Supplementation with mixed tocopherols increases serum and blood cell γ-tocopherol but does not alter biomarkers of platelet activation in subjects with type 2 diabetes1–3

Michael W Clarke, Natalie C Ward, Jason HY Wu, Jonathan M Hodgson, Ian B Puddey, and Kevin D Croft

ABSTRACT

Background: Some studies have shown potential benefit of vitamin E on platelet function, but several clinical trials failed to show improved cardiovascular outcome with α-tocopherol supplementation. γ-Tocopherol, a major dietary form of vitamin E, may have protective properties different from those of α-tocopherol.

Objective: We compared the effects of supplementation with α-tocopherol (500 mg) and a γ-tocopherol–rich compound (500 mg, containing 60% γ-tocopherol) on serum and cellular tocopherol concentrations, urinary tocopherol metabolite excretion, and in vivo platelet activation in subjects with type 2 diabetes.

Design: Fifty-eight subjects were randomly assigned to receive either 500 mg α-tocopherol daily, 500 mg mixed tocopherols daily, or matching placebo. Serum, erythrocyte, and platelet tocopherol and urinary metabolite concentrations were measured at baseline and after the 6-wk intervention. Soluble CD40 ligand, urinary 11-dehydro-thromboxane B₂, serum thromboxane B₂, soluble P-selectin, and von Willebrand factor were measured as biomarkers of in vivo platelet activation.

Results: Serum α-tocopherol increased with both tocopherol treatments. Serum and cellular γ-tocopherol increased 4-fold (P < 0.001) in the mixed tocopherol group, whereas red blood cell γ-tocopherol decreased significantly after α-tocopherol supplementation. Excretion of α-carboxyethyl-hydroxychroman increased significantly after supplementation with α-tocopherol and mixed tocopherols. Excretion of γ-carboxyethyl-hydroxychroman increased significantly after supplementation with mixed tocopherols and after that with α-tocopherol, which may reflect the displacement of γ-tocopherol by α-tocopherol due to incorporation of the latter into lipoproteins in the liver. Neither treatment had any significant effect on markers of platelet activation.


KEY WORDS α-Tocopherol, γ-tocopherol, diabetes, serum, erythrocytes, platelets, platelet function

INTRODUCTION

A number of large clinical trials and smaller supplementation studies investigated the link between α-tocopherol and cardiovascular disease (CVD) risk. The results from large clinical trials with α-tocopherol were largely negative (1–4). Other studies in subjects with enhanced oxidative stress associated with end-stage renal disease (5), in subjects with elevated cholesterol (6), and, in combination with vitamin C, in subjects undergoing transplant (7) were more promising.

γ-Tocopherol is the other major form of tocopherol in the diet. Some studies suggested a potential role for γ-tocopherol in preventing complications associated with diseases involving oxidative stress and inflammation (8). However, it was shown that supplementation with α-tocopherol can significantly reduce the plasma concentrations of γ-tocopherol (9, 10). Despite this finding, there is little information comparing changes in both plasma and cellular tocopherol concentrations after supplementation with either α- or γ-tocopherol.

Persons with type 2 diabetes have abnormal platelet function, which is characterized by increased interaction of platelets with von Willebrand factor (vWF) and with fibrin (11). Coagulation factors are increased and anticoagulant factors decreased, which leads to greater platelet activation and increased risk of thrombosis (11). Platelet activation is associated with plaque instability and represents an important therapeutic target in subjects at risk of thrombosis (12). Supplementation with α-tocopherol has been shown to inhibit platelet function in subjects with type 1 diabetes (13), type 2 diabetes (14), and hypercholesterolemia (15). In healthy subjects, a mixed tocopherol supplement composed predominantly of γ-tocopherol inhibited ADP-induced ex vivo platelet aggregation and increased endothelial constitutive nitric-oxide synthase activation and nitric oxide release, whereas synthetic α-tocopherol did not alter these endpoints (16).

In this study, our objective was to examine the relative serum and cellular uptake of the tocopherol isomers after supplementation with RRR-α-tocopherol or mixed tocopherols predominantly

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composed of γ-tocopherol. The excretion of the vitamin E metabolites α-, γ-, and δ-carboxyethyl-hydroxychroman (CEHC) was also measured for assessment of in vivo tocopherol status. We also sought to examine the effects of each supplement on in vivo markers of platelet activation in type 2 diabetic subjects, a group at increased risk of CVD.

