Vitamin E in fortified cow milk uniquely enriches human plasma lipoproteins[1–4]

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ABSTRACT

Background: Milk fat may contribute to atherogenesis in humans.

Objective: We sought to offset the atherogenic potential of milk fat by adding polyunsaturated fat and vitamin E to milk.

Design: We measured plasma lipids, lipoproteins, and tocopherol and LDL oxidation in normolipemic adults. In experiment 1 (n = 48), we compared delivery of 100 mg *all-rac*-α-tocopheryl acetate/d in capsules, skim milk, and 1%-fat milks containing soybean oil, milk fat, or both (1:1). In experiment 2 (n = 24), we compared delivery of natural (*RRR*-α-tocopheryl acetate) and synthetic (*all-rac*-α-tocopheryl acetate) vitamin E in milk with delivery of *all-rac*-α-tocopheryl acetate in orange juice (200 mg/d in each group). In experiment 3 (n = 7), we compared delivery of 30 mg *all-rac*-α-tocopheryl acetate/d in milks with and without added vitamins A and D.

Results: Enrichment of milk fat with soybean oil did not alter plasma lipoproteins. Microdispersion of vitamin E in milks increased the molar ratio of plasma tocopherol to cholesterol by >2-fold compared with the molar ratio after consuming vitamin E capsules, whereas the molar ratios were comparable after ingestion of orange juice and capsules. Synthetic and natural vitamin E performed comparably. The enhanced plasma vitamin E:cholesterol attributed to milk increased protection of LDL against oxidation. Vitamins A and D did not affect vitamin E delivery by milk.

Conclusions: Milk augments vitamin E transport by human lipoproteins at intakes of 100–200 but not 30 mg/d. This augmentation is independent of the presence and type of fat in milk, its vitamin A and D contents, and whether the vitamin E is natural or synthetic. *Am J Clin Nutr* 2001;74:211–8.

KEY WORDS Coronary heart disease, atherosclerosis, HDL, high-density lipoproteins, LDL, low-density lipoproteins, triacylglycerol, vitamin E, tocopherol, cow milk, fortification, fortified milk

INTRODUCTION

Vitamin E is an important lipid antioxidant involved in preventing oxidative damage to aging cells and macromolecules (1, 2). As a consequence, higher intakes of this vitamin have favorable effects on a variety of disease processes, such as deteriorating immune function (3), lens cataracts (4), the secondary effects of diabetes (5), and lipid peroxidation thought to occur in LDL during atherogenesis (6) or during initiation of certain cancers (7).

The ability of α-tocopherol to protect LDL against peroxidation was shown clinically. On the basis of ex vivo resistance of LDL to peroxidation, the benefit from extra dietary α-tocopheryl acetate appears positively related to intake until tocopherol saturation is reached at ≈70 μmol/L (≈3000 μg/dL) in plasma of normolipemic individuals (1, 6, 8). Vitamin E supplementation also depresses platelet aggregation and thrombosis while stabilizing endothelial function, all of which could reduce the long-term risk of developing coronary heart disease (CHD) (9). The latter hypothesis is supported by animal experiments (10) and by studies of CHD risk in humans (11–14). Furthermore, even 20–30 times the recommended dietary intake of vitamin E (30 IU) produced no adverse effects (15).

CHD is still the leading cause of morbidity and mortality in much of the world, and chronic oxidative deterioration in other tissues is becoming a monumental health challenge in aging populations worldwide. Therefore, the ability to enhance vitamin E uptake and its associated transport in lipoproteins by cost-effective and practical dietary means would have considerable public health benefit. Because milk is an effective delivery vehicle for the fat-soluble vitamins A and D, has a long-standing tradition of safety, and is a widely accepted food for enhancing normal growth and development, we reasoned that the fat in milk might also provide an effective delivery system for vitamin E, another fat-soluble vitamin. In addition, we wanted to reduce the potential of milk fat to raise plasma cholesterol by lowering its cholesterol and saturated fat contents while incorporating n–6 and n–3 polyunsaturated fatty acids (PUFAs) from vegetable oil.

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Received June 12, 2000.

Accepted for publication November 27, 2000.

SUBJECTS AND METHODS

Subjects

Normolipemic healthy men and women who did not smoke or use medications were enrolled in all 3 experiments. We recruited the subjects by advertising in the Brandeis University community. Potential subjects were screened for eligibility with a telephone questionnaire and face-to-face interview. Individuals with liver, kidney, intestinal, or cardiovascular disorders; lactose intolerance; or a history of vitamin or mineral supplementation were excluded.

Body weight, height, and age were recorded during the face-to-face interview, when the details of the study were explained. Subjects were told the basic objectives of the study, ie, to determine whether "the fat quality or quantity in milk would affect their blood cholesterol value or vitamin delivery." Aside from that general description, they knew nothing about the milk or vitamins under study. They were informed that they might be randomly assigned to a vitamin capsule control group. All subjects agreed to consume two 240-mL glasses of milk or one vitamin capsule daily for a period of 4 wk. All procedures were approved by the Institutional Review Board at Brandeis University and all subjects signed an approved consent form.

