Novel Tocotrienols of Rice Bran Inhibit Atherosclerotic Lesions in C57BL/6 ApoE-Deficient Mice

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ABSTRACT We are studying novel tocotrienols, which have a number of activities that might interfere with the formation of atherosclerotic plaques, including hypocholesterolemic, antioxidant, anti-inflammatory and antiproliferative effects. This study compared the effects of α-tocopherol, the tocotrienol-rich fraction (TRF25) and didesmethyl tocotrienol (d-P25-T3) of rice bran on the pathogenesis of atherosclerotic lesions in C57BL/6 apolipoprotein (apo)E-deficient (−/−) mice. These mice are an excellent model because they become hyperlipidemic even when they consume a low fat diet and they develop complex atherosclerotic lesions similar to those of humans. These compounds were also tested in wild-type C57BL/6 apoE (+/+ ) and (+/−) mice fed low or high fat diets. When a high fat diet was supplemented with α-tocopherol, TRF25 or d-P25-T3 and fed to mice (+/+) for 24 wk, atherosclerotic lesion size was reduced 23% (P = 0.33), 36% (P = 0.14) and 57% (P < 0.02), respectively, and in mice (+/−) for 18 wk, lesions were reduced by 19% (P = 0.15), 28% (P < 0.01) and 33% (P < 0.005), respectively, compared with mice fed a control diet. A low fat diet did not cause atherosclerotic lesions in these mice. The low fat diet supplemented with TRF25 or d-P25-T3 fed to apoE-deficient (−/−) mice for 14 wk decreased atherosclerotic lesion size by 42% (P < 0.04) and 47% (P < 0.01), respectively, whereas α-tocopherol supplementation resulted in only an 11% (P = 0.62) reduction. These results demonstrate the superior efficacy of tocotrienols compared with α-tocopherol. Although tocotrienols decreased serum triglycerides, total and LDL cholesterol levels, the decreases in atherosclerotic lesions seem to be due to the other activities. Serum tocot concentrations in various groups are also described. This is the first report of a significant reduction in the atherosclerotic lesion size in all three genotypes of apoE mice fed a novel tocotrienol (d-P25-T3) of rice bran. Dietary tocotrienol supplements may provide a unique approach to promoting cardiovascular health.


KEY WORDS: • C57BL/6 apoE-deficient (−/−) mice • novel tocotrienols (TRF25, d-P25-T3) • atherosclerotic lesions • serum cholesterol • triglycerides

Coronary heart disease is the result of a complex interaction among a number of different processes that affect either the acute phase of coronary disease or the initiation and growth of the atherosclerotic plaque (1–4). Lipoprotein metabolism is thought to be especially important in the initiation and growth of plaque, which involves complex cellular processes including the aggregation of “foam” cells, probably at a site of endothelial injury to initiate plaque formation (1–6). In the acute phase of coronary disease, aggregation of blood platelets, blood coagulation and fibrinolysis are of notable importance (1,2,4). The cardiovascular risk of a given patient is dependent on the interactions of all of these factors (1–6).

Recent studies have indicated that administration of hypocholesterolemic and antioxidant drugs and compounds can restrict the development of early atherosclerotic lesions in the aorta in various experimental models (7–11). Inhibition of lesion development in the carotid arteries of cholesterol-fed primates has been reported after administration of vitamin E (α-tocopherol) (12–14). The multitherapeutic properties of tocotrienols as hypocholesterolemic, antioxidant, antiinflammatory, anticancer (antiproliferative) and anti-inflammatory agents in various experimental animal models and humans have been reported (15–19).

Vitamin E will be used in this report to refer to α-tocopherol; however, it alternatively refers to a group of eight naturally occurring compounds with characteristic antioxidant activity (16–18). There are four tocopherols designated α-, β-, γ- and δ- and four corresponding tocotrienols. Tocotrienols differ from tocopherols (vitamin E) only in having three double bonds in the isoprene side chain. This unsaturation in the side chain is essential for inhibition of liver β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA)3 reductase (the rate-

3 Abbreviations used: apo, apolipoprotein; chol, cholesterol; d-P25-T3, desmethyl tocotrienol of stabilized and heated rice bran; d-P25-T3, desmethyl tocotrienol of stabilized and heated rice bran; H & E, hematoxylin and eosin; HMG-CoA, β-hydroxy-β-methylglutaryl coenzyme A; IDL, intermediate density lipoproteins; PKC, protein kinase C; Re-LPS, Escherichia coli lipopolysaccharide; TNF, tumor necrosis factor; TRF25, tocotrienol-rich fraction from stabilized and heated rice bran.
limiting enzyme in the synthesis of cholesterol) activity (16–18). Structure-function studies have revealed that the number and position of methyl substituents in different tocotrienols affect their hypocholesterolemic and antioxidant properties (16–18). δ-Tocotrienol (1 methyl group on benzene ring) is the most potent HMG-CoA reductase inhibitor among the four previously known tocotrienols (17). On the other hand, α-tocopherol has been shown to actually increase the activity of HMG-CoA reductase (17,20). Recently, we isolated and identified two novel tocotrienols, [desmethyl (d-P21-T3) and didesmethyl (d-P25-T3) tocotrienols] from stabilized and heated rice bran (17). These novel tocotrienols have superior efficacy in hypocholesterolemic, antioxidant, anti-inflammatory, antithrombotic and antiancancer activities compared with the known tocotrienols and vitamin E (17,20–22). The pharmacokinetics and bioavailability of various tocotrienols under fed and fasted conditions in humans have been reported recently (23).

Recently, the biological activities (antioxidant, antithrombotic, anti-inflammatory) of vitamin E (α-tocopherol) were reviewed by a number of investigators (24–29). The role of α-tocopherol in inhibiting the development of atherosclerotic lesions in the aorta has been attributed to the lower activity of protein kinase C (PKC) isoenzyme, which is caused by the higher concentration of α-tocopherol (30–33). PKC plays an important role in cellular signal transduction and serves as the major intracellular receptor for tumor promotion, cellular growth, differentiation, secretion and cellular proliferation (30,31). Control of the proliferation of aortic smooth muscle cells is especially important because hyperproliferation of these cells is associated with two vascular diseases, i.e., hypertension and atherosclerosis (32). The inhibition of aortic lesions by α-tocopherol in apolipoprotein (apo)E-deficient (−/−) mice has been reported to be due to inhibition of the activity of PKC by α-tocopherol, and is not related to its antioxidant activity (34,35). This reduction in lesions is achieved only by feeding a high level of α-tocopherol (21.0 mg/g in mice and 5–10 mg/g in rabbits) (9, 33–36); feeding a low level of α-tocopherol (500 µg/g) does not significantly inhibit the lesions in apoE-deficient (−/−) mice (37).

