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Novel tocotrienols of rice bran modulate cardiovascular disease risk parameters of hypercholesterolemic humans

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Tocotrienols inhibit cholesterol synthesis by post-transcriptionally suppressing β-hydroxy-β-methylglutaryl-coenzyme A reductase activity. A double blind, 12-week study was performed to investigate the effect of a novel tocotrienol-rich fraction (TRF_{25}; obtained by molecular distillation from specially processed rice bran oil) on cardiovascular disease risk factors of hypercholesterolemic human subjects (serum total cholesterol >5.69 mmol/L). After acclimation to an alcohol-free regimen (baseline) participants were assigned to the National Cholesterol Education Program (NCEP) Step-1 diet (saturated fat <19%, total fat <30% of total calories and cholesterol <7.76 mmol/L). The participants were evaluated after 4 weeks of exposure to the NCEP Step-1 diet; one group of 21 participants was continued on the NCEP Step-1 diet for 4 weeks receiving an additional 1.2 gm corn oil (placebo group) and a second group of 20 received 200 mg TRF_{25} dissolved in 1.0 gm corn oil (TRF_{25} group).

Serum total cholesterol and LDL-cholesterol levels of all the participants, stable during the baseline phase of the study, decreased 5% and 8%, respectively, during the 4-week NCEP Step-1 diet. Placebo continuing on the NCEP Step-1 diet for an additional 4 weeks experienced additional but modest decreases in serum total cholesterol (2%) and LDL-cholesterol (3%), yielding significant ($P < 0.05$) decreases when compared with the baseline values. These responses confirm the cholesterol-lowering action of a low fat, low cholesterol diet. Participants receiving TRF_{25} had 12% and 16% reductions ($P < 0.05$) in total cholesterol and LDL-cholesterol levels during the 4-week experimental phase; during the two phases (NCEP Step-1 diet plus treatment) the serum total cholesterol and LDL-cholesterol levels of these participants were decreased ($P < 0.05$) by 17% and 24%, respectively. TRF_{25}-mediated decreases in Apo B, Lp(a), platelet factor 4 and thromboxane B_2 (15%, 17%, 14%, and 31%, respectively) were significant ($P < 0.05$). There was no change in the levels of HDL-cholesterol and apolipoprotein A-I by this treatment. The treatments also resulted in remarkable increases in the levels of LDL-bound antioxidants, especially tocotrienols, which have substantially greater antioxidant activity than vitamin E. (J. Nutr. Biochem. 8:290–298, 1997) © *Elsevier Science Inc. 1997*

Introduction

Approximately one-half of the population enjoying the Western lifestyle will die of coronary heart disease (CHD) or stroke.[1] Dietary fat consumption is correlated with CHD and stroke mortality.[2] A diet rich in food of plant origin can significantly retard the development of CHD.[3,4] Rice bran with an extremely low β-glucan content, is as effective as high β-glucan oat and barley brans in lowering serum cholesterol.[5–9] The lowering of cholesterol levels by rice bran may be attributed to some of its minor lipid-soluble constituents.[5–9] These constituents are present in the unsaponifiable fraction of rice bran oil, consist primarily of phytosterols, triterpene alcohols, ferulic acid esters (γ-oryzanols), tocols (vitamin E, including tocopherols and tocotrienols), and other minor unidentified compounds.[6,10–13] The tocotrienol isomers differ from the tocopherol isomers only in having a side chain with three unsaturated bonds.

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The didesmethyl-tocotrienol (P_{25}) is a late-eluting component present in the tocotrienol-rich fraction (TRF) of oil from processed rice bran (the 25 min HPLC fraction). The designation TRF_{25} is used to distinguish this processed rice bran oil TRF from those isolated from commercial rice bran, palm and barley oils.

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Various isomeric forms of tocotrienols are distinguished by the number and location of methyl groups on the chroman rings. The α-, β-, γ- and δ-tocotrienols are α- (5, 7, 8-trimethyl), β- (5, 8-dimethyl), γ- (7, 8-dimethyl), and δ- (8-monomethyl), respectively, depending on the number and position of the methyl substituents on the benzene ring of the chroman moiety.[14] The two novel tocotrienols isolated from specially processed rice bran, eluting at 21 min (peak-21; P_{21}) and 25 min (peak-25; P_{25}) on high performance chromatography (HPLC) system have been characterized as desmethyl and didesmethyl (no methyl groups on the benzene ring of the chroman moiety) tocotrienols.[15] The unsaturated tocotrienols, unlike saturated tocopherols, exhibit varying degrees of cholesterol lowering activity depending on the isomer, i.e., β < α < γ < δ < desmethyl < didesmethyl.[14–17] The tocotrienols inhibit cholesterol synthesis by suppressing β-hydroxy-β-methylglutaryl-coenzyme A (HMG-CoA) reductase activity through two post-transcriptional actions, increasing the controlled degradation of reductase protein and decreasing the efficiency of the translation of HMG-CoA reductase messenger RNA (mRNA).[16,17]

The commercially stabilized rice bran and rice bran oil contains 125 to 830 μg/g tocols. Rice bran constitutes about 10% of rough rice grain and contains 18 to 22% oil. Milling activates an endogenous lipase activity resulting in the rapid deterioration of the oil, rendering it unsuitable for human consumption. The temperature-sensitive lipase is inactivated by a two-step process, a 3-second exposure to 135°C, followed by 3-min exposure to 100°C. This processing stabilizes the rice bran for use in high fiber cereal products.[18,19] Stabilizing the rice bran at high temperature (180°C) under vacuum (2 psi) for 60 min before extraction increases by 2 to 3 fold the quantities of the known tocotrienols and also produces six novel analogs of tocotrienols.[20] One of these novel analogs is didesmethyltocotrienol (P_{25}; no methyl groups on the chroman rings) in the oil, the proportion of which has been shown to increase with such processing to 20 to 30% of the total tocols. Molecular distillation of the oil yields four fractions, the first consisting of free fatty acids and the γ-oryzanols. The second, the novel tocotrienol-enriched fraction (TRF_{25}), consists of the P_{25} tocol complex (90%) with small quantities of sterols and triglycerides. Animal trials show P_{25}-tocotrienol to be the most efficacious mevalonate-suppressive, cholesterol-lowering tocotrienol isomer.[15]

As suggested in the foregoing discussion, the hypocholesterolemic action of tocotrienols affects endogenously synthesized cholesterol. TRF_{25} also provides antithrombotic and antioxidant activities.[15] Estimates of cardiovascular disease risk are nominally assessed by measures of serum total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, as well as apolipoproteins A-I (apo A-I), apo B, Lipoprotein(a) [Lp(a)], thromboxane B_2 (TxB_2), and platelet factor 4 (PF4). These considerations led us to test the effect of molecularly distilled, γ-oryzanol-free TRF_{25} on the cardiovascular risk profile of hypercholesterolemic human subjects.