Study design

Experiment 1

The first experiment examined absorption of synthetic vitamin E (100 mg/d) from a variety of milks and a standard soft-gel capsule. To recruit the subjects, we made ≈100 phone inquires that identified 65 potential subjects. At the initial interview, the potential subjects provided blood samples that were analyzed for plasma concentrations of total cholesterol (TC), LDL, HDL, and triacylglycerol and α-tocopherol status. The cutoffs for entry into the study were a TC concentration <5.82 mmol/L (225 mg/dL), an α-tocopherol concentration <29.0 μmol/L (1250 μg/dL), and a molar ratio of plasma tocopherol to cholesterol of <6.0. Forty-eight subjects with a mean (±SD) age of 21.5 ± 1.8 y (range: 18–40 y) were eventually enrolled in the first experiment; 6 groups of 8 subjects each were studied in parallel for a period of 4 wk. Group assignment was random except for distribution of subjects by sex. More women than men volunteered, so an attempt was made to place ≥3 men in each group of 8 subjects.

Three fat compositions were used in the milk formulations. The fats were a milk fat–based cream, soybean oil, and a 1:1 blend of these 2 fats. The fats were homogenized into a skim milk base to evaluate the effect of fat composition on blood lipid concentrations and absorption of *all-rac*-α-tocopheryl acetate. Group 1 received 1%-fat milk formulated with a 1:1 milk fat–soybean oil blend without added vitamin E. Group 2 received 1%-fat milk formulated with 100% milk fat and 200 mg oil-soluble vitamin E per 960 mL (50 mg, or 50 IU, per 240-mL serving); the vitamin E was dispersed in the milk fat before homogenization. Group 3 received 1%-fat milk formulated with a 1:1 milk fat–soybean oil blend and 200 mg oil-soluble vitamin E per 960 mL; the vitamin E was dissolved in the fat blend before homogenization. Group 4 received 1%-fat milk formulated with 100% soybean oil and 200 mg oil-soluble vitamin E per 960 mL; the vitamin E was dissolved in the soybean oil before homogenization. Group 5 received skim milk with 200 mg water-dispersible vitamin E per 960 mL; the vitamin E was blended into the skim milk before homogenization. Group 6 consumed a soft-gel capsule containing 100 mg *all-rac*-α-tocopheryl acetate (purchased from a local retail store) during breakfast. Thus, 2 servings (240 mL each) of fortified milk or the capsule provided ≈100 mg (100 IU) *all-rac*-α-tocopheryl acetate/d.

The skim milk we used was pasteurized skim milk containing 2000 IU (600 μg retinol equivalents) vitamin A palmitate and 400 IU (10 μg) vitamin D_2 per 960 mL (prepared by a local dairy processor). Both an oil-soluble vitamin E (*all-rac*-α-tocopheryl acetate; used in the 1%-fat milks) and a water-dispersible form carried on modified starch (used in the skim milk) were obtained from Watson Foods Co Inc (West Haven, CT). Losses of vitamin E in milk processing were measured and were found to be ≈25%. We replaced the lost vitamin E by including extra vitamin E, equal to the amount lost, in each milk formulation.

Pilot studies showed that the taste and mouthfeel of the various milks were acceptable and that the added vitamin E was stable. Subjects received their initial 2-d supply of milk servings under supervision in the assigned diet room so that they could become acclimated to the study and could ask questions. Thereafter, 1920 mL milk (8 servings for 4 d) was dispensed at one time.

Experiments 2 and 3

After experiment 1, we conducted 2 additional experiments. Experiment 2 addressed questions about the efficacy of a higher intake of natural vitamin E (*RRR*-α-tocopheryl acetate) compared with a lower intake of synthetic vitamin E (*all-rac*-α-tocopheryl acetate) and the potential difference between milk and orange juice as fluid delivery systems. Twenty-four subjects were assigned to 3 groups; 23 of the 24 subjects were re-enrolled from the first experiment after a 6-wk washout period. During the washout period, their plasma tocopherol concentrations returned essentially to baseline (21.2 compared with 22.0 μmol/L). One group drank 2 glasses of milk (1% fat with the 1:1 milk fat–soybean oil blend) containing 100 mg *RRR*-α-tocopheryl acetate/glass (200 mg/d, or 300 IU/d). Another group received milk containing 100 mg *all-rac*-α-tocopheryl acetate/glass (200 mg/d, or 200 IU/d). A third group drank orange juice providing 100 mg *all-rac*-α-tocopheryl acetate/serving (200 mg/d, or 200 IU/d). In addition, the antioxidant potential of the measured molar ratio in plasma of tocopherol to cholesterol was compared with an ex vivo measurement of LDL oxidation.

Experiment 3 was performed to assess the absorption of 30 mg vitamin E provided in 2 servings/d (15 mg, or 15 IU, in each 240-mL serving). The *all-rac*-α-tocopheryl acetate was added to milk containing either standard amounts of vitamins A and D [2000 IU (600 μg) and 400 IU (10 μg) per 960 mL, respectively] or no added vitamins A and D to determine whether their presence enhanced vitamin E uptake in the first experiment. The subjects (n = 7) were again recruited from experiments 1 and 2 after an appropriate 6-wk washout period during which plasma vitamin E concentrations returned to baseline (24.0 μmol/L).

