Celt Immuno Care®MD

PROUDLY MADE IN CANADA

While you might think you need to "boost" your immune system to minimize or prevent symptoms, the truth is you need to "regulate" your immune system to allow it to better respond to different conditions.

Celt Immuno-Care® is a patented and clinically tested plant sterol and antioxidant supplement that regulates your immune system by "up-regulating" it when its under-performing, such as when you're sick, and "down-regulating" it when its over reacting, such as when you're suffering from allergies.

Other supplements contain plant sterols, but there is a key reason why Celt Immuno-Care is simply better – it's the only "delayed release" plant sterol supplement available.

While other plant sterol products are destroyed in the harsh conditions of the stomach, the "delayed release" feature allows each capsule to arrive intact in the small intestine. There the capsule can release its maximum potential, and get to work regulating your immune system.

HOW IT WORKS

REGULATE YOUR IMMUNE SYSTEM*

Celt Immuno-Care® is a patented and clinically tested plant sterol and antioxidant supplement that regulates your immune system by "up-regulating" it when its under-performing, such as when you're sick, and "down-regulating" it when its over reacting, such as when you're suffering from allergies.

There are other supplements containing plant sterols, but there is a key reason why Celt Immuno-Care is simply better – it's the only "delayed release" plant sterol supplement available. While other plant sterol products are destroyed in the harsh conditions of the stomach, the "delayed release" feature allows each capsule to arrive intact in the small intestine. There the capsule can release its maximum potential, and get to work regulating your immune system.*

PLANT STEROLS*

Celt Immuno-Care® is a blend of plant sterols with a unique broad spectrum antioxidant in the form of pine bark

and an essential fatty acid complex (Cellasate™). Plant sterols, also known as phytosterols, are present in small amounts in all plants, including fruits and vegetables, seeds and legumes. It is difficult to get a sufficient amount in today's diet, so supplementation is required.

They have unique biomedical effects on animals and humans. Celt Immuno-Care contains 300mg of plant sterols, the amount shown in research to be an optimum daily dose, and an essential part of the human diet. (1)Rich in potent anti-oxidants, organic compounds and 100% natural, the proanthocyanidins and flavonoids, extracted from the bark of Radiata Pine trees, have been researched to help with heart health, allergies and many other health concerns. The powerful anti-oxidants may also help with anti- aging and DNA damage caused by free radicals.

These compounds consist of a highly active broad spectrum antioxidant mixture containing anti-inflammatory plant compounds, shown in studies to have many health supporting benefits. (2,3,4)

Cellasate™ is a proprietary patented blend of fruit and essential fatty acids sourced from selenium, flax seeds, bovine colostrum, bromelain, zinc and vitamin E. It is designed to increase the bioavailability and absorption of the nutrients contained in Celt Immuno-Care.*

1. The importance of sitosterol in human and animal nutrition. Pegel. S. African Jnl. Sc. 1997. Vol 93.
2. Pilot study on the clinical effects of dietary supplementation with Enzogenol, a flavonoid extract of pine bark and vitamin C. Shand et al, Phytother. Res. 2003; 17:490-494.
3. Comparison of antioxidant assays for flavonoid containing supplements. Free Gleseg S, Baird S. Rad Biol Med 1998; 25: S104.
4. Extractives of Pinus Radiata bark. Phenolic components. Markham KR, Porter LJ NZ Jnl. Sci. 1973 Dec; 16(4): 751-761.

IMMUNE SYSTEM – PHYTOSTEROLS AND T-HELPER CELL BALANCE
T-Helper cells are a very important part of the cell mediated response to disease and when the immune system is not in balance, we become subject to many of today's diseases. T-cells are the cells that trigger immune cells to go on a "packman" like mission, to seek and destroy viruses, fungi, yeast, parasites, especially the ones that live inside cells. Like modern day scud missiles, they lock onto cells and destroy them.*

Plant sterols have been shown to help balance the immune response, by enhancing T1- Helper cell proliferation and increasing the regulation of T2- Helper cells, to ultimately decrease conditions such as allergies, inflammation and may types of autoimmune conditions. (1,2,3).

1. The Importance of Sitosterol in Human and Animal Nutrition. Pegel et al, S. African Jnl. Sc. 1997. Vol 93.
2. Flow Cytometric Analysis of the Th1/Th2 Balance in Healthy Individuals. Breytenbach, Clark et al. Cell Biol. Int. 2001;25(1).
3. Effects of Increased Solar Radiation in Human Health. Longstreth, de Gruijl et al. United Nations Environmental Program. Ozone and Human Health Project.

CLINICAL STUDIES

IMMUNE PARAMETERS STUDY – CELT IMMUNO-CARE
CONDUCTED AT THE UNIVERSITY OF GUELPH, BY THE HUMAN NUTRACEUTICAL RESEARCH UNIT
The following randomized, double blind, placebo-controlled clinical trial with 20 subjects over 28 days demonstrated that Celt Immuno-Care® can have a beneficial effect on immune parameters. The clinical trial showed a reduction in basophils that release histamine typically found in allergic reactions. We are pleased to provide the findings of this important trial, and encourage you to contact us with any questions.

The Supplement Celt Immuno-Care

Human Nutraceutical Research Unit, J.T. Powell Bldg.
University of Guelph
Guelph, Ontario, Canada N1G 2W1
Tel:(519) 824-4120, ext. 5374
Fax: (519) 823-5247
Website: www.uoguelph.ca/hnru Maggie Laidlaw, M.Sc., Interim Director, HNRU.

OBJECTIVE:
This trial was to determine the effects on specific immune parameters and cardiovascular indices of the supplement Celt Immuno-Care containing plant sterols, pine bark antioxidants and an essential fatty acid complex. The trial was conducted at the University of Guelph as a randomized, double blind, placebo-controlled clinical trial with 20 subjects over 28 days.

ABSTRACT
A randomized, double blind, placebo-controlled clinical trial to determine the effects of Celt Immuno-Care 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.
The supplement appears to have effect on immune parameters, in particular basophils and Il-6 levels. Given these results this supplement would appear to have the potential to substantially alleviate allergic responses.

The supplement also significantly reduced circulating levels of LDL-cholesterol and increased circulating levels of HDL-cholesterol. There was a significant decrease in the ratio of TC/HDL and in the ratio of LDL/HDL-cholesterol, which corresponds to a decrease in cardiovascular risk, as these ratios are markers for a reduction in the risk for developing atherosclerosis.

