

Effects of adding tocotrienol-tocopherol mixed fraction and vitamin C on inflammatory status in hypercholesterolaemic patients in the low coronary risk category

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Abstract— Aim: This study is designed to investigate the effects of tocotrienol-tocopherol mixed fraction (TTMF), vitamin C and combined TTMF-vitamin C supplementations on serum lipids and biochemical markers of inflammation and endothelial activation in hypercholesterolemic subjects in the low-risk category. **Materials and Methods:** 78 hypercholesterolaemic subjects (total cholesterol of ≥ 5.2 mmol/L and low-density lipoprotein 3.4 – 4.9 mmol/L) in the low cardiovascular risk category according to the NCEP-ATP3 criteria were recruited. They were randomized into four treatment combination groups for a period of twelve months; (1) receiving TTMF and vitamin C, (2) receiving TTMF and placebo, (3) receiving vitamin C and placebo, and (4) receiving placebo for both. Serum fasting lipid profiles and levels of high-sensitivity C-reactive protein, interleukin-6, tumour necrosis factor- α , intercellular adhesion molecule, vascular cell adhesion molecule, E-selectin and homocysteine were measured at entry and multiple time points post-randomisation. **Results:** There were no significant differences in percentage changes of lipid profiles and inflammatory markers between treated and placebo groups for either single or combined antioxidants supplementations. **Conclusion:** TTMF, vitamin C and combined TTMF-vitamin C supplementations have neutral effects on lipid profiles and biochemical markers of inflammation and endothelial activation in low risk subjects, suggesting that they offer no added advantage in the low cardiovascular risk group.

Key words: Antioxidants, tocotrienols, hypercholesterolaemia, inflammation, atherosclerosis, low coronary risk.

INTRODUCTION

The use of vitamins C and vitamin E as dietary supplements due to their supposed benefits in health and disease is widespread. Both are antioxidants, and as such is thought to reduce oxidative stress and its implications for the human body (Breilmann J et al., 2010). One of such implications is atherosclerosis, of which hypercholesterolaemia is a risk factor. Low

density lipoproteins (LDL) that are elevated in hypercholesterolaemia are prone to oxidization (Palinski et al., 1989), thus initiating the cascade of atheroma formation by way of activating specific genes involved (Maziere and Maziere, 2009).

Elevated oxidized LDL (oxLDL) level is independently related to the risk of developing atherosclerosis and subsequent coronary heart disease (Toshima et al., 2000). Furthermore, it also contributes to atheroscle-

rotic plaque instability (Nishi et al., 2002; Okura et al., 2000), leading to thrombus formation and acute coronary events. Inflammation is considered to be the pathway by which oxLDL exerts its adverse cardiovascular effects (Bieghs et al., 2013; Shen et al., 2013). Additionally, oxLDL also promotes endothelial dysfunction via upregulation of its markers, such as the intercellular and vascular cell adhesion molecules (ICAM and VCAM, respectively) (Huang et al., 2013).

Efforts have been made to lower LDL levels by traditional medicine and/or medications to acceptable values for patients at risk of developing cardiovascular diseases, and the use of antioxidants to quench the oxidation of LDLs could prove to be a novel approach to this problem. However, research in this area is still not provided conclusive results to support their clinical use.

Tocotrienols and tocopherols are members of the vitamin E family. Tocotrienol has been proven to be a more potent antioxidant than tocopherols (Maniam et al., 2008). Furthermore, tocotrienol has been found to exert other effects outside of its antioxidant capabilities, such as cardioprotective (Das et al., 2007), neuro-regenerative (Khanna et al., 2005) and anti-cancer properties (Aggarwal et al., 2010). It was also found to directly inhibit HMG-CoA reductase, the predominant enzyme in cholesterol metabolism (Khor and Ng, 2000). Vitamin C is an established antioxidant and has been demonstrated to reduce oxidative stress induced by multiple mechanisms (El-Gendy et al., 2010; Tsovolas et al., 2008). The combination of vitamins C and E has been shown to be beneficial in combating oxidative stress induced by intense exercise (Naziroglu et al., 2010) and X-ray (Kayan et al., 2009).

Due to the potential benefit of tocotrienol in hypercholesterolaemia, we investigated the effects of tocotrienol-tocopherol mixed fraction (TTMF) of palm oil and vitamin C, either alone or in combination, on lipid profile and biomarkers of inflammation and endothelial activation in patients at low risk of cardiovascular disease.

