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Arterial Compliance and Vitamin E Blood Levels with a Self Emulsifying Preparation of Tocotrienol Rich Vitamin E

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The tocotrienol vitamin E has potent antioxidant property, however absorption is low due to high lipid solubility. A self emulsifying preparation of tocotrienol rich vitamin E (SF-TRE) had been reported to increase their bioavailability. This randomized, placebo controlled, blinded end point clinical study aimed to determine the effects of 50, 100 and 200 mg daily of SF-TRE and placebo for two months on arterial compliance and vitamin E blood levels. Assessment of arterial compliance by carotid femoral pulse wave velocity (PWV) and augmentation index (AI), plasma vitamin E, serum total cholesterol and low density lipoprotein cholesterol were taken before and after 2 months' treatment in 36 healthy males. Un-supplemented tocotrienol levels were low, after treatment, all SF-TRE treated groups had significantly higher plasma α, δ and δ tocotrienol concentrations compared to placebo. Augmentation index change from baseline to end of treatment for groups placebo, 50, 100, and 200 mg were 2.22±1.54, -6.59±2.84, -8.72±3.77, and -6.27±2.67% respectively (p=0.049, 0.049, and 0.047 respectively). Groups 100 and 200 mg showed significant improvement after treatment with pulse wave velocity reductions of 0.77 m/s and 0.65 m/s respectively (p=0.007 and p=0.002). There was no effect of SF-TRE on serum lipids. We conclude that there was a trend towards improvement in arterial compliance with 2 months' of SF-TRE.

Key words: Tocotrienols, Vitamin E, Arterial Compliance

INTRODUCTION

Vitamin E is the most abundant lipid soluble antioxidant in human plasma and cell membranes; it limits oxidation of LDL-C (O'Byrne et al*.,* 2000; Reaven et al*.,* 1993). Supplementary vitamin E was reported to be effective in reducing atherosclerosis progression in subjects with previous coronary artery bypass graft surgery not treated with lipid-lowering drugs (Kritchevsky et al*.*, 1995). However, apart from one secondary prevention study (Stephens et al*.,* 1996), recent large interventional clinical trials had not shown cardiovascular benefits by vitamin E supplementation. These clinical studies had used tocopherol as their supplemented vitamin E. Tocotrienols (T_3) are another class of vitamin E; they are found in high concentrations in palm oil and rice bran. Besides being potent antioxidants (Serbinova et al*.,* 1991; Serbinova et al*.,* 1992), they have the potential to lower serum cholesterol by inhibiting 3-hydroxy-3-methylglutyryl-coenzyme A reductase (HMG-CoA reductase), the enzyme responsible for *in vivo* cholesterol synthesis (Parker et al*.,* 1993; Pearce et al*.,* 1992). However, results from clinical studies on their lipid lowering property had been conflicting (O'Byrne et al*.,* 2000; Qureshi et al*.,* 2002).

However, the absorption of T_3, being highly fat soluble, is highly variable and dependent on physiological processes in the gastrointestinal tract (GIT). Only with sufficient food and fat intake will there be sufficient pancreatic juice and bile secreted to emulsify T_3 for satisfactory absorption. Self emulsifying preparation of tocotrienol vitamin E was reported to increase the bioavailability and blood levels of T_3 (Yap and Yuen, 2004). The preparation in the form of a soft gelatin capsule was supposed to be able to self emulsify under gentle agitation produced in the GIT.

Decreased arterial compliance or increased arterial stiff-

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ness is a predictor of cardiovascular events not only in diseases but also in normal subjects (Cruickshank et al., 2002; Laurent et al., 2001; Willum-Hansen et al., 2006). Arterial compliance relies on the presence of an intact endothelium; arterial compliance, as assessed by pulse wave velocity (PWV) and augmentation index (AI) can be improved even in normal subjects by certain dietary interventions (Vlachopoulos et al., 2005; Wilkinson et al., 1999). This clinical study primarily aims to determine the effects of a low, medium and high dose of a self emulsifying preparation of palm based tocotrienol rich vitamin E on T_3 blood levels and arterial compliance in healthy subjects. Serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) were also measured.