RESULTS
Immune Parameters

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 (FcepsilonRI) 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:** statistically significant, p<0.0

Immune Parameters	Immuno-Care Day 0	Immuno-Care Day 28	Immuno-Care Difference Day 28-Day 0	Control Day 0	Control Day 28	Control % Difference Day 28-Day 0
IgE	472	451	-4.4%	1335	1127	-15.6%
DHEA	6.44	6.44	0 %	4.93	4.77	-3.2%
Cortisol	507	584	15.2%	490	498	1.6%
Cortisol/DHEA	94.06	108.36	15.2%	160.66	141.44	-12%
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	2.16	2.24	3.7%	2.56	2.60	1.6%
Segmented 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%

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.

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 not statistically significant, a larger study with more subjects, over a longer period of time may show significance.

Celt Immuno-Care 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 recommended, with a larger patient participation and a longer trial period to investigate other areas of immunological response.

CARDIOVASCULAR PARAMETERS

The effects of the supplement on lipid and lipoprotein parameters and cardiovascular indices are illustrated in Tables 2 and 3

Table 2:The effects of Celt Immuno-Care on blood lipid parameters in experimental and placebo groups from day 0 to day 28. **statistically significant, p<0.05

Blood Lipid Parameters (mmol/L)	Immuno-Care Day 0	Immuno-Care Day 28	Immuno-Care % 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%

Table 3:The effects of Celt Immuno-Care supplementation on specific cardiovascular ratios in experimental and placebo groups from day 0 to day 28. **statistically significant, p<0.05

Cardio-vascular Parameter Ratios	Immuno-Care Day 0	Immuno-Care Day 28	Immuno-Care % 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%

The specific objective of this portion of the trial was to determine the effects of the supplement Celt Immuno-Care 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, though not statistically significant, 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 Celt Immuno-Care 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 Celt Immuno-Care could be very beneficial to the health of hypercholesterolemic individuals at risk of developing CVD.

CONCLUSIONS
Celt Immuno-Care and its components appear to have an effect on immune parameters and, in particular, in basophils and possibly IL-6 levels. Given these changes, Celt Immuno-Care would appear to have the potential to substantially alleviate allergic responses.

Celt Immuno-Care 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 Celt Immuno-Care 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 Immuno-Care 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 hypercholesterolemic individuals at risk of developing CVD.

CELLULAR ANTIOXIDANT PROTECTION STUDY – STEROL 117
GITTE S. JENSEN, PHD1 ABSTRACT
The objective of this study was to perform an assessment of the antioxidant protection provided at the cellular level by the product Sterol 117™. The CAP-e bioassay using human erythrocytes was used for this testing, aimed at assessing one important aspect of bioavailability. The water-soluble and water-insoluble components of Sterol 117 were tested in parallel. The data showed a dose- dependent protection against intracellular oxidative damage, verifying that antioxidants in Sterol 117 were able to protect living human cells from oxidative damage.

Keywords: Antioxidant capacity, bioassay, CAP-e, oxidative damage, plant sterols.

1. INTRODUCTION

The project involved a nutritional product, Sterol 117, containing a blend of antioxidants, essential fatty acids, plant sterols, and anti-inflammatory compounds, and which is marketed as support of immune function, and recovery from fatigue syndromes.

The ingredients include multiple aqueous and non-aqueous sources of antioxidants with various other properties, including immune activating and anti-inflammatory properties.

Celt Corp needs to build onto the portfolio of data on the blend of these ingredients. A sequential strategy has been discussed, starting with selected bioassays, and moving towards a human clinical pilot study.

Based on the broad spectrum of potent antioxidants in the blend, the CAP-e antioxidant bioassay was performed as part of an initial foundation to gain insight into the antioxidant availability to living cells.

1. Dr. Gitte S. Jensen, PhD (Immunology), Research Director, NIS Labs, 1437 Esplanade, Klamath Falls Oregon 97601.

2. MATERIALS AND METHODS

2.1. Preparation of Sterol 117 for in vitro bioassay work.

Sterol 117 (Celt Corp, Calgary Canada) was received as an encapsulated dry powder. Immediately prior to testing in biological assays, aqueous and ethanol extracts were prepared from the powder in the following manner: Five hundred milligrams powder was added to either 5mL phosphate-buffered saline (PBS) or 5mL ethanol. Further dilutions were prepared in PBS.

2.2. Chemicals and reagents.

The following buffers and reagents were obtained from Sigma-Aldrich (St. Louis, MO): phosphate-buffered saline (PBS), RPMI-1640 culture medium, Histopaque 1077, and Histopaque 1119. Dichlorofluorescein diacetate (DCF-DA) was obtained from Molecular Probes (Eugene, OR), a subdivision of Invitrogen (Carlsbad, CA).

2.3. Purification of red blood cells (RBC).

After obtaining informed consent as approved by the Sky Lakes Medical Center Institutional Review Board (Klamath Falls, OR), peripheral venous blood from healthy volunteers was drawn into sodium heparin and layered onto a double-gradient of Histopaque 1119 and 1077. The vials were centrifuged at 2400 rpm for 25 minutes. The RBC

fraction was washed twice in PBS without calcium or magnesium at 2400 rpm for 10 minutes. The packed RBC was transferred into new vials and again washed twice in PBS without calcium or magnesium at 2400 rpm for 10 minutes. The RBC aliquots were stored at 4oC until used in the CAP-e assay.

2.4. Cell-based antioxidant protection of erythrocytes (CAP-e) assays.

The CAP-e assay was conducted following the method published by Honzel et al (Honzel, Carter, Redman, Schauss, Endres, & Jensen, 2008), modified to an accelerated and more sensitive microplate-based protocol. The RBC cell suspension was prepared for the CAP-e assay by adding 0.1 mL packed RBC into 10 mL PBS. The cell suspension was distributed in a V-bottom 96-well microtiter plate. Twelve wells were not treated with any source of antioxidants, and served as negative controls (6 wells) and positive controls (6 wells) for minimum versus maximum oxidative damage. An additional twelve wells were treated with a standard source of Gallic Acid, a known antioxidant compound, across 6 different serial dilutions, where each dilution was tested in duplicate. The remaining wells were treated with the isolated compounds from Sterol 117, where each compound was tested at 6 serial dilutions, and each dilution was tested in duplicate.