Diet and diaries

Major dietary modifications were not introduced. However, all subjects were asked to refrain from consuming any milk or milk fat not provided by the study, especially ice cream, butter, and cheese (including cheese on pizza). Otherwise, participants were asked to maintain their usual diet and exercise regimens. Each subject was given a daily calendar for recording milk intake and any unusual events affecting their diet or health.

Calendars and exit interviews were used to evaluate compliance in relation to the plasma tocopherol response.

Blood analyses

After subjects fasted overnight for 12 h, blood samples (either 2 or 8 mL) were collected at 0, 2, and 4 wk by using standard sterile technique. Samples were placed into 0.01 parts of 10% EDTA. Plasma was isolated by centrifugation at 250 × *g* for 10 min at 4°C and was frozen at −80°C until analyzed in batches at the end of the study. TC and triacylglycerol concentrations were determined by enzymatic assays (Sigma Diagnostic kit, procedure nos. 352 for cholesterol and 336 for triacylglycerol; Sigma Chemical Co, St Louis). HDL cholesterol concentrations were measured after Mg^{2+} phosphotungstate precipitation by using Sigma HDL cholesterol reagent no. 352-4 (Sigma Chemical Co). LDL-cholesterol concentrations were estimated after adjustment for VLDL cholesterol on the basis of plasma triacylglycerol calculated by using the equation of Friedewald et al (16). Plasma retinol and α-tocopherol concentrations were determined by using HPLC according to the method of Bieri et al (17). Plasma retinol was recorded as a reference for individual sample reliability.

LDL oxidation

The resistance of LDL to oxidation was measured ex vivo by using LDL (density range: 1.019 to 1.063 kg/L) isolated from EDTA-treated plasma (10 mg/L) by ultracentrifugation (187 000 × *g* for 48 h at 16°C with an SW-41 rotor) in a Beckman L-60 ultracentrifuge (Beckman Instruments, Palo Alto, CA) according to Terpstra et al (18). Isolated LDL was stored at −80°C under nitrogen until used. The initiation and rate of LDL oxidation were measured by using the hemin + hydrogen peroxide system of Belcher et al (19) with modification related to LDL concentration. The final assay system contained 0.52 mmol LDL cholesterol/L, 5 μmol hemin/L and 50 μmol hydrogen peroxide/L in HEPES:NaCl (10:150 mmol/L) buffer at pH 7.4 in a final assay volume of 0.20 mL. Oxidation was initiated by adding hydrogen peroxide as the last system component. The LDL oxidation was monitored by measuring the decreasing absorbance of hemin at 405 nm (every 2 min for 180 min at 250°C) with a 96-well Thermomax microplate reader (Molecular Devices Co, Sunnyvale, CA). The resistance of LDL to oxidation was expressed as the lag time, which was defined as the time required to reach maximum oxidation velocity.

Statistical analyses

The results were evaluated by two-factor repeated-measures analysis of variance (ANOVA) with tests for time and diet and the time × diet interaction (experiments 1 and 2) or a two-tailed paired *t* test (experiment 3). When the ANOVA indicated a significant time × diet interaction ($P < 0.05$), it was followed by Tukey-Kramer post hoc test to establish between-group differences. The percentage difference between groups in the molar ratio of plasma tocopherol to cholesterol was assessed by one-way ANOVA (STATVIEW 5.0.1; SAS Institute Inc, Cary, NC).

RESULTS

The various types of milk and capsules in all 3 experiments were well accepted, with the exception of a few subjects in group 4 who received 1%-fat milk containing soybean oil and reported an "off" flavor; they returned the milk for fresh servings. This flavor problem did not occur with any other type of milk, including milks containing the 1:1 blend of milk fat and soybean oil.

Experiment 1

We obtained complete data from 28 women and 18 men. Their initial total plasma α-tocopherol concentrations and molar ratios of tocopherol to cholesterol (which were calculated to adjust for plasma cholesterol) were as expected for unsupplemented individuals (**Table 1**).

We evaluated compliance with the study protocol when subjects picked up their assigned volumes of milk and also when we reviewed their calendars and diaries, in which occasional deviations were recorded. Reporting by 2 subjects was sufficiently poor to deem them noncompliant, so their plasma samples were excluded from the study. An indirect marker of compliance for those receiving α-tocopheryl acetate was the typical increase in plasma α-tocopherol and decrease in γ-tocopherol during supplementation.

In experiment 1, plasma lipids were not affected by the treatment. All values for TC, LDL, HDL, and triacylglycerol were within the range predicted given the prescreening selection bias for normolipemic individuals. Despite the removal of dairy fat from the diet and the consumption of up to 4 g soybean oil/d in 2 glasses of milk in group 4, there was no treatment effect on the moderate-to-low initial plasma cholesterol values in these subjects (average of 4.01 mmol/L, or 155 mg/L). Plasma vitamin A was similarly unaffected by the treatment (initial average, 1.47 μmol/L).

