

EFFECT OF CITRUS FLAVONOIDS AND TOCOTRIENOLS ON SERUM CHOLESTEROL LEVELS IN HYPERCHOLESTEROLEMIC SUBJECTS

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Context • Preliminary studies have suggested that both citrus flavonoids and palm tocotrienols reduce cholesterol levels in laboratory animals.

Objective • To examine the effect of these nutrients in combination on blood levels of cholesterol and related cardiovascular disease risk factors.

Design • Two open-label studies and 1 double-blind study are reported.

Setting • Outpatient clinical research setting.

Patients • Three groups (n=10, n=10, n=120) of hypercholesterolemic men and women (cholesterol levels >230 mg/dL) between the ages of 19 and 65 years were recruited.

Intervention • Subjects were randomized to consume either 270 mg citrus flavonoids plus 30 mg tocotrienols (S) or placebo

(P) daily for a period of 4 weeks (group 1 [G1] and group 2 [G2]) or 12 weeks (group 3 [G3]).

Main Outcome Measures • Measurements of fasting levels of blood cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides were made at baseline and 4 weeks (all groups) and at 8 weeks and 12 weeks (G3).

Results • Daily treatment with S significantly improved cardiovascular parameters compared to P in all groups. Significant reductions were shown in total cholesterol (20%-30%), LDL (19%-27%), apolipoprotein B (21%), and triglycerides (24%-34%). HDL levels remained unchanged in G1 and G2 but increased 4% (nonsignificant) in G3 and was accompanied by a significant increase in apolipoprotein A1 (5%). (*Altern Ther Health Med.* 2007;13(6):44-48.)

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DISCLOSURE

The work was funded by KGK Synergize, London, Ontario, and performed at the Universities of Guelph and Western Ontario. Roza is an employee of SourceOne Global Partners, which distributes polymethoxyflavones and tocotrienols. Liu served as a paid consultant to SourceOne in the preparation of this manuscript but holds no other financial interest in the extracts studied or in either KGK Synergize or SourceOne. Guthrie is president/chief executive officer of KGK Synergize and holds patents related to the use of the studied extracts for cardiovascular disease.

Cardiovascular disease is the number one cause of death in America. According to the American Heart Association, approximately 20% of Americans have high blood cholesterol.¹ Lowering blood cholesterol is an advised strategy to help reduce the risk of cardiovascular disease. The National Heart, Lung, and Blood

Institute recently revised their guidelines for healthy cholesterol and triglyceride levels for the US population (Table 1).² By lowering the cut-off point for total and low-density lipoprotein (LDL) cholesterol, these recommendations suggest a need for greater focus by the US population on reducing and maintaining healthy cholesterol and triglyceride levels.

A number of epidemiological studies provide evidence that consumption of fruit and vegetables is associated with reduced risk of cardiovascular disease.³ This cardioprotective effect is thought to be due, at least in part, to minor components of fruit and vegetables called flavonoids.³ Previous studies have shown that flavonoids and limonoids from citrus, as well as unextracted citrus juices, have cholesterol-lowering properties.⁴⁻⁸ In a human liver cell line (HepG2), principal flavanones from oranges and grapefruit, hesperetin and naringenin, both reduced the medium content of apolipoprotein B, the main protein component of LDL, with IC₅₀ concentrations 43.0 and 48.5 mg/mL medium, respectively.⁴ A more pronounced reduction of medium apolipoprotein B in HepG2 cells has been observed for a principal citrus limonoid, limonin (IC₅₀ 20.5 mg/mL),^{4,5} and especially for polymethoxyflavones (PMFs) from tangerines, tangeretin (IC₅₀ 2.5 mg/mL).^{5,6}

In animal experiments, citrus flavonoids alone or in combination with limonoids also produced hypolipidemic responses in vivo.⁷ In hamsters fed a hypercholesterolemic diet for 5 weeks, dietary supplementation with 0.1%-1% citrus PMFs containing