In an earlier experiment, supplementation with tocotrienols (Palmvitee) from palm oil lowered serum total cholesterol and LDL-cholesterol levels by 16 to 20% in responding individuals (35 out of 47 hypercholesterolemic subjects). All the subjects in this trial were allowed to consume their normal diets (free-living). Because of the large number of nonrespondants, these effects were not statistically significant for the entire group.[21] Therefore, the present double-blind study was initiated to measure the effects of TRF_{25} on hypercholesterolemic subjects restricted to a controlled diet (NCEP Step-1 diet).

Methods and materials

TRF_{25}

γ-Oryzanol-free TRF_{25} was prepared by molecular distillation (Dr. Laxman Singh, Vitamins Inc., Chicago, IL USA) of the oil extracted from rice bran which was processed and supplied by Bionutrics, Inc. (Phoenix, AZ USA). The TRF_{25} consisted of 6% α-tocopherol, 12.5% α-tocotrienol, 21% γ-tocotrienol, 10% δ-tocotrienol, 4.5% d-tocotrienol, 17% P_{25}-tocotrienol (d-di-desmethyl tocotrienol; 3,4-dihydro-2-(4,8,12)-trimethyltrideca-3′ (E), 7′ (E). 11′-trienyl-2-H-1-benzopyran-6-ol), 18% unidentified tocotrienols, and 10% sterols and triglycerides.

Study population

Adults enrolling in this project were recruited from a hypercholesterolemic population screened at the University of Illinois, Chicago. Prospective participants were grouped according to cholesterol level (⟨median⟩) and subgrouped by sex. The members of each subgroup were randomized into two groups using a random number table. There were 21 participants in the placebo group, 9 males and 12 females, 42.8 ± 14.0 years of age, 27.3 ± 5.0 body mass index, and 6.75 ± 1.12 mmol cholesterol/L. There were 20 participants in TRF_{25} group, 10 males and 10 females, 41.5 ± 15.5 years of age, 25.5 body mass index and 6.57 ± 0.77 mmol cholesterol/L. There were 16 female participants over 50 years old (eight in Placebo and eight in TRF_{25} groups) and the remaining six were 33 to 37 years old (four in placebo and two in TRF_{25} groups). Exclusion criteria included weight (>125% of Metropolitan Life relative weights), use of cholesterol-altering medication, an elevated serum glutamate-pyruvate or glutamate-oxaloacetate transaminase activity, an elevated blood urea nitrogen or glucose value, diabetes, or a liver, renal, or hypertensive disease. All participants signed an informed-consent statement, which was approved by Institutional Review Board of the Medical Center of University of Illinois, Chicago.

Experimental design

The experiment consisted of three phases; the first, an alcohol-free, free choice diet phase (baseline) for a period of 4 weeks followed by a second phase (4 weeks) during which all participants were counseled to follow the National Cholesterol Education Program (NCEP) Step-1 diet. The participants were continued on the NCEP Step-1 diet during the third 4-week phase (treatment). During the treatment phase, participants assigned to the placebo group received four capsules, each containing 300 mg corn oil (tocopherol-stripped corn oil was used to prepare these capsules, Tekled Test Diets, Madison WI USA). Participants assigned to the TRF_{25} group received four capsules, each containing 50 mg TRF_{25} and 250 mg corn oil. In total, the TRF_{25} capsules supplied, daily, 25 mg α-tocotrienol, 42 mg γ-tocotrienol, 20 mg δ-tocotrienol, 34 mg P_{25}-tocotrienol, 9 mg d-tocotrienol,[21,22] 12 mg α-tocopherol, and 36 mg unidentified tocols. The participants recorded their daily food intakes during the final 3 days of each phase. Participants

were contacted by telephone during each phase for an unanticipated 24-hr recall of food intake. Diet records and 24-hr recalls were analyzed (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN USA); if required, a participant was individually counseled to modify food intake to meet the goals of the NCEP Step-1 diet or to maintain weight.

Initial measures included the participants' height, weight, blood pressure, history of significant diseases, medications, and alcohol use. Weights were recorded weekly. Venous blood samples (12-hr fast, 7:00–9:00 am) were drawn at screening. The participants were screened 1 to 3 weeks before obtaining the baseline values. At screening, the participants were counseled to follow their normal dietary patterns exclusive of alcohol consumption. Venous blood samples were drawn at the termination of the baseline phase at week 4, followed by NCEP Step-1 diet (weeks 7 and 8) and treatment (weeks 11 and 12) phases. Processed samples were coded and held at −72°C until analysis following the Treatment phase.

Analyses

Plasma and serum were frozen and shipped on dry ice to Advanced Medical Research, Madison, WI USA. The analyses of the coded samples were performed at Advanced Medical Research. Serum triglycerides, glucose, and cholesterol were estimated manually (incubation time was 10 min for each estimation of lipid parameters) with reagent kits from Sigma Chemical Co. (St. Louis, MO USA). LDL-cholesterol were precipitated from 200 μL of serum with 25 μL of a mixture of 9.7 mM phosphotungstic acid and 0.4 M $MgCl_2$. The preparation was mixed for 10 min at room temperature and then centrifuged at 12,000 × g for 10 min. The supernatant fraction was decanted and analyzed for HDL-cholesterol. The precipitate was dissolved in 200 μL of 0.1 M sodium citrate and the concentration of LDL-cholesterol could be determined. The LDL-cholesterol was estimated by Friedewald's formula[23] by subtracting the total cholesterol from (HDL-cholesterol + triglycerides/5). Serum apo A-I, apo B (Sigma Chemical Co., St. Louis, MO USA), platelet factor 4 (PF4; Abbott Laboratories, Chicago, IL USA) and thromboxane B_2 (TxB_2; Chemicon International, El Segundo, CA USA) concentrations were determined by radio immunoassay kits. Lp(a) was assayed with an immunoassay kit from Terumo Medical Corporation, Diagnostic Division (Elkton, MD USA).