Several investigators reported recently that tocotrienols have greater antioxidant activity than α-tocopherol (vitamin E), and protect more efficiently against some free radical–related diseases than does α-tocopherol (12,38–42). However, there is no report of the effect of tocotrienols on the activity of PKC although tocotrienols and α-tocopherol do share a common chromanol moiety in their structures (17). Moreover, TRF25 and didesmethyl tocotrienol (d-P25-T3) are more potent inhibitors of cholesterol synthesis than the known tocotrienols (17). Therefore, the present study was carried out to compare the effects of α-tocopherol, TRF25 and d-P25-T3 on the pathogenesis of atherosclerotic lesions in C57BL/6apoE-deficient (−/−) female mice fed a low fat diet with or without these compounds.

The normal rodent lipid profile is regarded as an HDL cholesterol model, because the HDL cholesterol level normally exceeds the level of LDL cholesterol, which is opposite to the human lipid profile (LDL cholesterol model). Recently, several useful models of atherosclerosis have been created by genetic alternation of lipid metabolism. The most widely used of these models involves a gene disruption of apolipoprotein E (43–46). Advantages of this model include the fact that the lesions develop at a much earlier age, exhibit more of the features of the so-called “complicated” lesions found in humans and can be induced by both low and high fat diets (47,48). Unlike the wild-type mice [C57BL/6apoE (+/+) or heterozygous mice [C57BL/6 apoE (+/−)], the homozygous female mice [C57BL/6 apoE-deficient (−/−)] have elevated LDL cholesterol levels [consisting mainly of VLDL + intermediate density lipoproteins (IDL)], similar to the high LDL cholesterol level of humans (43–48). They are also an especially excellent model with which to study atherosclerotic lesions because they become hyperlipidemic even when consuming a standard low fat rodent diet and develop large and complex atherosclerotic lesions that are similar to those of humans and 50 times larger than the lesions seen in wild-type mice fed a high fat diet (43–46).

The present study also compared the effects of α-tocopherol, TRF25 and d-P25-T3 in wild-type, C57BL/6apoE (+/+) and heterozygous, C57BL/6apoE (+/−) female mice fed low and high fat diets with or without the test compounds. Both wild-type C57BL/6apoE (+/+) and heterozygous (+/−) female mice fed a standard low fat diet have normal cholesterol levels and do not develop atherosclerosis. However, when they could access an alternative (high fat) diet, they develop spontaneous atherosclerotic lesions with extremely high serum total cholesterol levels (43–48).

MATERIALS AND METHODS

Sources of chemicals and diagnostic kits. Sources of chemicals, substrates, and diagnostic kits have been identified previously (21). α-Tocopherol was a gift from Archer Daniels Midland (Decatur, IL). Chemicals and solvents were of analytical grade. Sigma Diagnostic kits (Sigma Chemical, St, Louis, MO) were used to estimate serum total cholesterol and HDL cholesterol (kit 352; 500 nm), and triglycerides (kit 336; 500 nm).

Purification of γ-oryzanol-free TRF25 and d-P25-T3 by flash chromatography. The purification of large quantities of TRF25 (free from γ-oryzanol and most of α-tocopherol) and d-P25-T3 from stabilized and heated rice brans of ML-63 rice variety (supplied by M.Z. Wells, Riviana Foods, Abbeville, LA) was carried out by flash chromatography as described recently (21). The composition of various tocotocols in TRF25, 5.8% α-tocopherol, 14.9% α-tocotrienol, 1.9% γ-tocotrienol, 35.4% β-tocotrienol, 4.1% δ-tocopherol, 5.3% δ-tocotrienol, 14.3% d-desmethyl tocotrienol (d-P21-T3), 16.4% d-didesmethyl tocotrienol (d-P25-T3) and 1.9% unidentified tocotrienols. The molecular structures of desmethyl (d-P21-T3) and didesmethyl (d-P25-T3) tocotrienols have been established as 3,4-dihydro-2 (4,8,12-trimethyldeca-3(E),7(E),11’-triaryl)-2H-1-benzopyran-6-ol, and 3,4-dihydro-2-(4,8,12-trimethyldeca-3(E),7(E),11’-triaryl)-2H-1-benzopyran-6-ol, respectively (17).

Experimental design. Four experiments were carried out to study the effects of α-tocopherol, the tocotrienol-rich fraction (TRF25) and its pure component, d-P25-T3 (didesmethyl tocotrienol) purified from stabilized and heated rice bran, on the pathogenesis of atherosclerotic lesions in C57BL/6apoE-deficient (−/−) female mice fed low fat, specially prepared rodent diets. These compounds were also tested in wild-type C57BL/6apoE (+/+) and heterozygous C57BL/6apoE (+/−) female mice fed low and high fat diets.

Animals. The protocol was reviewed and approved by the University of Illinois at Chicago College of Medicine Animal Care Committee and the animal care was in accordance with institutional guidelines. The wild-type C57BL/6apoE (+/+), C57BL/6apoE (+/−) and C57BL/6apoE-deficient (−/−) female mice were obtained from Jackson Laboratories (Bar Harbor, MA). All mice were housed under pathogen-free conditions as previously described (49). Female mice were used in the present study because in the C57BL/6 hyperlipidemic model, female mice more rapidly develop much larger atherosclerotic lesions than male mice (50) except in apoE-deficient (−/−) mice in which the lesions are approximately equal in males and females.

Animal diets. The compositions of various diets are outlined in Table 1. The normal control diet (TD-5015), fed during propagation of the mice and before commencing experimental low or high fat diets contains 110 g/kg fat (Table 1). The standard low fat (Teklad...
The mice were transferred to the experimental low fat (5%) diet for 13 wk after weaning (3 wk) to permit atherosclerotic lesions to develop. Mice (n = 10/group) were then fed standard low fat diet supplemented with α-tocopherol, TRF25, or d-P25-T3 (100 μg/g) for the next 10 wk to evaluate the effects of these additives on the progression of atherosclerotic lesions. After 10 wk, mice were killed and sampled as described above for Experiment 1.