The RBC were incubated with the potential source of antioxidants for 20 minutes, based on testing of various incubation times on RBC antioxidant uptake of standard antioxidant compounds. Subsequently, antioxidants not absorbed by the cells were removed by washing twice in PBS at 2400 rpm for 2.5 min. The cells were lysed and the precursor dye was added to the wells. Incubation was performed at room temperature for 15 minutes. Oxidation was carried out June 2010. Cellular antioxidant protection by Sterol 117 3 of 5 using the peroxyl free radical generator AAPH for 1 hour. A measure of oxidative damage was the intensity of green fluorescence, as measured at 488 nm using a T ecan Spectrafluor plate reader (40 flashes, optimal gain). The inhibition of oxidative damage was calculated as the reduced fluorescence intensity of product-treated cells, compared to cells treated only with the oxidizing agent. The CAP-e value reflects the IC50 dose of the test product. This is then compared to the IC50 dose of the known antioxidant Gallic acid. The CAP-e value was expressed as Gallic acid equivalent (GAE) per gram.

3. RESULTS

Cell-based Antioxidant Protection assay Aqueous and ethanol extracts were prepared in parallel, to allow testing of both water-soluble and water-insoluble compounds in the test product.

The graphs in Figure 1 show the results of the CAP-e test on both the PBS and the ethanol extracts of Sterol 117. Cells cannot endure more than 2% ethanol, therefore the ethanol extract started at a lower concentration than the PBS extract and both were serially diluted 2-fold.

Both extracts showed a dose-dependent antioxidant protection of red blood cells. Thus, we can conclude that both water- soluble and water-insoluble antioxidants are present in the product in forms that are available to provide protection to live cells. The doses used of Sterol 117's PBS extract allowed the % inhibition to reach an IC50. The IC50 is a measure of the effectiveness of a compound in inhibiting (in the case of the CAP-e assay) oxidative damage. If

the product is potent enough to show more than 50% inhibition within the dose range tested, then an IC50 can be calculated.

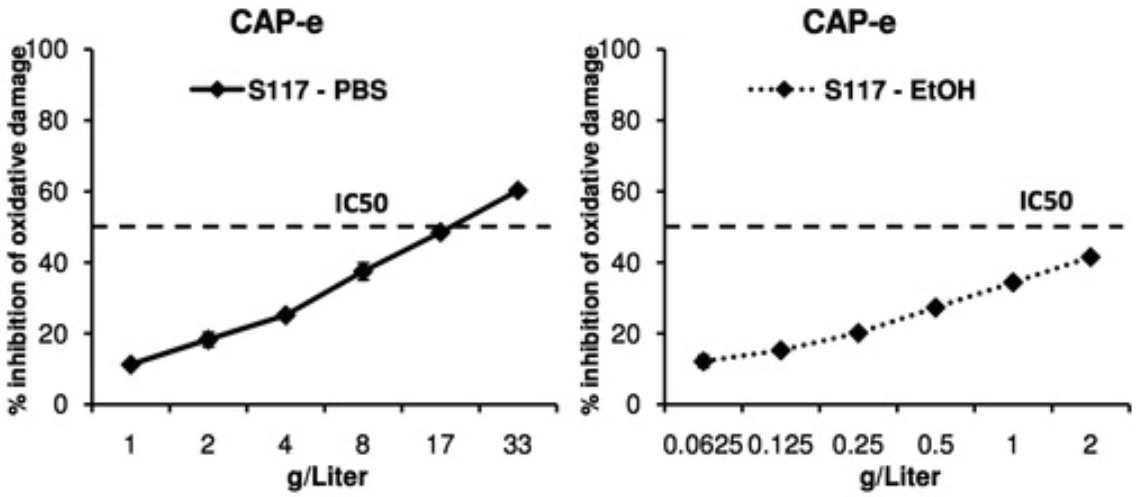


Figure 1. Dose dependent cellular antioxidant protection by Sterol 117, when using aqueous (left panel) versus non-aqueous (right panel) extraction. The dashed line indicates the level where 50% inhibition was reached (IC50).

The point on the graph where the dashed IC50 line intersects the curve reflects the IC50 dose of the test product, i.e. the dose that provided 50% inhibition of oxidative damage. This IC50 dose is compared to the IC50 dose of the known antioxidant Gallic Acid (which is used as a control in the assay), resulting in a CAP-e value reported in Gallic Acid equivalent units.

The PBS extract of Sterol 117 indeed reached an IC50 giving it a CAP-e value of 1.7 CAP-e units per gram.

Even though the diluted EtOH extract did not reach an IC50 at the doses tested, it was clearly more efficient – i.e. contained more antioxidants per weight, capable of providing a biological protection. This suggests that non-water-soluble antioxidants contribute more to the overall antioxidant protection than the water-soluble antioxidants. The ethanol extraction is not identical to the human digestion but allows assessment of compounds that are likely to be made available through the digestive process.

The graph below is a comparison of the two extracts from the test product. The ethanol extract shows a higher antioxidant protection since it provided greater inhibition of oxidative damage to the cells at the lower doses than the PBS extract. For example, this can be seen when comparing the antioxidant protection at 1g/L where the aqueous extract provided 11% protection, in contrast to the 34 % protection provided at the same 1 g/L dose of product in an ethanol extract.

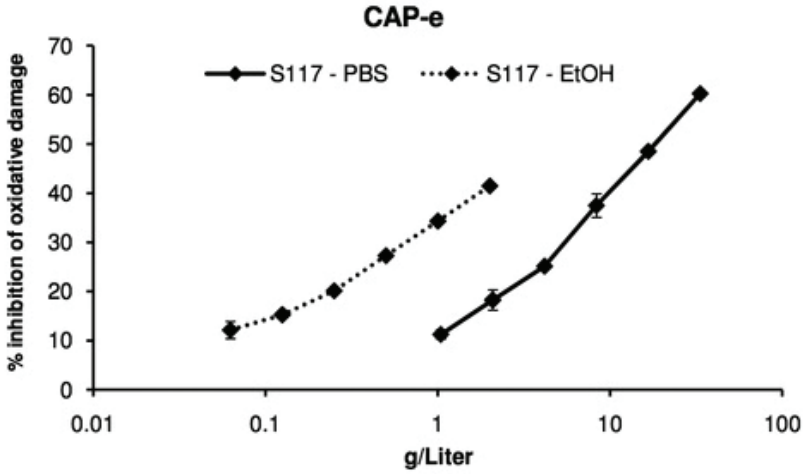


Figure 2. Comparison of the cellular antioxidant protection by Sterol 117, when using aqueous (solid line) versus non-aqueous (dashed line) extraction.

4. DISCUSSION

Antioxidant capacity of Sterol 117 was measured by the recently developed cell- based antioxidant protection (CAP-e) assay [1-4]. Data from the CAP-e assay reflects whether antioxidants can enter into and protect live cells from oxidative damage.

The rationale behind the CAP-e method is important: It allows assessment of anti- oxidant potential in a method that is comparable to the ORAC test, but only allows measurement of anti-oxidants that are able to cross the lipid bilayer cell membrane.

