Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (palmvitee)\textsuperscript{1-3}

Asaf A Qureshi, Nilofer Qureshi, JJK Wright, Z Shen, G Kramer, A Gapor, YH Chong, G DeWitt, ASH Ong, DM Peterson, and BA Bradlow

ABSTRACT A double-blind, crossover, 8-wk study was conducted to compare effects of the tocotrienol-enriched fraction of palm oil (200 mg palmvitee capsules/day) with those of 300 mg corn oil/d on serum lipids of hypercholesterolemic human subjects (serum cholesterol 6.21–8.02 mmol/L). Concentrations of serum total cholesterol (−15%), LDL cholesterol (−8%), Apo B (−10%), thromboxane (−25%), platelet factor 4 (−16%), and glucose (−12%) decreased significantly only in the 15 subjects given palmvitee during the initial 4 wk. The crossover confirmed these actions of palmvitee. Serum cholesterol concentrations of seven hypercholesterolemic subjects (> 7.84 mmol/L) decreased 31% during a 4-wk period in which they were given 200 mg γ-tocotrienol/d. This indicates that γ-tocotrienol may be the most potent cholesterol inhibitor in palmvitee capsules. The results of this pilot study are very encouraging.

KEY WORDS Palm oil, tocotrienols, γ-tocotrienol, vitamin E, serum lipids profile, platelet aggregation, thromboxane B\textsubscript{2}, platelet factor 4

Introduction

Coronary artery disease (CAD) is the leading cause of death in the United States and other developed countries. A relationship between CAD, elevated concentrations of serum cholesterol, and dietary fats and cholesterol has been established (1) and recent studies have shown that the risk of CAD can be reduced by lowering serum cholesterol concentrations (2–4). The reduction was achieved using clofibrate, cholestyramine, dietary manipulation (2, 3), and surgical-bypass procedures (4). The use of drug therapy or surgical-bypass procedures does involve potential side effects. Other approaches that use dietary manipulation or naturally occurring agents may be less likely to cause undesirable consequences.

We have demonstrated the hypocholesterolemic effect of tocotrienols isolated from barley (5), oats, rice, and palm oil in various animal models (AA Qureshi, N Qureshi, FE Weber, V Chaudhary, JJK Wright, BC Pearce, DM Peterson, and BC Wentworth, unpublished observations). The reduction in cholesterol is specifically in the serum low-density-lipoprotein (LDL) cholesterol and apolipoprotein B (Apo B) and was achieved by feeding different amounts of tocotrienols, ranging from 20 to 500 µg/g diet.

We found a dose-depandant (5–250 µg/g diet) effect of tocotrienol-rich fraction (TRF) from palm oil for lowering the serum cholesterol and LDL cholesterol in normolipemic and hypercholesterolemic swine, quail, and chicken (AA Qureshi, et al, unpublished observations). During these studies, the TRF was found to be more effective in hypercholesterolemic swine, quail, and chickens than in normolipemic animals and birds. Therefore, the present study was carried out to assess the effect of TRF (palmvitee) as a dietary supplement in hypercholesterolemic human subjects. Most of the variables associated with the lipid profile were estimated after feeding TRF (palmvitee capsules) for several weeks.

Subjects and methods

Design

A double-blind, crossover experimental design was used to control within-subject variability of cholesterol measurements and the effect of order of the administration of the two supplements (palmvitee and corn oil) for a placebo. Subjects were randomly assigned to one of two groups. In group I, each subject was observed for a 2-wk baseline period, a 4-wk palmvitee-capsule supplementation period, and a 4-wk corn-oil-capsule supplementation period. In group II, each subject was observed for a 2-wk baseline period, a 4-wk corn-oil-capsule supplementation period, and a 4–6-wk palmvitee supplementation period.

Subjects

Twenty-five subjects (aged 30–60 y; 14 male, 11 female) were screened from 60 volunteers who had cholesterol concentrations ranging from 6.21 to 8.02 mmol/L. Subjects were excluded on...
the basis of fasting serum cholesterol concentrations < 6.21 mmol/L; weight > 125% of Metropolitan Life (6) relative weights; use of cholesterol-altering medications; abnormally high values of SGOT, SGPT, glucose, or BUN; and known diabetes, liver, renal, or hypertensive diseases. Fifteen subjects were initially fed palmvitee capsules, and 10 subjects were fed corn-oil capsules. All subjects signed an informed-consent statement, which was approved by the Institutional Review Board (IRB) at Michael Reese Hospital and Medical Center. The study was also approved by the Institutional Review Board.

