters and natural organic acids prepared from the bark of *Pinus radiata* by a pure water extraction process (Shand et al. 2003). The extract has been shown to have *in vitro* antioxidant action as measured by inhibition of micelle oxidation, red blood cell hemolysis and a nitro blue tetra zolium enzymatic method (Giesege and Baird 1998; Wood et al. 2002).

Omega 3, 6 and 9 fatty acids, such as found in Cellasate™, have a structural role in phospholipids of all cell membranes in the body, influencing membrane viscosity and permeability (Drevon 1992). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are thought to be responsible for beneficial effects, such as prevention and management of coronary heart disease and hypertension (Simopoulos 1999).

2.0 PRODUCT CHARACTERISTICS

Product Composition

Sterol 117™ consists of an oval, gelatin capsule, filled with a creamy beige powder. The average mass of each capsule is about 0.4 grams. Each capsule of Sterol 117™ contains the ingredients plant phytosterols (183mg), beta-sitosterol (117mg), Cellasate™ (50mg), Enzogenol™ (20mg), and rice flour (30mg).

Plant sterols are extracted from non genetically modified soy beans (Glycine Max). Cellasate™ is a proprietary blend of GMO free natural ingredients extracted from fruit, specific oil producing seeds and plants, as well as essential fatty acids extracted from fish. Cellasate™ is 100% gluten free and does not contain any chemical or synthetic components. Enzogenol™ is an aqueous pine bark extract antioxidant, derived from

the New Zealand “*Pinus Radiata*” tree using pure water. Sterol 117™ has a shelf life of three to five years from date of manufacturing and should be stored in a cool, dark place with low humidity.

Manufacturing and Formulation

Sterol 117™ is manufactured in a pharmaceutically licensed facility in Canada. The facility complies with international regulations and standards for pharmaceutical manufacturing and, exceeds the GMP levels. Sterol 117™ was formulated by Celt Corporation in Calgary, Alberta Canada.

Dosage and Administration

The recommended adult dosing regimen is currently one capsule per day by oral ingestion. Sterol 117™ must be taken with water or juice (not milk), on an empty stomach 45 minutes before eating. One capsule a day normally maintains general health. More serious conditions may require two capsules per day, one in the morning and one at night. The dosing regimen will be changed to two capsules per day for the first week, followed by one capsule per day for the remainder of the trial.

Warnings and Contraindications

As indicated on the package label, Sterol 117™ **must not be taken by organ transplant recipients**, who normally have their immune system artificially depressed in order to prevent rejection of the transplanted organ. Diabetics should only take the supplement under the supervision of a physician, as it may lower their insulin requirement. Adults who are pregnant, nursing, multiple sclerosis patients, taking medication, or who are under a physicians care, should consult their physician before taking this supplement.

Pharmacology

Phytosterols:

Beta-sitosterol is not synthesized endogenously in mammals. Animal studies have demonstrated its intestinal absorption in mammals is minimal, possibly as little as 5% of total dietary beta-sitosterol consumed (Borgstrom 1968; Gould 1955). In contrast, intestinal absorption of cholesterol is 45-54% of intake (Plant Sterol Monograph 2001). Unlike cholesterol, beta-sitosterol is secreted into the bile and is esterified outside the intestinal wall at a much slower rate. After secretion into the bile, beta-sitosterol is stored in the gallbladder, then released intermittently into the duodenum, and subsequently incorporated into feces (Plant Sterol Monograph 2001).

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).

Cellasate™:

N-3 fatty acids from fish oils have been tested in acute and subchronic studies without toxic effects. Arterburn et al. conducted a teratogenic study using a DHA oil to ensure its safe use during pregnancy. Treatment with the DHA oil did not produce overt maternal toxicity or cause fetal malformations. Increased renal variations in development did occur in a non-dose dependent manner but this was not toxicologically significant (Arterburn et al. 2000). Conversely, adverse effects have been demonstrated using high doses of fish oil concentrate. In a study conducted by Rabbani et al., rats consumed high levels of fish oil concentrate (0.5% of body weight) on a subchronic basis. The use of the concentrate demonstrated benefits as well as potentially harmful effects. The high dose fish oil concentrate induced clinical abnormalities, including increased red cell deformity, increased relative liver and spleen weights, and reduced serum HDL cholesterol concentrations (Rabbani et al. 2001).