All 5 groups receiving 100 mg *all-rac*-α-tocopheryl acetate/d for 4 wk experienced an increase in plasma α-tocopherol from the initial average of 22.0 mmol/L (950 μg/dL) (Table 1). Control subjects with no added α-tocopheryl acetate in their milk (group 1) had no significant changes in plasma α-tocopherol or γ-tocopherol. In the capsule group, α-tocopherol increased 37% and γ-tocopherol decreased 75% (data not shown). All 4 milks that provided 100 mg of either the fat-soluble or water-dispersible form of *all-rac*-α-tocopheryl acetate/d increased the plasma concentration 2.0–2.5 times more (ie, 80–90% above baseline) than did the 100-mg capsule. No differences were noted between skim and 1%-fat milk or between the 3 fat compositions in this regard. Differences detected after 4 wk were also present at 2 wk (data not shown).

Because plasma cholesterol was not altered by the treatment, the increases in plasma tocopherol were directly reflected in the molar ratio of plasma tocopherol to cholesterol, which served as a measure of the potential for enhanced antioxidant protection against lipoprotein oxidation (Table 1).

Experiment 2

As shown in **Table 2**, there were no significant differences in any of the plasma variables between the milks containing the natural and synthetic isomers of α-tocopheryl acetate. Thus, although the intake of *RRR*-α-tocopheryl acetate (200 mg providing 300 IU/d) was 50% greater than the intake of *all-rac*-α-tocopheryl acetate (200 mg providing 200 IU/d), the 2 milks induced roughly the same increases in plasma α-tocopherol (≈110% above baseline values). The increase in plasma tocopherol with 200 IU *all-rac*-tocopheryl acetate/d in orange juice

TABLE 1
Plasma concentrations of lipids, lipoproteins, retinol, and tocopherol before and after subjects consumed milk or capsules providing 100 mg *all-rac*-α-tocopheryl acetate/d for 4 wk (experiment 1)[1]

	Milk no. 1 (1% fat blend,[2] no E)	Milk no. 2 (1% milk fat + E)	Milk no. 3 (1% fat blend + E)	Milk no. 4 (1% soybean oil + E)	Milk no. 5 (skim + E)	Capsule (control) (100 mg E)
Total cholesterol (mmol/L)						
0 wk	4.32 ± 0.59	3.80 ± 0.65	4.01 ± 0.75	4.06 ± 0.83	4.29 ± 0.47	3.54 ± 0.91
4 wk	4.34 ± 0.62	3.80 ± 0.72	3.78 ± 1.09	4.22 ± 1.01	4.11 ± 0.54	3.57 ± 0.88
HDL (mmol/L)						
0 wk	1.14 ± 0.34	1.16 ± 0.26	1.16 ± 0.21	1.29 ± 0.23	1.22 ± 0.21	1.09 ± 0.23
4 wk	1.11 ± 0.34	1.11 ± 0.28	1.22 ± 0.23	1.16 ± 0.21	1.16 ± 0.13	1.09 ± 0.21
LDL (mmol/L)						
0 wk	2.72 ± 0.49	2.22 ± 0.49	2.46 ± 0.57	2.28 ± 0.72	2.56 ± 0.47	2.09 ± 0.72
4 wk	2.77 ± 0.47	2.33 ± 0.59	2.17 ± 0.91	2.40 ± 0.88	2.46 ± 0.49	2.02 ± 0.72
LDL:HDL						
0 wk	2.56 ± 0.69	1.95 ± 0.45	2.14 ± 0.47	1.85 ± 0.71	2.16 ± 0.54	1.97 ± 0.70
4 wk	2.65 ± 0.73	2.20 ± 0.78	1.81 ± 0.68	2.03 ± 0.84	2.15 ± 0.45	1.91 ± 0.77
Triacylglycerol (mmol/L)						
0 wk	1.06 ± 0.28	0.91 ± 0.18	0.79 ± 0.28	1.15 ± 0.32	1.08 ± 0.32	0.80 ± 0.20
4 wk	0.98 ± 0.28	0.81 ± 0.21	0.86 ± 0.24	1.26 ± 0.55	1.04 ± 0.26	1.03 ± 0.43
Vitamin A (μmol/L)						
0 wk	1.40 ± 0.17	1.47 ± 0.35	1.43 ± 0.28	1.57 ± 0.24	1.71 ± 0.35	1.26 ± 0.21
4 wk	1.47 ± 0.24	1.43 ± 0.35	1.47 ± 0.24	1.64 ± 0.45	1.71 ± 0.42	1.29 ± 0.21
Vitamin E (μmol/L)[3]						
0 wk	23.3 ± 3.4	20.3 ± 3.9	21.0 ± 4.6	24.1 ± 4.8	23.3 ± 2.6	20.3 ± 4.9
4 wk	24.5 ± 4.7[a]	37.1 ± 4.2[a,b,4]	39.9 ± 12.4[b,c,4]	45.1 ± 10.3[b,4]	42.5 ± 4.7[b,c,4]	28.0 ± 8.9[a,c,4]
Vitamin E:cholesterol[3]						
0 wk	5.44 ± 0.77	5.38 ± 0.92	5.31 ± 1.10	5.96 ± 0.69	5.51 ± 0.94	5.82 ± 0.79
4 wk	5.62 ± 0.71[a]	9.96 ± 1.42[b,c,4]	10.62 ± 1.56[b,4]	10.86 ± 2.22[b,4]	10.53 ± 1.88[b,4]	7.76 ± 1.05[a,c,4]
Increase from 0–4 wk (%)	5 ± 15[a]	89 ± 41[b]	103 ± 33[b]	82 ± 29[b,c]	93 ± 32[b]	37 ± 36[a,c]