TABLE 1 Cholesterol and Triglyceride Guidelines*

Total Cholesterol Level	Total Cholesterol Category
<200 mg/dL	Desirable
200-239 mg/dL	Borderline high
≥240 mg/dL	High
LDL Cholesterol Level	LDL Cholesterol Category
<100 mg/dL	Optimal
100-129 mg/dL	Near optimal
130-159 mg/dL	Borderline high
160-189 mg/dL	High
≥190 mg/dL	Very high
HDL Cholesterol Level	HDL Cholesterol Category
<40 mg/dL	A major risk factor for heart disease
40-59 mg/dL	Adequate
≥60 mg/dL	Considered protective against heart disease
Triglyceride Level	Triglyceride Category
<150 mg/dL	Optimal
150-199 mg/dL	Borderline high
≥200 mg/dL	High

*LDL indicates low-density lipoprotein; HDL, high-density lipoprotein

largely tangeretin significantly reduced serum total and lipoprotein cholesterol as well as serum triacylglycerols.⁵ Consistent with these findings, improvement of blood lipid profile was also observed in animal studies and in human trials using citrus juices.^{6,7} In casein-fed rabbits, orange juice and grapefruit juice reduced LDL cholesterol by 43% and 32%, respectively.⁷ In moderately hypercholesterolemic human subjects, the inclusion of 3 glasses of orange juice per day for 4 weeks increased HDL cholesterol by 21% and reduced the LDL/HDL cholesterol ratio by 16%.⁸

In addition to lowering blood lipids, citrus PMFs have been shown to exert other biological activities that could potentially reduce the risk of heart disease. Recent studies show that PMFs strongly inhibit early stages of inflammation by reducing the release of inflammatory cytokines from stimulated human monocytes⁹ and possess anti-hypertensive effects in animals.¹⁰ Hypertension is known as an important risk factor for heart disease, and chronic systemic inflammation has been identified as a factor in the early stages of atherosclerotic lesion formation.¹¹ PMFs also may have other cardioprotective properties, as related flavonoids are known to improve endothelial function by increasing nitric oxide-dependent vasodilation.¹²

Tocotrienols are natural analogues of vitamin E found mainly in palm oil and cereal grains. Numerous laboratory and clinical studies have shown tocotrienols to possess cholesterol-lowering effects—chiefly via a suppression of cholesterol synthesis in the liver.^{13,14} Recent cell-culture studies have established that

tocotrienols reduce medium content of apolipoprotein B.¹⁴ Substantial cholesterol-lowering and anti-atherogenic responses also have been observed in animals fed tocotrienol-supplemented diets.¹⁵ The evidence for the cholesterol-lowering effect of tocotrienols in humans is less clear, with some trials showing a significant reduction of LDL cholesterol and related cardiovascular risk factors^{16,17} and others showing no effect.¹⁸

Tocotrienols, like PMFs, have been shown to produce cardioprotective effects beyond their lipid-lowering action.^{19,21} They are known to have strong antioxidant properties and therefore may protect LDL from oxidation and subsequent formation of atherosclerotic plaque.^{19,20} Recent reports have demonstrated that dietary tocotrienol supplements prevent development of high blood pressure in rats¹⁹ and that the ability of tocotrienols to prevent formation of atherosclerotic lesions may be via inhibition of monocyte adherence to the vascular endothelium.^{17,20}

OBJECTIVE

In this study, we assessed the cholesterol-reducing effects of a combination of PMFs (270 mg/d) and tocotrienols (30 mg/d) in 3 groups of hypercholesterolemic subjects (n=10, n=10, n=120). The selection of this combination was based on previous in vitro and in vivo research that demonstrated its effectiveness in improving lipid profiles.

PARTICIPANTS

Male and female adults between the ages of 19 and 65 years were recruited and provided informed consent. All work was done at the Universities of Guelph and Western Ontario in accordance with ethical treatment of human subjects in clinical research. Inclusion criteria called for total cholesterol levels above 230 mg/dL and LDL >155 mg/dL. Subjects were asked to limit alcohol intake to no more than 2 drinks per day, and alcohol consumption was monitored at each visit. All subjects had to be statin-free prior to participating. Those on cholesterol-lowering medications were required to undergo a 6-week washout period prior to supplementation.

Three groups of subjects were recruited through the use of advertisements and referrals made by general practitioners.