SUBJECTS AND METHODS

Study protocol

Fifty-eight men and women with type 2 diabetes were recruited from the Perth, Australia, general population to the School of Medicine and Pharmacology at the University of Western Australia. All participants had a previous diagnosis of type 2 diabetes or were taking oral hypoglycemic medication. All medication was taken as normal on the morning of each study visit. Exclusion criteria included body mass index (in kg/m²) > 35; use of insulin, nonsteroidal anti-inflammatory drugs, or nitrate medication; smoking; use of vitamin E supplements; any recent (in previous 6 mo) coronary or cerebrovascular event; impaired renal function (serum creatinine > 1.3 mg/dL); diabetes or were taking oral hypoglycemic medication. All medications were taken as normal on the morning of each study visit. All volunteers gave informed written consent. The study was approved by the University of Western Australia Human Ethics Committee.

Sample preparation

Blood and 24-h urine samples were collected from each subject at baseline and at the completion of the 6-wk intervention. The urine samples were aliquoted into 5-mL tubes without a preservative and frozen at −80 °C until they were analyzed. Blood was collected into 3-mL serum and citrate tubes and processed immediately. Serum samples for soluble CD40 ligand (sCD40L) and thrombomodulin (TM) were prepared for tocopherol analysis according to the method of Lehmann et al (19), with minor modifications. Briefly, we added 1000 mg mixed tocopherols/d (ie, 315 mg RRR-α-tocopherol, 110 mg δ-tocopherol, and 50 mg γ-tocopherol) to placebo (soybean oil containing 110 mg of α-tocopherol). Tablets were taken daily at breakfast and dinner as 500 mg RRR-α-tocopherol/d, or placebo (soybean oil containing 110 mg of α-tocopherol/d). Tablets were taken daily at breakfast and dinner as 500 mg of α-tocopherol/d.

Platelet tocopherols were extracted by using the method of Lehwah et al (19) with the following modifications. The prepared platelet pellets were sonicated with a Branson Sonifier 150 (Branson Ultrasonics Corp, Danbury, CT) for 5 quick pulses at a power setting of 3. We added 800 μL hexane and then 50 μL tocol (1 μg/mL). The samples were mixed for 60 s, and then 600 μL hexane was removed, evaporated under nitrogen, and reconstituted in 200 μL methanol. We injected 1 μL of the sample onto the column.

Erythrocyte tocopherols were extracted by using the method of Bieri et al (20), with minor modifications. Briefly, we added 500 μL ethanol to 500 μL washed erythrocytes and mixed the samples for 20 s. The samples were spiked with 50 μL tocol (10 μg/mL) and mixed again for 20 s. We added 2 mL hexane, mixed the samples for 60 s, and then centrifuged them at 2700 rpm for 15 min at 4 °C. Hexane (1 mL) was removed, dried under nitrogen, and reconstituted in 300 μL methanol. We injected 15 μL of the sample onto the column.

HPLC analysis of tocopherols with electrochemical detection

Tocol was used as an internal standard for the analysis of serum, platelet, and erythrocyte tocopherols. Serum tocopherols were extracted according to the method of Su et al (21), with minor modifications. In borosilicate glass tubes, 200 μL serum was spiked with 50 μL tocol (10 μg/mL) and mixed for 20 s; then 200 μL ethanol was added, and the solution was mixed again for 60 s and left in the dark for 5 min. The sample was extracted with 1 mL of hexane, and 600 μL of hexane was removed, evaporated under nitrogen, and reconstituted in 200 μL methanol. We injected 1 μL of the sample onto the column.

The tocopherols were separated on a Lichrospher 100 RP-18 (5 μmol/L) reverse-phase column (Agilent Technologies, Waldbronn, Germany) by using a mobile phase of 99% methanol and 1% water containing 10 μmol lithium acetate/L. Detection was performed on an ESA Coulochem III coulometric electrochemical detector (ESA Inc, Chelmsford, MA) set at 600 V and 1 μA for each assay, and the flow rate was 1 mL/min with the use of an Agilent 1100 HPLC. Recovery, precision, and detection limit studies were performed on all of the tocopherol assays. The recovery of α- and γ-tocopherols was between 95% and 105% by all methods. In-house quality controls were assayed in duplicate at the beginning, middle, and end of each assay. The interassay and intraassay CVs for all assays were < 10%. All determinations were performed in duplicate, and samples with a duplicate CV of > 10% were assayed again. Preintervention and postintervention samples from all subjects were assayed in the same run. The minimum limit of detection for either tocopherol for each method was 0.3 μmol/L packed erythrocytes, 0.03 nmol/mL platelets, and 1 nmol/L serum. The tocopherols were stable for ≥6 mo at −80 °C by all analytic methods, and all samples were assayed within 4 mo of collection. All reagents used were...
HPLC grade, and the standards for the tocopherol assays were supplied by Calbiochem (EMD Bioscience Inc, San Diego, CA).