Mutagenicity and Genotoxicity

Phytosterols:

Wolfreys et al. investigated the mutagenic activity of phytosterols and found no evidence of mutagenic activity in any of the assays conducted. There were no biologically or statistically significant increases in revertant bacterial colony numbers following treatment with phytosterols. Treatment of human peripheral blood lymphocytes with phytosterols showed no increase in the percentage of cells with aberrations. Furthermore, phytosterols showed no evidence of a dose-related, statistically significant increase in the mutant frequency at any concentrations in either the presence or absence of metabolic activation. As no evidence of mutagenic activity was observed in any of the assays, it can be concluded that phytosterols are not a genotoxic mutagen *in vitro*. In support of this conclusion, no evidence of mutagenic activity was observed in *in vivo* mutagenicity studies conducted on these phytosterols. Furthermore, the *in vivo* studies provided limited support to indicate that gut metabolism of these phytosterols did not produce mutagenic metabolites (Wolfreys et al. 2002).

Enzogenol™:

There does not appear to be any mutagenicity or genotoxicity associated with the use of flavonoids. A study

of the current literature available did not produce any information with regard to flavonoids and mutagenicity or genotoxicity.

Cellasate™:

Studies conducted in animals indicate that a high dietary intake of n-3 fatty acids increase lipid oxidation. It has been suggested that peroxidation products of n-3 fatty acids may exert cytotoxic effects and modulate the rate of tumour cell proliferation (Gonzalez et al. 1993; Gonzalez 1995). Further evidence is provided by *in vitro* studies suggesting that n-3 fatty acids have cytotoxic action against tumour cells and this activity may be due to their ability to enhance free radical generation and lipid peroxidation in these cells (Begin et al. 1986).

In a study conducted by Higgins et al., EPA and DHA did not show any genotoxic tendencies when supplemented on their own. However, when EPA and DHA were supplemented with genotoxins, such as hydrogen peroxide, the fatty acids enhanced genotoxic action (Higgins et al. 1999).

Anti-mutagens have been classified into two categories based on their modes of action: one represents desmutagenic, which inactivates mutagens chemically and/or enzymatically, precluding the production of DNA lesions (Kuroda et al. 2001). The other includes bio-antimutagens, which suppress mutation fixation after DNA is damaged by mutagens. Both EPA and DHA have been found to suppress mutagen-induced chromosome aberrations through their bio-antimutagenic action (Yu et al. 1994).

Kuroda et al. investigated the effects of EPA and DHA in cultured hamster cells. EPA and DHA had no mutagenicity in hamster cells when the cells were treated with these two fatty acids only. Cytotoxicity was induced by ethyl methanesulfonate (EMS), and DHA reduced the cell killing by EMS in simultaneous treatments. EPA inhibited EMS-induced cytotoxicity when used before, simultaneously with, or after treatment with EMS. The inhibitory effects of EPA and DHA suggest that the anti-mutagenic activity of EPA and DHA was partly due to protection against the entrance of the mutagen into cells or the modulation of the effect of the mutagen on DNA, or an effect on the repair action against the production of DNA damages induced by the mutagen (Kuroda et al. 2001).

Estrogenic/Testosterogenic Activity

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 testosterogenic 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 Cellasate™ 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

soybean oil lowered TC and LDL cholesterol concentrations by 8-13%, respectively. Margarines containing sheanut or rice bran oil sterols were not effective in lowering blood cholesterol concentrations.

Other related studies of interest:

Hallikainen MA, et al. Comparison of the effects of plant sterol ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolemic subjects on a low-fat diet. *Eur J Clin Nutr* 2000; 54:715-725.

Jones PJ, et al. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. *J Lipid Res* 2000; 41:697-705.

Vanstone CA, et al. Unesterified plant sterols and stanols lower low-density lipoprotein cholesterol levels equivalently in hypercholesterolemic individuals. *Am J Clin Nutr* 2002; 76:1272-1278.

Enzogenol™

Pilot study on the clinical effects of dietary supplementation with Enzogenol™, a flavonoid extract of pine bark and vitamin C Phytother Res 2003

In this open-labeled, uncontrolled investigation, 26 healthy males and females, between the ages of 55-75 years, consumed 120 mg of Enzogenol™ four times a day for 12 weeks. Venous blood samples were collected and various cardiovascular parameters were measured at week 0 (baseline) and at week 12. The results of the clinical, vascular and haemorheology data are presented in Table 1. The data of the biochemical and hematological safety indices are summarized in Table 2. Dietary supplementation with Enzogenol™ was not associated with any changes in glycemic control or renal and liver function. Therefore, from the results presented, dietary supplementation with Enzogenol™ in healthy people was associated with beneficial effects and appeared to be safe and well tolerated.

