

Clinical Investigation of the Protective Effects of Palm Vitamin E Tocotrienols on Brain White Matter

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Background and Purpose—Previous cell-based and animal studies showed mixed tocotrienols are neuroprotective, but the effect is yet to be proven in humans. Thus, the present study aimed to evaluate the protective activity of mixed tocotrienols in humans with white matter lesions (WMLs). WMLs are regarded as manifestations of cerebral small vessel disease, reflecting varying degrees of neurodegeneration and tissue damage with potential as a surrogate end point in clinical trials.

Methods—A total of 121 volunteers aged ≥ 35 years with cardiovascular risk factors and MRI-confirmed WMLs were randomized to receive 200 mg mixed tocotrienols or placebo twice a day for 2 years. The WML volumes were measured from MRI images taken at baseline, 1 year, and 2 years using a validated software and were compared. Fasting blood samples were collected for full blood chemistry investigation.

Results—According to per-protocol (88 volunteers) and intention-to-treat (121 volunteers) analyses, the mean WML volume of the placebo group increased after 2 years, whereas that of the tocotrienol-supplemented group remained essentially unchanged. The mean WML volume change between the 2 groups was not significantly different ($P=0.150$) at the end of 1 year but was significant at the end of 2 years for both per-protocol and intention-to-treat analyses ($P=0.019$ and $P=0.018$). No significant difference was observed in the blood chemistry parameters between the 2 groups.

Conclusions—Mixed tocotrienols were found to attenuate the progression of WMLs.

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Key Words: magnetic resonance imaging ■ tocotrienols

Vitamin E is a general term representing 2 classes of compounds, namely tocopherols and tocotrienols. Tocotrienols, but not tocopherols, have been reported to possess neuroprotective properties at concentrations within the physiological relevant range. Using hippocampal HT4 neuronal cells and immature primary cortical neurons, Sen et al¹ and Khanna et al² showed that α -tocotrienol at nanomolar concentrations was able to block glutamate-induced neuronal cell death by suppressing early activation of c-Src kinase and 12-lipoxygenase pathway, which is independent of its antioxidant property. In a later study using a stroke-induced rat model, rats supplemented with tocotrienols were found to have reduced infarct volume and brain injury after stroke induction compared with matched controls.³ This effect was similarly demonstrated in stroke-induced canine by Rink et al.⁴ Moreover, they also reported that tocotrienols were capable of preventing loss of white matter fiber tract connectivity after stroke with improved cerebrovascular collateral

circulation to the ischemic area. However, it is important to assess these findings in a prospective clinical trial.

Many compounds have been shown to display neuroprotective effects in animal models of stroke but failed in clinical trials.⁵ The use of patients with stroke for such studies has its limitations. The short treatment time window from the onset of clinical stroke makes recruitment of subject and logistics challenging. Also, during an ischemic stroke, poor perfusion may severely limit the amount of the administered compound from reaching the target tissues.⁶ It should be noted that in the animal studies that yielded positive outcomes,^{3,4} the test compounds were usually administered before stroke induction.

Other reasons for clinical failure have been attributed to the difference in the proportion of gray and white matter between human and animal brain. For example, rats have $\approx 10\%$ of white matter in the brain, whereas humans have $\approx 50\%$.⁷ Thus, positive outcome in studies using animals, such as rats, is mainly associated with protection of the gray matter, but

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the tested compounds may not necessarily be protective also of the white matter, which is a major component affected in human stroke.^{6,8,9} In view of this, a suitable model focusing on the white matter is warranted. Moreover, injury of the white matter has been reported to be the major cause of functional disability in cerebrovascular disease and hence the importance of white matter protection.¹⁰

White matter lesions (WMLs) are abnormal hyperintense regions observed in MRI T2-weighted sequences.¹¹ Often detected in elderly people, these hyperintense lesions are thought to be attributed to arteriosclerosis of small blood vessels in the brain, leading to chronic hypoperfusion and ischemic damage in the white matter.¹² Pathological findings corresponding to WMLs include myelin pallor, tissue rarefaction associated with loss of myelin and axon, mild gliosis, as well as fiber degeneration.¹¹ They reflect a spectrum of neuropathological changes with tissue damage of varying severity.¹³ It has been suggested that WML progression be used to complement the currently used primary outcome of cognitive impairment and dementia in neuroprotection trial. The potential of WMLs as a surrogate end point in intervention trials^{11,14} is mainly attributed to the fact that WMLs progress over time.¹⁴ Moreover, they have been shown to be associated with cardiovascular risk factors such as hypertension¹⁵ and diabetes mellitus.¹⁶ Furthermore, WMLs can be measured quantitatively and noninvasively on a large population, and thus WML progression has been advocated as a marker to evaluate the efficacy of various pharmacological substances in research and clinical settings.^{17,18}

Hence, the present study was conducted to investigate the protective effects of mixed tocotrienols in volunteers with WMLs.

Materials and Methods

Study Protocol

The present study was designed as a randomized, double-blind, and placebo-controlled trial. The trial was investigator initiated and undertaken at Universiti Sains Malaysia facilities in Hospital Kepala Batas, Penang, Malaysia. The study commenced in November 2007 with the first and the last patient randomized on November 6, 2007, and March 27, 2010, respectively. All volunteers with MRI-confirmed WMLs were subjected to a 2-year intervention period and were given either mixed tocotrienols (T3) 200 mg or matching placebo (edible palm oil) twice daily. Both placebo and T3 (Tocovid Suprabio) were soft gelatin capsules and were similar in terms of color, size, shape, and surface texture. They were purchased from Hovid Bhd (Ipoh, Malaysia).