**Urinary tocopherol metabolites**

The tocopherol metabolites were measured by using the highly sensitive gas chromatography–mass spectroscopy method of Galli et al (22) with some modifications. We added 5 nmol d9-α-CEHC (internal standard) in ethanol to 1 mL freshly thawed urine and then added 1 mL of 0.33 mol potassium phosphate buffer/L (pH 7.4) containing 350 units of *Escherichia coli* β-glucuronidase (type IX-A). The urine was incubated for 3.5 h at 37 °C in the dark, acidified with 0.25 mL of 6 mol HC1/L, and extracted with the use of 8 mL hexane/tert-butyl methyl ether (4:1 by vol). Urine extracts were dried under nitrogen and silylated with 50 μL anhydrous pyridine and 50 μL N,O-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane heated at 60 °C for 1 h. Samples were injected directly onto the gas chromatography–mass spectrometer (Agilent 5973) for analysis with the use of a DB-5MS column [25 m x 0.2 mm (internal diameter); 0.33-μm film thickness]. The initial oven temperature of 160 °C was held for 0.5 min, and then the temperature was increased by 20 °C/min to 300 °C and held at 300 °C for 10 min. Helium was used as the carrier gas, and the selected ion–monitoring mode was used to monitor the molecular ion and one major fragment ion (as a qualifying ion) of each metabolite as follows: d9-α-CEHC (internal standard), mass-to-charge ratio (m/z) 431, 246; α-CEHC, m/z 422, 237; γ-CEHC, m/z 408, 223; and β-CEHC, m/z 394, 209 (23).

**Enzyme-linked immunosorbent assays**

Serum and plasma concentrations of sCD40L were measured by using a module kit (BMS239MS; Bender MedSystems, Vienna, Austria) according to the manufacturer’s instructions. For the citrate plasma samples, the assay was modified to detect low concentrations of CD40L (24). Plasma P-selectin was measured by using a kit (R&D Systems, Minneapolis, MN). Serum TxB2 and urinary 11-dehydro-TxB2 were measured by using enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI). We measured the vWF by using the Stago Kit latex immunoassay on the STA-R automated coagulation analyzer (Diagnostica Stago, Asnieres, France). All assays had interassay and intraassay CVs of <10%, and all of the manual assay samples were assayed in duplicate.

We measured total cholesterol by using the enzymatic method of cholesterol oxidase-p-aminophenazone (HiCo; Merck, Darmstadt, Germany), triacylglycerol by using the enzymatic GOP-PAP assay, and HDL by using the direct method HDL-Plus. These kits were obtained from Boehringer Mannheim (Mannheim, Germany), and the assays were performed on a Hitachi 917 chemistry analyzer (Boehringer Mannheim). All assays had interassay and intraassay CVs of <6%.

**Statistical analysis**

Analyses were performed by using SPSS for WINDOWS software (version 12; SPSS Inc, Chicago, IL). All data are presented as means ± SEMs. Values not normally distributed were log transformed before analysis. Baseline characteristics were compared by using analysis of variance. General linear models were used to examine differences in postintervention values after adjustment for baseline values used as covariates. Post hoc comparisons were performed by using Bonferroni correction.