[1] $\bar{x}$ ± SD; n = 7 or 8.
[2] A 1:1 blend of milk fat and soybean oil.
[3] There was a significant time × diet interaction, $P < 0.05$ (repeated-measures ANOVA). Values in the same row with different superscript letters are significantly different, $P < 0.05$ (Tukey-Kramer post hoc test).
[4] Time effect was significant, $P < 0.05$.

was only 67%. Increases in plasma α-tocopherol were closely paralleled by increases in the molar ratio of plasma tocopherol to cholesterol, which in turn closely paralleled the increase in this ratio within the LDL fraction itself (data not shown; $r = 0.92$). However, this molar ratio in LDL was ≈30% lower than the corresponding ratio in plasma, suggesting a possible loss of tocopherol during storage and preparation of LDL. Nevertheless, the molar ratio in LDL was predictive of the LDL oxidation lag time, which was much better for milk ($r = 0.91$) than for orange juice ($r = 0.65$). Thus, the increased resistance of LDL to oxidation for all 3 groups after 4 wk reflected their respective increases in plasma α-tocopherol above baseline (Table 2).

Experiment 3

In this experiment we examined the possibility that the addition of vitamins A and D to milk would enhance vitamin E absorption. This 2-wk study tested the absorption of 30 mg vitamin E/d to determine whether enhanced vitamin E absorption would also occur with this low dose of vitamin E. Plasma α-tocopherol values for both groups (ie, those consuming milks with and without added vitamins A and D) increased slightly more (15%) than the amount predicted for an equivalent intake from capsules on the basis of the published literature (6). However, the superior delivery of vitamin E by milk at the higher intakes of α-tocopheryl acetate (in experiments 1 and 2) was not observed at this lower dose (**Table 3** and **Figure 1**).

Literature comparison

In Figure 1 we compare our results with findings published in the literature. Specifically, we show the increases in the molar ratios of plasma tocopherol to cholesterol after the different doses and delivery systems of α-tocopheryl acetate in this study compared with published results after supplementation with vitamin E capsules (6). First, no significant difference was observed between milk and capsules as delivery vehicles at intakes of 30 mg vitamin E/d, whereas the distinction between the 2 delivery systems was apparent at higher intakes. Second, the ability of milk to deliver 100–200 mg α-tocopheryl acetate/d to plasma lipoproteins exceeded that of capsules containing an equivalent dose by 2.0–2.5 fold. For example, ingestion of 200 mg *all-rac*-α-tocopheryl acetate/d in milk increased plasma tocopherol concentrations somewhat more than did 400 mg from capsules. Third, orange juice as a delivery vehicle was similar to capsules and was not as effective as milk. Fourth, plasma α-tocopherol concentrations were equivalent whether the vitamin E isomer delivered in milk was *RRR*-α- or *all-rac*-α-tocopheryl acetate (at a dose of 200 mg/d). Fifth, plasma α-tocopherol was raised to equivalent concentrations regardless of whether α-tocopheryl acetate (50 mg/serving, 2 servings/d) was homogenized into skim milk in a water-dispersible form or was in an oil-miscible form dispersed in 1%-fat milks containing milk fat, soybean oil, or the 1:1 soybean oil–milk fat blend.

TABLE 2
Plasma concentrations of lipids, lipoproteins, retinol, and tocopherol and LDL oxidative susceptibility for subjects receiving 200 mg *RRR*- or *all-rac*-α-tocopheryl acetate/d in milk or orange juice for 4 wk (experiment 2)[1]