TABLE 2 Participant Characteristics

Characteristic	Group 1 (n=10), 6 males, 4 females	Group 2 (n=10), 7 males, 3 females	Group 3 (n=120) S=30, P=30*
Age (y)			48.1±13.8
Height (cm)			169.9±10.2
Weight (kg)	86.3±12.5	80.8±10.9	82.9±18.4
Body mass index (kg/m ²)	28.8±4.6	27.4±1.8	28.7±5.5

*S indicates 270 mg citrus flavonoids plus 30 mg tocotrienols; P indicates placebo.

Participant characteristics are listed in Table 2. Participants in group 1 (n=10) and group 2 (n=10) received the supplement combination in an open-label fashion. Participants in group 3 (n=120) were randomized to receive either the supplement combination or a matching placebo in double-blind fashion. Participants in group 3 were part of a larger crossover design study that is ongoing. The first phase is reported here, with the full study results to follow in a future report. A dietary questionnaire was not included as part of this study but will be used in future investigations.

DESIGN/METHODS

Participants were asked to consume a combination of PMFs (270 mg/d) and tocotrienols (30 mg/d) (S) or a placebo (P) for 12 weeks (150 mg BID). Following informed consent and practical instructions on how to include supplements in a normal diet, blood samples (30 mL) were obtained from a forearm vein at baseline (all groups), week 4 (all groups), week 8 (group 3), and week 12 (group 3) after fasting. Total and HDL cholesterol and triglycerides were measured in whole plasma using enzymatic timed-endpoint methods (Cholesterol Kit K603-100, BioVision, Mountain View, California) in the Clinical Biochemistry Laboratory of the London Health Science Centre. The concentration of LDL cholesterol was calculated. Plasma concentrations of apolipoprotein B and apolipoprotein A1 were analyzed immunonephelometrically (BNII System, Dada-Behring Canada Inc, Mississauga, Ontario, Canada).

Statistical analysis was by analysis of variance with Dunnett's post-test. Data are reported as mean \pm standard deviation.

SAFETY/MAXIMUM TOLERATED DOSE

Prior to the PMF and tocotrienol group studies, an animal study was conducted to evaluate the highest effective dose of a combination of PMF and tocotrienols. Three groups (n=3) of mice were fed semi-purified diet containing 5% corn oil and a combination of PMFs and tocotrienols at 4 different levels (0.5%, 1.0%, 2.0%, and 4.0%). The animals were followed for 5 weeks

and observed for signs of toxicity, such as weight loss and inactivity. For each group, all animals were necropsied at termination, and tissues were examined grossly under a dissecting microscope for abnormalities. Liver, spleen, and kidney sections were examined histologically for signs of toxicity. Body weight, food consumption, and histological results indicate that the maximum tolerated dose of the supplement in the diet is 1.0%.

RESULTS

In group 1, 4 weeks of open-label treatment with the supplement combination resulted in a significant reduction in levels of total cholesterol (-25%), LDL cholesterol (-19%), and triglycerides (-24%). HDL levels were unchanged (Table 3, Figure 1).

In group 2, 4 weeks of open-label treatment with the supplement combination resulted in significant reductions in total cholesterol (-20%), LDL (-22%), apolipoprotein B, an important structural protein of LDL (-21%), and triglycerides (-28%). Additionally, subjects in group 2 showed a significant 5% increase in apolipoprotein A1, an important structural protein of HDL, but no significant change in HDL levels (Table 3, Figure 1).

In group 3, 12 weeks of double-blind, placebo-controlled treatment resulted in significant reductions in total cholesterol (-30%), LDL (-27%), and triglycerides (-34%). In addition, HDL levels increased 4% (ns), resulting in a 29% improvement in the LDL:HDL ratio (Table 4, Figure 1).

CONCLUSIONS

Approximately 80% of the body's cholesterol is produced by the liver. As a result, many patients find that attention to diet and exercise are not enough to keep cholesterol levels in a healthy range. This has led to a dramatic increase in prescriptions for drugs known as statins (eg, HMG-CoA reductase inhibitors). Statins are effective at lowering total cholesterol and LDL cholesterol levels, but they have been associated with certain negative side effects, including muscle and liver damage.^{21,22} Statins are also known to reduce tissue levels of coenzyme Q₁₀—a crucial cofactor for heart muscle contractile function.²³ Disillusioned

TABLE 3 Open Label Groups (Groups 1 and 2)—Changes in Cholesterol, Triglyceride, and Apolipoprotein Levels After 4 Weeks of Supplementation*