The volunteers were randomly assigned to study medications using a permuted block randomization. Each block of specified size contained allocation ratio of 1:1 (placebo:tocotrienols). The random allocation sequence was not made known to the researchers who were involved in the enrollment, management, and evaluation of volunteers. They were blinded throughout the study.

The study was approved by the by the Research Ethics Committee for Human Studies of Universiti Sains Malaysia, Malaysia (<http://www.crp.kk.usm.my/pages/jepem.htm>), and the volunteers provided informed consent before participation in the trial.

Study Population

Through leaflet distribution and public talks regarding this trial, 1300 volunteers aged ≥ 35 years from the northern region of peninsular Malaysia were screened for their full blood chemistry, lipid profile,

liver function, renal function, and fasting blood glucose. Demographic data including age, weight, height, ethnicity, social history, medical history, and blood pressure were also recorded. Those with normal renal and liver function were invited to undergo MRI for WMLs if they fulfilled ≥ 1 of the following criteria: total cholesterol level between 5.2 and 6.2 mmol/L and low-density cholesterol level between 2.6 and 4.2 mmol/L, body mass index of >25 kg/m², hypertension, or diabetes mellitus under medical supervision and treatment. Individuals with the above conditions were reported to be predisposed to the development of WMLs^{19–25} and hence were selected to increase our chances of recruiting volunteers with WMLs. Exclusion criteria included current use of vitamin E supplementation (tocopherols or tocotrienols) or within the past 3 months at the time of recruitment, self-reported intolerance/hypersensitivity to vitamin E, pregnant female, presence or history of neurological impairment, psychiatric disorders, stroke, transient ischemic attack, malignancy, and hematological disorders. Those taking cholesterol-lowering drugs or showing signs of alcohol and drug dependence or those who have contraindications to undergo MRI were also excluded.

A total of 390 volunteers who fulfilled the above inclusion and exclusion criteria underwent an MRI scan but were only recruited into the study if they had ill-defined hyperintensities ≥ 5 mm in diameter on both T2 and proton density/fluid-attenuated inversion recovery images. Lacunes were differentiated by well-defined areas with signal characteristics similar to cerebrospinal fluid. Lesions with these characteristics of ≤ 2 mm in diameter were excluded because they are perivascular spaces, including those around the anterior commissure, where perivascular spaces can be large.²⁶ Based on the MRI scan, 177 volunteers were considered as having WMLs and thus were eligible for recruitment, but only 121 agreed to participate in the study. On enrollment, the volunteers were dispensed study supplementations with instructions to take 1 capsule in the morning and another 1 capsule in the evening. They were also advised to maintain their habitual diet and physical activity and to report if they have been diagnosed with diseases or illnesses in between follow-up visits.

Clinical Evaluation Procedures

Volunteers were followed up every 3 months for a duration of 2 years. During each visit, body mass index, blood pressure, and lifestyle habits (such as food intake and physical activity) and adverse events were recorded. Fasting blood samples were obtained at 3, 6, 9, 12, and 24 months and analyzed for serum total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, apolipoprotein B, lipoprotein A, high-sensitivity C-reactive protein, fasting glucose, serum creatinine, alkaline phosphatase, alanine transaminase, aspartate transaminase, and γ -glutamyl transpeptidase. Brain MRI for WMLs was conducted at baseline, 1 year, and at the end of the 2-year study period. The magnetic resonance images were only analyzed on completion of the study by an independent radiologist who was blinded to the study medications.

Study supplementations sufficient for 3 months were dispensed to the volunteers during the follow-up visits. Compliance to the study supplements was assessed through patient recall, returned capsule count, and plasma levels of T3. Total plasma concentrations of T3 (α -, γ -, and δ -T3) were measured using a validated high-performance liquid chromatographic method²⁷ after completion of the study, until then the samples were kept in frozen condition at -80°C .

Brain MRI

MRI Parameters

Volunteers were imaged with a 1.5-T MRI machine (Model Signa HDx, General Electric, Milwaukee, WI) using an HD 8 ch NV array (Invivo corporation, Pewaukee, WI) radiofrequency coil. Padding was used to immobilize the head without causing discomfort. The scanner alignment light was used to align at the glabella. A rapid sagittal localizer scan was acquired as a reference image for planning.

Brain MRI images were taken at an axial, sagittal, and coronal plane of T₁-weighted, T₂-weighted, and a 3-dimensional volumetric

T₂ fluid-attenuated inversion recovery sequences with slice thickness of 5 mm. The pulse sequence parameters for T1-weighted images were repetition time, 440; echo time, 14; field of view, 24×18 cm; number of excitations, 2; and matrix size, 320×224. For the T2-weighted sequence parameters, the pulse sequence were repetition time, 4440; echo time, 100.4; field of view, 24×24 cm; number of excitations, 1; and matrix size, 512×256, followed by the T2 fluid-attenuated inversion recovery whereby the sequences were repetition time, 8002; echo time, 129; field of view, 24×24 cm; number of excitations, 1; and matrix size 320×224.