	Milk no. 6 (1% fat blend[2] + natural E)	Milk no. 8 (1% fat blend + synthetic E)	Orange juice (+ synthetic E)
Total cholesterol (mmol/L)			
0 wk	4.06 ± 0.75	4.58 ± 0.70	4.14 ± 0.70
4 wk	3.78 ± 0.57	4.16 ± 0.98	4.11 ± 1.47
HDL (mmol/L)			
0 wk	1.29 ± 0.31	1.29 ± 0.28	1.27 ± 0.26
4 wk	1.22 ± 0.31	1.40 ± 0.26	1.19 ± 0.21
LDL (mmol/L)			
0 wk	2.25 ± 0.47	2.61 ± 0.49	2.43 ± 0.62
4 wk	2.17 ± 0.47	2.33 ± 0.85	2.48 ± 0.78
LDL:HDL			
0 wk	1.84 ± 0.57	2.30 ± 0.37	2.04 ± 0.77
4 wk	1.89 ± 0.62	1.66 ± 0.46	2.16 ± 0.84
Triacylglycerol			
0 wk	0.91 ± 0.20	0.90 ± 0.28	0.98 ± 0.41
4 wk	0.86 ± 0.37	0.95 ± 0.37	0.97 ± 0.64
Vitamin A (μmol/L)			
0 wk	1.43 ± 0.24	1.36 ± 0.28	1.47 ± 0.31
4 wk	1.43 ± 0.35	1.33 ± 0.24	1.40 ± 0.28
Vitamin E (μmol/L)			
0 wk	20.0 ± 5.5	22.4 ± 3.9	22.5 ± 5.3
4 wk	41.5 ± 10.2[3]	48.9 ± 6.4[3]	38.3 ± 9.4[3]
Vitamin E:cholesterol			
0 wk	5.08 ± 1.17	4.96 ± 0.83	5.40 ± 0.80
4 wk[3]	11.09 ± 1.87	11.95 ± 3.46	9.39 ± 1.70
Increase from 0–4 wk (%)	122 ± 26[a]	137 ± 45[a]	75 ± 30[b]
LDL oxidation lag time (min)			
0 wk	46 ± 11	39 ± 10	43 ± 15
4 wk[3]	94 ± 14	85 ± 31	72 ± 20
Increase from 0–4 wk (%)	113 ± 43	119 ± 45	79 ± 68

[1] $\bar{x}$ ± SD; n = 7 or 8. Values in the same row with different supercript letters are significantly different, $P < 0.05$.
[2] A 1:1 blend of milk fat and soybean oil.
[3] Time effect was significant, $P < 0.05$.

DISCUSSION

Our objective was to render milk more heart-healthy by modifying the negative attributes of milk fat, namely reducing its cholesterol and saturated fat contents, while increasing its PUFA content. The 1:1 blend of milk fat and soybean oil reduced the cholesterol and saturated fat contents of milk by 50% and increased its PUFA content (both n–6 and n–3 fatty acids) by ≈10-fold. However, daily consumption of two 240-mL servings of this milk failed to affect plasma lipids. Vitamin E was added to the milk to counteract any negative effect on in vivo α-tocopherol reserves resulting from addition of PUFA to the diet (1). Surprisingly, α-tocopheryl acetate bioavailability (as evidenced by the plasma concentration) and lipoprotein antioxidant protection (as evidenced by resistance of LDL to oxidation) were increased more than expected when vitamin E (100–200 mg/d) was supplemented via microdispersion in milk, compared with providing the same dose in capsules or orange juice. Furthermore, neither the isomeric form used (*RRR* or *all-rac*) nor the dispersion of vitamin E in the fat or water component of milk had any significant effects on the measured endpoints.

The efficiency of α-tocopheryl acetate uptake from milk and its transfer into lipoproteins (LDL particles) did not depend on the amount or type of fat in the milk; similar responses were obtained with skim milk and milks containing soybean oil, milk fat, or a blend of both. The microdispersion of vitamin E in a fluid drink per se did not guarantee efficient uptake, as shown by the failure of orange juice to perform similarly to milk. Yet dispersion of the vitamin E within milk seems critical to its efficient uptake, given that other investigators who administered vitamin E acetate daily for 28 d in gelatin capsules together with a glass of either whole or skim milk failed to improve on plasma tocopherol concentrations expected from similar doses provided in capsules alone (20). However, the concomitant administration of fat is apparently not required to ensure enhanced vitamin E absorption from milk. This is because plasma α-tocopherol peaks 12–24 h after any given dose (20, 21), and therefore, 3–4 consecutive meals typically are consumed before absorption of vitamin E is complete. This suggests that α-tocopheryl acetate absorption, although fat-dependent, does not require that the vitamin be consumed simultaneously with fat.

The lower efficacy of orange juice than of milk for raising the plasma α-tocopherol concentration could not be attributed to use of the water-dispersible form of *all-rac*-α-tocopheryl acetate in the orange juice because skim milk with the same water-dispersible form was just as effective as 1%-fat milk (containing milk fat or soybean oil) into which oil-soluble *all-rac*-α-tocopheryl acetate had been homogenized. The lack of a difference in vitamin E

TABLE 3
Plasma concentrations of lipids, lipoproteins, retinol, and tocopherol for subjects receiving 30 mg *all-rac*-α-tocopheryl acetate/d in milk with or without vitamins A and D for 2 wk (experiment 3)[1]