Variable	Group 1		Group 2	
	Pre	Post	Pre	Post
Total cholesterol (mg/dL)	266.82 \pm 34.80	201.08 \pm 27.07 [†]	255.61 \pm 35.96	205.34 \pm 18.95 [†]
LDL-C (mg/dL)	181.75 \pm 30.94	146.95 \pm 27.07 [†]	197.60 \pm 34.03	154.29 \pm 16.63 [†]
HDL-C (mg/dL)	42.54 \pm 11.60	42.54 \pm 11.60	42.92 \pm 9.67	44.08 \pm 8.51
Total triglycerides (mg/dL)	96.68 \pm 19.34	73.47 \pm 19.34 [†]	77.34 \pm 29.78	55.41 \pm 13.64 [†]
Apo A-1 (g/L)	N/A	N/A	49.50 \pm 5.8	51.82 \pm 6.19
Apo B (g/L)	N/A	N/A	51.82 \pm 6.96	40.99 \pm 4.25

*LDL-C indicates low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Apo A-1, apolipoprotein A-1; Apo B, apolipoprotein B; N/A, not applicable.

[†]Significantly different between baseline (pre) and week 4 (post).

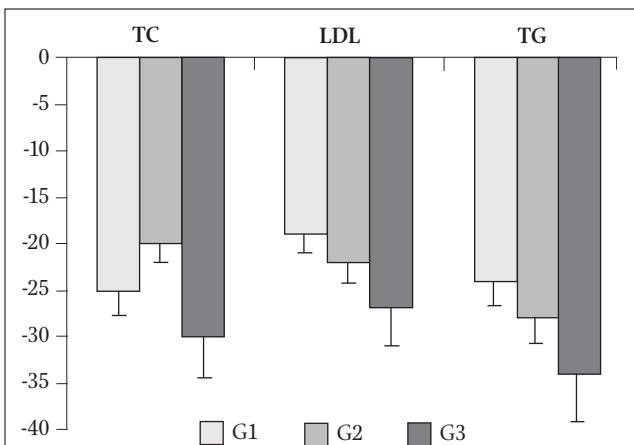


FIGURE 1 Change in Cholesterol and Triglyceride Levels—All Groups*

*TC indicates total cholesterol; LDL, low-density lipoprotein; TG, triglycerides; G1, group 1 change at 4 weeks; G2, group 2 change at 4 weeks; G3, group 3 change at 12 weeks.

with some of these potential side effects and the high cost of statins, cholesterol-conscious consumers are increasingly looking for alternatives.

PMFs are a subclass of citrus bioflavonoids, including nobiletin and tangeretin, that are distinguished by an extra methyl group. Both nobiletin and tangeretin have been studied for their anti-inflammatory and hypocholesterolemic effects.²⁴ Mechanistic studies have shown these PMFs to lower LDL levels by inhibiting the synthesis of both apolipoprotein B and triglycerides.²⁴⁻²⁷ Apolipoprotein B and triglycerides are considered to be primary “building blocks” of the LDL particle, with apolipoprotein B making up almost 90% of the LDL cholesterol complex.²⁵⁻²⁷

Palm tocotrienols are extracted from the fruit of the palm tree nut and are naturally occurring antioxidant analogs of tocopherols, a family of nutrients that includes natural Vitamin E. Tocotrienols are known to possess potent antioxidant and anti-inflammatory activity.²⁴ In addition, tocotrienols are known to regulate cholesterol production and exert a potent hypocholesterolemic effect via suppression of the HMG-CoA reductase enzyme.²⁸⁻³¹ Unlike statin drugs, which reduce the activity of the HMG-CoA reductase enzyme, however, tocotrienols appear to reduce total and LDL cholesterol levels by a novel post-transcriptional mechanism that regulates the degradation rate of the HMG-CoA reductase enzyme.²⁸⁻³¹

Other studies have demonstrated the antioxidant and anti-inflammatory benefits of PMFs and tocotrienols, and when combined with the current findings of a significant and repeatable reduction of total cholesterol, LDL cholesterol, and triglycerides, they lend further support to the overall cardioprotective benefits of these natural extracts. The results from this series of studies and others³² show that this combination of citrus PMFs plus palm tocotrienols is a promising natural option for controlling cholesterol and related cardiovascular risk factors in patients with elevated cholesterol levels. Integrative-minded physicians and allied healthcare practitioners should consider this combination of fruit extracts to be a safe and efficacious option for promoting cardiovascular wellness.