MATERIALS AND METHODS

Thirty six healthy male subjects participated in this randomized, placebo controlled, blinded end point clinical study. The study protocol had been approved by the Research and Ethical Committee of Universiti Sains Malaysia; all participating subjects gave their signed informed consent. Study subjects were participants who had responded to advertisement for healthy subjects (< 40 years old) placed at the Universiti Sains Malaysia Hospital. Subjects underwent medical examination with laboratory tests (blood count, renal function test, liver function test and serum TC) prior to participation. Subjects with a history of cardiovascular diseases including valvular heart disease, serum TC>6.5 mmol/L (>250 mg/dL), and allergies to vitamin E or palm oil were excluded. Subjects were not taking any chronic medications including dietary supplements and did not consume alcohol throughout study duration.

Subjects were entered into one of 4 study groups that were placebo daily, 50 mg SF-TRE, 100 mg SF-TRE, or 200 mg SF-TRE daily for 2 months. The tocol (vitamin E) content of this preparation (Tocovid Suprabio by Hovid Bhd, Malaysia) was α-T_3 23.54%, γ-T_3 43.16%, δ-T_3 9.83%, and α-tocopherol 23.5%. Similar looking capsules containing soybean oil devoid of vitamin E and prepared by Hovid Bhd, Malaysia were used as placebo. Subjects took the capsules daily with breakfast; pill counting was performed to monitor compliance; any adverse effects during the study were queried and recorded.

Before commencing study treatments, subjects attended a baseline session where study measurements were taken. After 2 months, subjects attended another study session where study parameters were repeated. On study days, subjects had breakfast together with the study medication 6 hours before appointment, after which they were asked to fast. Study sessions were conducted in the afternoon in a quite room with an average temperature of 25ºC. On arrival to the laboratory, bloods for plasma T_3, tocopherol, and serum TC and LDL-C were taken. Serum TC and LDL-C were measured at the Chemical Pathology laboratory of the Universiti Sains Malaysia Hospital using standard, validated methods. Plasma δ, γ and α-T_3 and α-tocopherol levels were quantified using the high performance liquid chromatography (Yap et al., 1999).

Subjects lie supine for 15 minutes before an average of two blood pressure (BP) recordings was obtained using a manual sphygmomanometer (Accoson, Finland). Arterial compliance was then performed by measuring the parameters aortic femoral PWV and AI using the Sphygmocor device (PWV Medical Pty Ltd – Australia). Carotid femoral PWV measures the velocity of the pulse wave travel along the carotid-femoral segment; a higher PWV indicated lower aortic compliance and vice versa. The repeatability of the measurement assessed by intraday and interday coefficient of variations was 2.0% and 2.6% respectively. Pulse wave analysis using the Sphygmocor was used to assess systemic arterial compliance (O'Rourke and Gallagher, 1996). Radial artery pressure waveform was recorded non-invasively by applanation tonometry using a micromanometer (Millar for Atcor Medical – US). A validated transfer function is used to derive the aortic pressure waveform, enabling aortic pressures & certain central arterial indices to be measured including the parameter of interest, augmentation index. Augmentation index is the proportion of central arterial pressure that results from arterial wave reflection and is a commonly used measure of arterial stiffness or reduced compliance (O'Rourke and Gallagher, 1996). A smaller AI value indicates better peripheral arterial compliance and vice versa. The repeatability of the AI measurement assessed as intra-observer coefficient of variance was 2.01%. Average of 2 readings were recorded for measurements of PWV and AI, all measurements were taken by a single investigator who was blinded to the study treatment.

Calculation of sample size was based on the method by Florey (1993), calculated to detect mean differences in PWV and AI of 1.0 m/s and 13.45% with standard deviations of 0.66 m/s and 9.7% (Rasool et al., 2006) with a power of 90% and 80% respectively at alpha of 0.05. Paired t-test was used to compare difference in a parameter after treatment compared to baseline. Changes in a parameter as the result of treatment, computed as difference between end of treatment values and baseline (for example, change in PWV = $PWV_{baseline} - PWV_{treatment}$) were computed for each parameter and compared between groups using analysis of variance and post-hoc analysis by Scheffe's test. Non parametric equivalent tests were used for non-normally distributed data. Results are presented as mean ± standard error of the mean [sem], and $p<0.05$ taken as the statistical significance level.