Table 1. Clinical, vascular and haemorheology data. Mean [SD]

Index	Baseline		Week 6		Week 12	
Anthropometrics						
BMI [kg/m ²]	26.1	[4.6]	25.8	[4.6] **	25.7	[4.7] ***
Waist circumference [cm]	90.4	[13.9]	88.9	[15.0] *	89.3	[15.1]
Body fat [%]	30.9	[13.4]	30.3	[13.2] *	29.3	[13.5] ***
Blood pressure						
Systolic [mmHg]	130	[18]	123	[15] **	123	[15] **
Diastolic [mmHg]	78	[11]	77	[11]	76	[10]
Heart rate [beats/min]	65	[8]	65	[10]	66	[11]
Plethysmography						
Basal flow [ml/min/dL]						
Left arm	1.98	[0.82]			2.32	[0.75] *
Right arm ‡	2.08	[1.02]			2.49	[0.96] **
Reactive hyperemic flow [ml/min/dL]						
Left arm	22.9	[7.5]			27.0	[7.2] **
Right arm – control basal flow	2.04	[1.13]			2.51	[1.07] *
Haemorheology profile						
Haematocrit	0.43	[0.03]			0.42	[0.03]
High shear rate blood viscosity [mPa.s]	3.98	[0.44]			3.90	[0.38]
RBC deformability Index [mPa.s]	4.22	[0.28]			4.27	[0.30]
RBC osmotic fragility						
Lysis at 0.475% NaCl [%]	13.5	[7.3]			13.7	[11.4]
Median RBC fragility [%NaCl]	0.438	[0.007]			0.440	[0.011]
Plasma viscosity [mPa.s]	1.64	[0.06]			1.58	[0.06] ***
Plasma total protein [g/L]	71.8	[3.1]			70.4	[3.3]**
Plasma albumin [g/L]	42.0	[1.8]			41.2	[1.7] *
Plasma globulins [g/L]	28.0	[3.7]			27.1	[3.4]
Plasma fibrinogen [g/L]	3.90	[0.63]			3.95	[0.73]
Albumin:fibrinogen ratio	11.1	[2.1]			10.6	[3.3]

p value of comparison with baseline. * =0.05 ** =0.01 *** =0.001

‡ Combined data of basal flow measurements and control measurements for hyperemic flow.

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

absorption of b-carotene, lycopene, and a-tocopherol (Hicks and Moreau 2001). Noakes et al. examined the effects of sterol and stanol ester spreads on carotenoid concentrations and found consumption of both spreads significantly lowered b-carotene concentration by 9% (Noakes et al. 2002). A key role for both b-carotene and a-tocopherol is to protect LDL-C from oxidation. Since sterols reduce the amount of LDL-C, it may be appropriate to normalize blood concentrations of these vitamins with respect to lower LDL-C concentrations. With this adjustment, sterols did not significantly lower blood concentration of a-tocopherol, but concentrations of b-carotene were still reduced by 8% to 19% (Hicks and Moreau 2001). Due to this observation, Hendriks et al. have suggested limiting daily dosage of sterol esters to about 1.6 g/day, a dose that gives LDL-C reductions without seriously affecting plasma carotenoid concentrations (Hendricks et al. 1999).

Enzogenol™:

Adverse reactions from flavonoids have been reported following administration of chronic pharmacological doses that exceed the estimated dietary intake. Toxic effects that have been documented from doses of 1-1.5 g/day of flavonoid drugs such as cyanidanol include hemolytic anemia, fever, and skin reactions (Cook and Samman 1996).