MATERIALS AND METHODS

Study design and endpoints

A randomized double blind placebo-controlled clinical trial was conducted in the Clinical Trial Center, Un-

iversiti Teknologi MARA in Selangor, Malaysia. Men and women ranged from 25 to 60 years old were screened for their cholesterol profile and assessed for their cardiovascular (CV) risk factors according to the National Cholesterol Education Plan-Adult Treatment Protocol 3 (NCEP-ATP3). Those with a LDL level of more than 3.4 mmol/l and one or less CV risk factor were recruited into the low risk category group. The exclusion criteria were: subjects with BMI >35kg/m², fasting triglycerides >4.5mmol/l, diabetes mellitus, uncontrolled hypertension (systolic blood pressure >150mmHg), established coronary heart disease or peripheral vascular disease, chronic inflammatory disorders or severe disease with poor prognosis.

Using the Open Epi statistical calculator (www.openepi.com), a sample size of 16 subjects was needed to achieve a power of 80% to 95% confidence interval. A total of 78 subjects who gave informed consent were recruited and subsequently randomized into four groups: (1) TRF 80mg/day and vitamin C 500mg/day, (2) Vitamin C 500mg/day and placebo TRF, (3) TRF 80mg/day and placebo C, and (4) placebo of both. Patients were given clear instruction to take their supplementation either before or after dinner. Those who regularly consume antioxidants or already on lipid lowering medications were subjected to a 4-week washout period before starting on their allocated supplementations.

Blood sampling, anthropometric measurements and medical consultation on therapeutic lifestyle changes were done at the randomization stage. After that, subjects were called back for consultation and blood sampling at 2 weeks, 3 months, 6 months and 12 months. The endpoints recorded were the levels of the outlined biochemical parameters, whether they were improved as compared to baseline levels, and percentage of treated subjects improving their parameters as compared to placebo.

The experimental protocol complied with the Helsinki Declaration and was approved by the institutional Research Ethics Committee prior to the commencement of the study. All patients gave written informed consent for their participation in this study.

Materials

TTMF was supplied by Golden Hope Bioganic Sdn. Bhd., Malaysia with the following composition: alpha tocotrienol (59.2mg, 22.1%), beta tocotrienol (4.5mg, 1.7%), gamma tocotrienol (58.6mg, 21.9%), delta tocotrienol (37.6mg, 14.0%), alpha tocopherol (44.0mg

23.9%) and palm super olein (128.0mg, 23.9%). Commercially available Vitamin C was used (Flavettes®, ECM Pharma Sdn. Bhd., Malaysia). The placebo was produced as soft-gel capsules with similar excipient of Vitamin E (palm super olein) but without TTMF.

Biochemical parameters and analysis

During the randomization stage and subsequent visits, 20mls of venous blood was obtained from the subjects. Besides routine biochemical testing (fasting blood glucose; FBG and serum lipids; FSL), levels of high sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), tumour necrosis factor- α (TNF- α), intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), E-selectin and homocysteine were also determined from these samples. Total cholesterol (TC), triglycerides (TG), HDL-c and hsCRP were measured on an automated chemistry analyzer (Cobas Integra, Roche Diagnostics, Basel, Switzerland). IL-6 and TNF- α were measured on automated immunoanalyser (Immulite 1000, Siemens Healthcare Diagnostics, USA). LDL-c concentration was derived by calculation using the Friedewald equation (Friedewald et al., 1972).

Statistical analysis

All data were entered and analyzed using Stata v11 software (StataCorp, Texas USA). All numerical variables were described as mean (SD) or median (IQR). Whereas categorical variables were summarized as frequencies and percentages. Normality of distribution was tested using the skewness and kurtosis test. Significance testing was done using the paired t-test for continuous variables and the chi-square test for categorical variables. P-values of less than 0.05 and 0.01 are considered significant and very significant, respectively.

Evaluation of the effects of TRF and vitamin C was done by combining treatment groups to increase the sample size of each treatment modality, with new groups designated A, B, C, D, E and F. Effects of TRF and/or vitamin C treatment on the various biochemical variables were evaluated by comparing group A (treatment groups 1 and 3) to group E (groups 2 and 4) to determine the effect of TRF, group B (groups 1 and 2) to group F (3 and 4) to determine the effect of vitamin C, and group C (groups 1, 2 and 3) to group D (group 4 only) to determine the effect of TRF and vitamin C in combination. Using these combinations, the sample size during each analysis of treatment effect is at least 38 subjects.