WML Volumetric Measurement

WML volumetric measurement was conducted using a validated software, Endeavor.²⁸ The software provided a quantitative analysis tools to quantify lesion loads and reconstruct brain tissue and lesions for 3-dimensional visualization. It is a fully automated adaptive technique to detect and segment WMLs in cranial magnetic resonance images without requiring any atlas or elaborated training procedures. It generates WML measurements in area (square millimeter) and volume (cubic millimeter) when the axial T1 and T2 fluid-attenuated inversion recovery images are loaded in the software. There are 3 main stages involved: (1) the preprocessing stage, which includes skull stripping and inhomogeneity correction; (2) the WMLs segmentation stage; and (3) the postprocessing stage, which includes normal brain tissue classification and morphological processing to remove false-positive WMLs.

Outcome Measures

The primary outcome measure was changes in WML volume or load after 1 year and 2 years from baseline. Secondary outcomes included an improvement in the total lipid profile and other markers associated with increased cardiovascular risks including apolipoprotein B, high-sensitivity C-reactive protein and lipoprotein A, and total lipid profile.

Statistical Analysis

All primary and secondary outcome measures were analyzed using the per-protocol approach which encompassed all 88 volunteers who have completed the 2-year follow-up period. However, intention-to-treat analysis was also performed on all randomized volunteers for WML changes. Missing data were imputed using last value carried forward.

The changes in WML volume at 1 year and 2 years from baseline were calculated for each volunteer. The data obtained were analyzed using an ANOVA procedure for a 2-factor split-plot experimental design.²⁹ If statistically significant difference was observed, a post hoc simple main effect test was used to locate the pair that gave rise to the difference observed.

Effect size analysis was also conducted in the WML volume change for the 2 groups.³⁰ Homogeneity of the baseline characteristics between the 2 groups was assessed using an independent Student *t* test. A statistically significant difference was considered at $P < 0.05$. All the statistical analyses were conducted using Statistical Package for Social Sciences (IBM SPSS) software version 19.

Results

Volunteers Flow and Follow-Up

Of the 121 volunteers who participated in the study, 62 received T3 and 59 received placebo. During the study period, 16 (25.8%) volunteers from the T3 group and 17 (28.8%) from the placebo group dropped out, with a total dropout of 27.3%. Of the 16 who dropped out in the T3 group, 9 withdrew their consent, 2 were noncontactable, 4 were excluded because of protocol violation by taking antihyperlipidemic medications, and 1 became pregnant. Of the 17 who dropped out in the placebo group, 13 withdrew their consent, 1 was noncontactable,

and 3 were excluded because they started on antihyperlipidemic drugs (Figure 1). Among the 33 who dropped out from the study, 5 from the T3 group and 7 from the placebo group had undergone only the baseline brain MRI scan and came for ≥ 1 consecutive follow-up, whereas 11 from the T3 group and 10 from the placebo group had repeated their brain MRI scan at the 1-year follow-up period. Thus, 109 volunteers completed the first year of study and 88 volunteers completed the entire study period of 2 years. Hence, 121 volunteers were included in the intention-to-treat analysis, whereas 88 in the per-protocol analysis.

Baseline Characteristics

Baseline characteristics of the 121 volunteers are shown in Table 1. No statistically significant difference was observed between the baseline data of the T3 and placebo groups. Furthermore, the 2 groups have comparable baseline mean WML volume of $1627 \pm 154 \text{ mm}^3$ and $1732 \pm 197 \text{ mm}^3$, respectively ($P = 0.67$). The baseline total plasma T3 levels were below detection limit ($0.04 \mu\text{g/mL}$).

WML Volume Changes

In the per-protocol analysis, 46 were on T3 group and 42 on placebo. The mean WML volume (\pm SEM) of the T3 group remained essentially unchanged with values of $1523 \pm 182 \text{ mm}^3$ at baseline, $1532 \pm 167 \text{ mm}^3$ at 1 year, and $1494 \pm 179 \text{ mm}^3$ at 2 years. In contrast, the mean WML volume of the placebo group showed a

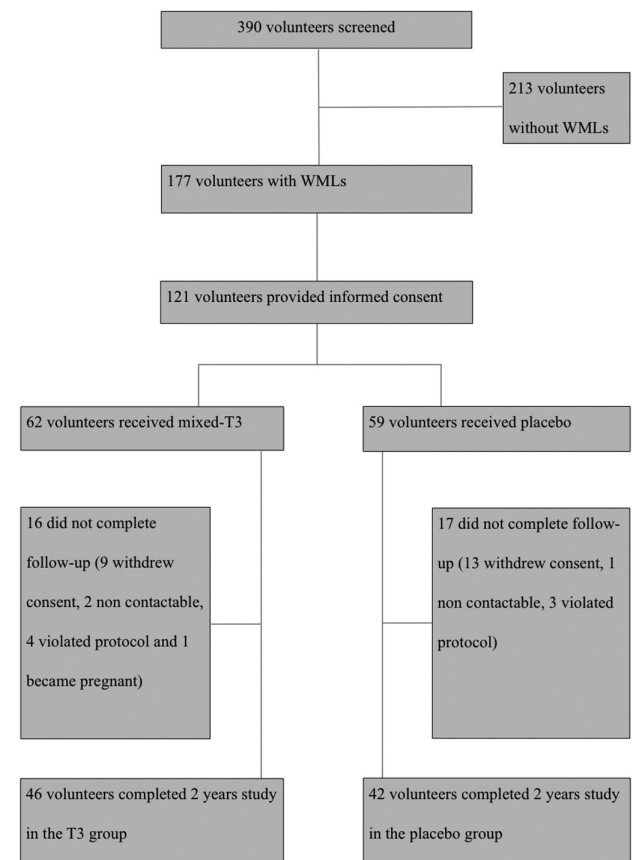


Figure 1. Participant flowchart. T3 indicates tocotrienol; and WML white matter lesion.