**Dietary supplements**

A highly concentrated fraction of tocotrienols (α-tocopherol, 15-20%; α-tocotrienol, 12-15%; γ-tocotrienol, 35-40%; and δ-tocotrienol, 25-30%), TRF can be separated from palm oil. Capsules containing TRF (50 mg) were mixed with 250 mg palm superolein and called palmvitee.

For the experimental period (4 wk), subjects were asked to take four capsules containing either 50 mg TRF in 250 mg palm superolein (palmvitee; 15 subjects) or 300 mg corn oil (40 subjects) after breakfast and supper every day. No other changes in dietary habits were suggested. TRF, which consists of a mixture of α-tocopherol and α-, γ-, and δ-tocotrienols, was extracted from palm oil. The corn oil was obtained from a commercial source.

**Measures**

Initial measures included the subject’s height, weight, blood pressure, and history of significant diseases, medications, and alcohol use. At the end of each week during the experiment, the weight was recorded and a venous blood sample was drawn for analysis after an overnight fast. The venous blood sample was analyzed for total cholesterol (TC), high-density-lipoprotein (HDL) cholesterol (HDL-C), triglyceride (TG), LDL cholesterol (LDL-C), glucose, apolipoprotein B (Apo B), apolipoprotein A-I (Apo A-I), thromboxane B2 (TXB2), platelet factor 4 (PF4) and platelet aggregation in platelet-rich plasma against adenosine diphosphate (ADP), epinephrine (EPI), collagen, and tocotrienol.

At baseline and the end of each experimental period, a screening for glucose, BUN, creatinine, and liver-function studies was performed. All blood samples were kept frozen at -70°C as plasma or serum until analyzed. All samples were analyzed for each subject as a block at the end of the experiment.

**Serum lipid assays**

Total cholesterol (7) TGs (8), and HDL-C (9) were estimated in serum after an overnight fast. Serum cholesterol concentrations were estimated by use of a Sigma Cholesterol-Reagent kit. LDLs and very-low-density lipoproteins (VLDLs) were isolated from the serum (100 μL) by precipitation with a mixture of 10 μL each of 9.7 mmol phosphotungstic acid/L and 0.4 mol MgCl₂/L. After standing for 5 min at room temperature, the serum was centrifuged at 12,000 × g for 10 min. The supernatant was decanted and analyzed for HDL-C concentration. The precipitate was dissolved in 100 μL of a solution of 0.1 mol sodium citrate/L, and the concentration of cholesterol (VLDL + LDL) was determined (9, 10). Serum glucose and TGs were determined by using Sigma kits.

**Chemical determinations of Apo A-I, Apo B, TXB₂, PF₄ in serum or plasma**

Apo A-I and Apo B were determined by a radioimmunoassay (RIA) methods by using a Sigma kit. PF₄ and TXB₂ were determined by RIA by using commercially available standardized kits for each component. The PF₄ kit from Abbott Laboratories, North Chicago, IL uses a [125I]PF₄ to compete with nonradioactive PF₄ on PF₄ antiserum. For TXB₂, [125I]antibody to TXB₂ from Chemicon International was used.

Platelet aggregation was measured by the method of Born and Cross (11) in platelet-rich plasma (PRP) and whole blood (WB) using a Chronolog model 530 WB aggregometer and a model 330 platelet aggregometer for PRP (Chronolog Corp and Sigma Chemical Co). ADP and EPI were obtained from Sigma Chemical Company. Aggregation to ADP and EPI was measured at final concentrations of 5 and 20 μmol/L for PRP and aggregation to collagen, at 4.0 and 10.0 μmol/L in PRP and WB. All aggregation studies were completed within 3 h of blood collection. Aggregation responses were quantitated by measuring the percentage aggregation at 6 min, with the platelet-poor-plasma (PPP) and PRP readings as 100% and 0% aggregation, respectively.

Platelets were counted by using a standard counting chamber in a 1:100 dilution of PRP.

**Estimate of plasma tocopherol and tocotrienol concentrations after feeding corn oil or palmvitee capsules**

The total serum tocopherol and different tocotrienols were estimated by using the HPLC method of Hakkarainen et al (12).

**Statistical analysis**

Statistical comparison of results was performed by a one-way or two-way analysis of variance. When the F test indicated a significant effect, the differences between the means were analyzed by a protected least-significant-difference (LSD) test (13).