Sera were processed for the determination of tocols. Serum (1.0 ml) was transferred to a 20-mL culture tube, 8 mL hexane was added, mixed for 20 min, centrifuged for 10 min at 850 × g, and the hexane layer was transferred to a 15-mL conical tube. The hexane extraction was repeated, the hexane extracts containing the tocols were combined and evaporated under vacuum at 40°C. The aqueous layer was taken to dryness in a vacuum oven (2 psi) and held at 180°C for 1 hr. The serum residue was then extracted with 8 mL methanol. The methanol was removed under vacuum to measure the tocols bound to the protein. Tocols extracted by each procedure were dissolved in 200 μL hexane, transferred to injecting vials, the vials were capped and centrifuged for 4 min. The hexane and methanol soluble tocols present in the HDL and LDL (separated by phosphotungstic acid and $MgCl_2$) fractions were similarly extracted. The clear solutions were analyzed by slight modification of the well established HPLC method of Hakkarainen et al.[24,25]

The HPLC system consisted of a continuous-flow 307 pump (Gilson, Madison, WI USA), flow rate, 1.3 mL/min, pressure 0.4 psi, and a Gilson's Model 231/401 auto-sampler, loop size (20 μL), a Shimadzu Model RF-535 fluorescence monitor set at an excitation wavelength of 295 nm and an emission wavelength of 330 nm, and for UV monitoring, Shimadzu UV-VIS SPD-10 AV scanning stop-flow detector set at 295 nm and peak areas were determined by using a Shimadzu Model C-R3A integrator (Shimadzu, Wood Dale, IL USA). A 10 micron, 30 cm × 4.0 mm i.d., normal-phase silica column (Waters Associates, Milford, MA USA) was used to separate various tocols. The eluting solvent was 0.5% (vol/vol) isopropyl alcohol in hexane.[25] Retention time of the individual peaks of the unknown tocols were compared against the retention time of the pure standard tocols. The tocols were eluted under these conditions in the sequence; α-tocopherol (4.6 min), α-tocotrienol (5.3 min), β-tocopherol (6.4 min), γ-tocopherol (7.4 min), β-tocotrienol (8.6 min), γ-tocotrienol (9.4 min), δ-tocopherol (14.6 min), δ-tocotrienol (16.2 min), desmethyl tocotrienol (21.2 min; P_{21}), and didesmethyl tocotrienol (25.3 min; P_{25}) as reported earlier from cereals and serum samples.[14,15,21,25,26] The pure tocols (tocopherols and tocotrienols) were provided by Dr. B. Pearce of Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT USA.

Statistical analyses

The data were analyzed by using the GLM procedure of SAS (Statistical Analysis System) for personal computers to test the study hypothesis. Duncans multiple-range test was used to test whether the treated groups differed from the placebo for serum lipid parameters. Repeated-measures, two-way ANOVA was used to test whether changes in serum lipid parameters occur in the course of supplementation, and whether there were between- and within-subjects differences; because all observations were required, available degrees of freedom were reduced by this statistical approach. The treatment effects on cholesterol were also evaluated using the paired, two-tailed *t*-test (StatView, Abacus Concepts, Berkeley, CA USA). Data are reported as mean ± SD in the text. The statistical significance level was set at 5%.

Results

The intervention for all subjects during the last two phases (weeks 5 to 12) was the NCEP Step-1 diet. The analysis of all the subjects during this phase reveals that the subjects significantly decreased their reported intakes of energy (23%, $P < 0.01$), carbohydrate (19%, $P < 0.05$), fat (40%, $P < 0.03$), saturated fatty acids (38%, $P < 0.01$), and cholesterol (33%, $P < 0.04$) as shown in *Table 1*. The detailed dietary intake of protein, fat, carbohydrate, cholesterol, fiber and alcohol at various phases has been outlined in *Table 1*. Moreover, the body weight in Placebo and Treatment groups showed no significant difference during NCEP Step-1 diet and NCEP Step-1 diet + TRF_{25} as compared to Baseline (*Table 1*). The sole restriction during the acclimation phase, that of alcohol abstention, had little effect, as shown by week 4 (Baseline) values for all parameters from Screening values (*Tables 2–4*). The dietary restriction (tests at weeks 7 and 8; NCEP Step-1 diet) alone lowered serum total cholesterol and LDL-cholesterol and apo B 5%, 8%, and 8% (NS) respectively, from Baseline levels (*Table 2*). TRF_{25} supplement (tests at weeks 11 and 12) decreased total cholesterol, LDL-cholesterol, and apo B 16%, 23%, and 15% ($P < 0.05$) respectively, from Baseline values. There were further decreases in the Placebo group of 2%, 3%, and 3% ($P < 0.05$), respectively, in these parameters caused by the NCEP Step-1 diet at 12-week period (*Table 2*).

The dietary modification of these subjects did not produce any change in the serum Lp(a) levels in either group

Table 1 Effects of National Cholesterol Education Program (NCEP = AHA) Step-1 Diet on dietary intake during weeks 5 to 12 of hypercholesterolemic human subjects

Dietary intake	n*	Week-4 Baseline	Week-8 NCEP Step-1 Diet	Week-12 NCEP Step-1 Diet + TRF25	t#	W-4 vs W-8	W-4 vs W-12 P##	W-8 vs W-12
Energy (Kj/d)	39	7714 ± 3195	6629 ± 1796	6295 ± 2517	0.317	0.01	0.05	NS###
Protein (g/d)	39	71.5 ± 30.0	65.5 ± 28.0	67.0 ± 29.5	0.557	NS	NS	NS
Fat (g/d)	39	70.0 ± 43.5	51.0 ± 26.5	45.0 ± 25.0	0.063	0.05	0.04	NS
Carbohydrate (g/d)	39	220.0 ± 87.5	194.0 ± 79	211.0 ± 95.5	0.441	0.05	0.05	NS
Alcohol (g/d)	39	9.0 ± 16.0	2.0 ± 5.0	2.5 ± 6.0	0.053	0.05	0.05	NS
Protein En. %	39	16.5 ± 6.7	18.1 ± 6.3	18.1 ± 6.3	0.555	NS	NS	NS
Fat En. %	39	32.4 ± 9.9	35.9 ± 12.2	25.0 ± 14.7	0.146	NS	NS	NS
Carbohydrate En. %	39	49.0 ± 12.0	51.9 ± 14.2	56.1 ± 14.2	0.234	NS	NS	NS
Alcohol En. %	39	2.9 ± 5.35	1.0 ± 2.1	1.5 ± 5.35	0.358	0.05	NS	NS
Cholesterol (g/d)	39	231.0 ± 176	145.0 ± 103	158.0 ± 126	0.258	0.05	0.05	NS
SFA** (g/d)	39	22.0 ± 9.0	16.0 ± 9.0	13.5 ± 7.0	0.077	0.01	0.001	NS
MUSA*** (g/d)	39	27.0 ± 18.5	18.0 ± 9.5	16.5 ± 11.0	0.092	0.05	0.05	NS
PUFA**** (g/d)	39	14.5 ± 11.0	12.5 ± 8.0	10.5 ± 7.5	0.346	NS	NS	NS
SFA En. %	39	10.9 ± 4.5	9.7 ± 5.2	8.5 ± 4.2	0.195	NS	NS	NS
MUFA En. %	39	12.4 ± 4.3	10.8 ± 4.4	9.5 ± 4.5	0.081	NS	NS	NS
PUFA En. %	39	6.6 ± 3.4	7.9 ± 5.5	6.0 ± 3.6	0.397	NS	NS	0.05
Fiber total (g/d)	39	15.1 ± 7.5	15.0 ± 6.9	16.8 ± 10.9	0.689	NS	NS	NS
Soluble (g/d)	39	5.5 ± 3.6	5.1 ± 3.2	8.6 ± 3.9	>0.800	NS	NS	NS
Insoluble (g/d)	39	9.3 ± 4.3	9.6 ± 4.3	11.0 ± 7.4	0.468	NS	NS	NS
Body weight (kg) (Corn oil, n = 20)		78.9 ± 15.5!	78.0 ± 15.9	78.0 ± 16.2	0.0035	NS	NS	NS
Body weight (kg) (TRF25, n = 19)		73.6 ± 12.7	72.8 ± 11.7	72.4 ± 11.9	0.0013	NS	NS	NS

t# = paired, two-tailed *t*-test; P## + Probability; NS### = not significant; n* = Subjects in corn oil group = 20 and in TRF25 group 19; **SFA = saturated fatty acid; ***MUFA = monounsaturated fatty acid; ****PUFA = polyunsaturated fatty acid; !No significance differences between corn oil and TRF25 at any time point by one-way ANOVA; carried out at Medical School, University of Illinois, Chicago.