Table 1. Baseline Characteristics of Volunteers Recruited Into the Study (Mean±SD)

Characteristics	Total (n=121)	T3 Group (n=62)	Placebo Group (n=59)	P Value
Sex (male)	48 (39.7%)	27 (43.5%)	21 (35.6%)	
Age, y	52±8.5	52±8.8	52±8.2	0.66
WML volume, mm ³	1678±1361	1627±1211	1732±1511	0.67
BMI, kg/m ²	26±4.30	26.12±4.32	26.16±4.28	0.95
TC, mmol/L	5.70±0.70	5.76±0.63	5.63±0.67	0.27
LDL, mmol/L	3.60±0.60	3.57±0.50	3.56±0.62	0.90
HDL, mmol/L	1.42±0.32	1.43±0.34	1.41±0.30	0.81
TG, mmol/L	1.57±0.90	1.69±1.15	1.45±0.64	0.16
Systolic blood pressure, mm Hg	129±16	126±16	131±16	0.08
Diastolic blood pressure, mm Hg	78±9	76±9	79±9	0.07
Lp(A), mg/dL	19.50±16.20	19.61±16.60	19.47±15.90	0.96
ApoB, g/L	1.30±0.20	1.29±0.19	1.23±0.21	0.18
hs-CRP, mg/L	3.50±6.60	3.50±7.40	3.45±5.70	0.97
Serum creatinine, μmol/L	82.80±13.90	83.2±13.50	82.4±14.40	0.76
ALP, IU/L	74.20±19.10	73.7±21.20	74.7±16.70	0.77
AST, IU/L	32.20±10.20	32.2±11.40	32.2±8.70	0.97
ALT, IU/L	30.70±15.90	30.0±14.60	31.4±17.30	0.65
GGT, IU/L	25.70±15.40	25.5±13.70	26.0±17.20	0.83
Fasting glucose, mmol/L	5.60±1.23	5.37±0.70	5.74±1.60	0.10
Smokers, current	18 (14.9%)	11 (17.7%)	7 (11.9%)	0.45
Hypertension only	17 (14.0%)	8 (12.9%)	9 (15.3%)	
Diabetes mellitus only	8 (6.6%)	3 (4.8%)	5 (8.5%)	
Hypertension and diabetes mellitus	5 (4.1%)	3 (4.8%)	2 (3.4%)	

ALP indicates alkaline phosphatase; ALT, alanine transaminase; ApoB, apolipoprotein B; AST, aspartate transaminase; BMI, body mass index; GGT, γ -glutamyl transpeptidase; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; Lp(A), lipoprotein A; T3, tocotrienol; TC, total cholesterol; TG, triglycerides; and WML, white matter lesion.

gradual increase over time (1633±232 mm³ at baseline, 1804±246 mm³ at 1 year, and 2013±305 mm³ at 2 years), being in accord with the progression of WML reported in other studies.^{14,18,20,31} As observed from Figure 2A, the T3 group showed a negligible change in the mean WML volume from baseline at 1 year (9±67 mm³; 0.6%) and 2 years (-29±119 mm³; -1.9%). For the placebo group, the mean WML volume was increased by 171±81 mm³ (10.5%) after 1 year and 380±123 mm³ (23.3%) after 2 years. Increase in the mean WML volume was significantly different statistically between the 2 groups at end of 2 years ($P=0.019$), but was not significant after 1 year ($P=0.150$). This could be because of the slow, natural progression of WMLs.^{24,25}

A moderate effect size of 0.3 ($P=0.06$) at 1 year and 0.5 ($P=0.004$) at 2 years from baseline was obtained with the placebo group, being in accord with the observed WML progression in this group. In comparison, the effect size in T3 group was 0.02 ($P=0.88$) at 1 year and 0.06 ($P=0.81$) at 2 years, inferring that WML progression was negligible.

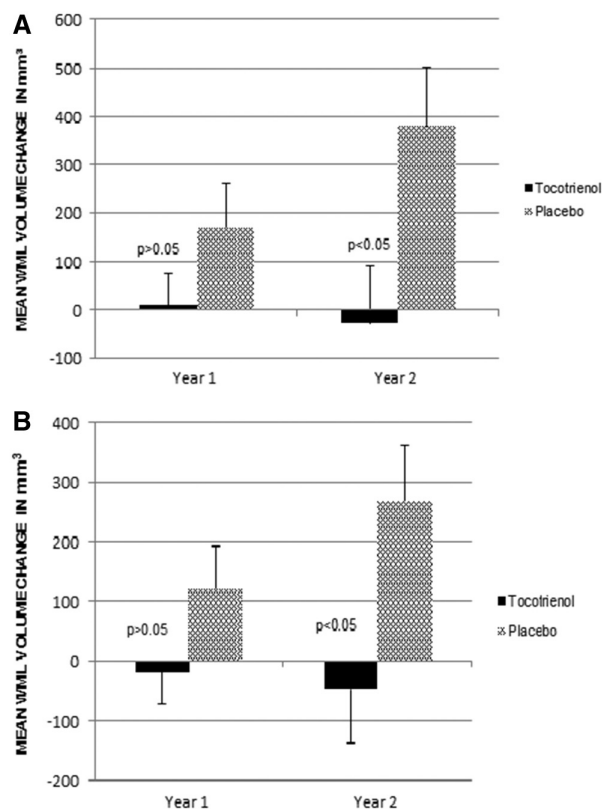


Figure 2. A, Mean white matter lesion (WML) volume change from baseline of tocotrienol and placebo groups using per-protocol analysis. **B,** Mean WML volume change from baseline of tocotrienol and placebo groups using intention-to-treat analysis.