### RESULTS

**Serum and cellular tocopherol concentrations**

Fifty-five subjects completed the study. Three withdrew because of changes in treatment by their primary care physician during the trial. The subjects were well matched at baseline, and their characteristics are presented in Table 1. Serum, erythrocyte, and platelet tocopherol concentrations after intervention are presented in Figure 1, Figure 2, and Figure 3, respectively. Supplementation with α-tocopherol significantly increased serum, red blood cell, and platelet α-tocopherol concentrations. Supplementation with mixed tocopherols increased serum α-tocopherol concentration, but the increase in platelet α-tocopherol was not statistically significant. Supplementation with mixed tocopherols led to a 4-fold increase in serum, erythrocyte, and platelet γ-tocopherol concentrations. Conversely, supplementation with α-tocopherol led to a significant decrease in erythrocyte γ-tocopherol, but the decrease in serum and platelet γ-tocopherol concentrations was not statistically significant. The serum and cellular tocopherol concentrations did not change significantly in the placebo group. During the 6-wk intervention, the serum cholesterol, HDL, and triacylglycerol concentrations in the α-tocopherol, mixed tocopherol, and placebo groups did not change significantly: postsupplementation values were 4.94 ± 0.2, 4.84 ± 0.3, and 4.71 ± 0.2 mmol/L; 1.29 ± 0.08, 1.28 ± 0.06, and 1.33 ± 0.10 mmol/L; and 1.87 ± 0.22, 1.76 ± 0.17, and 1.59 ± 0.24, respectively.

**Urinary tocopherol metabolite excretion**

The excretion of the 3 major tocopherol metabolites—α-, γ-, and δ-CEHC—corrected for creatinine excretion is shown in Table 2. At baseline, low concentrations of α-CEHC could be detected in most subjects, possibly reflecting reasonably high serum α-tocopherol concentrations. Very low concentrations of δ-CEHC were present at baseline, and the excretion of this metabolite increased significantly after supplementation with mixed tocopherols (containing 110 mg δ-tocopherol). Significant increases in the excretion of α- and δ-CEHC were observed after supplementation with α-tocopherol and mixed tocopherols.

### Table 1

Baseline characteristics of study subjects by treatment group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RRR-α-tocopherol (n = 18)</th>
<th>Mixed tocopherols (n = 19)</th>
<th>Placebo (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>64 ± 71</td>
<td>58 ± 7</td>
<td>62 ± 7</td>
</tr>
<tr>
<td>Men/women</td>
<td>13/5</td>
<td>12/7</td>
<td>16/2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29 ± 0.5</td>
<td>28 ± 0.6</td>
<td>28 ± 1.0</td>
</tr>
<tr>
<td>Hypertension (n)</td>
<td>9</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Statin use (n)</td>
<td>11</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Aspirin use (n)</td>
<td>8</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4.71 ± 0.23</td>
<td>4.70 ± 0.25</td>
<td>4.62 ± 0.16</td>
</tr>
<tr>
<td>LDL</td>
<td>2.67 ± 0.17</td>
<td>2.64 ± 0.23</td>
<td>2.63 ± 0.18</td>
</tr>
<tr>
<td>HDL</td>
<td>1.27 ± 0.08</td>
<td>1.35 ± 0.07</td>
<td>1.35 ± 0.11</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.70 ± 0.16</td>
<td>1.50 ± 0.16</td>
<td>1.38 ± 0.18</td>
</tr>
</tbody>
</table>

*There were no significant differences between the groups.

^2 x ± SEM (all such values).
respectively. It is interesting that the excretion of γ-CEHC also increased significantly after supplementation with α-tocopherol. This suggests that the decrease in plasma and cellular γ-tocopherol seen with α-tocopherol supplementation may be the result of increased γ-tocopherol metabolism to γ-CEHC.

Markers of platelet and endothelial function

As shown in Table 3, none of the tocopherol treatments had a significant effect on sCD40L, serum TxB₂, urinary 11-dehydro-TxB₂, soluble P-selectin, or vWF. As expected, baseline values of both serum TxB₂ and urinary 11-dehydro-TxB₂ concentrations were significantly lower in aspirin-treated (serum TxB₂: 5.2 ± 1.8 ng/mL; urinary 11-dehydro-TxB₂: 838 ± 217 ng/d) than in non-aspirin-treated (serum TxB₂: 59.6 ± 6 ng/mL; urinary 11-dehydro-TxB₂: 1018 ± 264 ng/d) subjects. Neither aspirin nor statin use differed significantly between the treatment groups (P = 0.421 for aspirin; P = 0.351 for statin). Moreover, adjustment for aspirin and statin use did not influence the results.

DISCUSSION

The current controlled intervention trial shows that high-dose α-tocopherol supplementation over 6 wk caused α-tocopherol concentrations in serum and cells to more than double and γ-tocopherol concentrations in red blood cells to decrease. Previous studies that examined the effects of α-tocopherol supplementation on serum and cellular tocopherol concentrations found significant increases in α-tocopherol and a corresponding decrease in γ-tocopherol (9, 19). Our study in a diabetic population resulted in similar changes in tocopherol concentrations. Taken together, these studies conclusively show that serum and cellular concentrations of γ-tocopherol are lower after α-tocopherol supplementation.