Quantitative assessment of atherosclerotic lesions. A slightly modified method of Paigen et al. (51) was used to quantitate the areas of athero sclerosis for each mouse. These areas were measured by analytical morphometry in five 10-μm sections through the region of the aortic valve at 100-μm intervals. The mean of five sections was calculated instead of using the entire arterial tree. Lesions in wild-type mice C57BL/6 apoE (+/+) and heterozygous mice [apoE (+/−)] were found only in the aortic valve. Lesions in homozygous C57BL/6 apoE-deficient (−/−) mice had developed extensively throughout the aorta (52). It has been reported that in C57BL/6 apoE-deficient (−/−) mice, there is a significant correlation between the extent of lesions in the entire aorta (measured as the percentage of surface area) and that at the aortic origin (measured as the averaged lesion area per cross section) (52). The detailed procedures are described below.

The aorta was removed after the mice were killed, rinsed in saline, and then fixed on two groups of polylysine-coated slides, placing the aortic valve sinus. Once this section was located, 10-μm sections through the region of the aortic valve at 100-μm intervals. The mean of five sections was calculated instead of using the entire arterial tree. Lesions in wild-type mice C57BL/6 apoE (+/+) and heterozygous mice [apoE (+/−)] were found only in the aortic valve. Lesions in homozygous C57BL/6 apoE-deficient (−/−) mice had developed extensively throughout the aorta (52). It has been reported that in C57BL/6 apoE-deficient (−/−) mice, there is a significant correlation between the extent of lesions in the entire aorta (measured as the percentage of surface area) and that at the aortic origin (measured as the averaged lesion area per cross section) (52). The detailed procedures are described below.

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in lesions stained with Oil Red O with the morphological details better appreciated with the H & E-stained sections. To quantitate the degree of atherosclerosis in each mouse, the areas of the atherosclerotic lesions of five of the sections stained by Oil Red O, sections 1, 6, 11, 16 and 24, were examined. These sections were ~100 μm apart and covered a span of ~400 μm of the aorta. If any of the designated sections were folded or torn, the section preceding it was used. The area of the lesions within each Oil Red O section was determined by point counting using a squared grid ocular graticule (Graticules, Townbridge, UK) at 40X magnification (53,54). The two-dimensional area of the lesions per section were then determined by the following formula: \( A = \mu \cdot \mu' \), where \( \mu \) is the area in \( \mu^2 \), \( \mu' \) is the number of points falling within the lesions and \( \mu \) is the distance between two neighboring points (i.e., \( \mu' \) will equal the area associated with each point). In the present system, \( \mu = 50 \mu m \) as determined by a reference grid; therefore, \( \mu'^2 = 2500 \mu m^2 \) at X40 magnification. The aortas of each mouse provided five independent data points for evaluation. The mean value of the five points was then used as the final value for each mouse. The morphometric studies were performed on frozen sections stained with Oil Red O and the photomicrographs were taken from frozen sections stained with H & E as described in detail previously (51).

**Serum lipid analyses.** Blood samples were obtained from the mice after overnight food deprivation (12 h) by orbital puncture under ether anesthesia. The serum total cholesterol, HDL cholesterol and triglycerides levels were estimated using Sigma Kits (Cat. no. 280-06-10) and assayed using a kit for triglycerides (55). The LDL cholesterol (LDL chl), and VLDL cholesterol (VLDL chl), and the phosphotungstic acid and 0.4 mol MgCl\(_2\) with gentle shaking for 10 min at room temperature, followed by centrifugation at 12,000 × g for 10 min. The supernatant, containing HDL chl, was analyzed with Sigma reagents (kit 532) (55). LDL chl was estimated according to Friedewald’s formula by subtracting the total cholesterol from the HDL chl triglycerides (56).

**Estimation of tocols of serum.** The separation and quantitation of tocols (tocopherols and tocotrienols) of serum were carried out by HPLC as reported recently in cereals and serum (57).

**Expression of data and statistical analysis.** Treatment-mediated differences in atherosclerotic lesion size in aorta and serum lipid analyses were identified with one- or two-way ANOVA to compare group means of main effects by ANOVA; when the F-test indicated a significant effect, the differences between the means were analyzed by Fisher’s Protected Least Significant Difference (LSD) test (Abacus Concepts, Berkeley, CA). Differences were considered significant at \( P < 0.05 \) (58).

**RESULTS**

**Atherosclerotic lesion size in aorta**

**Experiment 1.** The wild-type C57BL/6 apoE (+/+) female mice fed the low fat diet with or without \( \alpha \)-tocopherol, TRF\(_{25}\) or \( d\)-P\(_{25}\)-T3 for 24 wk showed no lesions in the aortas (Table 2). However, lesions were observed in the mice fed the high fat diet with or without supplements. The control group had maximum lesions in the aorta, and supplementation of the high fat diet with \( d\)-P\(_{25}\)-T3 reduced (57%; \( P < 0.02 \)) the size of the lesions compared with the control group (Table 2). Supplementation of the high fat diet with \( \alpha \)-tocopherol or TRF\(_{25}\) resulted in decreases of 23% (\( P = 0.33 \)) and 36% (\( P = 0.14 \)), respectively, compared with the control group (Table 2).

**Experiment 2.** No lesions were detected in the aortas of the heterozygous C57BL/6 apoE (+/-) female mice fed the low fat diet for 18 wk, but all of those fed the high fat diet had lesions. The size of the lesions in the control group was 50-fold greater than the lesions in the wild-type C57BL/6 apoE (+/+) female mice fed the same diet (Table 2).

Large lesions were found in heterozygous apoE (+/-) mice fed the high fat diet and supplementation with \( \alpha \)-tocopherol, TRF\(_{25}\) or \( d\)-P\(_{25}\)-T3 reduced lesion size 19% (\( P = 0.15 \)), 28% (\( P < 0.01 \)) and 33% (\( P < 0.005 \)), respectively, compared with the control group (Table 2). The experimental period was 18 wk.