RESULTS

Thirty six subjects participated in this study (mean age 23.9±0.39 years); there was overall good tolerability to the study medications, with no adverse effects needing drug withdrawal. There were no significant difference between groups in their baseline age, body mass index, BP, serum TC and LDL-C (Table I).

Baseline δ-T_3 concentrations were low and only detectable in 11 subjects, while baseline γ-T_3 and α-T_3 concentrations can be detected in 35 and 21 out of the 36 subjects. There were significant differences between placebo and all SF-TRE treated groups in their δ, γ, and α-T_3 concentrations at the end of 2 months ($p<0.0001$). There appears to be a linear dose and blood level relationship for all T_3 isomers with administration of 50, 100 and 200 mg of SF-TRE daily; meaning that as the dose was increased, the concentration also increased (Fig. 1a, b and c).

Alpha tocopherol levels were detected in all subjects before treatment. Although groups 100 and 200 mg showed rises in concentration after treatment, change in α-tocopherol after treatment compared to baseline was

Table I. Baseline and end of 2 months values for study parameters. No significant difference was seen between groups for their baseline age and BMI. Treatment had no effect on systolic blood pressure (SBP), diastolic blood pressure (DBP), serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C).

Parameter	Placebo		50 mg SF-TRE		100 mg SF-TRE		200 mg SF-TRE	
Age (years)	24.1±0.96		24.1±0.81		23.8±0.91		23.4±0.38	
Body Mass Index (kg/m^2)	22.6±0.7		23.6±1.39		21.2±1.52		25.2±1.22	
	baseline	2 months	baseline	2 months	baseline	2 months	baseline	2 months
SBP (mmHg)	125.2 ±3.1	123.9 ±3.7	122.2 ±3.5	122.7 ±3.8	120.9 ±3.3	116.5 ±3.1	124.6 ±3.7	123.4 ±2.9
DBP (mmHg)	75.8 ±2.3	72.5 ±2.8	74.1 ±2.6	73.0 ±2.7	75.0 ±2.7	73.0 ±3.1	76.4 ±3.7	73.7 ±3.6
TC (mmol/L)	5.26±0.30	5.23±0.16	4.75±0.32	4.88±0.28	4.79±0.27	5.01±0.21	5.06±0.23	5.18±0.25
LDL-C (mmol/L)	3.60±0.23	3.26±0.19	3.14±0.24	3.09±0.21	3.11±0.29	3.26±0.19	3.44±0.21	3.35±0.24