As a model cell type, we use the red blood cell (RBC). This is an inert cell type, in contrast to other cell types such as PMN cells, where pro-inflammatory compounds may induce the reactive oxidative burst, or anti-inflammatory compounds may perform cellular signaling and change the behavior of the PMN cell, at doses many times below levels of detection for antioxidants. We developed this assay particularly to be able to assess antioxidants from complex natural products in a cell-based system [1].

The data showed that Sterol 117 contained both water-soluble and water- insoluble antioxidants capable of providing a biologically meaningful antioxidant protection of living cells, in that they were able to protect living human cells form oxidative stress-related damage, and warrants further clinical study in humans.

REFERENCES

1. Honzel D., Carter S. G., Redman K. A., Schauss A. G., Endress J. R., & Jensen G. S. (2008). Comparison of chem-

ical and cell-based antioxidant methods for evaluation of foods and natural products: generating multifaceted data by parallel testing using erythrocytes and polymorphonuclear cells. Journal of Agricultural and Food Chemistry, 56(18), 8319-8325.

2. Jensen G. S., Wu X., Patterson K. M., Barnes J., Carter S. G., Scherwitz L., et al. (2008). In vitro and in vivo antioxidant and anti-inflammatory capacities of an antioxidant-rich fruit and berry juice blend. Results of a pilot and randomized, double-blinded, placebo- controlled, crossover study. Journal of Agricultural and Food Chemistry, 56(18), 8326-8333.

3. Schauss A. G., Jensen G. S., Wu X., & Scherwitz L. (2009). Increased antioxidant capacity and inhibition of lipid

peroxidation in healthy adults consuming Monavie Active, an acai (Euterpe oleracea) fruit based juice. Acat horticulturae, 841, 97-100.

4. Jensen G. S., Schauss A. G., Beaman R., Ager D. M. (2009). Acai fruit (Euterpe oleracea Mart.): systematic and collaborative study of the phytochemistry, nutrient composition, and in vitro and in vivo bioactivities of the Amazonian palm fruit in humans. Alternative Therapies in Health and Medicine, 15(3), S90-S91.

IMMUNE MODULATION STUDY – STEROL 117

Gitte S. Jensen, PhD1 Abstract

Two bioassays targeted at key aspects of the human innate immune defense were used to examine effects of Sterol 117™ on the function of select subsets of human immune cells. The polar (water-soluble) and non-polar (water-insoluble) components of Sterol 117 were tested in parallel. The data showed that Sterol 117 activated human natural killer cells in vitro, and contains compounds that support phagocytosis as an important part of anti-bacterial immune defense. Keywords: Immune modulation, Natural Killer cells, Phagocytes, anti-viral, anti-bacterial.

1. INTRODUCTION

The project involved a nutritional product, Sterol 117, containing a blend of antioxidants, essential fatty acids, plant sterols, and other anti-inflammatory compounds, and which is marketed as support of immune function and recovery from fatigue syndromes. The ingredients include multiple aqueous and non- aqueous sources of antioxidants with various other properties, including immune activating and anti-inflammatory properties.

The initial testing conducted at NIS Labs, showed that the product contains antioxidants capable of protecting live cells from free radical damage when the cells were exposed to oxidative stress. An additional pilot test provided data that suggested support of anti-viral immune defense mechanisms, since both the aqueous and ethanol extracts induced activation of Natural Killer (NK) cells.

This report presents data that further documents NK cell activation by Sterol 117 in more detail, regarding this important aspect of anti-viral immune defense. The report also documents the effect on a key aspect of our anti-bac-

terial immune defense mechanisms, phagocytosis.

1. Dr. Gitte S. Jensen, PhD (Immunology), Research Director, NIS Labs, 1437 Esplanade, Klamath Falls Oregon 97601.

2. MATERIALS AND METHODS

2.1. Preparation of Sterol 117 for in vitro bioassay work.

Sterol 117 (Celt Corp, Calgary Canada) was received as an encapsulated dry powder. Immediately prior to testing in biological assays, aqueous and ethanol extracts were prepared from the powder in the following manner: Five hundred milligrams powder was added to either 5mL phosphate-buffered saline (PBS) or 5mL ethanol. Further dilutions were prepared in PBS at physiological pH.

2.2. Chemicals and reagents.

The following buffers and reagents were obtained from Sigma-Aldrich (St. Louis, MO): phosphate-buffered saline (PBS), RPMI-1640 culture medium, Histopaque 1077, and Histopaque 1119. Dichlorofluorescein diacetate (DCF-DA) was obtained from Molecular Probes (Eugene, OR), a subdivision of Invitrogen (Carlsbad, CA).

2.3. Purification of white blood cells.

After obtaining informed consent as approved by the Sky Lakes Medical Center Institutional Review Board (Klamath Falls, OR), peripheral venous blood from healthy volunteers was drawn into sodium heparin and layered onto a double-gradient of Histopaque 1119 and 1077. The vials were centrifuged at 2400 rpm for 25 minutes. The peripheral blood mononuclear cell (PBMC) and polymorphonuclear (PMN) fractions were harvested and washed twice in PBS without calcium or magnesium at 2400 rpm for 10 minutes. The cells were used immediately for testing in the NK activation and phagocytosis bioassays.

2.4. Evaluation of activation status of human NK cells in vitro.

Freshly purified human PBMC were used for these assays. The cells were plated in micro-well plates. Negative control samples in quadruplicate were left untreated. Positive control samples were treated with Interleukin-2 (IL-2). Two sets of samples were treated with serial dilutions of test product. One set was only treated with test product to evaluate the direct effect of the test product on NK cell activation. The second set was also treated with IL-2, to see whether the test product affects the response of NK cells to the known NK cell activator IL-2.
After 18 hours of culture, cells were stained with fluorescent markers for the T cell antigen CD3, the antigen CD56, and the activation molecule CD69. The expression level of CD69 on the surface of CD3-negative, CD56-positive NK cells was analyzed as a measure of mean fluorescence intensity (MFI). The analysis allows us to detect if compounds

in a test product directly activates NK cells in vitro. It also provides indication of whether a test product enhances or otherwise affects the response of NK cells to IL-2. This would be an important indication of whether the product may support an already ongoing anti-viral immune response.

2.5. Evaluation of phagocytic activity.

Freshly purified peripheral blood PMN cells were pretreated with test products for 3 minutes, and then introduced to fluorescent micro-particles mimicking August 2010. Immune modulation by Sterol 117 3 of 8 bacteria. The cells were allowed to ingest particles for 2 minutes, after which free micro-particles were removed by centrifugation. The fluorescence intensity of phagocyte cells was then evaluated by flow cytometry.