**TABLE 2**

**Effects of the tocotrienol-rich fraction (TRF\(_{25}\)) from stabilized and heated rice bran and its component didesmethyl tocotrienol (\( d\)-P\(_{25}\)-T3) on atherosclerotic lesion size in aorta of three genotypes of C57BL/6 apoE female mice fed low or high fat diets\(^1,2\)**

<table>
<thead>
<tr>
<th>Diet</th>
<th>C57BL/6 apoE (+/-) mice</th>
<th>C57BL/6 apoE (-/-) mice</th>
<th>C57BL/6 apoE-deficient (-/-) mice</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Feeding period</td>
<td></td>
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<tr>
<td></td>
<td>Low fat diet</td>
<td>High fat diet</td>
<td>Low fat diet</td>
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<tr>
<td></td>
<td>10-34 wk = 24 wk</td>
<td>6-24 wk = 18 wk</td>
<td>6-20 wk = 14 wk</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Control (low or high fat)</td>
<td>None</td>
<td>2.22 ± 0.86(^a) (100)(^3)</td>
<td>191.98 ± 53.83(^a) (100)</td>
</tr>
<tr>
<td></td>
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<td>[10]</td>
<td>[10]</td>
</tr>
<tr>
<td>( d)-( \alpha )-Tocopherol(^5)</td>
<td>1.71 ± 0.88(^a) (77)(^8)</td>
<td>154.59 ± 54.39(^a) (81)(^9)</td>
<td>171.22 ± 40.43(^a) (89)(^9)</td>
</tr>
<tr>
<td></td>
<td>1.43 ± 0.85(^a) (64)(^8)</td>
<td>137.86 ± 26.53(^a) (72)(^9)</td>
<td>111.72 ± 46.39(^a) (58)(^9)</td>
</tr>
<tr>
<td>TRF(_{25})(^5)</td>
<td>1.43 ± 0.85(^a) (64)(^8)</td>
<td>128.12 ± 30.57(^a) (67)(^9)</td>
<td>101.59 ± 29.16(^a) (53)(^9)</td>
</tr>
<tr>
<td>( d)-P(_{25})-T3(^3)</td>
<td>0.96 ± 0.51(^b) (43)(^8)</td>
<td>128.12 ± 30.57(^a) (67)(^9)</td>
<td>177.38 ± 25.85(^a) (76)(^9)</td>
</tr>
</tbody>
</table>

\(^1\) Data are means ± SD, \( n = 5-10 \) per group; \( \alpha \) = values in a column with a different superscript letter differ, \( P < 0.05 \).

\(^2\) Wild-type C57BL/6 apoE (+/+) and heterozygous (+/-) mice were fed normal control diet (fat 11%) for 10 and 6 wk and then transferred to experimental low fat or high fat diets containing fat at 5 and 15%, respectively. The homozgyous C57BL/6 apoE-deficient (-/-) mice were fed only standard low fat (5%) diet throughout the experiment.

\(^3\) Percentages of treatment values compared with control value are in parentheses.

\(^4\) Numbers of mice in each group are in brackets.

\(^5\) Control (low or high fat) diets were supplemented with 100 \( \mu g \) \( d\)-\( \alpha \)-tocopherol, TRF\(_{25}\) or \( d\)-P\(_{25}\)-T3 (didesmethyl tocotrienol)/g.
wk with these mice vs. 24 wk in the wild-type apoE (+/+)
female mice, which may explain in part the smaller reduction of
atherosclerotic plaques with d-P25-T3 (33 vs. 57%).

**Experiment 3.** C57BL/6 apoE-deficient (−/−) mice fed
the low fat diet with α-tocopherol, TRF25 or d-P25-T3 for 14
wk had 11% (P = 0.62), 42% (P < 0.04) and 47% (P < 0.01)
altering lesions, respectively, compared with the control group (Table 2). The average lesion size in the control group fed the
low fat diet was similar to that of heterozygous apoE (+/−)
mice fed the high fat diet.

**Experiment 4.** The reductions of atherosclerotic lesions
were 8% (P = 0.73), 22% (P = 0.42) and 24% (P = 0.31),
respectively, compared with the control group (Table 2) in homozygous apoE-deficient (−/−) female mice fed the standard low
fat diet for 16 wk and then the low fat diet supplemented with
α-tocopherol, TRF25 or d-P25-T3 for 10 wk, respectively. No
samples were taken to measure the extent of plaques at 16 wk
(the start of the therapy trial), but we speculate that the 22 or
24% reductions relative to 26-wk controls, if real, would represent
a slowing of plaque growth rather than a reversal (Table 2).

**Morphological studies**

Representative histologic sections from the control and
d-P25-T3 groups of heterozygous apoE (+/−) mice are illustrated in
**Figure 1** (Experiment 2). A low power view of a control lesion
shows a large extensive covering (arrow head) over 95% of the
circumference of the aortic wall (Fig. 1A). A high power view of
one of these lesions shows marked thickening of the subendothelial
intimal space with extensive deposition of extracellular lipids
and connective tissue matrix, and multiple scattered mononuclear
cells, including foams cells (Fig. 1B). In addition, a few
scattered spindle-shaped cells with elongated nuclei suggestive of
vascular smooth muscle cells or fibroblasts were seen. The repre-
sentative lesions from the d-P25-T3 group were somewhat smaller
than those of the control group, covering (arrow head) ~70% of
the circumference of the aortic wall (Fig. 1C). The histologic
features of this lesion seen in high power were similar to those of
the control group (Fig. 1D).

Representative histologic sections from the control and
d-P25-T3 groups of homozygous apoE-deficient (−/−) mice (Ex-
periment 3) were similar to those described in the apoE (+/−)
group. They show lesions covering (arrow head) >90% in the
control (Fig. 2A), and the representative lesions from the
d-P25-T3 group were smaller than those of the control group,
covering (arrow head) ~60% of the circumference of the aortic
wall (Fig. 2C).

**Serum lipids: total cholesterol**

**Experiment 1.** Feeding the wild-type C57BL/6, apoE (+/+)
female mice the high fat diet for 24 wk resulted in a twofold
higher serum total cholesterol level than in those fed the low fat
diet in the control groups (Table 3). Supplementing either diet with
α-tocopherol, TRF25 or d-P25-T3 for 24 wk had 2% (P = 0.25), 11% (P = 0.14) and 30% (P < 0.004) lower levels,respectively, than the control group (Table 3). The decreases in
those fed the high fat diet (24 wk) supplemented with these
components were only 5% (P = 0.10), 9% (P < 0.03) and 6% (P =
0.10), respectively (Table 3).