RESULTS

Baseline characteristics

Table 1 shows the baseline characteristics of the patients recruited in this clinical trial according to their treatment group. From the table, the distribution of patients in the different treatment groups was similar as there were no significant differences in the baseline characteristics (**Table 1**).

Effect of supplements on fasting serum lipid profile:

Table 2 summarises the comparison between different treatment modalities in terms of the outcome of the fasting serum lipid parameters. For the TTMF (groups A vs E), there were trends of lowering of the plasma levels of TC, LDL, HDL and TG after the 12-month treatment as compared to baseline. Except for TG lowering ($p = 0.05$), the other observations were not statistically significant. Compared with TTMF placebo, a bigger percentage of TTMF treated subjects lowered their LDL, HDL and TG, with the percentage experiencing HDL-lowering reaching statistical significance ($p = 0.05$) (**Table 2**).

When combined with vitamin C (groups C vs D), TTMF modestly but significantly lowered TC ($p = 0.02$), HDL ($p < 0.01$) and TG ($p = 0.03$) after 12 months of treatment compared to the baseline. A similar but insignificant trend was also observed for LDL. The percentage of subjects improving the combined treatment was higher compared to placebo for all the lipid parameters measured.

Effects of supplements on inflammatory mediators and markers for endothelial activation:

Tables 3 and **4** summarise the comparison between the treatment groups in terms of assessing inflammation and endothelial activation. As for the inflammatory mediators (**Table 3**), subjects taking TTMF showed a tendency of lowering of all the inflammatory mediators after 12 months of treatment. When coupled with vitamin C, a similar trend was observed for IL6 and hsCRP. However, the percentage of treated subjects (TTMF and TTMF with vitamin C) improving their inflammatory mediator levels was smaller when compared to placebo over the same time period (**Table 4**).

TTMF treatment markedly increased homocysteine levels after 12 months when compared to placebo ($p = 0.01$). The combination of TTMF with vitamin C also showed a similar increment of homocysteine ($p = 0.04$). Among the additional endothelial activation biomark-

ers, 12-month treatment with TTMF showed a beneficial lowering trend only for VCAM. In terms of proportion of subjects improving their biomarkers on treatment, the percentage was smaller for TTMF treated subjects compared to placebo. When TTMF treatment is combined with vitamin C, a bigger percentage of subjects improved their biomarkers over the 12-month period, with the exception of E-selectin, where the percentage is significantly smaller ($p = 0.05$) (Table 4).

DISCUSSION

After 12 months of treatment with TTMF, either alone or in combination, we found that despite the encouraging trends, TTMF had a neutral effect on the lipid parameters when compared to placebo. The utilisation of a mixture of tocotrienol and tocopherol, rather than a pure tocotrienol compound, could be the reason be-

hind this observation. Although other human studies have demonstrated the benefit of this mixed fraction in lowering plasma lipids (Qureshi et al., 2001, 2002), the fraction of tocopherol in the mixture used by them yielded a lower tocopherol fraction, compared to our TTMF (8% vs 26%, respectively).

The effect of tocopherol on plasma lipids has been positively established (de Oliveira et al., 2011). However, its utilization in a mixture with tocotrienol has yielded conflicting results (Fu et al., 2014). One study suggested that tocopherol has a negative impact on the lipid lowering property of tocotrienols (Qureshi et al., 1995), via its stimulating effect on HMG-CoA reductase, the rate-limiting enzyme in the cholesterol synthesis pathway, thus attenuating the inhibitory effect exerted by tocotrienols (Qureshi et al., 1996). It was determined that in a mixed preparation of tocotrienol and tocopherol, 20% or more of the latter leads to attenuation of the former's hypocholesterolaemic effects (Qureshi et al., 1995; Qureshi et al., 1991).