REFERENCES

1. American Heart Association. What Your Cholesterol Levels Mean. Available at: www.americanheart.org/presenter.jhtml?identifier=183. Accessed August 24, 2007.
2. *Your Guide to Lowering Your Cholesterol With TLC*. Washington, DC: US Dept of Health and Human Services; 2005. NIH publication 06-5235.
3. Cook NC, Samman S. Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. *J Nutr Biochem*. 1996;7(2):66-76.

TABLE 4 Placebo-Controlled Group (Group 3)—Changes in Cholesterol and Triglyceride Levels After 12 Weeks of Supplementation*

Measure	Treatment	Baseline	4 weeks	8 weeks	12 weeks	Δ 0-12 weeks	% change from P	% change within group
Total cholesterol (mg/dL)	P	287±33	297±36	293±32	295±34	8		+3%
	S	293±32	219±31	216±32	215±34	-78	-30%†	-27%‡
LDL-C (mg/dL)	P	204±32	209±33	206±34	207±33	3		+1.7%
	S	208±27	161±29	159±31	156±30	-52	-27%†	-25%‡
Total triglycerides (mg/dL)	P	104±19	105±19	106±20	107±19	3		+3%
	S	105±19	74±17	71±17	73±18	-32	-34%†	-31%‡
HDL-C (mg/dL)	P	45±10	44±10	45±10	45±10	NC		NC
	S	43±11	45±11	44±12	45±12	2	4%	+4%
LDL:HDL ratio	P	4.53	4.75	4.58	4.60	0.07		+1.7%
	S	4.84	3.58	3.61	3.47	-1.37	-30%†	-28%‡

*P indicates placebo; S, supplement; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; NC, no change from baseline; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

†Significantly different from placebo, $P \leq .05$.

‡Significantly different within same group, $P \leq .05$.