3. RESULTS

Effect on activation of NK cells: CD69 expression levels. Treatment of PBMC, which is the white blood cell fraction containing the NK cell subset, resulted in increased expression of CD69 on the surface of the NK cells. Both S117 PBS and S117 EtOH increased the expression of CD69 on the cell surface of CD3- CD56+ NK cells.

This effect was dose-dependent for both product preparations with S117 EtOH performing better than S117 PBS. For S117 EtOH the three highest concentrations of product, and for S117 PBS the two highest concentrations.

The treatment with test product may result in more phagocytes deciding to engage in phagocytosis, and may also lead to a faster or stronger rate of phagocytosis, resulting in higher numbers of fluorescent micro-particles per cell. The data analysis examined both aspects of the phagocytic activity of the cells produced statistically significant increases in CD69 expression above baseline ($P<0.05$). When the effects of S117 PBS and S117 EtOH on NK cell activation were assayed in the presence of the known NK cell activator IL-2, both products showed synergy with IL-2 and increased CD69 expression to levels higher than those resulting from IL-2 treatment alone. This effect was very pronounced with the S117 EtOH product where the 0.25 g/L and 0.063 g/L doses resulted in a doubling of CD69 expression on NK cells ($P<0.02$).

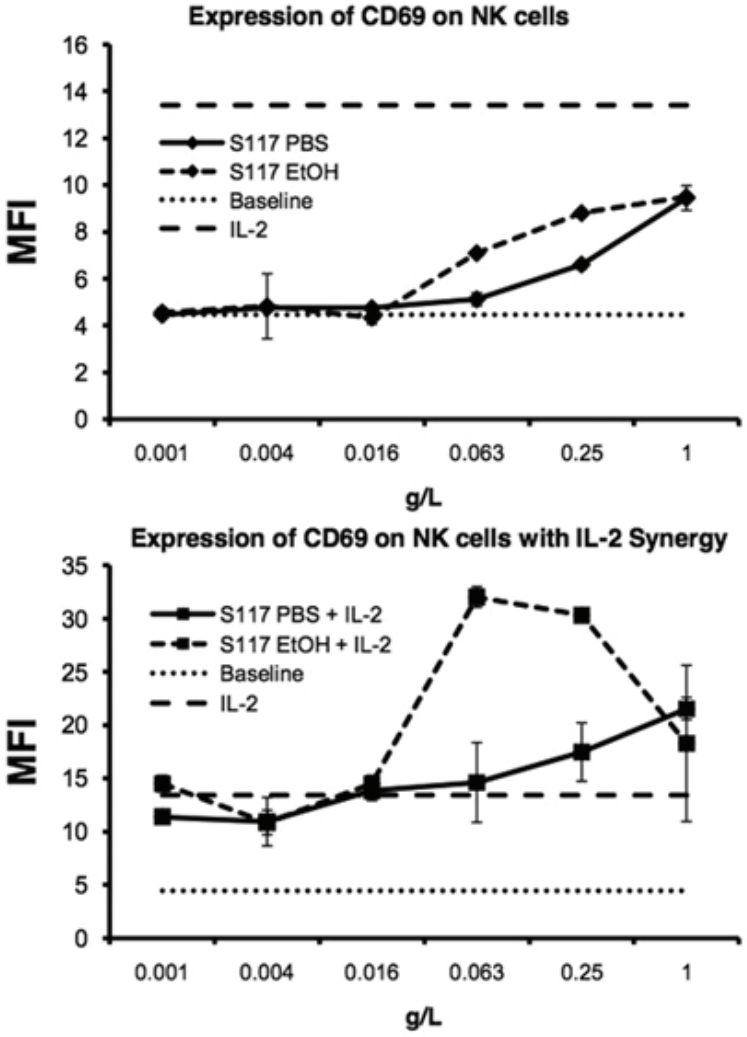


Figure 1. A) Sterol 117 directly activates human NK cells in vitro. Dose dependent activation of NK cells by Sterol 117 was seen both when using aqueous (PBS) versus non-aqueous (EtOH) extraction. B) Synergistic enhancement of NK cell activation in the presence of IL-2. The dotted line indicates the baseline level of CD69 expression on the NK cells. The broadly dashed horizontal line indicates the level of CD69 expression achieved when NK cells were activated by the known stimulus IL-2. In addition to evaluating the mean fluorescence intensity (MFI) of the CD69 activation marker, analysis was also performed to evaluate if the relative number of CD69-positive NK cells changed with treatment by test product. The highest dose of S117 PBS also produced a statistically significant increase in the percent of NK cells result when NK cells were treated with expressing CD69 that was greater than IL-2 alone ($P<0.05$).