(Placebo and Treatment), which is consistent with the published observations.[27,28] The administration of TRF_{25} produced a decrease of 17% ($P < 0.05$) in the Lp(a) level as compared to Baseline values (*Table 2*).

To check the influence of sex on the serum total cholesterol and HDL-cholesterol levels, values were calculated separately for male and female subjects at Baseline (week 4), NCEP Step-1 diet (week 8), and NCEP Step-1 diet + TRF_{25} (week 12) for Placebo group (corn oil) and the Treatment group (TRF_{25}) as shown in *Table 3*. It is evident that males and females respond similarly in each phase of the present short-term study. Moreover, the percentage of

Table 2 Effects of NCEP Step-1 diet and tocotrienol-rich fraction (TRF_{25}) from rice bran on serum total cholesterol, LDL-cholesterol, apolipoprotein B, and lipoprotein (a) in hypercholesterolemic human subjects

Lipid Parameters	1- to 3-week Screening	4-week Baseline	NCEP Step-1 Diet 7-week	NCEP Step-1 Diet 8-week	Treatment 11-week	Treatment 12-week
Total cholesterol (mmol/L)						
Placebo (corn oil)	7.07 ± 0.87[a]	7.02 ± 0.81[a*] (100)[†]	6.78 ± 0.82[a] (96)	6.67 ± 0.84[a] (95)	6.63 ± 0.87[a] (94)	6.53 ± 0.84[a] (93)
TRF_{25} capsules	6.56 ± 0.80[a]	6.59 ± 0.86[a]	6.39 ± 0.79[a] (97)	6.26 ± 0.79[a] (95)	5.74 ± 0.64[b] (87)	5.48 ± 0.66[b] (83)
LDL-cholesterol (mmol/L)						
Placebo (corn oil)	5.08 ± 0.78[a]	5.07 ± 0.72[a]	4.77 ± 0.72[a] (94)	4.66 ± 0.75[a] (92)	4.64 ± 0.76[ab] (92)	4.53 ± 0.75[b] (89)
TRF_{25} capsules	4.53 ± 0.78[a]	4.54 ± 0.80[a]	4.35 ± 0.71[a] (96)	4.18 ± 0.72[a] (92)	3.71 ± 0.60[b] (82)	3.48 ± 0.61[b] (77)
Apolipoprotein B (g/L)						
Placebo (corn oil)	0.98 ± 0.09[a]	0.99 ± 0.12[a]	0.93 ± 0.09[a] (94)	0.91 ± 0.08[a] (92)	0.89 ± 0.08[b] (90)	0.88 ± 0.09[b] (89)
TRF_{25} capsules	0.94 ± 0.07[a]	0.94 ± 0.06[a]	0.89 ± 0.06[a] (95)	0.87 ± 0.06[a] (93)	0.83 ± 0.05[b] (88)	0.80 ± 0.06[b] (85)
LP(a) (g/L)						
Placebo (corn oil)	—	0.39 ± 0.07[a]	—	0.38 ± 0.07[a] (100)	—	0.37 ± 0.07[a] (95)
TRF_{25} capsules	—	0.40 ± 0.09[a]	—	0.40 ± 0.09[a] (100)	—	0.33 ± 0.08[b] (83)

Time of drawing blood was 0800. The subjects fasted for 12 hr before samples were taken.
*$\bar{x}$ ± SD (mean ± standard deviation).
†Percentages with respect to baseline values are in parentheses.
[a–b]Values in a row not sharing a common superscript letter are significantly different, $P < 0.05$.

Table 3 Effects of NCEP Step-1 diet and tocotrienol-rich fraction (TRF_{25}) from rice bran on serum total cholesterol and HDL-cholesterol in male and female hypercholesterolemic human subjects

Lipid Parameters	Week-4 (Baseline)	Week-8 (NCEP Step-1 Diet)	Week-12 (Treatment)
Total cholesterol (mmol/L)			
Male subjects			
Placebo (corn oil)	7.49 ± 1.18[a*] (100)[†]	7.09 ± 1.14[a] (95)	6.98 ± 1.15[a] (93)
TRF_{25}	7.29 ± 0.77[a]	7.06 ± 0.69[a] (96)	6.19 ± 0.68[b] (85)
Females subjects			
Placebo (corn oil)	8.11 ± 0.59[a]	7.67 ± 0.66[a] (95)	7.62 ± 0.68[a] (94)
TRF_{25}	7.23 ± 1.09[a]	6.77 ± 1.24[a] (94)	5.95 ± 0.79[b] (83)
HDL-cholesterol (mmol/L)			
Male subjects			
Placebo (corn oil)	1.27 ± 0.26[a]	1.27 ± 0.19[a] (100)	1.27 ± 0.24[a] (100)
TRF_{25}	1.19 ± 0.26[a]	1.25 ± 0.22[a] (105)	1.28 ± 0.24[a] (108)
Female subjects			
Placebo	1.34 ± 0.23[a]	1.36 ± 0.25[a] (101)	1.37 ± 0.26[a] (102)
TRF_{25}	1.31 ± 0.22[a]	1.39 ± 0.21[a] (106)	1.42 ± 0.24[a] (108)

*X + SD (mean + standard deviation); †Percentages with respect to Baseline values are in parentheses; [a–b]Values in a row not sharing a common superscript letter are significantly different, $P < 0.05$.

decrease for serum total cholesterol level during NCEP Step-1 diet and Treatment groups was similar as described above in *Table 2* (4% ~ 5% during NCEP Step-1 diet and 6% and 17% for the Placebo and the TRF_{25} treatment groups, respectively). No significant difference was found in HDL cholesterol levels either in any of these phases in these groups (*Table 3*).

