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Short term effects of palm-tocotrienol and palm-carotenes on vascular function and cardiovascular disease risk: A randomised controlled trial

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ABSTRACT

Background and aims: *In vitro*, *ex vivo* and animal studies suggest palm-based tocotrienols and carotenes enhance vascular function, but limited data in humans exists. The aim was to examine the effects of palm-tocotrienols (TRF- 80) and palm-carotene (CC-60) supplementation on vascular function and cardiovascular disease (CVD) risk factors in adults at increased risk of impaired vascular function.

Methods: Ninety men and women (18–70 yr, 20–45 kg/m^2) with type 2 diabetes, impaired fasting glucose and/or elevated waist circumference were randomised to consume either TRF-80 (420 mg/day tocotrienol + 132 mg/day tocopherol), CC-60 (21 mg/day carotenes) or placebo (palm olein) supplements for 8 weeks. Brachial artery flow-mediated dilation (FMD), other physiological and circulatory markers of vascular function, lipid profiles, glucose, insulin and inflammatory markers were assessed pre- and post-supplementation. Pairwise comparisons were performed using mixed effects longitudinal models (n = 87, n = 3 withdrew before study commencement).

Results: Plasma α- and β-carotene and α-, δ- and γ-tocotrienol concentrations increased in CC-60 and TRF-80 groups, respectively, compared to placebo (mean ± SE difference in total plasma carotene change between CC-60 and placebo: 1.5 ± 0.13 μg/ml, $p < 0.0001$; total plasma tocotrienol change between TRF-80 and placebo: 0.36 ± 0.05 μg/ml, $p < 0.0001$). Neither FMD (treatment x time effect for CC-60 *vs.* placebo, $p = 0.71$; TRF-80 *vs.* placebo, $p = 0.80$) nor any other vascular function and CVD outcomes were affected by treatments.

Conclusions: CC-60 and TRF-80 supplementation increased bioavailability of palm-based carotenes and tocotrienols but had no effects, superior or detrimental, on vascular function or CVD risk factors.

1. Introduction

Impaired vascular function is an early event in atherosclerotic development [1] and an important target for cardiovascular disease (CVD) prevention. Abnormalities in vascular endothelial function in several physiological and disease states (e.g. pre-diabetes, diabetes, hypertension, hypercholesterolemia, abdominal obesity) is predictive of CVD outcomes [2]. Growing evidence suggests supplementation with natural antioxidants are effective in preserving vascular endothelial function, due to their ability to reduce reactive oxygen species burden and promote nitric oxide production and bioavailability [3,4].

Palm oil-based antioxidants include a bouquet of compounds, including tocotrienols (an isoform of vitamin E) and carotenes [5,6]. *In vitro, ex vivo,* animal studies and observational studies have demonstrated several vascular benefits of these preparations

Abbreviations: AI, augmentation index; BMI, body mass index; CTAB, citrate, theophylline, adenosine and dipyridamole; CV, coefficient of variance; CVD, cardiovascular disease; EFSA, European Food Safety Authority; eGFR, estimated global filtration rate; FID, flow-independent dilation; FMD, flow-mediated dilation; HbA1c, glycated haemoglobin; HDL-C, high density lipoprotein cholesterol; HOMA2-IR, homeostasis assessment model 2 Insulin resistance; hsCRP, high sensitivity C-reactive protein; HMG-C0A, 3-hydroxy-3-methylglutaryl-coenzyme A; ICAM-1, intercellular adhesion molecule-1; IFG, impaired fasting glucose; IMVS, Institute of Medical and Veterinary Science; IL-6, interleukin-6; IR, insulin resistance; LDL-C, low density lipoprotein cholesterol; NOAEL, no-observed-adverse-effect level; NSAID, non-steroidal anti-inflammatory drugs; PAI-1, plasminogen activator inhibitor 1; PWV, pulse wave velocity; SDT, suggested dietary target; T, tocopherol; T_3, tocotrienol; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides; TNFα, tumor necrosis factor alpha; tPA, tissue plasminogen activator; TRF, tocotrienol-rich fraction; VCAM-1, vascular cell adhesion molecule-1; WC, waist circumference.

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[6–12] including free radical scavenging actions, anti-inflammatory properties, modulation of signaling mechanisms and improved nitric oxide bioavailability that could lead to physiological outcomes such as improved vascular relaxation and health [6,13,14]. This suggests that supplementation of palm-tocotrienols and carotenes could be a simple dietary strategy to improve vascular function and reduce CVD risk. Despite this, there remains a paucity of data in humans examining these effects on vascular function markers [12] including physiological measures such as brachial artery flow-mediated dilatation (FMD), recognized as a good prognostic predictor of future cardiac events [15] and circulating biomarkers including adhesion molecules (intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], E-Selectin), thrombotic or inflammatory cytokines. Hence the purpose of this study was to evaluate the effects of supplementation with palm-based tocotrienols and carotenes (rich in both α and β forms) on vascular function and CVD risk in individuals with increased risk of impaired vascular function and CVD risk. It was hypothesized that palm-based tocotrienol and carotene supplementation will improve vascular function and CVD risk profiles compared to a placebo.