**Experiment 2.** Similar decreases of 9% (P = 0.17), 19% (P
< 0.02) and 23% (P < 0.02) in serum total cholesterol were also observed when the low fat diet supplemented with
α-tocopherol, TRF25 or d-P25-T3 for 24 wk had 2% (P = 0.25), 11% (P = 0.14) and 30% (P < 0.004) lower levels,respectively, than the control group (Table 3). The decreases in
those fed the high fat diet (24 wk) supplemented with these
components were only 5% (P = 0.3), 6% (P < 0.02) and 8% (P < 0.02), respectively (Table 3).

**Experiments 3 and 4.** The C57BL/6 apoE-deficient
(−/−) female mice fed the low fat diet for 10 wk (Table 3).
Serum lipids: LDL cholesterol

**Experiment 1.** The decreases in serum total cholesterol levels in these mice were also reflected in the serum LDL cho-
lesterol (mainly VLDL + IDL) levels (Table 3). The wild-type
C57BL/6 apoE (+/+) female mice fed the low fat diet supple-
mented with α-tocopherol, TRF25 or d-P25-T3 for 24 wk had 2% (P
= 0.25), 11% (P = 0.14) and 30% (P < 0.004) lower levels, respectively, than the control group (Table 3). The decreases in
those fed the high fat diet (24 wk) supplemented with these
components were only 5% (P = 0.10), 9% (P < 0.03) and 6% (P
= 0.10), respectively (Table 3).

**Experiments 1–4.** Serum HDL cholesterol was not af-
fected in mice fed the low or high fat diets containing α-toc-
opherol, TRF25 or d-P25-T3 (Table 4). The HDL cholesterol
levels in all three genotypes of apoE female mice fed control and
experimental diets (0.84–1.26 mmol/L) are similar to those of humans (1.29 mmol/L HDL cholesterol), despite the fact that the normal rodent pattern is the high HDL choles-
terol model (HDL cholesterol level > 3.62–4.65 mmol/L).

Serum triglycerides concentrations were not affected by any
of these treatments in wild-type and heterozygous apoE mice
fed control and experimental diets (Tables 4). Serum triglyc-
erde concentrations were reduced 9% (P < 0.01) and 6% (P
= 0.1) in homozygous apoE-deficient (−/−) mice fed the low
fat diet supplemented with TRF25 or d-P25-T3 for 14 and 10
wk, respectively, compared with control groups (Table 4).

**Serum tocols (tocopherols + tocotrienols)**

**Experiments 1–4.** The HPLC analyses of serum samples for
tocols obtained from the wild-type mice [apoE (+/+) and
heterozygous mice [apoE (+/−)] fed the control low fat or high fat diets or supplemented with α-tocopherol showed the
presence of α-, γ-, and δ-tocopherols only (Tables 5 and 6).
The samples of the α-tocopherol-supplemented diets had
~25% higher concentrations of these tocopherols compared
with samples from the control groups fed each diet (Tables 5, 6). There were also 20–25% higher concentrations of these
FIGURE 1  Morphology of atherosclerotic lesions in heterozygous apolipoprotein (apo)E (+/−) mice in Experiment 2 fed high fat diets. (A) Low power view through the upper portion of the aortic valve of a control mouse fed only the high fat diet showing lesions (arrow head) involving >95% of the circumference of the aortic wall; hematoxylin and eosin (H & E)-stained, magnification X120. (B) High power view of one of the lesions showing marked thickening of the subendothelial intimal space, extensive deposition of extracellular lipids and connective tissue matrix and multiple scattered mononuclear cells, including foam cells. There are also a few scattered spindle-shaped cells with elongated nuclei suggestive of vascular smooth muscle cells or fibroblasts; H & E-stained, magnification X270. (C) Low power view through the upper portion of the aortic valve of a mouse fed the high fat diet supplemented with didesmethyl tocotrienol of stabilized and heated rice bran (d-P25-T3) showing lesions (arrow head) covering ~70% of the circumference of the aortic wall; H & E-stained, magnification X90. (D) High power view of one of the lesions showing changes similar to those in (B); H & E-stained, magnification X270.

FIGURE 2  Morphology of atherosclerotic lesions in apolipoprotein (apo)E-deficient (−/−) mice in Experiment 3 fed low fat diets. (A) Low power view through the upper portion of the aortic valve of a control mouse fed the standard low fat diet showing multiple lesions (arrow head) covering >90% of the circumference of the aortic wall; hematoxylin and eosin (H & E)-stained, magnification X100. (B) High power view of one of the lesions displaying marked thickening of the subendothelial intimal space, extensive deposition of extracellular lipids and connective tissue matrix and multiple scattered mononuclear cells, including foam cells. There are also a few scattered spindle-shaped cells with elongated nuclei suggestive of vascular smooth muscle cells or fibroblasts; H & E-stained, magnification X300. (C) Low power view through the upper portion of the aortic valve of a mouse fed the standard low fat diet plus didesmethyl tocotrienol of stabilized and heated rice bran (d-P25-T3) showing lesions (arrow head) covering ~60% of the circumference of the aortic wall; H & E-stained, magnification X90. (D) High power view of one of the lesions showing changes similar to those in (B); H & E-stained, magnification X270.
Effects of the tocotrienol-rich fraction (TRF25) from stabilized and heated rice bran and its component didesmethyl tocotrienol (d-P25-T3) on serum total cholesterol and LDL cholesterol levels of three genotypes of C57BL/6 apoE female mice fed low or high fat diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Wild-type C57BL/6 apoE (+/+) mice</th>
<th>C57BL/6 apoE (+/-) mice</th>
<th>C57BL/6 apoE-deficient (-/-) mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low fat diet</td>
<td>High fat diet</td>
<td>Low fat diet</td>
</tr>
<tr>
<td></td>
<td>Low fat diet</td>
<td>High fat diet</td>
<td>Low fat diet</td>
</tr>
<tr>
<td>Feeding period</td>
<td>10–34 wk = 24 wk</td>
<td>6–24 wk = 18 wk</td>
<td>6–20 wk = 14 wk</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (low or high fat)</td>
<td>2.15 ± 0.09a (100)</td>
<td>6.15 ± 0.29a (100)</td>
<td>2.47 ± 0.123a (100)</td>
</tr>
<tr>
<td>d-(\alpha)-Tocopherol(6)</td>
<td>2.10 ± 0.10a (98)</td>
<td>5.94 ± 0.24a (97)</td>
<td>2.37 ± 0.10a (99)</td>
</tr>
<tr>
<td>TRF25(5)</td>
<td>2.03 ± 0.07b (94)</td>
<td>5.76 ± 0.20b (94)</td>
<td>2.21 ± 0.06b (93)</td>
</tr>
<tr>
<td>d-P25-T3(3)</td>
<td>1.99 ± 0.10b (93)</td>
<td>5.82 ± 0.20b (95)</td>
<td>1.94 ± 0.10c (88)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(VLDL + IDL(6))</td>
<td>0.72 ± 0.09a (100)</td>
<td>5.20 ± 0.32a (100)</td>
<td>1.08 ± 0.11a (100)</td>
</tr>
<tr>
<td>d-(\alpha)-Tocopherol(6)</td>
<td>0.70 ± 0.12a (98)</td>
<td>4.95 ± 0.22b (95)</td>
<td>0.98 ± 0.08a (91)</td>
</tr>
<tr>
<td>TRF25(5)</td>
<td>0.64 ± 0.04b (89)</td>
<td>4.83 ± 0.24b (91)</td>
<td>0.88 ± 0.07b (81)</td>
</tr>
<tr>
<td>d-P25-T3(3)</td>
<td>0.53 ± 0.04c (70)</td>
<td>4.87 ± 0.19b (94)</td>
<td>0.84 ± 0.26b (77)</td>
</tr>
</tbody>
</table>