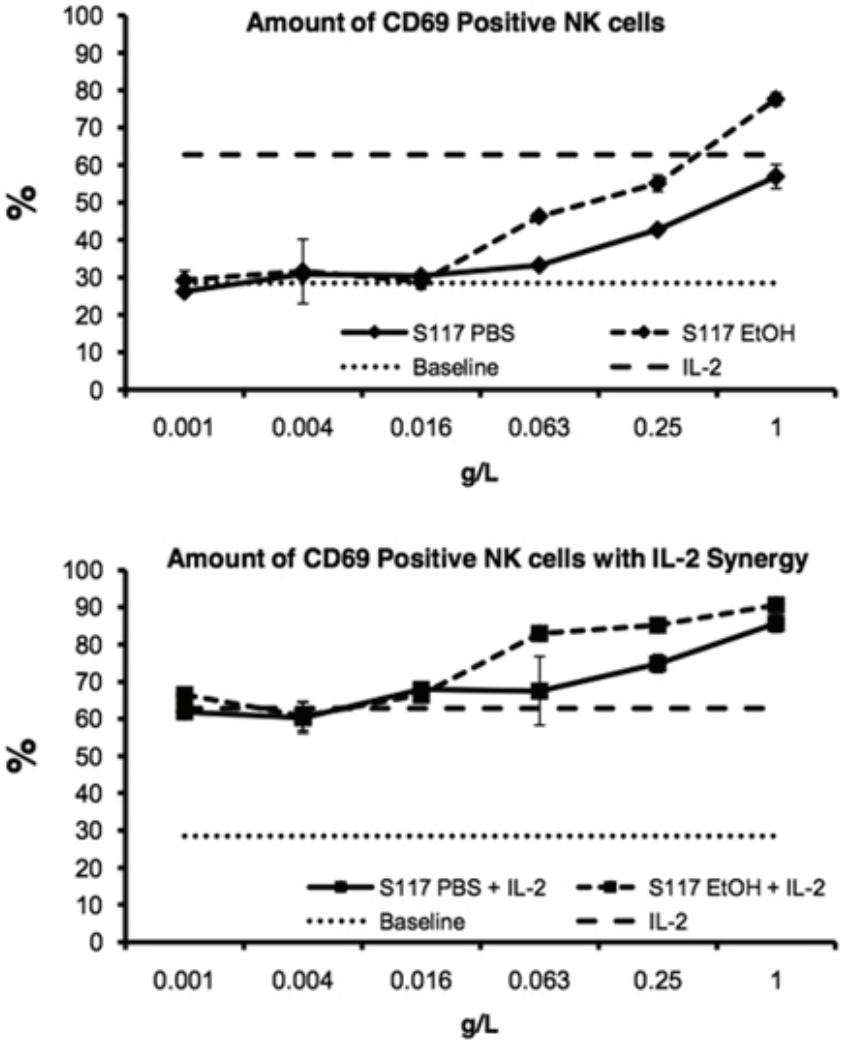


Figure 2. Sterol 117 increases the numbers of NK cells that express the CD69 marker, both in a direct manner (top graph) and in synergy with IL- 2 (bottom graph). A dose dependent increase was seen both when using aqueous (PBS) versus non-aqueous (EtOH) extraction.

Effect on activation of Natural Killer T (NKT) cells

Cell subset called NKT cells is almost In most normal healthy human donors, non-detectable. During analysis, we the proportion of a rare CD3+ CD56+ found that two out of the three donors had detectable levels of NKT cells. This allowed us to gather pilot data on how the test product may affect the activation status of this type of cell, known to conduct rapid immune modulating effects including cytokine secretion.
Expression of CD69 on NKT cells was increased by treatment of cells with S117 PBS and S117 EtOH.

In the presence of IL-2, S117 EtOH nearly quadrupled the expression level of CD69 on NKT cells when assayed at the 0.25 g/L concentration. This increase was much higher than the effect of IL-2 alone and was highly statistically significant (P<0.003).

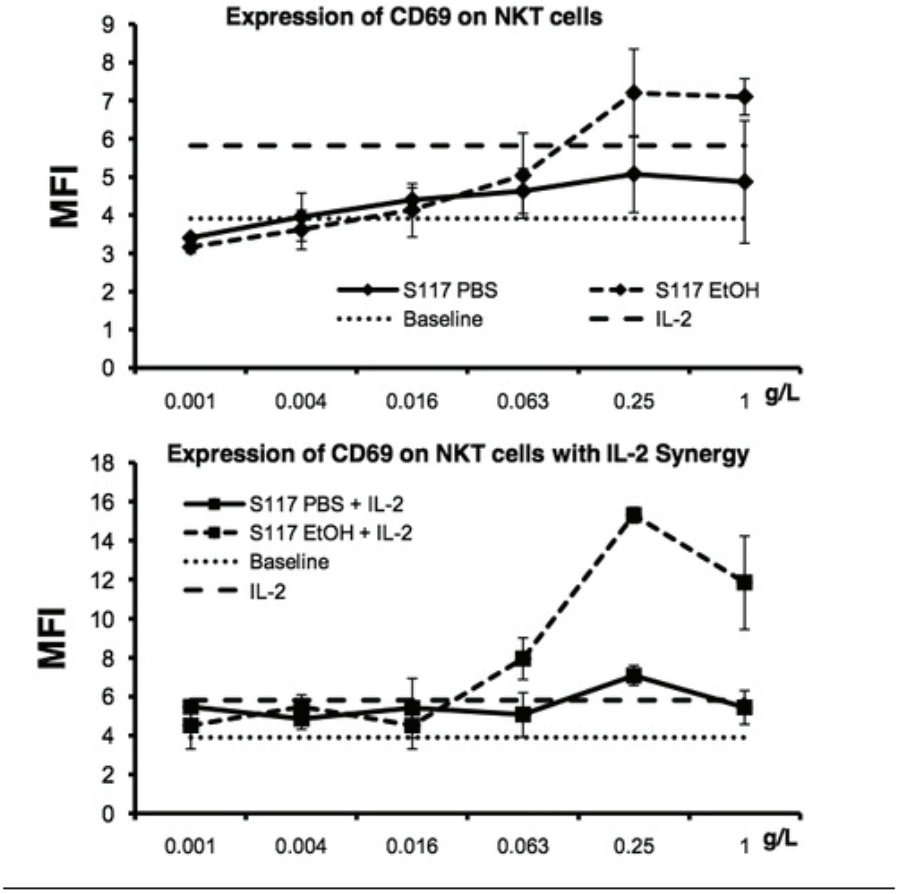


Figure 3. A) Sterol 117 directly activates human NKT cells in vitro. Dose dependent activation of NKT cells by Sterol 117 was seen both when using aqueous (PBS) versus ethanol (EtOH) extraction. B) Synergistic enhancement of NTK cell activation in the presence of IL-2 was seen for the EtOH extract.

Phagocytic activity.

When measuring the percent of PMN cells that were actively engaged in phagocytic activity, it was seen that treatment with S117 EtOH at the two highest doses tested, showed an effect on increasing this percent. Treatment of cells with S117 EtOH also increased the number of particles ingested per phagocytic PMN cell (indicated by an increase in fluorescence intensity).

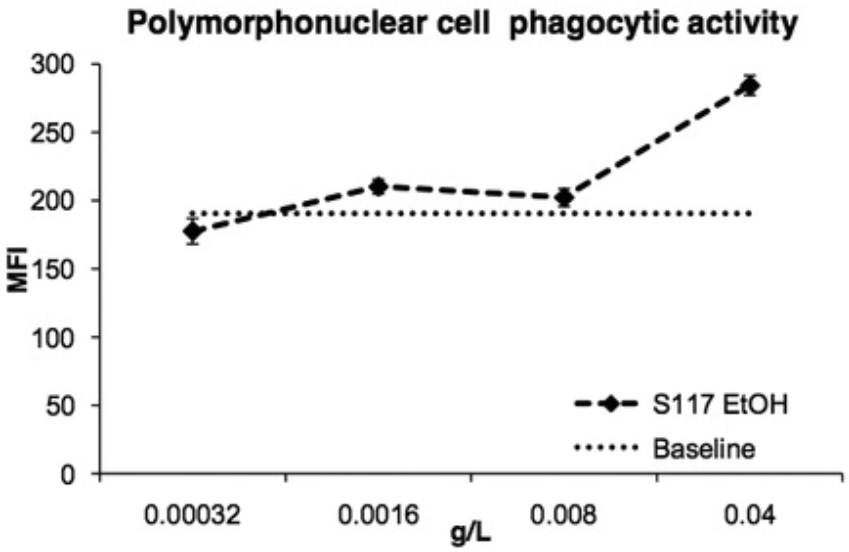


Figure 4. The treatment of human phagocytes with the EtOH extract of Sterol 117 resulted in an enhanced function of these cells in terms of the anti-bacterial behavior of engulfing foreign particles.