The substantial effect of TRF_{25} on total cholesterol levels during the Treatment phase is more evident by performing a second more sensitive paired *t*-analysis of the individual subject responses (evaluation of paired differences) to treatment. The two groups responded uniformly during the NCEP Step-1 diet phase. During the Treatment phase the cholesterol level of the Placebo group decreased by 0.16 ± 0.13 mmol/L ($P < 0.001$) whereas that of the TRF_{25} group decreased by 0.73 ± 0.32 mmol/L ($P < 0.001$) as shown in *Table 4*.

Neither the NCEP Step-1 diet nor TRF_{25} had an effect on HDL cholesterol. Baseline and Treatment HDL cholesterol levels for the Placebo group were 1.18 ± 0.22 and 1.20 ± 0.23 mmol/L, respectively, and for the TRF_{25} group, 1.14 ± 0.29 and 1.22 ± 0.18 mmol/L, respectively (*Table 5*). In parallel, apo A-I levels for both groups remained stable throughout the study (*Table 5*). Similarly, triglycerides remained constant (Baseline, 1.95 ± 0.50; terminal Treatment, 1.74 ± 0.52 mmol/L) and glucose decreased from a Baseline 5.95 ± 0.94 to a terminal Treatment 5.12 ± 0.73 mmol/L (*Table 5*).

The other parameters directly associated with cardiovascular risk, thromboxane B_2 (TxB_2) and platelet factor 4 (PF4) similarly decreased (each $P < 0.05$) in response to the TRF_{25}, but not to the Step-1 diet (*Table 6*).

The estimates of hexane-extracted serum tocols (~13 mg/L, *Table 7*) are in general agreement with the tocol levels reported for healthy adults.[29] Because of our finding that processing increased by 3-fold the quantity of tocols extracted from rice bran, aliquots of serum were exposed to heat under negative pressure. This is the first report demonstrating a 13-fold increase in the estimate of serum tocols after this procedure (~170 mg/L, *Table 6*) and our findings suggest that the standard procedure markedly underestimates serum tocol concentrations. The HDL and LDL moieties extracted with hexane and methanol showed similar results (*Table 8*). Moreover, more than 70% of the tocols are found in LDL moiety as compared with the HDL fraction. The TRF_{25} supplementation has nearly doubled the concentration of serum total tocols with the tocotrienols accounting for the major increases (*Table 8*).

Table 4 Changes in serum total cholesterol levels (mmol/L) during different phases of the study of hypercholesterolemic human subjects

		Paired Differences[1]								
		Baseline - NCEP Step-1 Diet			Baseline - Treatment[2]			NCEP Step-1 Diet - Treatment[2]		
Treatment	*n*	Change	t[#]	P[†]	Change	t	P	Change	t	P
Placebo	21	0.40 ± 0.29	6.08	0.0001	0.51 ± 0.37	7.83	0.0001	0.16 ± 0.13	4.51	0.0002
TRF_{25}	20	0.53 ± 0.39	3.89	0.001	1.05 ± 0.53	8.39	0.0001	0.73 ± 0.32	9.59	0.0001

[1]Week 4 analyses of each phase
[2]Tocotrienol Rich Fraction (TRF_{25}) of oil extracted from thermal/vacuum-processed rice bran
[#]t = paired two-tailed *t*-test
[†]P = probability

Table 5 Effects of NCEP Step-1 diet and tocotrienol-rich fraction (TRF_{25}) from rice bran on HDL-cholesterol, apolipoprotein A-1, triglycerides, and glucose in hypercholesterolemic human subjects

Lipid Parameters	1 to 3-week Screening	4-week Baseline	NCEP Step-1 Diet		Treatment	
			7-week	8-week	11-week	12-week
HDL-Cholesterol (mmol/L)						
Placebo (corn oil)	1.19 ± 0.24[a]	1.18 ± 0.22[a] *(100)[†]	1.18 ± 0.21[a] (100)	1.19 ± 0.21[a] (101)	1.19 ± 0.23[a] (101)	1.20 ± 0.23[a] (102)
TRF_{25} capsules	1.13 ± 0.20[a]	1.14 ± 0.20[a]	1.16 ± 0.19[a] (103)	1.21 ± 0.20[a] (107)	1.21 ± 0.22[a] (107)	1.22 ± 0.18[a] (108)
Apolipoprotein A-I (g/L)						
Placebo (corn oil)	1.14 ± 0.11[a]	1.13 ± 0.11[a]	1.14 ± 0.12[a] (100)	1.14 ± 0.12[a] (100)	1.14 ± 0.12[a] (100)	1.14 ± 0.12[a] (100)
TRF_{25} capsules	1.11 ± 0.07[a]	1.11 ± 0.08[a]	1.11 ± 0.08[a] (100)	1.11 ± 0.08[a] (100)	1.12 ± 0.08[a] (101)	1.12 ± 0.08[a] (101)
Triglycerides (mmol/L)						
Placebo (corn oil)	1.87 ± 0.38[a]	1.86 ± 0.39[a]	1.80 ± 0.36[a] (97)	1.78 ± 0.36[a] (96)	1.74 ± 0.37[a] (94)	1.75 ± 0.36[a] (94)
TRF_{25} capsules	1.95 ± 0.49[a]	1.95 ± 0.50[a]	1.92 ± 0.51[a] (98)	1.89 ± 0.51[a] (97)	1.79 ± 0.51[a] (92)	1.74 ± 0.52[a] (89)
Glucose (mmol/L)						
Placebo (corn oil)	5.91 ± 0.73[a]	5.96 ± 0.77[a]	5.78 ± 0.73[a] (97)	5.59 ± 0.64[a] (94)	5.41 ± 0.59[a] (92)	5.38 ± 0.64[a] (91)
TRF_{25} capsules	5.91 ± 0.95[a]	5.95 ± 0.94[a]	5.67 ± 0.86[a] (96)	5.71 ± 0.86[a] (97)	5.31 ± 0.69[ab] (90)	5.12 ± 0.73[b] (87)

Time of drawing blood was 0800. The subjects fasted for 12 hr before samples were taken.
*$\bar{x}$ ± SD (mean ± standard deviation).
[†]Percentages with respect to baseline values are in parentheses.
[a–b]Values in a row not sharing a common superscript letter are significantly different, $P < 0.05$.

Discussion

This study adds support for the role played by dietary guidance patterned after the NCEP Step-1 diet in the management of hypercholesterolemia. Energy, fat, and cholesterol intakes and percent energy provided by saturated and monounsaturated fatty acids during the NCEP Step-1 diet and Treatment phases were significantly lower than the Baseline values. Differences in values between Placebo and TRF_{25} groups and between NCEP Step-1 diet and Treatment phases were not significant. The dietary guidance alone caused changes in serum total cholesterol (−5%), LDL cholesterol (−8%), and apo B (−7%) levels. The HDL-cholesterol, apo A-I, and triglycerides values remained relatively constant across the various phases of the study. The other risk parameters (Lp (a), TxB_2, and PF4) were resistant to dietary guidance-initiated change.