Table 1. Baseline characteristics of subjects compared across the treatment groups

Parameters	Group 1 (E80+C500)	Group 2 (PE+C500)	Group 3 (E80+PC)	Group 4 (PE+PC)	All	p-value
n	19	21	19	19	78	
Age (years)	42.4 (6.6)	46.7 (5.7)	46.9 (9.8)	41.3 (9.5)	44.4 (8.3)	NS
Male frequency (%)	52.6	47.6	52.6	36.8	46.3	NS
Smoker percentage (%)	0	0	0	0	0	
Anthropometrics						
Waist circumference (cm)	81.9 (9.3)	84.4 (11.3)	83.9 (9.6)	80.1 (10.4)	82.7 (10.1)	NS
Waist-Hip ratio	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	NS
BMI (kg/m ²)	25.3 (4.4)	26.1 (3.1)	25.6 (4.3)	25.4 (2.8)	25.6 (3.7)	NS
Systolic BP (mmHg)	113.7 (12.7)	119.3 (15.1)	118.6 (15.3)	116.3 (19.6)	117.05 (15.7)	NS
Diastolic BP (mmHg)	71.1 (11.3)	75.5 (9.2)	72.3 (9.2)	72.0 (8.5)	72.7 (9.5)	NS
Lipid profile						
TC (mmol/l)	6.2 (0.8)	6.4 (0.6)	6.3 (0.6)	5.9 (0.5)	6.2 (0.6)	NS
HDL (mmol/l)	1.3 (0.2)	1.5 (0.3)	1.4 (0.2)	1.3 (0.2)	1.3 (0.2)	NS
LDL (mmol/l)	4.2 (0.8)	4.2 (0.6)	4.2 (0.6)	4.1 (0.4)	4.1 (0.6)	NS
TG (mmol/l)	1.4 (0.6)	1.6 (0.9)	1.5 (0.7)	1.3 (0.4)	1.4 (0.6)	NS

Table 2. Effect of different treatment modalities on fasting serum lipid parameters in low cardiovascular risk patients

Parameter	Timeline	Groups								
		TTMF vs placebo TTMF		Vitamin C vs placebo vitamin C		TTMF+Vitamin C vs placebo				
		A	vs	E	B	vs	F	C	vs	D
TC	BL	6.4±0.8		6.0±0.5	6.3±0.8		6.2±0.6	6.3±0.8		6.0±0.5
	12 months	6.1±0.8		5.7±0.7	6.0±1.0		5.9±0.5	5.9±0.9		5.9±0.5
	<i>p</i> within group	0.08		0.04*	0.09		0.02*	0.02*		0.3
	<i>p</i> between groups		NS			NS			NS	
	% improved on treatment	63.2		64.7	58.8		68.4	68.0		54.6
	<i>p</i> between groups		0.4			0.3			0.1	
LDL	BL	4.2±0.7		4.0±0.5	4.0±0.8		4.2±0.6	4.1±0.7		4.1±0.6
	12 months	4.1±0.8		4.0±0.5	4.1±0.8		4.0±0.4	4.0±0.7		4.1±0.4
	<i>p</i> within group	0.3		0.5	0.3		0.07	0.2		0.8
	<i>p</i> between groups		NS			NS			NS	
	% improved on treatment	63.2		47.1	47.1		63.2	60.0		45.5
	<i>p</i> between groups		0.4			0.4			0.7	
HDL	BL	1.4±0.2		1.4±0.3	1.4±0.3		1.4±0.2	1.4±0.3		1.4±0.3
	12 months	1.3±0.2		1.3±0.4	1.4±0.4		1.2±0.2	1.3±0.3		1.2±0.3
	<i>p</i> within group	0.06		1.0	0.07		0.001**	0.006**		0.02*
	<i>p</i> between groups		NS			NS			NS	
	% improved on treatment	31.6		5.9	29.4		10.5	24.0		9.1
	<i>p</i> between groups		0.05*			0.2			0.3	
TG	BL	1.6±0.8		1.4±0.7	1.7±0.8		1.3±0.8	1.7±0.8		1.2±0.7
	12 months	1.5±0.6		1.3±0.6	1.4±0.6		1.4±0.7	1.4±0.6		1.3±0.7
	<i>p</i> within group	0.05*		0.3	0.02*		0.3	0.03*		0.06
	<i>p</i> between groups		NS			NS			NS	
	% improved on treatment	63.2		35.3	64.7		36.8	60.0		27.3
	<i>p</i> between groups		0.1			0.09			0.07	

TC, total cholesterol; LDL, low-density lipoproteins; HDL, high-density lipoproteins; TG, triglycerides; TTMF, tocotrienol-tocopherol mixed fraction vitamin E; BL, baseline. All measurements are in mmol/L.