1 Data are means ± so, n = 5–10 per group; a–c values in a column with a different superscript letter differ; P < 0.05.
2 Wild-type C57BL/6 apoE (+/+), and heterozygous (+/-) mice were fed normal control diet (fat 11%) for 10 and 6 wk and then transferred to experimental low or high fat diets containing 5% and 15%, respectively. The homozygous C57BL/6 apoE-deficient (-/-) mice were fed only standard low fat (5%) diet throughout the experiment.
3 Percentages of treatment values compared with control value are in parentheses.
4 Numbers of mice in each group are in brackets.
5 Control (low or high fat) diets were supplemented with 100 \(\mu\)g d-\(\alpha\)-tocopherol, TRF25, or d-P25-T3 (didesmethyl tocotrienol)/g.
6 IDL, intermediate density lipoprotein.

A striking result of these experiments is that the novel tocotrienol d-P25-T3 can substantially reduce the growth of atherosclerotic plaques in all three of the tested mouse genotypes and diet combinations that produce plaques. For instance, wild-type mice [apoE (+/+) ] fed a high fat diet showed a 57% reduction, heterozygous mice [apoE (+/-) ] fed a high fat diet showed a 33% reduction, and homozygous defective mice [apoE (-/-) ] fed a low fat diet showed a 47% reduction in plaque size. Intervention before the plaques form or early in their growth is suggested to be important because when the treatment of apoE (-/-) mice was delayed from 6 to 16 wk, the reduction in plaque amount dropped from 47% (\(P < 0.01\)) [Experiment 3, treatment from 6 to 20 wk (Table 2)] to 24% (\(P = 0.31\)) [Experiment 4, treatment from 16 to 26 wk (Table 2)].
TRF25 also had substantial effects. TRF25 is a mixture of tocotrienols, enriched to obtain a high tocotrienol/tocopherol ratio, purified from rice bran. The TRF25 preparation used in these experiments contains >88% tocotrienols and only 10% tocopherols. It contains 16.4% d-\(\alpha\)-tocotrienol (\(\text{d-P25-T3}\)); thus the mice supplemented with 100 \(\mu\)g TRF25/g were receiving only one sixth the amount given to the groups supplemented with pure \(\text{d-P25-T3}\). Yet, TRF25 also substantially reduced atherosclerotic plaque formation. Comparing the effects with TRF25 vs. \(\text{d-P25-T3}\), we observed 26 vs. 57% for (+/+) mice (high fat), 28 vs. 33% for (+/-) mice (high fat), and 42 vs. 47% for (-/-) mice (low fat) (Table 2). We doubt that these TRF25 results are due only to the 600% reduced amount of \(\text{d-P25-T3}\) and suggest that the other tocotrienols in the TRF25 mixture probably also played important roles.

At this dose, \(\alpha\)-tocopherol did not significantly reduce plaque with any genotype/diet combination. The apoE-deficient (-/-) mice fed a low fat diet are perhaps of greatest importance because of the resemblance of this model to the human disease. Here \(\alpha\)-tocopherol produced a reduction of only 11% compared with 42 and 47% with TRF25 and \(\text{d-P25-T3}\), respectively. Thus, these results clearly demonstrate a superior efficacy of tocotrienols compared with \(\alpha\)-tocopherol (vitamin E) (Table 2).

This is the first report to describe the significant reduction in atherosclerotic lesions in C57Bl/6 apoE-deficient (-/-) mice after feeding TRF25 and a novel tocotrienol (\(\text{d-P25-T3}\)) of rice bran. As mentioned earlier, apoE-deficient (-/-) mice provide an excellent model because they become hyperlipidemic even when consuming a standard low fat mouse diet (47,48). These mice have increased oxidation-specific autoantibodies and develop complex atherosclerotic lesions that are similar to those of humans (59,60). The normal serum cholesterol level is four times greater in these mutant mice than normal mice, which show a high LDL cholesterol (consisting of VLDL + IDL) pattern atypical of normal mice. The progression of lesion formation is rapid in the apoE-deficient (-/-) mice, even when they consume a low fat diet; they develop foam cell–rich deposits in their proximal aorta by age 3 mo, after which lesions progress spontaneously and completely clog the coronary artery by 8 mo (43–48).

