

Removal of Fat from Cow's Milk Decreases the Vitamin E Contents of the Resulting Dairy Products*

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Abstract:

The present study was undertaken to determine whether decreases in fat contents result in lower vitamin E contents. Milk samples of varying fat contents (half and half, whole milk, reduced-fat milk, low-fat milk, and nonfat milk) were obtained from a local dairy on six different occasions. α -Tocopherol was the major form of vitamin E (>85%); γ -tocopherol and α -tocotrienol were present to a lesser extent. As the fat contents of milk products decreased from 11 to 0.3%, the vitamin E contents decreased. For example, raw milk as compared to nonfat milk had both higher α -tocopherol contents (45.5 ± 4.6 vs. 4.5 ± 0.5 $\mu\text{g}/100$ g; P:5 0.0001) and higher total lipids (3.46 ± 0.49 vs. 0.30 ± 0.07 g/100 g; P:5 0.0001). Vitamin E, cholesterol, and total lipids increased as cream was added back to nonfat milk during production. For every 1 mg cholesterol increase, there was an increase of approximately 4 μg of α -tocopherol; for every 1 g total lipids increase, the α -tocopherol content increased by 17 μg . These data demonstrate that removal of milk fat markedly decreases the vitamin E content of various milk products.

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Article:

In recent years, the term “antioxidant vitamins” has caught the attention of consumers. This is in part due to an increase in the research and understanding of the significant roles of antioxidant vitamins in disease processes. Vitamin E is one such essential lipid-soluble, chain-breaking antioxidant. Several prospective studies have suggested inverse associations between dietary intakes or plasma concentrations of antioxidants and cardiovascular disease (1,2). In some studies, this association has been observed for dietary vitamin E, but in other instances the relationship was seen only in persons taking high doses of vitamin E as supplements (3,4).

* Abbreviations: FDA, Food and Drug Administration; HPLC, high-performance liquid chromatography; MI, myocardial infarction.

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There have been three major intervention studies testing the effects of vitamin E on coronary heart disease risk. The Cambridge Heart Antioxidant Study, a double-blind placebo controlled study, looked at the effect of vitamin E (α -tocopherol) in the secondary prevention of coronary heart disease. The study demonstrated a 76% reduction in second nonfatal heart attacks in patients, who previously had had one heart attack (5). The GISSI-Prevenzione trial was a study of more than 11,000 individuals with a recent myocardial infarction (MI) who were randomly assigned to consume fish oil, vitamin E, or both, in a 2 x 2 factorial design study. The results were favorable for the fish oil supplement and neutral for vitamin E, but a post-hoc statistical analysis suggested a positive outcome for vitamin E supplementation (7). However, the Heart Outcomes Prevention Evaluation Trial of more than 9,000 subjects with vascular disease and diabetes also did not show a benefit for vitamin E, although the angiotension-converting enzyme inhibitor ramipril was shown to be beneficial in reducing cardiovascular risk (8). In contrast, in a trial with 196 patients with endstage renal disease, vitamin E (800 IU) compared to placebo showed a 46% reduction in the primary end point (cardiovascular disease including sudden death), and a reduction in MI by 70% (9). Thus, the role of vitamin E in decreasing coronary heart disease risk remains controversial.

Both vitamin E and fat are important in human health, but may have opposite effects. The American public has focused on decreasing dietary saturated fat to benefit health. However, modifying dairy product fat contents by either reducing total fat or altering the kind of fat may alter the vitamin E contents. Since vitamin E is a fat-soluble vitamin, it is likely to be removed by fat-modification of dairy foods. Is this detrimental because it reduces vitamin E intake? The present study was undertaken to analyze vitamin E, total lipid, and cholesterol in regular and fat-modified dairy products to assess whether decreases in fat result in lower vitamin E contents.

MATERIALS AND METHODS

Selection of analytical samples

Milk samples were obtained from Loch Mead Dairy (Junction City, OR) on six different occasions. Briefly, raw milk was processed by the dairy to separate cream and nonfat milk. Depending on the type of milk being produced, a varying amount of fat in the form of cream was added back to the nonfat milk; homogenization and pasteurization followed immediately. The U.S. Food and Drug Administration (FDA) regulations define the names for various milk products based on the fat contents (half and half, 11%; whole milk, 3%; reduced-fat milk, 2%; low-fat milk, 1%; and nonfat milk, 0.5% fat).

Six aliquots of each milk sample were obtained for analysis from a single batch of raw milk being processed on the same day. All the samples were transferred from the dairy to the laboratory on ice and analyzed within 24 h from the time of collection. Six different batches were analyzed.

Laboratory analyses.