	Milk no. 20 (synthetic E, no A, D)	Milk no. 21 (synthetic E, + A, D)
Total cholesterol (mmol/L)		
0 wk	4.27 ± 1.11	3.96 ± 0.65
2 wk	4.19 ± 0.93	3.90 ± 0.70
HDL (mmol/L)		
0 wk	1.29 ± 0.28	1.24 ± 0.21
2 wk	1.27 ± 0.34	1.27 ± 0.21
LDL (mmol/L)		
0 wk	2.51 ± 1.01	2.30 ± 0.70
2 wk	2.51 ± 0.78	2.22 ± 0.62
LDL:HDL		
0 wk	2.03 ± 0.92	1.91 ± 0.71
2 wk	2.06 ± 0.75	1.78 ± 0.56
Triacylglycerol (mmol/L)		
0 wk	1.03 ± 0.40	0.94 ± 0.37
2 wk	0.85 ± 0.27	0.91 ± 0.27
Vitamin A (μmol/L)		
0 wk	1.40 ± 0.21	1.47 ± 0.28
2 wk	1.36 ± 0.28	1.50 ± 0.24
Vitamin E (μmol/L)		
0 wk	24.6 ± 4.6	24.1 ± 3.1
2 wk[2]	30.5 ± 6.2	28.0 ± 3.4
Vitamin E:cholesterol		
0 wk	5.91 ± 1.06	6.15 ± 0.86
2 wk[2]	7.40 ± 1.46	7.22 ± 0.73
Increase from 0–2 wk (%)	25 ± 10	19 ± 14

[1] $\bar{x}$ ± SD; n = 7.
[2] Time effect was significant, $P < 0.05$.

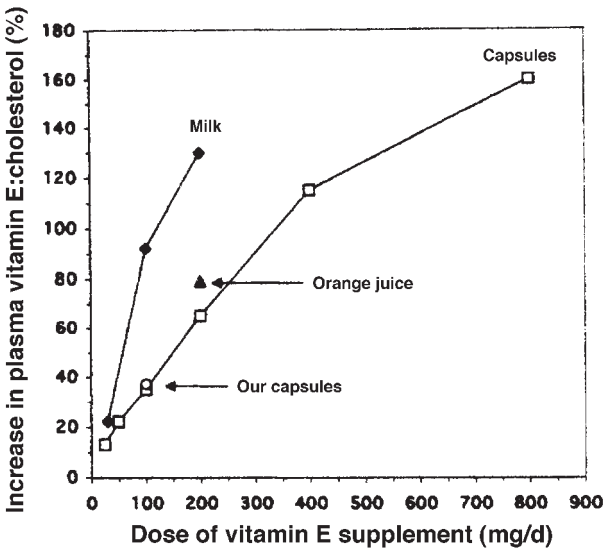

FIGURE 1. Increases from baseline in the molar ratio of plasma tocopherol to cholesterol (μmol:mmol) in response to milk (◆) and to capsules (□, from reference 6; ○, capsules in the present study) providing supplemental vitamin E. The responses to 200 mg *RRR*- and *all-rac*-α-tocopheryl acetate (experiment 2) were similar and were averaged to create one point. The response to 200 mg vitamin E in orange juice (▲) was similar to the response to capsules. At vitamin E intakes of 100–200 mg/d, the response to supplemented milk was more than twice the response to an equivalent dose in capsules, whereas the response to a vitamin E intake of 30 mg in milk was not distinct from that anticipated with capsules.

absorption between the fat-soluble and water-dispersible forms of α-tocopheryl acetate in our study contrasts with the results of Dimitrov et al (21). They reported that encapsulated fat-soluble *RRR*-α-tocopheryl acetate was absorbed significantly more effectively than was the equivalent *RRR*-α-tocopheryl succinate in glycol solubilized in water. This discrepancy between studies supports the theory that the inherent chemistry of milk elicits a physiologic response that enhances vitamin E uptake relative to uptake from orange juice, capsules, or water.

Two points concerning the intake and bioavailability of α-tocopheryl acetate seem noteworthy. First, the net efficiency of vitamin E absorption and incorporation into lipoproteins decreases progressively as dietary intake of this vitamin increases. Otherwise, increasing amounts of dietary α-tocopheryl acetate would raise plasma concentrations linearly and proportionately for an indefinite period of time; this does not happen (Figure 1). A plateau in the plasma concentration is reached, typically at 45–70 μmol/L (2000–3000 μg/dL) in normolipemic individuals (6, 8, 15). Second, food containing natural vitamin E produces remarkable plasma concentrations with minimal intakes. For example, the average daily intake of vitamin E from foods is <10 mg, yet subjects entered our study with an average plasma α-tocopherol concentration of 14–23 μmol/L (600–1000 μg/dL); these individuals were not taking supplements. In contrast, ≈200–400 mg must be ingested from capsules (this is 20–40 times the average intake from food) to double this basal plasma concentration (1, 6, 8, 20).

These observations imply that a control mechanism exists for regulating α-tocopherol uptake and incorporation into lipoproteins and tissues, and that this control mechanism can be modified and is saturable. Such a system has been described and involves the modulation of α-tocopherol transfer protein (α-TTP) synthesized by the liver (22). This protein preferentially binds the natural *RRR*-α-tocopherol isomer (22). Our results suggest that this transport system may be enhanced by one or more factors in milk other than fat, in effect rendering the system substantially more efficient. Potential candidates may be the special proteins or peptides produced during the digestion and absorption of milk.