The loss of γ-tocopherol from serum or cells may have adverse health consequences (8). Low serum concentrations of γ-tocopherol but not of α-tocopherol were observed in subjects with coronary artery disease (25, 26). Therefore, supplementation with α-tocopherol alone may have disadvantages over mixed tocopherols that are rich in γ-tocopherol. The current study is the first to examine changes in serum and cellular tocopherols after supplementation with γ-enriched tocopherols. Previous studies examined the effect of deuterated γ-tocopherol on γ-CEHC production (27) and, to evaluate platelet function, the effects of a mixed tocopherol supplement in subjects with end-stage renal disease (28) and in healthy subjects (16).

Supplementation with mixed tocopherols in the current study resulted in a significant increase in serum α-tocopherol, together with 4-fold increases in γ-tocopherol in serum, erythrocytes, and
Urinary excretion of tocopherol metabolites

TABLE 2

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Preintervention</th>
<th>Postintervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-CEHC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed tocopherols</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (n = 18)</td>
<td>α-CEHC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.21 ± 0.04</td>
<td>7.84 ± 1.4²</td>
</tr>
<tr>
<td></td>
<td>0.22 ± 0.03</td>
<td>1.29 ± 0.21²</td>
</tr>
<tr>
<td></td>
<td>0.19 ± 0.06</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>γ-Tocopherol (n = 19)</td>
<td>α-CEHC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.65 ± 0.09</td>
<td>0.88 ± 0.11³</td>
</tr>
<tr>
<td></td>
<td>0.63 ± 0.07</td>
<td>10.46 ± 1.61²</td>
</tr>
<tr>
<td></td>
<td>0.51 ± 0.08</td>
<td>0.52 ± 0.06</td>
</tr>
<tr>
<td>δ-Tocopherol (n = 18)</td>
<td>α-CEHC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.08 ± 0.01</td>
<td>3.49 ± 0.48²</td>
</tr>
<tr>
<td></td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>

¹ All values are ± SEM. Baseline-adjusted postintervention differences were analyzed by using general linear models and Bonferroni correction.

² P < 0.001.

³ P = 0.01.

platelets. The response to supplementation with mixed tocopherols appeared greater in platelets than in serum and erythrocytes. Higher uptake of α-tocopherol by platelets during supplementation was observed previously (19). Our study found a higher uptake of γ-tocopherol by platelets after mixed tocopherol supplementation, which suggested that platelets provided an excellent marker of intake. It is important that all of the subjects in the current study had normal fasting plasma cholesterol concentrations. This may be important in tocopherol transport, given that the absorption of α-tocopherol into plasma and cells is inhibited in hyperlipidemic subjects (29).

The excretion of tocopherol metabolites in urine provides a good marker of vitamin E status. The metabolites are formed via cytochrome P450-mediated ω-oxidation that is followed by β-oxidation in the liver (30–32). The excretion of each metabolite strongly reflected the composition of the tocopherol supplement. It is interesting to note that supplementation with α-tocopherol caused an increase in the excretion of γ-CEHC along with a decrease in the γ-tocopherol concentration in plasma and red blood cells. This result suggests that the decrease in cellular γ-tocopherol was an effect of a greater metabolism to γ-CEHC, which possibly reflected the displacement of γ-tocopherol by α-tocopherol due to the former’s incorporation into lipoproteins in the liver. This finding is in contrast to that of Bruno et al (33), who saw no correlation between plasma α-tocopherol and γ-CEHC after supplementation with a lower dose of α-tocopherol, 75 mg/d, for 6 d.