Cellasate™:

In 1991 and 1993, the U.S. Food and Drug Administration comprised a list of n-3 fatty acid safety concerns. These safety concerns included: (1) increased bleeding times, (2) the possibility of hemorrhagic stroke, (3) oxidation of n-3 fatty acids forming biologically active oxidation products, (4) increased levels of low-density lipoproteins (LDL) cholesterol or apoproteins associated with LDL cholesterol among diabetics and hyperlipidemics, and (5) reduced glycemic control among diabetics. These safety concerns were taken into consideration and the U.S. FDA ruled that marine n-3 fatty acids are GRAS (Generally Recognized As Safe) for inclusion in the diet (Dept. of Health and Human Services, US FDA 1997). Bleeding times were reviewed and it was concluded that there was no significant risk for increased bleeding time beyond the normal range, provided consumption of marine oils is limited to 3 grams or less per person per

day of EPA and DHA (Office of Nutritional Products, US FDA 2000). Furthermore, the FDA has approved a qualified health claim for EPA and DHA n-3 fatty acids in dietary supplements (Office of Nutritional Products, US FDA 2002). The U.S. FDA recommends that consumers not exceed more than a total of 3 grams per day of EPA and DHA n-3 fatty acids, with no more than 2 grams per day from a dietary supplement (Dept. of Health and Human Services, US FDA 2004).

5.0 Objectives of the University of Guelph pilot study:

This pilot study was designed to investigate whether supplementation with Sterol 117™ comprising plant sterols, antioxidants and essential fatty acids could, in subjects with non-food allergies:

- a) Decrease immunological response to allergens
- b) Beneficially modify blood lipid parameters

Subjects

Twenty subjects consisting of 16 females and 4 males, aged from 20-50, and who fit specific inclusion/exclusion criteria were recruited. The inclusion/exclusion criteria were as follows:

Inclusion Criteria:

- Male and female subjects aged 18 years or older
- For females, confirmation (via pregnancy test) of non-pregnancy at baseline, and use of acceptable birth control method in females of childbearing potential
- No food allergies
- Willing and able to give informed written consent

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.

Table 1. The effects of Sterol 117™ supplementation on specific immune parameters in experimental and placebo groups from day 0 to day 28

Immune Parameters	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
IgE	472.00	451.00	-4.4%	1335.00	1127.00	-15.6%
DHEA	6.44	6.44	0.0 %	4.93	4.77	-3.2%
Cortisol	507.00	584.00	15.2%	490.00	498.00	1.6%
Cortisol/DHEA	94.06	108.36	15.2%	160.66	141.44	-12.0%
IL-6	1.261	0.937	-25.7%	1.318	1.179	10.5%
WBC	7.41	7.24	-2.3%	7.28	7.13	-9.8%
Lymphocyte Count Segmented	2.16	2.24	3.7%	2.56	2.60	1.6%
Neutrophil Count	4.65	4.39	-5.6%	4.11	3.97	-3.4%
Monocytes	0.33	0.34	3.0%	0.35	0.31	-11.4%
Eosinophils	0.24	0.20	-16.7%	0.23	0.20	-13.0%
Basophils	0.23	0.01	-95.6%**	0.13	0.04	-69.0%

** statistically significant, p<0.05

The participants in the treatment group, when compared to the control group, *showed a significant reduction in basophil count*, while the reduction seen in the control group was non-significant. A reduction in basophil count may indicate a reduction in histamine release.

Interleukin-6

The immune system also responds to stressors by causing certain immune cells to secrete the pro-inflammatory cytokines, Interleukin-1 (IL-1) and Interleukin-6 (IL-6). These cytokines are both involved in inflammation and IL-6 in particular is thought to worsen the symptoms of autoimmune diseases and fibromyalgia. Interleukin-6 has been found to act as a growth factor in several tumors and some viruses also use IL-6 to replicate. Interleukin-6 also causes calcium to be released from bone, promoting osteoporosis. We must control the release of these cytokines if we want to enhance immunity and reduce degenerative diseases.

It was noted in the pilot trial that the pro-inflammatory cytokine IL-6 levels showed a substantial, reduction in the treated group when compared to the control group. Although the drop in the IL-6 levels in the treatment group was significant, a larger study with more subjects may show statistic significance.

Sterol 117™ has demonstrated that it has an effect on histamine-containing basophil counts and a reduction of IL-6 levels, and consequently may substantially alleviate symptoms associated with airborne allergens, asthma and allergic rhinitis. Further studies are recom-

mended, with a larger patient participation and a longer trial period to investigate other areas of immunological response.

Cortisol/DHEA Levels

The body has developed mechanisms to protect it from the damaging effects of stress. The “fight-or-flight” response is one way the body deals with extreme situations of stress. Upon realizing we are in danger, the brain sounds an alarm, telling our adrenal glands to secrete adrenaline and cortisol, which mobilizes the body to fight or run. This response is supposed to be a short-lived reaction yet today most of us are in and out of this state continually. As a result, our immune system becomes imbalanced, sending out too many inflammatory cytokines. Our adrenal glands become exhausted, weakening several body systems, especially the cardiovascular and endocrine systems.