4. DISCUSSION

Our body's primary defense mechanisms towards cancers and viral diseases involve a group of cells called NK cells. These cells travel in our blood stream in a state of rest, but can be immediately recruited into tissues by chemical signals and activated through various mechanisms to a) kill cancer cells, b) divide and make more NK cells, and c) secrete substances that attract other cells into the site.

The data showed that Sterol 117 contained both water-soluble and water- insoluble antioxidants capable of providing a significant increase in the activation marker CD69 on both NK cells and NKT cells. Expression of the CD69 marker on NK cells has been equated to an increased cytotoxic capacity, in terms of the capacity of the NK cells to kill transformed target cells [1].

Phagocytosis of microbial particles is an important part of the innate (immediate) immune response. It is a rapid process, and the effect of test products on enhancing this cellular function is often almost immediate Sterol 117 contains compounds that are extractable by ethanol, i.e. non-polar compounds, that induce an enhanced phagocytic capacity in vitro. This may suggest that ingestion of Sterol 117 supports phagocytes in the gut mucosal lining, and possibly be supportive of anti- bacterial defense mechanisms at the gut interface.

These findings taken together suggest that Sterol 117 contains compounds that are capable of supporting two distinct function of the human innate immune defense.

REFERENCES

1. Clausen J, Vergeiner B, Enk M, Petzer AL, Gastl G, Gunsilius E. Functional significance of the activation-associated receptors CD25 and CD69 on human NK-cells and NK-like T-cells. Immunobiology. 2003;207(2):85-93.

BENIFITS

SEASONAL ALLERGIES*
Basophils produce histamine, which can lead to seasonal allergy symptoms like runny nose, itching eyes, sneezing and watery eyes.

A double blind, placebo-controlled clinical study conducted on the effects of Celt Immuno-Care® in allergic individuals, showed that over a 28 day period, users experienced a 96% decrease in basophils compared with 69% decrease in the control group.* (1)

The release of histamine can also lead to asthma, due to its ability to constrict smooth muscle. The muscle around the airways in the lungs constricts, narrowing the airways and causing shortness of breath or even complete tracheal closure, a medical emergency. (2)

Flavanoids have also been shown to be effective at decreasing allergy symptoms, Celt Immuno-Care contains Enzogenol a powerful broad spectrum antioxidant rich in flavanoids. Enzogenol™ works synergistically with the plant sterols to accomplish the goal of symptom relief. (3)

1. Pilot Clinical Trial on Celt Immuno-Care. Laidlaw M. Dept of Human Nutraceutical Res, University of Guelph, March 2005.
2. The Role of Basophils in Allergic Disease. Knol, Mul et al, Eur Respir Jnl Suppl. 1996 Aug. 22:126s-131s.
3. Flavonoids and Related Compounds as Anti–allergic substances. Kawai, Hirano et al, Allergol Int. 2007 June;56 (2):113-23.

FIBROMYALGIA*
Fibromyalgia is thought to be a disease in which the immune system is chronically overactive. One of the main culprits is a pro- inflammatory cytokine IL-6, believed to be involved in the over activity of the immune system in people with fibromyalgia. Plant sterols have been shown to decrease IL-6 by approx 25%. (1)

Fibromyalgia has also been theorized to be partially due to an increase in free radicals, with fewer antioxidants and more oxidative damage being measured in people with fibromyalgia. Celt Immuno-Care® contains important antioxidants (Enzogenol™) capable of reducing free radicals that contribute to the development of muscle pain and fatigue disorders.* (2,3)

1. Pilot study conducted at the University of Guelph, by Human Nutraceutical Research Unit on the supplement Celt Immuno-Care. Laidlaw M. Pilot Clinical Trail, March 2005.
2. Free radicals and antioxidants in primary fibromyalgia; An antioxidant stress disorder. Bagis S, Tamer L et al, Rheumatol Int. 2005 Apr;25 (3); 188-90.
3. Effects of flavonoid extract Enzogenol with vit “C” on protein oxidation and DNA damage in older human subjects. Nutr Res. 2003;(23); 1199-1210.

CHOLESTEROL*

There is no doubt that much research now confirms that the consumption of plant sterols will beneficially effect cholesterol concentrations and reduce the negative effects on cardiovascular disease.

In a double blind study at the University of Guelph, Celt Immuno-Care® significantly reduced circulating levels of LDL-cholesterol (the bad cholesterol) and increased circulating levels of HDL-cholesterol (good cholesterol). There was a significant decrease in the ratio of TC/HDL and in the ratio of LDL/HDL cholesterol, which corresponds to a decrease in cardiovascular risk, as these ratios are markers for a reduction in the risk for developing atherosclerosis. (1,2,3)

Plant sterols are safe and easy to use, with no known side effects.

The effects of Celt Immuno-Care on blood lipid parameters in experimental and placebo groups from day 0 to day 28.*