Group A: all groups taking TTMF 80mg/day, B: taking vitamin C 500mg/day, C: taking TTMF 80mg/day and/or vitamin C 500mg/day, D: taking placebo TTMF and placebo vitamin C, E: taking placebo TTMF, F: taking placebo vitamin C.

* statistically significant

** statistically very significant

Table 3. Effects of different treatment modalities on levels of inflammatory mediators in low cardiovascular risk patients

Parameter	Timeline	Groups					
		TTMF vs placebo TTMF		Vitamin C vs placebo Vitamin C		Combination vs placebo	
		A	E	B	F	C	D
IL6	BL	10.3±21.0	10.9±8.9	20.4±28.0	5.0±6.1	10.5±19.2	10.4±11.8
	12 months	3.5±2.0	3.6±1.5	3.5±1.4	3.5±2.2	3.7±1.9	2.3±0.4
	p within group	0.2	0.1	0.1	0.2	0.1	0.3
	% improved on treatment	30.0	50.0	60.0	22.2	33.3	50.0
	p between groups	0.5		0.2		0.6	
TNF- α	BL	10.8±7.6	11.8±5.3	13.4±10.0	9.8±3.6	11.2±7.3	11.1±3.6
	12 months	10.5±2.9	18.3±24.4	18.5±24.4	10.4±3.0	14.4±16.4	9.5±2.3
	p within group	0.5	0.3	0.3	0.3	0.3	0.2
	% improved on treatment	30.0	66.7	50.0	40.0	38.5	66.7
	p between groups	0.2		0.7		0.4	
hsCRP	BL	2.2±2.6	2.4±3.0	2.7±3.1	2.0±2.5	2.6±2.9	0.9±0.4
	12 months	2.1±1.4	2.5±2.2	2.7±2.2	1.9±1.3	2.4±1.8	1.4±0.9
	p within group	0.4	0.4	0.5	0.5	0.4	0.2
	% improved on treatment	38.5	62.5	50.0	46.2	47.1	50.0
	p between groups	0.3		0.9		0.9	

IL6, interleukin-6; TNF- α , tumour necrosis factor-alpha; hsCRP, high-sensitivity C-reactive protein; TTMF, tocotrienol-tocopherol mixed fraction vitamin E; BL, baseline.

Group A: all groups taking TTMF 80mg/day, B: taking vitamin C 500mg/day, C: taking TTMF 80mg/day and/or vitamin C 500mg/day, D: taking placebo TTMF and placebo vitamin C, E: taking placebo TTMF, F: taking placebo vitamin C.

* Statistically significant

** Statistically very significant

Table 4. Effects of different treatment modalities on levels of markers for endothelial activation in low cardiovascular risk patients

Parameter	Timeline	Groups					
		TTMF vs placebo TTMF		Vitamin C vs placebo Vitamin C		Combination vs placebo	
		A	E	B	F	C	D
ICAM	BL	299.1±98.0	299.3±91.9	280.6±62.0	311.2±109.3	296.0±91.1	310.7±111.1
	12 months	311.7±116.6	278.0±65.8	272.5±64.1	312.9±113.2	301.4±103.4	281.5±79.2
	p within group	0.2	0.2	0.3	0.5	0.3	0.3
	% improved on treatment	38.5	50.0	66.7	28.6	44.4	40.0
	p between groups	0.6		0.07		0.9	
VCAM	BL	926.8±340.3	1002.5±711.8	953.3±208.1	953.2±365.6	981.2±512.4	795.1±288.9
	12 months	911.9±339.5	776.8±450.1	733.5±412.2	971.9±97.3	855.2±406.3	918.2±123.8
	p within group	0.4	0.3	0.2	0.4	0.2	0.2
	% improved on treatment	46.2	57.1	55.6	45.5	52.9	33.3
	p between groups	0.6		0.7		0.5	
E-selectin	BL	41.7±11.6	55.8±30.2	47.3±17.7	47.4±26.2	44.1±15.9	64.1±40.4
	12 months	41.5±17.8	52.2±21.6	49.0±18.9	42.4±20.8	44.9±18.0	50.2±30.2
	p within group	0.5	0.3	0.4	0.09	0.4	0.06
	% improved on treatment	53.3	60.0	46.2	66.7	47.6	100.0
	p between groups	0.7		0.3		0.05*	
Homocysteine	BL	7.9±1.7	7.8±3.4	8.2±3.4	7.6±1.7	8.1±2.6	6.9±2.0
	12 months	9.7±2.5	8.3±2.3	9.1±2.7	9.1±2.4	9.2±2.7	8.6±1.2
	p within group	0.01**	0.2	0.2	0.007**	0.04*	0.01**
	% improved on treatment	16.7	33.3	44.4	8.3	29.4	0.0
	p between groups	0.4		0.06		0.2	

ICAM, inter-cellular adhesion molecule; VCAM, vascular cell adhesion molecule; TTMF, tocotrienol-tocopherol mixed fraction vitamin E; BL, baseline.