Such results raise the question of whether unique foods, such as milk, may indirectly influence vitamin E homeostasis with significant consequences for the host. It is noteworthy that colostrum enhances vitamin E uptake in newborn calves (23), possibly to ensure superior absorption and utilization of this important antioxidant for rapidly growing cells, tissues, and developing organ systems in milk-dependent suckling newborns, for whom milk is the natural delivery vehicle. Presumably, this highly efficient α-tocopherol transport system has a built-in upper threshold for transport on the basis of α-TTP dynamics and available space in the lipoprotein phospholipid surface coat, where most lipoprotein α-tocopherol presumably resides after absorption (24). Milk may simply enhance the efficiency of this system such that at higher intakes (100–200 mg/d) of supplemental α-tocopheryl acetate, the fat-soluble and water-dispersible forms and the natural and synthetic isomers appear to be absorbed equally well.

As shown in Figure 1, milk was not detectably better than were capsules as a delivery system at intakes of 30 mg vitamin E/d. In contrast, the difference between the 2 delivery modes was clearly apparent at vitamin E intakes of 100 and 200 mg/d. Milk performed 2.0–2.5 times more efficiently than did capsules or orange juice at these higher intakes. More data are needed at intakes >200 mg/d and <100 mg/d to clearly define the saturation curve for plasma α-tocopherol when milk is the delivery system.

With regard to the latter point, it was noteworthy that 200 mg of the synthetic form of vitamin E performed as well as did 200 mg of the natural form in experiment 2. The natural form was shown to be more bioavailable than the synthetic form at lower intakes (20–50 mg/d) when single oral doses were used (22, 25). Also, the literature clearly indicates that natural vitamin E has superior biological activity during demanding metabolic circumstances (eg, direct competition for α-TTP in the liver when the absolute mass of the various α-tocopherol isomers is limited). With our experimental design, this differential was not observed, presumably because competition between natural and synthetic vitamin E was not an issue and tocopheryl acetate was present in relative abundance in both test milks. Thus, it is possible that the enhanced bioavailability of vitamin E associated with microdispersion in milk, coupled with improved utilization of the natural (D-isomer) portion of the racemic (synthetic) preparation, allowed vitamin E saturation of lipoproteins at 200 mg (200 IU) *all-rac*-α-tocopheryl acetate/d. The additional 100 IU D-isomers/d provided by 200 mg of the natural *RRR* isomer may have been superfluous when delivered in milk. If so, further research should be done to identify the point at which the saturation curve for lipoprotein transport eventually discriminates between the *RRR* and *all-rac* isomeric forms at lower doses of tocopheryl acetate in milk.

One inference we can draw from these data is that fortification of milk with vitamin E could have major public health implications not unlike those currently acknowledged for vitamins A and D in milk and folic acid in flour. Specifically, the combination of enhanced vitamin E absorption and supplemental amounts slightly higher than the current recommended dietary intake might have

major positive health benefits in the general population for prevention of CHD and certain forms of cancer, given the protective benefits associated with higher plasma tocopherol concentrations (1, 7, 11–13). Vitamin E fortification of milk may offer a simple, economical means for reaching all age groups of the milk-consuming population, particularly children. It is important to note that atherosclerosis is thought to begin in childhood.

Vitamin E is considered safe, even at sustained intakes of 400 mg/d for 6 y (26) or 800 mg/d for 4 mo (15). As described above, vitamin E has a built-in plasma transport threshold (limited by α-TTP dynamics). For most individuals, this transport system reaches saturation near 70 μmol/L (3000 μg/dL) or at a molar ratio of tocopherol to cholesterol near 1:75 in plasma. Thus, the health of the milk-drinking public should be improved by vitamin E fortification of dairy products consumed in conventional quantities.

In addition to the potential public health benefits to be gained from these findings, the results are also novel in their comparison of the bioavailability of identical forms and amounts of tocopheryl acetate (both natural and synthetic isomers) from a particular food (ie, milk) as compared with capsules. The comparisons were made within the same clinical setting, and in many cases within the same individuals. Few previous studies have even described α-tocopheryl acetate bioavailability from foods. One clinical study concluded that 31 mg α-tocopheryl acetate/d dispersed in margarine delivered α-tocopherol to plasma with an efficiency expected of capsules, ie, a 16% increase over basal values (27). Similar results were reported for rats consuming vitamin E in cereals (28).

This study showed that α-tocopheryl acetate in milk was significantly more bioavailable than was the same form and amount of vitamin E in capsules. In addition, our assessment of ex vivo LDL oxidation showed the potential for a direct health benefit. LDL oxidation is considered a putative indicator of the atherogenic potential of LDL and has been related to CHD risk (6, 8, 29). Although this relation still needs to be confirmed (30), enhanced resistance of LDL to oxidation measured in subjects consuming vitamin E in milk (compared with capsules) directly reflected the superior transport of plasma LDL α-tocopherol with milk as the delivery system.

In summary, we discovered a novel means of enhancing vitamin E bioavailability by 2-fold via microdispersion in milk. We predict that the resulting enhanced transport of α-tocopherol by lipoproteins could confer significant antioxidant-related health benefits.

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