**Results**

Dietary supplementation of TRF (palmvitee capsules) to hypercholesterolemic subjects caused a drop in serum total cholesterol of 13% and 15% after 14 and 28 d, respectively, as compared with the baseline values (Table 1). This drop in serum cholesterol after 14 and 28 d was also reflected in a fall in LDL-C concentration of 4% and 8% and in Apo B of 7% and 10%, respectively (Table 1). Moreover, the TRF (palmvitee capsule) supplementation showed a significant decrease in PF₄ (16%) after 4 wk, as shown in Table 2. TXB₂ fell by 25% but this was not statistically significant (P = 0.05).

Decreases were also observed in platelet aggregation determined in PRP to ADP (9-17%), EPI (6-14%), and collagen (17-28%) as well as in WB (33-37%, to collagen only) with TRF (palmvitee capsules) as compared with baseline values (Table 3). Some of these differences were statistically significant but the physiological effect of even those that did is uncertain and possibly of little or no importance. TRF also caused a drop in serum glucose concentration of 12% (Table 4), but this was not statistically significant.

The serum HDL-C, Apo A-I, and the TG concentrations showed no significant changes compared with the baseline values.
TABLE 1
Effects of palmvite on serum lipid concentrations in hypercholesterolemic humans

<table>
<thead>
<tr>
<th>Nutritional state</th>
<th>Baseline value (first 2 wk):</th>
<th>Feeding period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>time 0</td>
<td>14 d</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (corn-oil capsules)</td>
<td>7.50 ± 0.802±</td>
<td>7.39 ± 0.698*</td>
</tr>
<tr>
<td>Palmvitee capsules</td>
<td>7.60 ± 0.8799 (100)</td>
<td>6.65 ± 0.8799(87)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (corn-oil capsules)</td>
<td>6.34 ± 0.6989</td>
<td>6.36 ± 0.6469*</td>
</tr>
<tr>
<td>Palmvitee capsules</td>
<td>6.41 ± 0.7249 (100)</td>
<td>6.13 ± 0.7769(96)</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (corn-oil capsules)</td>
<td>1.80 ± 0.1699</td>
<td>1.84 ± 0.1599*</td>
</tr>
<tr>
<td>Palmvitee capsules</td>
<td>1.71 ± 0.1499 (100)</td>
<td>1.60 ± 0.1599(93)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (corn-oil capsules)</td>
<td>1.24 ± 0.2079</td>
<td>1.22 ± 0.2339*</td>
</tr>
<tr>
<td>Palmvitee capsules</td>
<td>1.21 ± 0.2079 (100)</td>
<td>1.29 ± 0.1299(107)</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (corn-oil capsules)</td>
<td>0.35 ± 0.0199</td>
<td>0.35 ± 0.0299*</td>
</tr>
<tr>
<td>Palmvitee capsules</td>
<td>0.35 ± 0.0299 (100)</td>
<td>0.35 ± 0.0199(101)</td>
</tr>
</tbody>
</table>

* x ± SD. Percentages of increases or decreases with respect to baseline value in parentheses. Time of drawing blood was 0800. The 15 subjects fasted for 12 h before samples were taken.
† Values of three poor respondents (>2 SD) were not included in statistical calculations.
‡ Values in a row not sharing a common superscript letter are significantly different, P < 0.05.

The placebo group fed diets supplemented with corn-oil capsules showed no change as compared with baseline values in the variables described above (Tables 1–4).

In the crossover study, the dietary supplementation with corn-oil capsules of the subjects previously fed TRF (palmvitee capsules) showed continued lower serum cholesterol concentrations even after 6 wk of supplementation with corn-oil capsules (Table 5). The reverse was true for the placebo group. After ingesting palmvitee capsules 6 wk, the group showed a drop in serum cholesterol concentrations as shown in Table 5.

TABLE 3
Effects of palmvite on plasma thromboxane B2 and platelet factor 4 concentrations in hypercholesterolemic humans

<table>
<thead>
<tr>
<th>Nutritional state</th>
<th>Baseline value (first 2 wk):</th>
<th>Feeding period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>time 0</td>
<td>14 d</td>
</tr>
<tr>
<td>Thromboxane B2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (corn-oil capsules)</td>
<td>166 ± 1799*</td>
<td>162 ± 2299</td>
</tr>
<tr>
<td>Palmvitee capsules</td>
<td>169 ± 2199 (100)</td>
<td>144 ± 369(85)</td>
</tr>
<tr>
<td>Platelet factor 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (corn-oil capsules)</td>
<td>286 ± 299*</td>
<td>276 ± 199</td>
</tr>
<tr>
<td>Palmvitee capsules</td>
<td>299 ± 199 (100)</td>
<td>262 ± 29(88)</td>
</tr>
</tbody>
</table>