Analysis according to intention-to-treat principle also yielded similar outcomes. As shown in Figure 2B, the T3 group showed a negligible change in the mean WML volume from baseline at 1 year (-17.1±56 mm³; -1.1%) and at 2 years (-46.1±92 mm³; -2.8%), whereas the placebo group recorded an increase of 122±71 mm³ (7.0%) and 270±95 mm³ (15.6%), respectively. Again, the increase in the mean of WML volume was significantly different statistically at the end of 2 years ($P=0.018$) but was not statistically significant after 1 year ($P=0.120$). Effect size attributed to the placebo group was 0.2 ($P=0.09$) at 1 year and 0.4 ($P=0.006$) at 2 years, whereas insignificant effect size was observed with the T3 group with respective values of 0.04 ($P=0.76$) and 0.07 ($P=0.62$).

Clinical Parameters

Table 2 shows the blood chemistry profiles of the 88 volunteers at baseline, 3, 6, 9, 12, and 24 months. For all parameters, there was no significant change in the mean levels from baseline throughout the study period for both treatment groups, albeit an increase in the mean high-sensitivity C-reactive protein was observed at 24th month in the placebo group. This was because of unexplained sudden increase observed in the high-sensitivity C-reactive protein of 2 volunteers in the placebo group at this interval. The results suggested that T3 did not seem to have any influence on all these biochemical parameters. Moreover, there was also no statistically significant difference between the 2 groups in all the parameters at

Table 2. Blood Biochemistry Profiles of Volunteers (Per-Protocol Analysis; Mean±SD)

Parameters	Month	T3 (n=46)	Placebo (n=42)	P Value
TC, mmol/L	Baseline	5.79±0.68	5.64±0.69	0.32
	3	5.76±0.61	5.52±0.63	0.08
	6	5.84±0.67	5.56±0.69	0.06
	9	5.75±0.69	5.52±0.57	0.08
	12	5.78±0.51	5.60±0.56	0.11
	24	5.84±0.65	5.70±0.62	0.29
HDL, mmol/L	Baseline	1.44±0.37	1.42±0.32	0.77
	3	1.45±0.38	1.47±0.35	0.77
	6	1.46±0.43	1.48±0.43	0.81
	9	1.42±0.32	1.40±0.33	0.76
	12	1.41±0.33	1.38±0.33	0.68
	24	1.31±0.30	1.40±0.36	0.21
LDL, mmol/L	Baseline	3.58±0.53	3.57±0.65	0.96
	3	3.55±0.51	3.39±0.67	0.19
	6	3.68±0.56	3.44±0.58	0.06
	9	3.68±0.57	3.48±0.49	0.07
	12	3.66±0.45	3.47±0.64	0.10
	24	3.79±0.57	3.61±0.72	0.19
TG, mmol/L	Baseline	1.71±1.23	1.43±0.72	0.20
	3	1.62±1.10	1.40±0.57	0.25
	6	1.58±1.05	1.36±0.62	0.24
	9	1.43±0.76	1.37±0.71	0.69
	12	1.63±1.19	1.30±0.58	0.11
	24	1.64±0.96	1.37±0.37	0.10
ApoB, g/L	Baseline	1.30±0.19	1.22±0.21	0.06
	3	1.27±0.16	1.26±0.13	0.61
	6	1.31±0.17	1.26±0.16	0.17
	9	1.33±0.16	1.24±0.16	0.01
	12	1.27±0.18	1.24±0.17	0.40
	24	1.24±0.22	1.22±0.22	0.61
Lp(A), mg/dL	Baseline	18.4±13.5	17.4±15.9	0.76
	3	19.1±12.9	16.9±15.1	0.46
	6	19.6±13.6	16.0±16.5	0.27
	9	19.0±12.6	16.1±15.2	0.33
	12	19.9±13.0	16.5±15.2	0.25
	24	19.4±12.6	17.5±14.7	0.50
hs-CRP, mg/L	Baseline	2.94±6.35	3.32±6.44	0.78
	3	2.28±2.62	3.58±4.59	0.10
	6	2.46±3.11	3.39±8.17	0.47
	9	2.30±2.91	3.54±4.44	0.12
	12	2.48±3.16	3.43±4.95	0.29
	24	2.37±3.15	5.44±15.2	0.18

ApoB indicates apolipoprotein B; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; Lp(A), lipoprotein A; T3, tocotrienol; TC, total cholesterol; and TG, triglycerides.

each follow-up period, with the exception of apolipoprotein B levels at the ninth month follow-up interval. Hence, the above findings tend to suggest that T3 have no effect on cardiovascular risk factors.