Group A: all groups taking TTMF 80mg/day, B: taking vitamin C 500mg/day, C: taking TTMF 80mg/day and/or vitamin C 500mg/day, D: taking placebo TTMF and placebo vitamin C, E: taking placebo TTMF, F: taking placebo vitamin C.

* Statistically significant

** Statistically very significant

A worrying observation is the consequence of HDL lowering with TTMF in this study. HDL, which is a plasma cholesterol scavenger, is positively correlated with a reduction of adverse cardiovascular (CV) risk and CV mortality (Moradi et al., 2014). Whilst there is another study that reported a similar observation (Tan et al., 1991), most reported a neutral effect of tocotrie-

nols on HDL levels (Baliarsingh et al., 2005; Mensink et al., 1999; Qureshi et al., 1995). Another study demonstrated that the same TTMF preparation was able to significantly increase plasma HDL after 6 months of treatment when compared to baseline levels (Chin et al., 2011). However, the same study showed that the HDL increment was only observed to be significantly

better than placebo in the older age group (>50 years) compared to those aged between 35 – 49 years, a group where most of our subjects were located in.

Despite this observation with regard to the HDL levels, a bigger percentage of subjects on TTMF (alone or in combination with vitamin C) improved their lipid profiles. This trend is encouraging, and supports the notion that tocotrienols should be an adjunct therapy for attenuating hypercholesterolaemia. This is due to the inhibitory effect that tocotrienol has on HMG-CoA reductase (Khor and Ng, 2000), the same enzyme that is inhibited by the statin group of drugs. Owing to their synergistic interaction, the combination of lovastatin and tocotrienol-rich fraction (TRF) has been shown to be effective in lowering plasma lipid parameters (Qureshi et al., 2001). OxLDL predisposes to inflammation (Bieghs et al., 2013) and endothelial dysfunction (Huang et al., 2013). In our study, we demonstrated that TTMF treatment had a neutral effect on hsCRP and TNF- α , and a tendency for lowering of IL6 levels, thus proving to be potentially beneficial in quenching oxLDL-mediated inflammation. Similarly, one study has reported that TRF has a neutral effect on CRP and IL6 in haemodialysis patients (Daud et al., 2013), but another reported that the level of CRP precursor protein was down-regulated in healthy individuals treated with TRF (Heng et al., 2013).

TTMF was also found to have a neutral effect on biomarkers of endothelial activation in the current study. Even though many in-vitro studies have reported tocotrienol's beneficial effect on adhesion molecules expression (Ahn et al., 2007; Naito et al., 2005; Theriault et al., 2002), ours is the only study that evaluated its role in human. However, the homocysteine elevation seen with TTMF treatment when compared to the baseline is in disagreement with a study in animals, which have demonstrated a beneficial lowering of homocysteine with tocotrienol treatment (Norsidah et al., 2013). One study in humans yielded a neutral effect of tocopherol, rather than tocotrienol, on homocysteine levels (Breilmann et al., 2010). Although the effect observed here is minimal, due to the correlation of homocysteine to cardiovascular risk (Nygård et al., 1995), further studies should aim to confirm this finding and look at the possible mechanisms involved, if any.

CONCLUSION

Treatment for 12- months with TTMF, either alone or in combination with vitamin C supplementations have neutral effects on lipid profiles and biochemical markers of inflammation and endothelial activation in low risk subjects, suggesting that they offer no added advantages in the low cardiovascular risk group. The utilization of TTMF with its high tocopherol fraction (26%) has probably impeded the improvement observed, due to antagonistic interaction between tocopherols and tocotrienols, although worrying, the negative impact of TTMF on plasma HDL and homocysteine is quite minimal. In the future, more studies will be needed to assess the effect of tocotrienols in human, especially on biomarkers for endothelial activation or dysfunction.

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Competing interests

The authors declare that they have no competing interests.

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