Because subjects with type 2 diabetes are thought to have high platelet activation, we examined the potential effects of tocopherol supplementation on biomarkers of platelet activation. CD40 ligand is a transmembrane protein primarily found within platelets (34), and it has the capacity to initiate inflammatory reactions when bound to CD40 that is present on some cells, including endothelial cells (35). The soluble form, sCD40L, can be detected in plasma, and its importance in thrombosis was recently highlighted in subjects with acute coronary syndromes (36) and in healthy women at risk of cardiovascular events (37). Concentrations of sCD40L are elevated in diabetic patients (38, 39) and have been reduced in type 2 diabetics after treatment with peroxisome proliferator–activated receptor agonists (38, 40). Statin therapy can lower the elevated sCD40L concentrations in hypercholesterolemic subjects (41, 42), independent of cholesterol lowering (43). Treatment with aspirin has also led to reductions in sCD40L in stroke patients (44). It has been shown that CD40L expression in primary human T cells is regulated by the nuclear factor-κB transcription factor (45). The activation of this transcription factor can be induced in the retina of diabetic rats and inhibited by supplementation with antioxidants such as dl-α-tocopherol acetate (46). Given that γ-tocopherol can inhibit the oxidized LDL-mediated activation of the nuclear factor-κB transcription factor in endothelial cells (47), we hypothesized that one or both of our tocopherol supplements could inhibit the production and hence the release of sCD40L into the circulation. However, we saw no effect with either tocopherol supplement on
sCD40L release in serum or plasma in subjects with type 2 diabetes. We cannot rule out a treatment effect on platelet CD40L expression, but it seems unlikely, given that platelet CD40L has been shown to significantly correlate with sCD40L (43).

Platelet activation and subsequent adhesion to dysfunctional endothelium are important steps in thrombus formation and the formation of thromboxane A₂ (12). A metabolite of thromboxane A₂, 11-dehydro-TxB₂, can be measured in urine and provides a good indication of in vivo thromboxane A₂ production (48). Neither treatment affected 11-dehydro-TxB₂ excretion or whole-blood TxB₂ production.

Membrane-bound P-selectin is a cell adhesion molecule that mediates the adhesion of leukocytes to endothelial cells and platelets and subsequently has an important role in thrombosis (49), and the soluble form is readily detectable in plasma. vWF is stored with P-selectin in Weibel-Palade bodies, and both vWF and P-selectin are released during stimulation with prothrombotic stimuli (50). α-Tocopherol has been shown to inhibit P-selectin expression in human platelets (51). A small uncontrolled study in subjects with hypercholesterolemia found significant reductions in sP-selectin, vWF, and urinary 11-dehydro-TxB₂ after 2-wk supplementation with 600 mg α-tocopherol acetate/d (15). A subsequent study, also in 2 groups of subjects with hypercholesterolemia, observed a significant reduction in vWF after supplementation with α-tocopherol (400 and 800 IU) (52). High-dose (1200 IU/d) RRR-α-tocopherol given to diabetic subjects for 3 mo was also shown to reduce soluble P-selectin concentrations (14). The lack of treatment effect on soluble P-selectin and vWF observed in our study may be related to circulating cholesterol and hence to background oxidized LDL concentrations. Twenty-nine of our subjects were taking statins as part of their usual therapy for cholesterol management, and 21 were taking aspirin. Although adjustment for statin or aspirin use did not affect the results after adjustment for baseline values, it is possible that the current study lacked sufficient power to establish whether the tocopherol treatments have any potential therapeutic benefits in subjects not taking either treatment.

The aim of our study was to examine subjects in a realistic clinical setting in an attempt to ascertain the potential benefits of vitamin E supplements. Amid recent concerns that high-dose α-tocopherol (≥400 IU/d) may increase all-cause mortality (53) and the risk of heart failure (54), there seems little benefit in taking high-dose α-tocopherol supplements to prevent CVD and inhibit platelet function. Data regarding the safety in large doses of a mixed tocopherol supplement enriched in γ-tocopherol and any potential benefit of that supplement on CVD are lacking at this time. Our findings suggest that γ-tocopherol supplements do not inhibit platelet function or sCD40L release in subjects with well-controlled type 2 diabetes. However, mixed tocopherol treatment does result in higher concentrations of both α- and γ-tocopherol in serum without the reduction in red blood cell γ-tocopherol seen with high-dose α-tocopherol supplementation. Future studies should ascertain whether this supplement can prevent sequelae associated with inflammation and oxidative stress.

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KDC, JMH, and IBP were involved in the initial conception of the study, analysis of the data, and writing of the manuscript. NCW was involved in patient recruitment, overall study management, data analysis, and intellectual input into the manuscript. MWC conducted all the tocopherol and platelet biomarker assays and drafted the manuscript. JHYW measured urinary tocopherol metabolites and was involved in data analysis and manuscript preparation. None of the authors had a personal or financial conflict of interest.

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