The early termination of treatment in Experiment 3 [20 wk for apoE-deficient (-/-) mice compared with 34 wk for wild-type apoE (+/+) mice in Experiment 1] was dictated in part by the aggressiveness of stenosis in the apoE-deficient (-/-) mice. At close to 34 wk, the untreated apoE-deficient (-/-) mice would be reaching total occlusion of their coronary arteries (45,46). It would be interesting to carry out further experiments in which the treatment interval in Experiment 4 was changed to start at weaning (3 wk) and continue until either 20 or 28 wk. Would the 3- to 20-wk treatments with TRF25 or \(\text{d-P25-T3}\) retard the progression of lesion formation in these apoE-deficient (-/-) mice? This question is of great interest, particularly because it has been suggested that tocotrienols reduce the amount of damage (as a percentage of the control group) more than the 3- to 20-wk treatment, suggesting the degree to which TRF25 or \(\text{d-P25-T3}\) can retard the progression of development of atherosclerotic plaques? Finally, if the results of the preceding experiment are positive, it would be...
than the values in the fasting state (23). Moreover, the mean values of all three tocotrienols in the fed state were higher than in the fasting state. The mean concentration (d-P25-T3) on the concentrations of various tocotrienols (T) and tocopherols (T3) in serum of wild-type C57BL/6 apoE (+/−) female mice fed low or high fat diets (Experiment 1).

**TABLE 5**

Effects of the tocotrienol-rich fraction (TRF25) from stabilized and heated rice bran and its component didesmethyl tocotrienol (d-P25-T3) on the concentrations of various tocols (tocopherols (T) + tocotrienols (T3)) in serum of wild-type C57BL/6 apoE (+/−) female mice fed low or high fat diets (Experiment 1).

Experiment 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>Low fat diet</th>
<th>High fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding period</td>
<td>10–34 wk = 24 wk</td>
<td>10–34 wk = 24 wk</td>
</tr>
<tr>
<td>Tocols</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α-Tocopherol</td>
<td>α-Tocotrienol</td>
</tr>
<tr>
<td>Control diet3</td>
<td>11.87 ± 1.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.46 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>d-α-tocopherol3</td>
<td>22.59 ± 1.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.25 ± 2.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TRF25&lt;sup&gt;3&lt;/sup&gt;</td>
<td>14.93 ± 0.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.09 ± 1.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>d-P25-T3&lt;sup&gt;3&lt;/sup&gt;</td>
<td>11.28 ± 1.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.23 ± 1.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

|                          | μmol/L                  |                          |
| Total tocopherol         |                        |                          |
| Control diet3            | 24.75 ± 1.04<sup>c</sup> | 39.26 ± 1.88<sup>a</sup> |
| d-α-tocopherol3          | 22.08 ± 0.53<sup>b</sup> | 39.26 ± 1.88<sup>a</sup> |
| TRF25<sup>3</sup>        | 23.22 ± 0.74<sup>a</sup> | 46.16 ± 3.62<sup>a</sup> |
| d-P25-T3<sup>3</sup>     | 18.52 ± 1.18<sup>b</sup> | 46.16 ± 3.62<sup>a</sup> |
| Total tocotrienol        |                          |                          |
| Control diet3            | 39.26 ± 1.88<sup>a</sup> | 39.26 ± 1.88<sup>a</sup> |
| d-α-tocopherol3          | 39.26 ± 1.88<sup>a</sup> | 39.26 ± 1.88<sup>a</sup> |
| TRF25<sup>3</sup>        | 39.26 ± 1.88<sup>a</sup> | 39.26 ± 1.88<sup>a</sup> |
| d-P25-T3<sup>3</sup>     | 39.26 ± 1.88<sup>a</sup> | 39.26 ± 1.88<sup>a</sup> |
| Total T + T3             | 78.52 ± 3.75<sup>a</sup> | 78.52 ± 3.75<sup>a</sup> |

1 Data are means ± SD, n = 5; <sup>a</sup>–<sup>c</sup> values in a column with a different superscript letter differ, P < 0.05.
2 All mice were fed normal control diet (fat 11%) for 10 wk and then transferred to experimental low or high fat diets containing fat of 5 and 15%, respectively.
3 Control (low or high fat) diets were supplemented with 100 μg d-α-tocopherol, TRF25 or d-P25-T3 (didesmethyl tocotrienol)/g.

The treatment of hypercholesterolemia with diet and lipid-lowering agents has been the mainstay of the treatment of atherosclerosis (7–11). There are a number of possible mechanisms that could prevent the development of atherosclerosis (1–4). Atherosclerosis is a disease of injury, chronic inflammation, accumulation of cholesterol and foam cells within the arterial wall and altered lipoprotein cholesterol metabolism (1–4). Physiologic and genetic factors also contribute to the progression of the fatty streaks into an atherosclerotic lesion (3). The development of atherosclerotic lesions could be prevented by modifying the response to injury or the response to retention of lipoproteins, or by the oxidative modification of lipoproteins (5,64–66).

Endothelial injury causes the formation of fatty streaks by increasing the permeability of the endothelium to lipoproteins and macrophages (3). The increased endothelial permeability leads to the accumulation of lipoproteins; subsequently, foam cells within the subendothelial space start forming atherosclerotic plaque (5,64–66). The oxidation of lipoproteins, particularly LDL, is responsible for the inflammatory response seen in atherosclerosis (9,64). Most lipoprotein oxidation occurs...
within the arterial wall (9,64). There is growing evidence that some immunological factors may also play a role and that heat shock proteins or oxidized lipoproteins may be targets of an autoimmune response (10,67). Recent reports indicate that atherosclerotic lesions are due primarily to the proliferation of smooth muscle cells in the arterial intima where they accumulate, surrounded by connective tissue, lipid-loaded macrophages and lymphocytes, and are responsible for vascular occlusions (10,67–69).

Vitamin E (α-tocopherol) protects against free radical damage and inhibits aortic smooth muscle cell proliferation and platelet aggregation by decreasing the activity of PKC (25,70,71). Although the exact mechanism of inhibition of atherosclerotic lesions by tocotrienols has not been elucidated, there is evidence suggesting that tocotrienols affect several distinct steps in the pathways leading to formation of complex atherosclerotic lesions. The effect of tocotrienols on the activity of PKC has not been reported; recently, however, we and several other investigators reported that tocotrienols have greater antioxidant activity than α-tocopherol (vitamin E), and are more effective than α-tocopherol in protecting against some free radical–related diseases (15–18). Moreover, tocotrienols are also beneficial for the prevention of oxidative LDL modification and are potent hypcholesterolemic and anti-inflammatory agents (15,19,22).