DHEA is an abbreviation for dehydroepiandrosterone. It is a hormone made primarily by the adrenal or stress glands. Hormones are messenger molecules that influence the function of cells and tissues all over the body. DHEA and cortisol are the body’s long-acting stress hormones and are antagonistic to each other to some degree. Whereas DHEA has an anabolic or building influence, cortisol has a catabolic or tearing down effect on the body. *Both of these effects are essential and these two hormones must be in proper balance for optimal health.* How do these hormones become imbalanced? By stress maladaptation. Stress maladaptation is the

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, $p < 0.05$

Table 3. The effects of Sterol 117™ supplementation on specific cardiovascular ratios in experimental and placebo groups from day 0 to day 28.

Cardiovascular Parameters Ratios	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
TC/HDL	2.88	2.61	-9.4%**	3.50	3.56	1.7%
LDL/HDL	1.58	1.30	-17.7%**	2.11	2.10	-0.5%
TG/HDL	0.66	0.68	3.0%	0.85	1.02	20.0%

** statistically significant, p<0.05

Cholesterol is an extremely important biological molecule that has roles in membrane structure as well as being a precursor for the synthesis of the steroid hormones and bile acids. Both dietary cholesterol and that synthesized de novo are transported through the circulation in lipoprotein particles. The same is true of cholesterol esters, the form in which cholesterol is stored in cells.

The synthesis and utilization of cholesterol must be tightly regulated in order to prevent over-accumulation and abnormal deposition within the body. Of particular importance clinically is the abnormal deposition of cholesterol and cholesterol-rich lipoproteins in the coronary arteries. Such deposition, eventually leading to atherosclerosis, is the leading contributory factor in coronary artery disease.

Cholesterol is minimally soluble in water; it cannot dissolve and travel in the water-based blood stream. Instead, it is transported in the blood stream by lipoproteins, i.e. protein “molecular-suitcases” which are water soluble and carry cholesterol and fats internally. The proteins form part of the surface of the given lipoprotein particle and determine from what cells cholesterol will be removed and where it will be supplied.

The largest lipoproteins, which primarily transport fats from the intestinal mucosa to the liver are called chylomicrons. They carry mostly triglyceride fats and cholesterol (both from food and especially internal cholesterol secreted by the liver into the bile). In the liver, chylomicron particles give up triglycerides and some cholesterol and are converted into low-density lipoprotein (LDL) particles which carry triglycerides and cholesterol on to other body cells. In healthy individuals the low-density lipoprotein (LDL) particles are large and relatively few in number. Conversely, large numbers of small low-density lipoprotein (LDL) particles are strongly associated with promoting atheromatous disease within the arteries.

High-density lipoprotein (HDL) particles transport cholesterol back to the liver for excretion, but vary considerably in their effectiveness for doing this. Having large numbers of large HDL particles correlates with better health outcomes. Conversely, having small amounts of large HDL particles is strongly associated with atheromatous disease progression within the arteries.

The cholesterol in LDL cholesterol and the cholesterol in HDL cholesterol are identical. The only difference between the two is the carrier protein molecules (i.e. the lipoprotein).

High cholesterol levels has been shown in many trials to be the source of cardiovascular disease. High cholesterol is the best known of all the many threats to a healthy heart. When excess amounts of this waxy, fat-like substance build up along the walls of the arteries, you face a dramatically higher risk of a complete blockage, leading to a heart attack or stroke.

At normal levels, cholesterol is not a bad thing. On the contrary, it’s an essential raw material used by the body to build cell walls and produce hormones such as estrogen and testosterone. The body produces its own supply of cholesterol in the liver, and it’s found naturally in all animal products (such as meats, eggs, milk, and cheese). It poses a problem only when the body is unable to use or eliminate excessive supplies.

As one of a variety of fatty substances in the body, cholesterol is classified as a lipid. It is carried through the bloodstream attached to proteins, forming complexes called lipoproteins. There are two major types of lipoproteins: the low-density lipoproteins (LDL) commonly known as “bad” cholesterol, and the high-density lipoproteins (HDL) usually dubbed “good” cholesterol. It’s the “bad” LDL cholesterol that tends to form deposits on the artery walls. HDLs, on the other hand, help to clear excess cholesterol from the bloodstream. The ideal situation to aim for, then, is a low

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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