The TRF_{25} supplement had an add-on effect for serum total cholesterol (−16%) and LDL cholesterol (−23%) values. Thromboxane B_2, platelet factor 4, and Lp(a) clearly responded to supplemental TRF_{25}. The 17% decrease in Lp(a), seen in *Table 2* was unexpected, as neither diet nor the hypocholesterolemic drugs is likely to have an impact on serum Lp(a) levels.[27,28]

The study design provides a basis for isolating the TRF_{25} effect from that of the dietary counseling. In the present study, the males and females in Placebo and Treatment groups were almost in equal numbers. Moreover, most of the females (16 out of 22) were over 50 years of age. They were selected because it is well established that premenopausal females are protected from heart disease because of their estrogen level. Although the remaining six female subjects were premenopausal, their serum total cholesterol levels were >5.69 mmol/L, presumably because of high dietary fat and cholesterol intake or because of heriditary reasons. The effects of NCEP Step-1 diet with or without TRF_{25} were calculated on the serum total cholesterol and HDL cholesterol levels in male and female subjects separately in Placebo and TRF_{25} groups. The present data did

Table 6 Effects of NCEP Step-1 diet and tocotrienol-rich fraction (TRF_{25}) from rice bran on plasma thromboxane B_2 and platelet factor 4 concentrations in hypercholesterolemic subjects

Lipid Parameters	1 to 3-week Sreening	4-week Baseline	NCEP Step-1 Diet		Treatment	
			7-week	8-week	11-week	12-week
Thromboxane B_2 (pg/L)						
Placebo (corn oil)	313 ± 33[a]	315 ± 29[a] *(100)[†]	312 ± 30[a] (99)	309 ± 30[a] (98)	309 ± 31[a] (98)	306 ± 31[a] (97)
TRF_{25} capsules	316 ± 21[a]	315 ± 19[a]	313 ± 17[a] (99)	311 ± 20[a] (98)	240 ± 17[b] (76)	218 ± 13[c] (69)
Platelet Factor 4 (pg/L)						
Placebo (corn oil)	56 ± 4[a]	56 ± 4[a]	56 ± 5[a] (100)	56 ± 4[a] (99)	56 ± 6[a] (99)	56 ± 5[a] (99)
TRF_{25} capsules	56 ± 3[a]	56 ± 2[a]	56 ± 4[a] (100)	56 ± 8[a] (100)	50 ± 4[b] (89)	48 ± 3[c] (86)

Time of drawing blood was 0800. The subjects fasted for 12 hr before samples were taken.
*$\bar{x}$ ± SD (mean ± standard deviation).
[†]Percentages with respect to baseline values are in parentheses.
[a–c]Values in a row not sharing a common superscript letter are significantly different, $P < 0.05$.

Table 7 Hexane- and methanol-extracted tocopherols (T) and tocotrienols (T3) in serum of corn oil (Placebo) and tocotrienol-rich fraction from rice bran (TRF_{25}) treated hypercholesterolemic human subjects

Treatments	Hexane-Extracted			Methanol-Extracted			Grand Total
	T	T3	Total	T	T3	Total	
	mg/L						
Placebo (corn oil)							
4-week (Baseline)	13.66 ± 3.42†	0.36 ± 0.11	14.02	151.17 ± 17.83	1.94 ± 0.81	153.11	167.13
8-week (NCEP Step-1 diet)	11.11 ± 3.71	0.17 ± 0.18	11.28	140.21 ± 19.17	3.17 ± 1.42	143.38	154.66
12-week (corn oil)	11.27 ± 4.13	0.50 ± 0.15	11.77	138.71 ± 16.75	4.66 ± 1.64	143.37	155.14
Percentage T3 to Total		(4.25%)			(3.25%)		
TRF_{25} capsules							
4-week (Baseline)	12.36 ± 2.84	0.55 ± 0.17	12.91	164.27 ± 21.23	2.89 ± 0.74	167.16	180.07
8-week (NCEP Step-1 diet)	12.94 ± 3.45	0.51 ± 0.19	13.45	166.71 ± 19.67	4.27 ± 0.98	170.98	184.43
12-week (TRF_{25})	37.59 ± 7.94	6.68 ± 2.2	44.27	192.42 ± 26.51	73.52 ± 11.69	265.94	310.21
Percentage T3 to Total		(15.09%)			(27.64%)		

†$\bar{x}$ ± SD (mean ± standard deviation).

not show any difference between the two sexes as far as the serum total cholesterol or HDL cholesterol levels are concerned. This might be because the present study was performed only for a very short period (8 weeks). The modest (7%) cholesterol-lowering action of the NCEP Step-1 diet became significant ($P < 0.05$) at Treatment week 4 for the Placebo group, 8 weeks after the introduction of the diet by using a second evaluation, the paired *t*-test, which compares two measurements taken from the same participant. This approach assumes that one measurement of each pair essentially serves as the control for the second. This test shows that the 5% and 7% decreases in cholesterol levels observed after 4 and 8 weeks of exposure to the NCEP Step-1 diet (Placebo group) were significant. Total cholesterol levels of the TRF_{25} group were decreased by 5% and 17%, respectively, during the NCEP Step-1 diet and Treatment phases of the study. The difference in the responses of the two groups, a 7% (Placebo) and 17% (TRF_{25}) lowering of total cholesterol during the Treatment phase after the first 4-week NCEP Step-1 diet phase, reflects the impact of the TRF_{25} on total cholesterol levels. As in previous studies of the cholesterol-lowering efficacy of Palmvitee (TRF; tocotrienol-rich fraction from palm oil) in general, 25% of participants proved to be resistant to the cholesterol-lowering action of the Palmvitee.[21] Participants whose hypercholesterolemia traces to an error in cholesterol transport or in cholesterol degradation would, we anticipate, not respond to an agent whose action is limited to one aspect[17] of the multivalent regulation[30] of cholesterol synthesis.