Compliance

Compliance with the study protocol and supplementation regimen was satisfactory, as evidenced by the returned capsule count. In addition, the mean total T3 plasma levels of the T3 group were markedly and consistently higher compared with those of the placebo group which were below detection limit (Figure 3).

Adverse Events

Throughout the study period, no adverse event related to the consumption of T3 was reported except for 5 complaints of mild diarrhea or loose stools during the first week of supplementation (T3: n=5; placebo: n=0). Their conditions were fully resolved without the need to initiate drug intervention or the volunteers withdrawing from the study.

Discussion

This is the first clinical trial to investigate the protective effect of T3 on brain white matter, and the results showed that T3 could attenuate the progression of WML. It was previously reported that the neuroprotective properties of T3 was essentially via attenuation of glutamate-induced excitotoxicity.^{1,2,32} Recent studies tend to suggest that compounds including T3, which can attenuate the neurotoxic effects of glutamate, are protective not only of neuron bodies (gray matter) but also of the white matter region. These studies demonstrated that glutamate is also released in the white matter region during cerebral ischemia³³ and exerting similar excitotoxicity via N-methyl-D-aspartate glutamate receptors which are now known to be present in the white matter.³⁴

Dufouil et al³⁵ and Godin et al³⁶ demonstrated that antihypertensive treatment reduced WML progression. In addition, Mok et al³⁷ also showed that antihyperlipidemic treatment could slow down the progression of WML in those with severe WML at baseline. Hence, in the present study, only those volunteers with well-controlled hypertension and diabetes mellitus were recruited. Moreover, their blood pressure and fasting blood glucose were closely monitored and were found to be essentially unchanged throughout the study period (systolic blood pressure was <140 mmHg, diastolic blood pressure

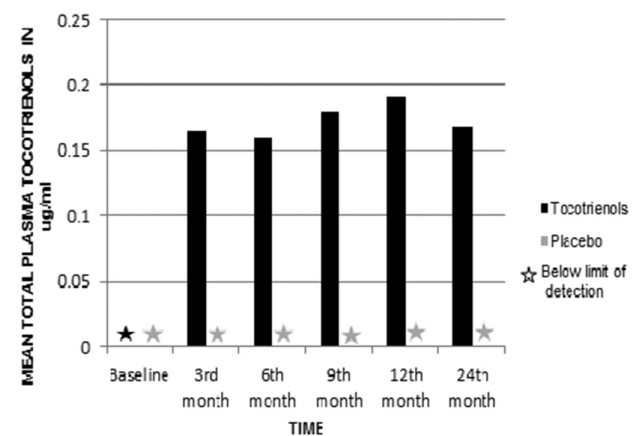


Figure 3. Mean total plasma tocotrienol concentrations of volunteers at baseline, 3, 6, 9, 12, and 24 months for tocotrienol and placebo groups.

<90 mmHg, and fasting blood glucose was <7 mmol/L). In the case of mildly hypercholesterolemic volunteers, their total cholesterol levels were <5.84 mmol/L and the low-density lipoprotein levels were <3.79 mmol/L and they were not on any antihyperlipidemic medications. Those who were started on antihyperlipidemic treatment were withdrawn from the study. All these measures were aimed to avoid any confounding effects on the results of our study.

The present investigation must also be differentiated from several recent studies evaluating antihyperlipidemic^{38,39} and antihypertensive drugs^{35,36,40} on WML progression. These studies were basically investigating WML progression via controlling the risk factors of hyperlipidemia and hypertension rather than the protective effects of these agents. In contrast, our study was primarily aimed at investigating the protective effects of T3 on WML progression.

Our volunteers have a relatively smaller mean baseline WML volume compared with those of Godin et al³⁶ (1.68 versus 5.31 cm³) in a white population. This could be attributable to the fact that our volunteers were younger (mean age of 52 versus 72 years). Moreover, a relatively low percentage of our volunteers were presented with hypertension (14%), diabetes mellitus (6.6%), or both (4.2%), whereas in the study by Godin et al,³⁶ 75% of the volunteers were hypertensive. However, the mean baseline WML volume reported by Mok et al³⁷ in an Asian population was comparable with that of our study, although the median age of their volunteers was 63 years.

A validated, fully automated plug-in software²⁸ was used to compute the volume of WMLs. It has the advantage of minimizing discrepancies that might occur using methods which are semiautomated or those based on visual rating scales to assess the WMLs.^{35,39} In general, visual rating scales are insensitive for measuring changes in WML severity and are subjected to significant ceiling or floor effects during the grading of WMLs. Limitations of visual scales to evaluate WML severity, as well as WML changes over time, have been extensively discussed.^{35,36,41–44}

The findings from our present study indicated that T3 could attenuate the progression of WMLs. Because they are natural vitamins and shown to be well tolerated, they can be used as a long-term supplement that may help to minimize injury of brain tissues particularly in the white matter region during an ischemic event. Also, the present study demonstrated that WMLs can be used for investigating the prophylactic effects of potential neuroprotectants with objective measurements.

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Disclosures

Dr Wong and Ng are employees of Hovid Research Sdn Bhd, a subsidiary of Hovid Bhd. Dr Yuen is an Executive Director of Hovid Bhd. The other authors report no conflicts.