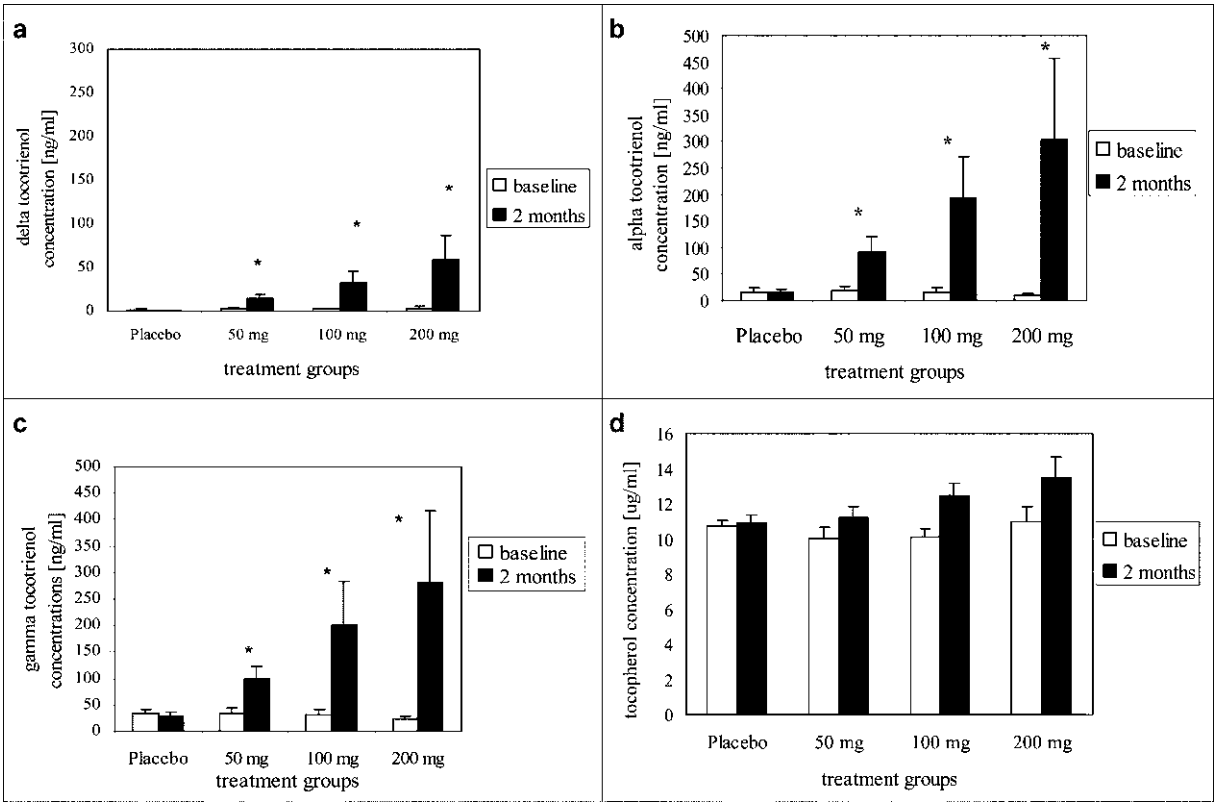

Fig. 1. a, b, c and d shows δ-T_3, γ-T_3, α-T_3, and α-tocopherol levels at baseline and after treatment for each study group. There were significant differences between placebo and all SF-TRE treated groups in their δ, γ and α-T_3 levels at the end of treatment. * indicates significant difference between placebo and treated groups for the individual T_3 isomer concentration at 2 months.

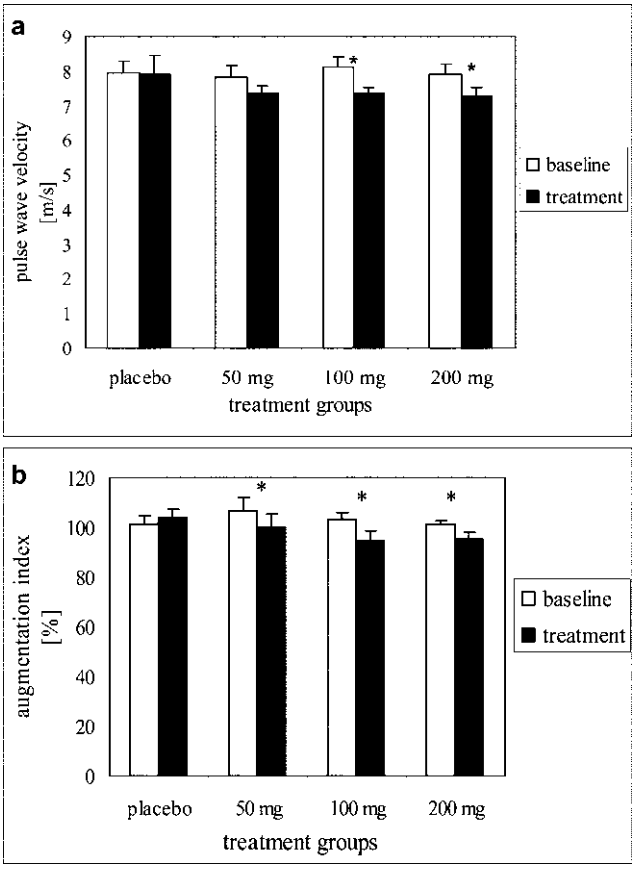

Fig. 2. a and b shows pulse wave velocity and augmentation index values at baseline and after 2 months treatment for each study group. Lower PWV and AI values indicate better arterial compliance. * indicates significant reduction in parameter after treatment compared to baseline.

not significantly different between groups. There was also no difference between the SF-TRE treated groups and placebo in their α-tocopherol levels at 2 months (Fig. 1d).