Pretreatment with novel tocotrienols reduced the induction of tumor necrosis factor (TNF) in response to Escherichia coli lipopolysaccharide (Re-LPS) in mice (19). The inhibition of TNF levels in serum was 72 and 82% with TRF25 and d-P25-T3, respectively, compared with the control group. A corresponding rise was observed in the plasma levels of corticosterone and adrenocorticotropic hormone (19). These results suggest that treatment of mice with tocotrienols blocked the rapid and transient rise in TNF caused by Re-LPS. TRF25 and d-P25-T3 also lowered arachidonic acid in various tissues of hereditary hypercholesterolemic swine (17,22). Thus, there is an overall reduction in prostanoids and leukotrienes, both of which are synthesized from arachidonic acid, and thus a possible reduction in interleukin-1.

The inhibition of TNF by novel tocotrienols is accompanied by a decrease in inflammation, by inhibition of the respiratory burst of neutrophils or by free radical scavenging, the decrease in the secretion of TNF by tocotrienols could be due to the rise in endogenous corticosteroids, which modulate the synthesis of inflammatory cytokines. This property of tocotrienols might be effective in reducing acute and chronic inflammation, and in reducing the size of atherosclerotic lesions in the arteries of humans.

Now it is of great interest to study the effect of various tocotrienols on the activity of PKC to understand its role in the inhibition of development of atherosclerotic lesions. As mentioned earlier, atherosclerotic lesions are due primarily to the proliferation of smooth muscle cells (67–69). Tocotrienols, particularly novel tocotrienols, have significantly greater potency in inhibiting proliferation of tumor cells compared with α-tocopherol (17). It is possible that tocotrienols inhibit

### TABLE 6

Effects of the tocotrienol-rich fraction (TRF25) from stabilized and heated rice bran and its component didesmethyl tocotrienol (d-P25-T3) on the concentrations of various tocols [tocopherols (T) + tocotrienols (T3)] in serum of C57BL/6 apoE (+/−) female mice fed low or high fat diets (Experiment 2).1,2

<table>
<thead>
<tr>
<th>Experiment 2</th>
<th>Diet</th>
<th>Low fat diet</th>
<th>High fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding period</td>
<td>(6–24 wk - 18 wk)</td>
<td>(6–24 wk - 18 wk)</td>
<td></td>
</tr>
<tr>
<td>Tocols</td>
<td>α-Tocopherol</td>
<td>α-Tocotrienol</td>
<td>γ-Tocopherol</td>
</tr>
<tr>
<td>Control diet3</td>
<td>15.44 ± 1.44a</td>
<td>15.49 ± 1.13a</td>
<td>5.27 ± 0.63b</td>
</tr>
<tr>
<td>d-α-Tocopherol3</td>
<td>15.02 ± 0.98a</td>
<td>15.53 ± 1.23b</td>
<td>2.58 ± 0.65</td>
</tr>
<tr>
<td>TRF253</td>
<td>15.05 ± 1.09</td>
<td>11.35 ± 0.86</td>
<td>14.35 ± 0.67b</td>
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<tr>
<td>d-P25-T33</td>
<td>11.84 ± 1.18c</td>
<td>17.32 ± 0.35a</td>
<td>3.88 ± 0.60c</td>
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<tr>
<td>Control diet3</td>
<td>15.20 ± 0.49c</td>
<td>17.62 ± 0.72b</td>
<td>6.87 ± 0.32b</td>
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<tr>
<td>d-α-Tocopherol3</td>
<td>21.64 ± 1.04a</td>
<td>16.32 ± 0.81c</td>
<td>9.36 ± 0.81a</td>
</tr>
<tr>
<td>TRF253</td>
<td>17.23 ± 0.65b</td>
<td>8.29 ± 0.56</td>
<td>18.53 ± 1.02b</td>
</tr>
<tr>
<td>d-P25-T33</td>
<td>15.65 ± 1.09c</td>
<td>19.55 ± 0.65a</td>
<td>4.18 ± 0.46c</td>
</tr>
</tbody>
</table>

1 Data are means ± SD, n = 5; a–c values in a column with a different superscript letter differ, P < 0.05.
2 All mice were fed normal control diet (fat 11%) for 6 wk and then transferred to experimental low or high fat diets containing fat at 5 and 15%, respectively.
3 Control (low or high fat) diets were supplemented with 100 μg d-α-tocopherol, TRF25 or d-P25-T3 (didesmethyl tocotrienol)/g.
Effects of the tocotrienol-rich fraction (TRF25) from stabilized and heated rice bran and its component didesmethyl tocotrienol (d-P25-T3) on the concentrations of various tocols [tocopherols (T) + tocotrienols (T3)] in serum of C57BL/6 apoE-deficient (−/−) female mice fed low fat diet (Experiments 3 and 4)1,2

Table 7

<table>
<thead>
<tr>
<th>Experiment 3</th>
<th>Diet</th>
<th>Low fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding period</td>
<td>(6–20 wk = 14 wk)</td>
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</tr>
<tr>
<td>Tocols</td>
<td>α-</td>
<td>α-</td>
</tr>
<tr>
<td>Control diet3</td>
<td>12.19 ± 0.77c</td>
<td>20.06 ± 1.13a</td>
</tr>
<tr>
<td>d-α-Tocopherol3</td>
<td>20.06 ± 1.21a</td>
<td>20.62 ± 0.99a</td>
</tr>
<tr>
<td>TRF253</td>
<td>14.49 ± 0.79b</td>
<td>12.98 ± 0.86</td>
</tr>
<tr>
<td>d-P25-T33</td>
<td>12.52 ± 0.58c</td>
<td>2.99 ± 0.37</td>
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</tbody>
</table>

<table>
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<tr>
<th>Experiment 4</th>
<th>Diet</th>
<th>Low fat diet</th>
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</thead>
<tbody>
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<td>(16–26 wk = 10 wk)</td>
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<tr>
<td>Tocols</td>
<td>α-</td>
<td>α-</td>
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</tr>
<tr>
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<td>21.11 ± 0.87a</td>
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<td>TRF253</td>
<td>13.79 ± 0.67b</td>
<td>8.29 ± 0.56</td>
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<td>d-P25-T33</td>
<td>13.84 ± 0.86b</td>
<td>16.99 ± 0.74c</td>
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</tbody>
</table>

1 Data are means ± SD, n = 5; a–c values in a column with a different superscript letter differ, P < 0.05.
2 All mice were fed only standard low fat diet (fat 5%) throughout the experiment.
3 Control (low fat, 5%) diet was supplemented with 100 μg d-α-tocopherol, TRF25 or d-P25-T3 (didesmethyl tocotrienol)/g.

LITERATURE CITED

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