References

- Sen CK, Khanna S, Roy S, Packer L. Molecular basis of vitamin E action. Tocotrienol potentially inhibits glutamate-induced pp60(c-Src) kinase activation and death of HT4 neuronal cells. *J Biol Chem.* 2000;275:13049–13055.
- Khanna S, Roy S, Ryu H, Bahadduri P, Swaan PW, Ratan RR, et al. Molecular basis of vitamin E action: tocotrienol modulates 12-lipoxygenase, a key mediator of glutamate-induced neurodegeneration. *J Biol Chem.* 2003;278:43508–43515.
- Khanna S, Roy S, Slivka A, Craft TK, Chaki S, Rink C, et al. Neuroprotective properties of the natural vitamin E α -tocotrienol. *Stroke.* 2005;36:e144–e152.
- Rink C, Christoforidis G, Khanna S, Peterson L, Patel Y, Khanna S, et al. Tocotrienol vitamin E protects against preclinical canine ischemic stroke by inducing arteriogenesis. *J Cereb Blood Flow Metab.* 2011;31:2218–2230.
- O'Collins VE, Macleod MR, Donnan GA, Horky LL, van der Worp BH, Howells DW. 1,026 experimental treatments in acute stroke. *Ann Neurol.* 2006;59:467–477.
- Gladstone DJ, Black SE, Hakim AM; Heart and Stroke Foundation of Ontario Centre of Excellence in Stroke Recovery. Toward wisdom from failure: lessons from neuroprotective stroke trials and new therapeutic directions. *Stroke.* 2002;33:2123–2136.
- Zhang K, Sejnowski TJ. A universal scaling law between gray matter and white matter of cerebral cortex. *Proc Natl Acad Sci U S A.* 2000;97:5621–5626.
- Correa F, Gauberti M, Parcq J, Macrez R, Hommet Y, Obiang P, et al. Tissue plasminogen activator prevents white matter damage following stroke. *J Exp Med.* 2011;208:1229–1242.
- Ho PW, Reutens DC, Phan TG, Wright PM, Markus R, Indra I, et al. Is white matter involved in patients entered into typical trials of neuroprotection? *Stroke.* 2005;36:2742–2744.
- Goldberg MP, Ransom BR. New light on white matter. *Stroke.* 2003;34:330–332.
- Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ.* 2010;341:c3666.
- Yamauchi H, Fukuda H, Oyanagi C. Significance of white matter high intensity lesions as a predictor of stroke from arteriolosclerosis. *J Neurol Neurosurg Psychiatry.* 2002;72:576–582.
- Gouw AA, Seewann A, Vrenken H, van der Flier WM, Rozemuller JM, Barkhof F, et al. Heterogeneity of white matter hyperintensities in Alzheimer's disease: post-mortem quantitative MRI and neuropathology. *Brain.* 2008;131(pt 12):3286–3298.
- Gouw AA, van der Flier WM, Fazekas F, van Straaten EC, Pantoni L, Poggesi A, et al; LADIS Study Group. Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. *Stroke.* 2008;39:1414–1420.
- Liao D, Cooper L, Cai J, Toole JF, Bryan NR, Hutchinson RG, et al. Presence and severity of cerebral white matter lesions and hypertension, its treatment, and its control. The ARIC Study. Atherosclerosis Risk in Communities Study. *Stroke.* 1996;27:2262–2270.
- Perros P, Deary IJ, Sellar RJ, Best JJ, Frier BM. Brain abnormalities demonstrated by magnetic resonance imaging in adult IDDM patients with and without a history of recurrent severe hypoglycemia. *Diabetes Care.* 1997;20:1013–1018.
- Kuller LH, Longstreth WT Jr, Arnold AM, Bernick C, Bryan RN, Beauchamp NJ Jr, et al. White matter hyperintensity on cranial magnetic resonance imaging: a predictor of stroke. *Stroke.* 2004;35:1821–1825.
- Schmidt R, Berghold A, Jokinen H, Gouw AA, van der Flier WM, Barkhof F, et al; LADIS Study Group. White matter lesion progression in LADIS: frequency, clinical effects, and sample size calculations. *Stroke.* 2012;43:2643–2647.
- Breteler MM, van Swieten JC, Bots ML, Grobbee DE, Claus JJ, van den Hout JH, et al. Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. *Neurology.* 1994;44:1246–1252.
- Schmidt R, Fazekas F, Kleinert G, Offenbacher H, Gindl K, Payer F, et al. Magnetic resonance imaging signal hyperintensities in the deep and subcortical white matter. A comparative study between stroke patients and normal volunteers. *Arch Neurol.* 1992;49:825–827.
- Fazekas F, Niederkorn K, Schmidt R, Offenbacher H, Horner S, Bertha G, et al. White matter signal abnormalities in normal individuals:

- correlation with carotid ultrasonography, cerebral blood flow measurements, and cerebrovascular risk factors. *Stroke*. 1988;19:1285–1288.
22. Ylikoski A, Erkinjuntti T, Raininko R, Sarna S, Sulkava R, Tilvis R. White matter hyperintensities on MRI in the neurologically nondiseased elderly. Analysis of cohorts of consecutive subjects aged 55 to 85 years living at home. *Stroke*. 1995;26:1171–1177.
 23. Schmitz SA, O'Regan DP, Fitzpatrick J, Neuwirth C, Potter E, Tosi I, et al. White matter brain lesions in midlife familial hypercholesterolemic patients at 3-Tesla magnetic resonance imaging. *Acta Radiol*. 2008;49:184–189.
 24. Taylor WD, MacFall JR, Provenzale JM, Payne ME, McQuoid DR, Steffens DC, et al. Serial MR imaging of volumes of hyperintense white matter lesions in elderly patients: correlation with vascular risk factors. *AJR Am J Roentgenol*. 2003;181:571–576.
 25. Schmidt R, Fazekas F, Kapeller P, Schmidt H, Hartung HP. MRI white matter hyperintensities: three-year follow-up of the Austrian Stroke Prevention Study. *Neurology*. 1999;53:132–139.
 26. Wahlund LO, Barkhof F, Fazekas F, Bronge L, Augustin M, Sjögren M, et al; European Task Force on Age-Related White Matter Changes. A new rating scale for age-related white matter changes applicable to MRI and CT. *Stroke*. 2001;32:1318–1322.
 27. Yap SP, Julianto T, Wong JW, Yuen KH. Simple high-performance liquid chromatographic method for the determination of tocotrienols in human plasma. *J Chromatogr B Biomed Sci Appl*. 1999;735:279–283.
 28. Ong KH, Ramachandram D, Mandava R, Shuaib IL. Automatic white matter lesion segmentation using an adaptive outlier detection method. *Magn Reson Imaging*. 2012;30:807–823.
 29. Kirk RE. Split-plot design-factorial design with block-treatment confounding. In: Kirk RE, ed. *Experimental Design: Procedures for the Behavioral Sciences*. Pacific Grove, CA: Cole Publishing Company; 1968:245–318.
 30. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Hillsdale, NJ: Erlbaum; 1988.
 31. Whitman GT, Tang Y, Lin A, Baloh RW, Tang T. A prospective study of cerebral white matter abnormalities in older people with gait dysfunction. *Neurology*. 2001;57:990–994.
 32. Khanna S, Parinandi NL, Kotha SR, Roy S, Rink C, Bibus D, et al. Nanomolar vitamin E alpha-tocotrienol inhibits glutamate-induced activation of phospholipase A2 and causes neuroprotection. *J Neurochem*. 2010;112:1249–1260.
 33. Tekkök SB, Ye Z, Ransom BR. Excitotoxic mechanisms of ischemic injury in myelinated white matter. *J Cereb Blood Flow Metab*. 2007;27:1540–1552.
 34. Lipton SA. NMDA receptors, glial cells, and clinical medicine. *Neuron*. 2006;50:9–11.
 35. Dufouil C, Chalmers J, Coskun O, Besançon V, Bousser MG, Guillon P, et al; PROGRESS MRI Substudy Investigators. Effects of blood pressure lowering on cerebral white matter hyperintensities in patients with stroke: the PROGRESS (Perindopril Protection Against Recurrent Stroke Study) Magnetic Resonance Imaging Substudy. *Circulation*. 2005;112:1644–1650.
 36. Godin O, Tzourio C, Maillard P, Mazoyer B, Dufouil C. Antihypertensive treatment and change in blood pressure are associated with the progression of white matter lesion volumes: the Three-City (3C)-Dijon Magnetic Resonance Imaging Study. *Circulation*. 2011;123:266–273.
 37. Mok VC, Lam WW, Fan YH, Wong A, Ng PW, Tsoi TH, et al. Effects of statins on the progression of cerebral white matter lesion: Post hoc analysis of the ROCAS (Regression of Cerebral Artery Stenosis) study. *J Neurol*. 2009;256:750–757.
 38. ten Dam VH, van den Heuvel DM, van Buchem MA, Westendorp RG, Bollen EL, Ford I, et al; PROSPER Study Group. Effect of pravastatin on cerebral infarcts and white matter lesions. *Neurology*. 2005;64:1807–1809.
 39. Bernick C, Katz R, Smith NL, Rapp S, Bhadelia R, Carlson M, et al; Cardiovascular Health Study Collaborative Research Group. Statins and cognitive function in the elderly: the Cardiovascular Health Study. *Neurology*. 2005;65:1388–1394.
 40. Weber R, Weimar C, Blatchford J, Hermansson K, Wanke I, Möller-Hartmann C, et al; PROFESS Imaging Substudy Group. Telmisartan on top of antihypertensive treatment does not prevent progression of cerebral white matter lesions in the prevention regimen for effectively avoiding second strokes (PROFESS) MRI substudy. *Stroke*. 2012;43:2336–2342.
 41. van Straaten EC, Fazekas F, Rostrup E, Scheltens P, Schmidt R, Pantoni L, et al; LADIS Group. Impact of white matter hyperintensities scoring method on correlations with clinical data: the LADIS study. *Stroke*. 2006;37:836–840.
 42. Scheltens P, Erkinjuntti T, Leys D, Wahlund LO, Inzitari D, del Ser T, et al. White matter changes on CT and MRI: an overview of visual rating scales. European Task Force on Age-Related White Matter Changes. *Eur Neurol*. 1998;39:80–89.
 43. Kapeller P, Barber R, Vermeulen RJ, Adèr H, Scheltens P, Freidl W, et al; European Task Force of Age Related White Matter Changes. Visual rating of age-related white matter changes on magnetic resonance imaging: scale comparison, interrater agreement, and correlations with quantitative measurements. *Stroke*. 2003;34:441–445.
 44. Pantoni L, Simoni M, Pracucci G, Schmidt R, Barkhof F, Inzitari D. Visual rating scales for age-related white matter changes (leukoaraiosis): can the heterogeneity be reduced? *Stroke*. 2002;33:2827–2833.

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