Baseline PWV and AI were not significantly different among the groups. After treatment, significant reduction in PWV was seen, reductions of 0.77 m/s (10% reduction compared to baseline) and 0.65 m/s were observed for groups 100 and 200 mg ($p=0.007$ and $p=0.002$) respectively (Fig. 2a). There were reductions in AI from baseline of 6.59%, 8.72%, and 6.27% after 2 months treatment for groups 50, 100, and 200 mg respectively ($p=0.049$, $p=0.049$, and $p=0.047$) (Fig. 2b); change in AI due to treatment was significantly different between groups ($p=0.048$). Placebo group did not show any reduction in their PWV and AI after treatment compared to baseline.

The effects of the SF-TRE on other study parameters were non remarkable; no change in BP and serum TC and LDL-C were observed due to treatment (Table I).

DISCUSSION

This study showed that the individual δ, γ, and α-T_3 isomers of the self emulsifying preparation of tocotrienol rich vitamin E were bioavailable 6 hours after supplementation from as low as 50 mg daily. Previously there were doubts about the ability of T_3 to be absorbed and remain in systemic circulation, as some human and animal studies had failed to detect T_3 isomers even on supplementation (Chen, 2004; Khor and Chieng, 1996; Khor et al., 1995; Tomeo et al., 1995). Thus, doubts were expressed by some investigators on the ability of T_3 to exert any biological effect *in vivo*.

Levels of α and γ-T_3 at 2 months appears fairly similar, although the capsules contain a higher percentage of γ-T_3 (43%) compared to α-T_3 (24%). This suggests that α-T_3 may have better absorption and bioavailability compared to γ-T_3; an observation supported by O'Byrne, and Ikeda (Ikeda et al., 1996; O'Byrne et al., 2000). As expected, δ-T_3 concentration was lowest as the percentage of δ-T_3 in this preparation was lowest, being only 9.83% of the whole tocol content.

Pharmacodynamically, there appears to be a trend of improved arterial compliance in the treated groups. For systemic arterial compliance as assessed by AI, groups 50, 100, and 200 mg all showed reductions in AI compared to baseline. There were significant reductions in PWV after treatment compared to baseline for the 100 and 200 mg groups. Treatment however, has no significant effect on blood pressure and serum TC and LDL-C.

The exact mechanisms whereby antioxidants such as T_3 may improve arterial compliance had not been determined. Because improvement could occur within weeks, vascular functional improvement is probably involved rather than structural mechanisms. Among possible mechanisms for the improvement in arterial compliance include firstly; reduction in BP contributing to improvement in arterial compliance. However, in our study no difference was seen in BP with treatment. Secondly, mechanisms involving nitric oxide (NO) production by the endothelium may be involved. Nitric oxide relaxes vascular smooth muscle, evidence also suggests NO to be important in regulating arterial stiffness (Wilkinson et al., 2002). Newaz et al. reported that γ-T_3 increased endothelial nitric oxide synthase (NOS) activity in spontaneously hypertensive rats (SHR) with a concomitant reduction in BP (Newaz et al., 2003). Endothelial nitric oxide synthase enzyme mediates conversion of arginine to NO *in vivo*. Thirdly, free radicals, had been reported to inactivate endothelium derived relaxing factor (Rubanyi and Vanhoutte, 1986), which may lead to increased peripheral resistance and arterial stiffness. Antioxidants neutralize free radicals thus may preserve the action of EDRF and maintaining

arterial compliance.