The NCEP Step-1 diet effectively increased the apo A-I/B ratio from 1.09 to 1.26 (Placebo group) and from 1.18 to 1.27 (TRF_{25} group). During the Treatment phase, the increase in the ratio for the Placebo group, 1.26 to 1.30, was far surpassed in the TRF_{25} group (1.27 to 1.46). Using the aforementioned rationale, the TRF_{25} accounted for a 0.15 increase in ratio for this important predictor of cardiovascular disease risk. Our findings that other cardiovascular disease risk factors, TxB_2 and PF4, also responded to TRF_{25} seem to be consistent with recent reports that the tocotrienols suppress platelet aggregation.[31,32] The thromboxanes induce platelet aggregation and vasoconstriction. The prostacyclins inhibit platelet aggregation and produce vasodilation. These investigators attribute these tocotrienol-mediated actions to their unique in situ antioxidant activity of tocols.[31–34]

The hexane-extracted and methanol-extracted tocols of HDL and LDL moieties of the participants receiving the

Table 8 Hexane- and methanol-extracted tocopherols (T) and tocotrienols (T3) in serum of high-density lipoprotein (HDL-) and low-density lipoprotein (LDL-) of corn oil (placebo) and tocotrienol-rich fraction from rice bran (TRF_{25}) treated hypercholesterolemic human subjects

Treatments	HDL (T and T3)			LDL (T and T3)			Grand Total
	HE*	ME**	Total	HE	ME	Total	
	mg/L						
Placebo (corn oil)							
4-week (Baseline)	4.69 ± 0.67†	19.12 ± 3.67	23.81	8.96 ± 2.12	126.18 ± 19.21	135.14	158.95
8-week (NCEP Step-1 diet)	4.16 ± 0.81	16.15 ± 4.12	20.31	8.24 ± 3.34	119.89 ± 15.41	128.13	148.44
12-week (corn oil)	4.18 ± 1.12	14.24 ± 2.67	18.42	8.15 ± 2.15	123.74 ± 16.11	131.89	150.31
TRF_{25} capsules							
4-week (Baseline)	2.63 ± 0.44	27.18 ± 5.15	28.81	7.32 ± 1.67	123.89 ± 11.89	131.21	161.02
8-week (NCEP Step-1 diet)	3.79 ± 0.39	31.48 ± 6.67	35.27	6.89 ± 1.12	122.22 ± 17.84	127.11	162.38
12-week (TRF_{25})	16.21 ± 1.85	55.67 ± 11.21	71.89	22.89 ± 4.72	193.26 ± 22.75	216.15	288.03

*HE = hexane-extracted
**ME = methanol-extracted
†$\bar{x}$ ± SD (mean ± standard deviation).

TRF_{25} (178 mg tocols/day) were 4-, 4-fold (in HDL moiety), and 3-, 1.6-fold (in LDL moiety), respectively, higher than the Placebo values. Differences in the transport and tissue uptake of the saturated and unsaturated tocols have been reported by various investigators.[35,36] A hepatic binding protein with high specificity for α-tocopherol in the liver results in the subsequent enrichment of this tocol in the VLDL moiety and as a consequence, the LDL moiety. The tocotrienols on the other hand are transported nonspecifically like other lipid-soluble compounds.[35,36] We interpret our findings to show that the TRF_{25}-receiving group substantially increased hexane-extracted tocols (mostly in tocotrienols) of the HDL moiety (4-fold as compared with the Placebo group), but there was not as great an increase in the tocols (2.9 fold) of the LDL moiety of the treatment group. This might indicate that the tocol compartment in the LDL moiety was saturated under normal dietary conditions.

The HMG-CoA reductase-suppressive action of the tocotrienols[16,17,37] was manifested in a significant reduction in serum cholesterol. The post-transcriptional action of the tocotrienols argues for their use in combination with the competitive inhibitors of reductase activity.[17] We note that human subjects do not respond uniformly to the cholesterol-lowering action of the tocotrienols,[21] particularly when cholesterol and alcohol intakes are not controlled. Our present finding that 4 of 20 participants with restricted alcohol and cholesterol intakes failed to respond to the tocotrienols likely suggests their hypercholesterolemia traces to a metabolic error other than a failure to downregulate cholesterol synthesis. A second factor confounding the tocotrienol effect is the attenuating action of α-tocopherol.[38] Although the tocols are widely distributed in cereal grains, it is important to note that cultivars of both barley and oats differ substantially in their tocol profiles. The finding that the tocols of some cultivars are enriched in α-tocotrienol[26] likely explains the conflict in reports of the effect of oat bran on serum cholesterol levels. The rice bran processing method described above increased by 2 to 3 fold the quantity of the recovered tocotrienols; and, most importantly, P_{25}-tocotrienol. Tocotrienol fractions isolated from commercial rice bran had little cholesterol-suppressive potency.[15]

Ischemic heart disease mortality has been shown to have a 79% predictive correlation with serum cholesterol levels together with inverse levels of lipid standardized serum vitamin-E.[39] Critical elements in the progression of atherosclerosis and the occurrence of myocardial infarction and stroke appear to include high serum LDL-cholesterol, oxidation of LDL-cholesterol, and development of acute coronary thrombi. These results suggest reduction of the LDL-cholesterol substrate of plaque, and inhibition of both LDL-cholesterol oxidation and occlusive thrombi formation, are possibly three important health consequences of a diet high in tocotrienols and other isoprenoid constituents of foods. The effect of the TRF_{25} was not confined to the cholesterol component of the risk profile of the hypercholesterolemic participants. The TRF_{25}-mediated decreases in LP(a), TxB_2, and PF4 levels may be meaningful in the prevention of cardiovascular disease processes in normocholesterolemic individuals. If so, these results fall in line with epidemiological studies showing that diets high in cereal grains, fruits, and vegetables, the dietary sources of the tocotrienols, provide protection against cardiovascular disease. This is the first reported clinical study on TRF_{25} (tocotrienols) from rice bran that has a high effect on human cardiovascular health.

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References

1 Heart Memo, National Heart, Lung and Blood Institute. (1994). National Institutes of Health, Spring

2 National Research Council Committee on Diet and Health. Diet and health: implications for reducing chronic disease risk. (1989). Washington DC: National Academy of Sciences Press

3 Grundy, S.M. (1986). Cholesterol and coronary heart disease: a new era. *J. Am. Med. Assoc.* **256,** 2849–2858

4 Nutrition Committee, American Heart Association. (1988). Dietary guidelines for healthy American adults. *Circulation* **77,** 721–724A

5 Kahlon, T.S., Chow, F.I., Sayer, R.N., and Betschart, A.A. (1992). Cholesterol-lowering in hamster fed rice bran at various levels, defatted rice bran and rice bran oil. *J. Nutr.* **122,** 513–519

6 Rukmini, C., and Raghuram, T.C. (1991). Nutritional and biochemical aspects of the hypolipidemic action of rice bran oil (a review). *J. Am. Coll. Nutr.* **10(6),** 593–601

7 Kahlon, T.S., Saunders, R.M., Chow, F.I., Chiu, M.M., and Betschart, A.A. (1990). Influence of rice bran, oat bran, and wheat bran on cholesterol and triglycerides in hamsters. *Cereal Chem.* **67,** 439–441

8 Seetharamaiah, G.S. and Chandrasekhara, N. (1989). Studies of the hypocholesterolemic activity of rice bran. *Atherosclerosis* **78,** 219–223

9 Sharma, R.D. and Rukmini, C. (1987). Hypocholesterolemic activity of unsaponifiable fraction of rice bran oil. *Ind. J. Med. Res.* **85,** 278–281.