2. Patients and methods

The study (http://www.anzctr.org.au; ACTRN12613001041741) was conducted at the Commonwealth Scientific and Industrial Research Organization (CSIRO) Clinical Nutrition and Health Research Clinic, Adelaide, South Australia between March 2014 and August 2015 according to the ethical guidelines of the National Health and Medical Research Council of Australia. Ethical approval was obtained from the CSIRO Human Ethics Committee and written informed consent was obtained from all participants.

2.1. Participants

Ninety participants (aged:18–70 yrs, body mass index [BMI]:20–45 kg/m^2) at increased risk of impaired vascular function and CVD, were recruited by public advertisement and a pre-existing participant database. Participants with type 2 diabetes (T2DM) (defined as taking diabetes medication or glycated haemoglobin [HbA1c] ≥7%), impaired fasting glucose (IFG) (plasma glucose ≥5.6 mmol/L) and/or elevated waist circumference (WC, men: ≥120 cm; women ≥88 cm) were recruited as these conditions have been associated with impaired vascular function [16–18].

Exclusion criteria were: Type 1 diabetes; smoking; proteinuria (urinary albumin:creatinine ratio ≥30 mg/mmol; serum estimated globular filtration rate [eGFR] <60 ml/min); abnormal liver enzymes; any overt endocrinopathy (other than stable treated thyroid disease as determined by the study physician); history of malignancy, respiratory disease, gastrointestinal disease, coronary artery disease or cardiac abnormalities, CVD or peripheral vascular disease; pregnancy or lactating; taken vitamin E, carotene supplements, oral contraceptives or hormone replacement therapy, or participation in regular vigorous physical exercise >1 h per week or >2 × 30 min sessions/week during the 3 months prior to study; taken supplements known to affect outcome measures (i.e. fish oil, vitamin C, 3 months or 2 weeks prior to study commencement, respectively, except if participants were stable on supplements for at least 3 months prior to study commencement (no changes in type and dose) with no intention to change during study, they were included; used nitrate or non-steroidal anti-inflammatory drugs (NSAID) (excluding stable [at least 3 months] aspirin use); history of heavy alcohol consumption (>5 standard drinks/day); followed a weight reducing diet or having an eating disorder.

2.2. Study design

A randomised, placebo-controlled, double-blind study design was used. Eligible participants were randomly assigned to one of three intervention groups: palm carotenes (CC-60), palm-tocotrienols (TRF-80) or placebo supplementation for 8 weeks. Participants were requested to consume the supplements once per day with their largest meal. Randomization was performed by computer generation (http://www.randomisation.com) using stratified random assignment on the basis of metabolic state (T2DM, IFG, and elevated WC), age, gender, weight and diabetes medication usage. A third party packed the supplements according to the random sequence and held the random codes until after data analysis. Identical supplement containers were distributed to participants marked with only the participant's ID, hence research staff distributing supplements had no knowledge of supplement identity. All capsules were identical in shape, color and size ensuring blinding of participants. Research staff involved in outcome assessments and statistical analysis were blinded until after data analysis.

Participants were asked to maintain their usual diet and exercise patterns and keep medication/supplement intakes constant for the study duration. Medications/supplements and dosages were documented at baseline and any changes recorded.

After an overnight fast, outcome measures were assessed at baseline and after 4 and 8 weeks. Baseline dietary intakes were assessed using an online validated food frequency questionnaire [19]. Participants were asked to refrain from caffeine, alcohol or strenuous exercise for 24 h, and to cease supplementation 12 h before assessments to minimize any acute effects on vascular assessments.

2.3. Supplements

All supplements including placebo were supplied and analysed by Carotino Sdn Bhd, Malaysia and distributed to participants during their baseline and week 4 visits. Participants were requested to return left-over supplements to assess compliance by capsule counting. Compliance was also assessed by daily log and analysis of plasma tocotrienol and carotene concentrations.

CC-60: 3 × 483 mg capsules were consumed per day. Each capsule contained 7 mg carotenes dissolved in palm olein (refined palm oil devoid of carotenes and low in vitamin E); i.e. 21 mg carotenes daily intake. The supplement is commercially available and the dosage represents ~8-fold the mean daily β-carotene intake in the US population [20]. A no-observed-adverse-effect level (NOAEL) or tolerable upper level have not been defined for carotenes [21,22].

TRF-80: 2 × 510 mg capsules were consumed per day. Each capsule contained 210 mg tocotrienols plus 66 mg tocopherols (thus, a total daily intake of 420 mg tocotrienols + 132 mg tocopherols = 552 mg total vitamin E) dissolved in palm olein. Vitamin E isomers per capsule were: α-tocotrienol (T_3) = 67.6 mg (24.5%); β-T_3 = 9.7 mg (3.5%); γ-T_3 = 97.7 mg (35.4%); δ-T_3 = 35 mg (12.7%); α-tocopherol (T) = 66 mg (23.9%). Tocotrienol supplements are commercially available. Currently no NOAEL for tocotrienol in humans have been defined. The European Food Safety Authority (EFSA) concluded from sub-chronic toxicity studies in rats with tocotrienol-rich palm oil extract that a NOAEL of 120 and 130 mg/kg body weight/day for male and female rats respectively can be derived with no observed adverse effects [23]. Dosages used in the current study (7 mg tocotrienol/kg body weight for a 60 kg person) are ~18 times lower than this level. Human studies suggest doses up to 5 mg/kg body weight/day were of no safety concern which is slightly lower than dosages in the current study [23].