We are unable to directly compare the results of our current study with a previous one looking at the effect of a non self emulsifying preparation of tocotrienol on arterial compliance (Rasool et al., 2006). In that study, the group given 160 mg of the normal tocotrienol preparation had improved augmentation index which is associated with a reduction in aortic systolic blood pressure after 2 months' of treatment. The self emulsifying preparation currently used, was not available at that time for direct comparison study between the two preparations. The preparation used in that study has α-tocotrienol as the highest content tocotrienol (35%) followed by α-tocotrienol (25%) and the least being δ-tocotrienol. Alpha tocopherol content was 26%. The current self emulsifying preparation predominantly contained γ-tocotrienol at 43% followed by α-tocotrienol at 24% and δ-tocotrienol the least, this preparation also has less tocopherol at 23% of tocol content.

Thus, in conclusion, treatment with a self emulsifying preparation of mixed tocotrienols at doses of 50, 100, and 200 mg produced significantly higher plasma δ, α and γ-T_3 concentrations compared to placebo. There is linear dose-concentration relationship for all the isomers. There was a trend towards improvement in arterial compliance after 2 months of treatment. Treatment has no effect on BP and serum TC and LDL-C.

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REFERENCES

Chen, C. K., Effects of vitamin E [palm vitee] supplementation on exercise induced lipid peroxidation and endurance performance in the heat. PhD thesis, Universiti Sains Malaysia (2004).

Cruickshank, K., Riste L., Anderson, S. G., Wright, J., Dunn, G., and Gosling, R. G., Aortic pulse wave velocity and its relationship to mortality in diabetes and glucose intolerance; and integrated index of vascular function. *Circulation.* 106, 2085-2090 (2002).

Florey, C. V., Sample size for beginners. *BMJ.* 306, 1181-4 (1993).

Ikeda, I., Imasato, Y., Sasaki, E., and Sugano, M., Lymphatic transport of alpha, gamma and delta tocotrienols and alpha tocopherol in rats. *Int. J. Vit. Nutr. Res*., 66, 217-221 (1996).

Khor, H. T. and Chieng, D. Y., Effect of dietary supplementation of tocotrienols and tocopherols on serum lipids in the hamster. *Nutr. Res.,* 16, 1393-1401 (1996).

Khor, H. T., Chieng, D. Y., and Ong, K. K., Tocotrienols inhibit liver HMG CoA Reductase activity in the guinea pig. *Nutrition Research,* 15, 537-544 (1995).

Kritchevsky, S. B., Shimakawa, T., Tell, G. S., Dennis, B., Carpenter, M., Eckfeldt, J. H., Peacher-Ryan, H., and Heiss, G., Dietary antioxidants and carotid artery wall thickness. The ARIC study. *Circulation*, 92, 2142-2150 (1995).

Laurent, S., Boutouyrie, P., Asmar, R., Gautier, I., Laloux, B., Guize, L., Ducimetiere, P., and Benetos, A., Aortic stiffness is an independent predictor of all cause and cardiovasular mortality in hypertensive patients. *Hypertension* 37, 1236-1241 (2001).

Newaz, M. A., Yousefipour, Z., Nawal, N., and Adeeb, N., Nitric oxide synthase activity in blood vessels of spontaneously hypertensive rats: antioxidant protection by gamma-tocotrienol. *J. Physiol. Pharmacol*., 54, 319-327 (2003).

O'Byrne, D., Grundy, S., Packer, L., Devaraj, S., Baldenius, K., Hoppe, P., Kramer, K., Jialal, I., and Traber, M. G., Studies of LDL oxidation following alpha, gamma or delta tocotrienol acetate supplementation of hypercholesterolemic humans. *Free Radic. Biol. Med*., 29, 834-845 (2000).

O'Rourke, M. and Gallagher, D. E., Pulse wave analysis. *J. Hypertens,* 14, S147-S157 (1996).

Parker, R. A., Pearce, B. C., Clark, R. W. D., Gordon, A., and Wright, J. J., Tocotrienols regulate cholesterol production in mammalian cells by post transcriptional suppression of 3 hydroxy-3methylglutaryl-coenzyme A reductase. *J. Biol. Chem*., 268, 11230-11238 (1993).