4. Borradaile NM, Carroll KK, Kurowska EM. Regulation of HepG2 cell apolipoprotein B metabolism by the citrus flavanones hesperetin and naringenin. *Lipids*. 1999;34(6):591-598.
5. Kurowska EM, Manthey JA, Hasegawa S, Manners GD, Vandenberg TA. Cholesterol-lowering effects of citrus juices, flavonoids and limonoids. Paper presented at: International Conference and Exhibition on Nutraceuticals and Functional Foods; September 13-17, 2000; Houston, TX.
6. Kurowska EM, Banh C, Hasegawa S, Manners GD. Regulation of Apo B production in HepG2 cells by citrus limonoids. In: Berhow MA, Hasegawa S, eds. *Citrus Limonoids: Functional Chemicals in Agriculture and Foods*. ACS Symposium Series. Washington, CD: American Chemical Society; 2000:175-184.
7. Kurowska EM, Borradaile NM. Hypocholesterolemic effects of dietary citrus juices in rabbits. *Nutr Res*. 2000;20:121-129.
8. Kurowska EM, Spence JD, Jordan J, et al. HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia. *Am J Clin Nutr*. 2000;72(5):1095-1100.
9. Manthey JA, Guthrie N, Grohmann K. Biological properties of citrus flavonoids pertaining to cancer and inflammation. *Curr Med Chem*. 2000;8(2):135-153.
10. Kumamoto H, Matsubara Y, Iizuka Y, Okamoto K, Yokoi K. Structure and hypotensive effect of flavonoid glycosides in orange (*Citrus sinensis* OSBECK) peelings. *Agric Biol Chem*. 1986;50(3):781-783.
11. Kullo IJ, Gau GT, Tajik AJ. Novel risk factors for atherosclerosis. *Mayo Clin Proc*. 2000;75(4):369-380.
12. Lemos VS, Freitas MR, Muller B, Lino YD, Queiroga CE, Côrtes SF. Dioclein, a new nitric oxide-and endothelium-dependent vasodilator flavonoid. *Eur J Pharmacol*. 1999;386(1):41-46.
13. Parker RA, Pearce BC, Clark RW, Gordon DA, Wright JJ. Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J Biol Chem*. 1993;268(15):11230-11238.
14. Guthrie N, Kurowska EM. Anti-cancer and cholesterol-lowering activities of tocotrienols. In: Wildman REC, ed. *Handbook of Nutraceuticals and Functional Foods*. Boca Raton, FL: CRC Press; 2000:269-280.
15. Black TM, Wang P, Maeda N, Coleman RA. Palm tocotrienols protect ApoE +/- mice from diet-induced atheroma formation. *J Nutr*. 2000;130(10):2420-2426.
16. Qureshi AA, Bradlow BA, Brace L, et al. Response of hypercholesterolemic subjects to administration of tocotrienols. *Lipids*. 1995; 30(12):1171-1177.
17. Qureshi AA, Bradlow BA, Salsler WA, Brace LD. Novel tocotrienols of rice bran modulate cardiovascular disease risk parameters of hypercholesterolemic humans. *Nutr Biochem*. 1997;8(5):290-298.
18. Mensink RP, van Houvelingen AC, Kromhout D, Hornstra G. A vitamin E concentrate rich in tocotrienols had no effect on serum lipids, lipoproteins, or platelet function in men with mildly elevated serum lipid concentrations. *Am J Clin Nutr*. 1999;69(2):213-219.
19. Newaz MA, Nawal NN. Effect of gamma-tocotrienol on blood pressure, lipid peroxidation and total antioxidant status in spontaneously hypertensive rats (SHR). *Clin Exper Hypertens*. 1999;21(8):1297-1313.
20. Theriault A, Chao JT, Wang Q, Gapor A, Adeli K. Tocotrienol: a review of its therapeutic potential. *Clin Biochem*. 1999;32(5):309-319.
21. Smogorzewski M. The myopathy of statins. *J Ren Nutr*. 2005;15(1):87-93.
22. Silver M, Langsjoen P, Szabo S, Patil H, Zelinger A. Effect of atorvastatin on left ventricular diastolic function and ability of coenzyme Q10 to reverse that dysfunction. *Am J Cardiol*. 2004;94(10):1306-1310.
23. Nawarskas JJ. HMG-CoA reductase inhibitors and coenzyme Q10. *Cardiol Rev*. 2005;13(2):76-79.
24. Manthey JA, Grohmann K, Guthrie N. Biological properties of citrus flavonoids pertaining to cancer and inflammation. *Curr Med Chem*. 2001;8(2):135-153.
25. Jamil H, Gordon DA, Eustice DC, et al. An inhibitor of the microsomal triglyceride transfer protein inhibits apoB secretion from HepG2 cells. *Proc Natl Acad Sci U S A*. 1996;93(21):11991-11995.
26. Gordon DA. Recent advances in elucidating the role of the microsomal triglyceride transfer protein in apolipo-protein B lipoprotein assembly. *Curr Opin Lipidol*. 1997;8(3):131-137.
27. Kurowska EM, Manthey JA, Casaschi A, Theriault AG. Modulation of HepG2 cell net apolipoprotein B secretion by the citrus polymethoxyflavone, tangeretin. *Lipids*. 2004;39(2):143-151.
28. Parker RA, Pearce BC, Clark RW, Gordon DA, Wright JJ. Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J Biol Chem*. 1993;268(15):11230-11238.
29. Pearce BC, Parker RA, Deason ME, Qureshi AA, Wright JJ. Hypercholesterolemic activity of synthetic and natural tocotrienols. *J Med Chem*. 1992;35(20):3595-3606.
30. Qureshi AA, Pearce BC, Nor RM, Gapor A, Peterson DM, Elson CE. Dietary alpha-tocopherol attenuates the impact of gamma-tocotrienol on Hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in chickens. *J Nutr*. 1996;126(2):389-394.
31. Theriault A, Chao JT, Wang Q, Gapor A, Adeli K. Tocotrienol: a review of its therapeutic potential. *Clin Biochem*. 1999;32(5):309-319.
32. Kurowska EM, Laidlaw M, Barber J, et al. Sytrinol™, a novel cholesterol-lowering supplement. Paper presented at: Canadian Federation of Biological Sciences Annual Meeting; June 21-25, 2005; Guelph, Ontario, Canada.