10 Seetharamaiah, G.S. and Chandrasekhara, N. (1988). Hypocholesterolemic activity of oryzaniol in rats. *Nutr. Rep. Int.* **38,** 927–935

11 Yoshino, G., Kazumi, T., Amano, M., Tateiwa, M., Yamasaki, T., Takashima, S., Iwai, M., Hatanaka, H., and Baba, S. (1989). Effects of gamma-oryzaniol on hyperlipidemic subjects. *Cur. Ther. Res. Clin. Exp. (USA)* **45,** 543–552

12 Nicolosi, R.J., Ausman, L.M., and Hegsted, D.M. (1991). Rice bran oil lowers serum total and low density lipoprotein cholesterol and apo B levels in nonhuman primates. *Atherosclerosis* **88,** 133–142

13 Rogers, E.J., Rice, S.M., Nicolosi, R.J., Carpenter, D.R., McClelland, C.A., and Romanczyk, L.J. (1993). Identification and quantitation of γ-oryzanol components and simultaneous assessment of tocols in rice bran oil. *J. Am. Oil Chem. Soc.* **70,** 301–307

14 Qureshi, N. and Qureshi, A.A. (1993). Tocotrienols, novel hypocholesterolemic agents with antioxidant properties. In: *Vitamin E in Health and Disease*, p. 247–268, (Packer, L. and Fuchs, J., eds.), Marcel Dekker: New York, NY USA

15 Qureshi, A.A., Lane, R.H., and Salser, A.W. (1993). Tocotrienols and tocotrienol-like compounds and methods for their use. International Patent Application, No. PCI/US92/10277

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

17 Parker, R.A., Pearce, B.C., Clark, R.W., Gordon, D.A., and Wright, J.J.K. (1993). Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-

methylglutarylcoenzyme A reductase. *J. Biol. Chem.* **268,** 11230–11238

18 Randall, J.M., Sayre, R.N., Schultz, W.G., Fong, R.Y., Mossman, A.P., Tribelhorn, R.E., and Saunders, R.M. (1985). Rice bran stabilization by extrusion cooking for extraction of edible oil. *J. Food Sci.* **50,** 361–363

19 Saunders, R.M. (1985). Rice bran, Composition and potential food use. *Food Rev. Int.* **1,** 465–495

20 Qureshi, A.A., Becker, K.W., Wells, D.M., and Lane, R.H. (1990). Process for recovering tocotrienols, tocopherols and tocotrienol-like compounds. International Patent Application, No. PCI/US 91/03626

21 Qureshi, A.A., Qureshi, N., Wright, J.J.K., Shen, Z., Kramer, G., Gapor, A., Chong, Y.H., DeWitt, G., Ong, A.S.H., Peterson, D.M., and Bradlow, B.A. (1991). Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (palmvitee). *Am. J. Clin. Nutr.* **53,** 1021S–1026S

22 Qureshi, N., Hofman, J., and Qureshi, A.A. (1993). Inhibition of LPS-induced tumor necrosis factor and necrosis factor synthesis and hypocholesterolemic effects of novel tocotrienol. *Proc PORIM Internat Palm Oil Conf,* Sept 20–25, Kuala Lumpur Abstr. 19.

23 Friedwald, W.T., Levy, R.I., and Fredrickson, D.S. (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of preparative ultracentrifuge. *Clin. Chem.* **18,** 499–502

24 Hakkarainen, R.V.J., Tyoppnen, J.T., and Bengtsson, S.G. (1983). Changes in the content and composition of vitamin E in damp barley stored in airtight bins. *J. Sci. Food Agri.* **34,** 1029–1038

25 Lehmann, J.W., Putnam, D.H., and Qureshi, A.A. (1994). Vitamin E isomers in grain amaranths (Amaranthus spp). *Lipids* **29,** 177–181

26 Peterson, D.M. and Qureshi, A.A. (1993). Genotype and environment effects on tocols of barley and oats. *Cereal Chem.* **72,** 157–162

27 McLean, J.W., Tomlinson, J.E., Kuang, W.J., Eaton, D.L., Chen, E.Y., Fless, G.M., Scanu, A.M., and Lawn, R.M. (1987). cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature (London)* **300,** 132–135

28 Haberland, M.E., Fless, G.M., Scanu, A.M., and Fogelman, A.M. (1992). Malondialdehyde modification of lipoprotein(a) reduces avid uptake by human monocyte-macrophages. *J. Biol. Chem.* **267,** 4143–4151

29 Machlin, L.J. and Brin, M. (1980). Vitamin E. In: Human Nutrition: A Comprehensive Treatise. 3B. Nutrition and the Adult, Micronutrients. Plenum: (Alfin-Slater, R.B., Kritchevsky, D. eds.) New York, NY USA

30 Goldstein, J.S. and Brown, M.S. (1990). Regulation of the mevalonate pathway. *Nature (London)* **343,** 425–430

31 Serbinova, E., Kagan, V., Han, D., and Packer, L. (1991). Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol, and alpha-tocotrienol. *Free Radic. Biol. Med.* **10,** 263–275

32 Kooyenga, D.K., Geller, M., Watkins, T.R., Gapor, A. Diakoumakis, E., and Bierenbaum, M.L. (1996). Antioxidant effects of tocotrienols in patients with hyperlipidemia and carotid stenosis. *PORIM International Palm Oil Congress*, p. 143–151, September 23–28, 1996. Kaula Lumpur, Malaysia. Nutrition Conference

33 Traber, M.G., Podda, M., Weber, C., Thiele, J., Rallis, M., and Packer, L. (1996). Diet-derived and topically applied tocotrienols accumulate in skin and protect the tissue against ultraviolet light-induced oxidative stress. *PORIM International Palm Oil Congress*, p. 152–160, September 23–28, 1996. Kuala Lumpur, Malaysia. Nutrition Conference

34 Richardson, P.D. and Steiner, M. (1993). Adhesion of human platelets inhibited by vitamin E. In: *Vitamin E in Health and Disease* (Packer, L. and Fuchs, J., eds.), p. 297–311, Marcel Dekker: New York

35 Kayden, H.J. and Traber, M.G. (1993). Absorption, lipoprotein transport, and regulation of plasma concentration of vitamin E in Humans. *J. Lipid Res.* **34,** 343–358

36 Pearson, C.K. and Barnes, M. McC. (1970). The absorption and distribution of the naturally occurring tocochromanols in the rat. *Br. J. Nutr.* **24,** 581–587

37 Qureshi, A.A., Burger, W.C., Peterson, D.M., and Elson, C.E. (1986). The structure of an inhibitor of cholesterol biosynthesis isolated from barley. *J. Biol. Chem.* **261,** 10544–10550

38 Qureshi, A.A., Pearce, B.C., Nor, R.M., Gapor, A., Peterson, D.M., and Elson, C.E. (1996). α-Tocopherol attenuates the impact of γ-tocotrienol on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in chickens. *J. Nutr.* **126,** 389–394

39 Gey, K.F., Puska, P., Jordan, P., and Moser, U.K. (1991). Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am. J. Clin. Nutr.* **53,** 326S–334S