Pearce, B. C., Parker, R. A., Deason, M. E., Qureshi, A. A., and Wright, J. J., Hypocholesterolemic activity of synthetic and natural tocotrienols. *J. Med. Chem*., 35, 3595-3606 (1992).

Qureshi, A. A., Sami, S. A., Salser, W. A., and Khan, F. A., Dose-dependent suppression of serum cholesterol by tocotrienol-rich fraction (TRF25) of rice bran in hypercholesterolemic humans. *Atherosclerosis,* 161, 199-207 (2002).

Rasool, A. H., Yuen, K. H., Khalid, Y., Wong, A. R., and Rahman, A. R. A., Dose dependent elevation of plasma tocotrienol levels and its effect on arterial compliance, plasma total antioxidant status and lipid profile in healthy humans supplemented with tocotrienol rich vitamin E. *J Nutr Sci Vitaminol.*, 52, 473-478 (2006).

Reaven, P. D., Khouw, A., Beltz, W. F., Parthasarathy, S., and Witzturo, J. L., Effect of antioxidant combinations inhumans; protection of LDL by vitamin E but not by Beta Carotene. *Arterioscler Thromb Vasc Biol*., 13, 590-600 (1993).

Rubanyi, G. M. and Vanhoutte, P. M., Superoxide anion and hyperoxia inactivate endothelial derived relaxing factor. *Am. J. Physiol*., 25, 822H-827H (1986).

Serbinova, E., Kagan, V., Han, D., and Packer, L., Free radical recycling and intramembrane mobility in the antioxidant properties of alpha tocopherol and alpha-tocotrienol. *Free*

Radic. Biol. Med. 10, 263-275 (1991).

Serbinova, E., Khwaja, S., Catudioc, J., Ericson, J., Torres, Z., Gapor, A., Kagan, V., and Packer, L., Palm oil vitamin E protects against ischemia/reperfusion injury in the isolated perfused Langendorff heart. *Nutr. Res.,* 12, S203-S215 (1992).

Stephens, N. G., Parsons, A., Schofield, P. M., Kelly, F., Cheeseman, K., Mitchinson, M. J., and Brown, M. J., Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet*, 347, 781-786 (1996).

Tomeo, A. C., Geller, M., Watkins, T. R., Gapor, A., and Bierenbaum, M. L., Antioxidant effects of tocotrienols in patients with hyperlipidemia and carotid stenosis. *Lipids*, 30, 1179-1183 (1995).

Vlachopoulos, C., Aznaouridis, K., Alexopoulos, N., Economou, E., Andreadou, I., and Stefanadis, C., Effect of dark chocolate on arterial function in healthy individuals. *Am. J. Hypertens*, 18, 785-791 (2005).

Wilkinson, A. B., Megson, I. L., MacCallum, H., Sogo, N., Cockroft, J. R., and Webb, D. J., Oral vitamin C reduces arterial stiffness and platelet aggregation in humans. *J. Cardiovasc Pharmacol*., 34, 690-93 (1999).

Wilkinson, I. B., Qasem, A., McEniery, C. M., Webb, D. J., Avolio, A. P., and Cockroft, J. R., Nitric oxide regulates local arterial distensibility in vivo. *Circulation*, 105, 213-17 (2002).

Willum-Hansen, T., Staessen, J. A., Torp-Pedersen, C., Rasmussen, S., Thijs, L., Ibsen, H., and Jeppesen, J., Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation*, 113, 601-3 (2006).

Yap, S. P., Julianto, T., Wong, J. W., and Yuen, K. H., Simple high performance liquid chromatographic method for the determination of tocotrienols in human plasma. *Journal of Chromatography*, 735, 279-283 (1999).

Yap, S. P. and K. Yuen, H., Influence of lipolysis and droplet size on tocotrienol absorption from self-emulsifying formulations. *Int. J. Pharm*., 281, 67-78 (2004).