* x ± SD. Percentages of increases or decreases with respect to baseline value in parentheses. Time of drawing blood was 0800. The 15 subjects fasted for 12 h before samples were taken.
† Values in a row not sharing a common superscript letter are significantly different, P < 0.05.
The different concentrations of plasma tocotrienols and tocopherol were estimated by HPLC after 4 wk of feeding corn-oil or palmvitee capsules as shown in Table 6. The HPLC analysis revealed that the total tocopherols and tocotrienols were 16.09 ± 0.70 and 0.26 ± 0.001 μmol/L, with corn oil and 13.21 ± 0.76 μmol/L and 21.51 ± 0.69 μmol/L with palmvitee capsules, respectively, as shown in Table 6. The ratios of tocotrienols to tocopherol were 0.012 and 1.57 with these treatments, respectively, indicating the 130-fold increase of tocotrienols with respect to tocopherol were estimated by HPLC after 4 wk of feeding corn-oil capsules, then palmvitee capsules, as shown by the large SDs. In 3 (of 15) subjects, the baseline value is the value at the end of 4 wk of corn-oil or palmvitee capsules (see also Table 1). There was considerable individual variation in the response and percentage of increases or decreases with respect to baseline value in parentheses. Time of drawing blood was 0800. The subjects fasted for 12 h before samples were taken.

Discussion

Many studies have established that saturated fatty acids from animal and vegetable sources in human diets tend to cause an elevation of serum cholesterol concentrations (14). An exception to this general proposition is palm oil, which has a lipid-lowering effect despite a high concentration of saturated fatty acids (50% palmitic acid) (15, 16). A possible explanation for this apparently paradoxical action is the high content of tocotrienols present in palm oil (780-1080 μg/g). Animal studies (5) have demonstrated that tocotrienols derived from barley oil (which contains 44000 μg toco-trienols/g lower serum cholesterol concentrations and inhibit enzymes of the cholesterol biosynthetic pathway, including 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase and cholesterol 7α-hydroxylase.

The effects of a TRF isolated from palm oil (palmvitee capsules) in human subjects are described in this study. There were significant decreases in serum total cholesterol, LDL-C, TBX2, platelet aggregation, PF4, and glucose concentrations when the TRF was administered to hypercholesterolemic subjects for 4 wk. Interestingly, this effect persisted for ≥ 2 wk after the capsules were discontinued in one experiment and for ≥ 6 wk in the second experiment. It is possible that the tocotrienols are stored in the lipids and are protected from oxidant effects, perhaps by tocopherols.

There was considerable individual variation in the response to the palmvitee capsules, as shown by the large SDs. In 3 (of 15) subjects, the baseline value is the value at the end of 4 wk of corn-oil or palmvitee capsules (see also Table 1). There was considerable individual variation in the response to the palmvitee capsules, as shown by the large SDs. In 3 (of 15) subjects, the baseline value is the value at the end of 4 wk of corn-oil or palmvitee capsules (see also Table 1). There was considerable individual variation in the response to the palmvitee capsules, as shown by the large SDs. In 3 (of 15) subjects, the baseline value is the value at the end of 4 wk of corn-oil or palmvitee capsules (see also Table 1). There was considerable individual variation in the response to the palmvitee capsules, as shown by the large SDs. In 3 (of 15) subjects, the baseline value is the value at the end of 4 wk of corn-oil or palmvitee capsules (see also Table 1). There was considerable individual variation in the response to the palmvitee capsules, as shown by the large SDs. In 3 (of 15) subjects, the baseline value is the value at the end of 4 wk of corn-oil or palmvitee capsules (see also Table 1). There was considerable individual variation in the response to the palmvitee capsules, as shown by the large SDs. In 3 (of 15) subjects, the baseline value is the value at the end of 4 wk of corn-oil or palmvitee capsules (see also Table 1).
subjects, possibly due to lack of dietary control. A follow-up study in which cholesterol and fat intakes are limited to the recommendation of the American Heart Association and in which graded doses of TRF are administered may yield additional information that would define the value of these interesting compounds more accurately. Further studies of the effects on platelets, fibrinolysis, and coagulation would also be of interest.

It is possible that the tocotrienols have an important role in the control of many forms of hypercholesterolemia. They are derived from natural food products, are easily administered, are readily accepted by human subjects, and may well prove to have fewer side effects that do many other medications. Future studies may define their role more clearly, whether as single agents or as adjuvants to other cholesterol-lowering therapies, including dietary manipulation.

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References