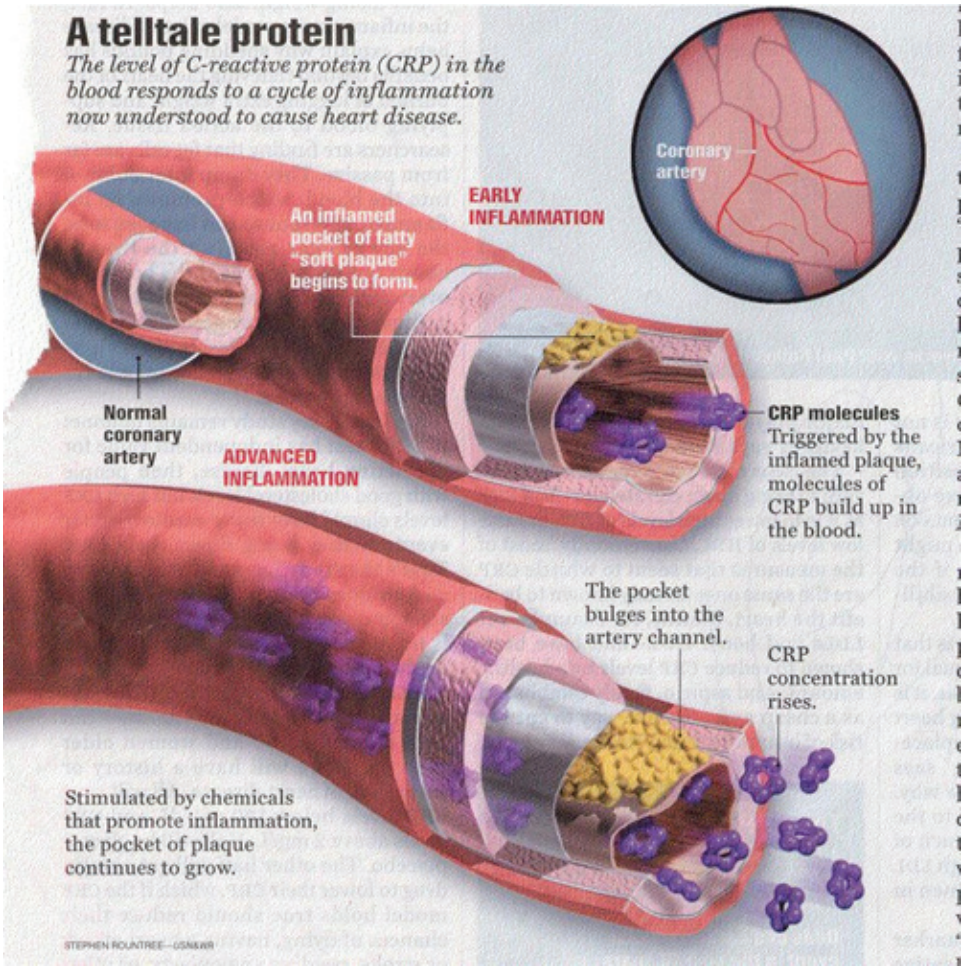
Blood Lipid Parameters (mmol/L)	Immuno-Care Day 0	Immuno-Care Day 28	Immuno-Care % Difference Day 28-Day 0	Control Day 0	Control Day 28	Control % Difference Day 28-Day 0
Total Cholesterol	4.36	4.13	-5.3%	4.87	4.91	8.2%
LDL	2.27	1.93	-15.0%**	2.85	2.87	0.7%
HDL	1.63	1.70	4.3%	1.48	1.41	-4.7%
TG	1.00	1.09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

**statistically significant, $p<0.05$

1. Pilot Clinical Trial on Celt Immuno-Care. Laidlaw M Dept Human. Nutraceutical Research, University of Guelph, March 2005.
2. Plant Sterols and Stanols Lower LDL-cholesterol. Concentrations in Hypercholesterolemic Persons. Vanstone, Raeini-Sarjaz et al. An. Jnl. Nutr 2002 Dec:76(8).
3. Optimising the Effect of Plant Sterols on Cholesterol. Absorption in Man. Mattson Grundy et al, Am. Jnl. Clin. Nutr. 1082 Apr;35 (4).

INFLAMMATORY DISEASE*

Chronic inflammation is involved in diseases as diverse as hardening of the arteries, heart valve dysfunction, obesity, diabetes, rheumatoid arthritis and others. Whenever there is an increase in IL-6, there is trouble. There is good correlation between spiked levels of IL-6 and the conditions above. Celt Immuno-Care® showed in a clinical trial a reduction in IL-6.

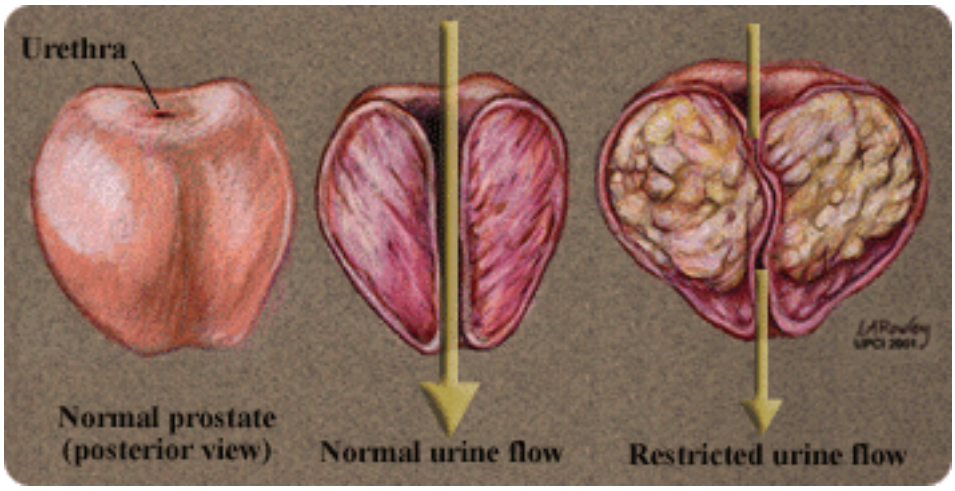


The other marker that affects disease is C-reactive protein, a molecule that is released in the blood in response to inflammation. This marker is now being used to determine the amount of inflammation in the body. It is being used in conjunction with other tests especially in determining the risk factors for CVD. Plant sterols have been shown to reduce CRP.*

1. Pilot Clinical Trial on Celt Immuno-Care. Laidlaw M. Dept Human Nutraceutical Research, University of Guelph, March 2005.
2. C-Reactive Protein, Interleukin-6 as Predictors of Coronary Heart Disease.Tge Prime Study. Artt. Throm. Luc, Bard et al. Vasc Biol. 2003;23;1255.

3. Direct Proinflammatory Effect of C-Reactive Protein on Human Endothelial Cells. Pasceri, Willerson et al. Circulation 2000:102:2165.
4. Relations of C-Reactive Protein and Interleukin-6 with Silent Brain Infarction. Hoshi, Kitagawa et al. Stroke 2005;36:768.

PROSTATE ENLARGEMENT (BPH)*
Plant sterols and in particular betasitosterol, have been found in numerous studies to decrease symptoms of an enlarged prostate. Clinical studies show that betasitosterol, a major component in Celt Immuno-Care®, is one of the most effective plant-derived options available for BPH. It can increase urinary flow, help prevent frequent bathroom visits.*



1. Klippel. Double Blind clinical trial of beta sito sterol for BPH. BR.J.UROL.1997 sept 80 (3).

*DISCLAIMER
The statements made about specific products on this web site have not been evaluated by the United States Food and Drug Administration (FDA) and are not intended to diagnose, treat, cure or prevent disease. All information provided on this web site is for informational purposes only and is not intended as a substitute for advice from your physician or other health care professional. You should not use the information on this web site for diagnosis or treatment of any health problem. Always consult with a healthcare professional before starting any new vitamins, supplements.
Immuno-Care is available for sale in the USA under the name tradename – Sterol 117™