(i) Vitamin E

The distribution of α - and γ -tocopherols and a- and γ -tocopherols in half and half, raw, whole, reduced-fat, low-fat, and nonfat milk was determined as previously described (10). Briefly, the milk vitamin E was extracted with hexane [n-hexane high-performance liquid chromatography (HPLC) grade; EM Science, Cherry Hill, NJ] following saponification with ethanolic potassium

hydroxide. An aliquot of the hexane layer was evaporated under nitrogen and the residue was resuspended in 1:1 ethanol/ methanol. An appropriate aliquot was then injected onto the HPLC. The HPLC was configured with an SIL-10AD_{VP} autoinjector with a sample cooler (Shimadzu, Kyoto, Japan) consisting of a SCL-10A system controller, a LC-10AD_{VP} HPLC series isocratic pump, a Beckman Ultrasphere (ODS C-18 column, 4.6 mm i.d., 25 cm, 5 μm particle size) with a Waters® Spherisorb ODS guard column and detected using LC-4C amperometric detector with a glassy carbon electrode (Bioanalytical Systems Inc., Lafayette, IN). The mobile phase was a mixture of methanol/water (99:1, % vol/vol) and 0.1% (wt/vol) lithium perchlorate. The total run time for the assay was approximately 12 min. Peaks were integrated using Shimadzu Class-VP automated software program (Columbia, MD). Authentic α- and γ-tocopherols and α- and γ-tocopherols were used as external standards for quantification of vitamin E (Cognis, La Grange, IL). No γ-tocopherols was detected in the samples.

(ii) Cholesterol.

The cholesterol content of the milk sample was measured using a cholesterol kit from Sigma Diagnostics (Procedure No. 352, St. Louis, MO) in an aliquot of hexane saved from the extract described for vitamin E analysis.

(iii) Total lipids.

Total lipids were extracted from milk samples using methanol and chloroform (11). The total lipids were calculated from the weight of the dried aliquot and expressed as weight in grams per 100 g milk.

Statistical analysis.

Data were analyzed using a one-way analysis of variance model using StatView (SAS Institute Inc., Cary, NC). Differences between means were considered statistically significant if $P < 0.05$. If significant differences were found, then Fisher's post-hoc tests were used for making pairwise comparisons. Here differences between means were considered statistically significant if $P < 0.01$.

RESULTS

Milk vitamin E, cholesterol, and total lipids.

Routine processing of milk involves separation of the cream from raw milk and later adding the cream back to nonfat milk in appropriate amounts depending on the dairy product. The total lipid, cholesterol, and vitamin E contents (α- and γ-tocopherols and α-tocotrienol) of half and half, raw, whole milk, reduced-fat milk, low-fat milk, and nonfat milk were analyzed and results are shown in Table 1. α-Tocopherol was the most abundant form of vitamin E in all types of milk—it represented from 84 to 92% of the vitamin E, while γ-tocopherol and α-tocotrienol were each roughly 5%.

The total lipids content of milk increased from 0.30 ± 0.07 g/100 g in nonfat to 11.6 ± 0.53 g/100 g in half and half with the addition of increasing amounts of cream to nonfat milk. The cholesterol contents of milk also varied with the amount of cream added, with the highest concentrations in the products with the highest fat contents. Similarly, the α- and γ-tocopherols and α-tocotrienol contents varied in the different products apparently depending on the fat

content. Half and half contained the most fat and had the highest amount of α -tocopherol ($193 \pm 1.66 \mu\text{g}/100 \text{g}$) among all the products tested. The other products had lower fat and α -tocopherol contents. For example, the α -tocopherol concentration of whole milk ($43.9 \pm 2.2 \mu\text{g}/100 \text{g}$) was

TABLE 1
Total Lipid, Cholesterol, and Vitamin E Concentrations in Milk

	Total lipids	Cholesterol	α -Tocopherol	γ -Tocopherol	α -Tocotrienol ($\mu\text{g}/100 \text{g}$) ^d
Raw	3.4 \pm 0.49	16. \pm 1.5	45.5 \pm 4.6	1.92 \pm 0.44 ^c	1.96 \pm 0.51 ^c
Whole	3.4 \pm 0.11	14. \pm 1.4	43.9 \pm 2.2	2.06 \pm 0.38 ^d	1.76 \pm 1.76 ^d
Reduced-fat	2.1 \pm 0.15	7.0 \pm 0.5	26.4 \pm 3.9	1.34 \pm 0.58 ^e	1.09 \pm 0.31 ^e
Low-fat	1.1 \pm 0.15	3.5 \pm 0.4	14.2 \pm 1.7	0.96 \pm 0.31 ^f	0.59 \pm 0.09 ^f
Nonfat	0.3 \pm 0.07	1.7 \pm 0.3	4.5 \pm 0.5	0.62 \pm 0.11 ^g	0.14 \pm 0.03 ^g
Half and	11. \pm 0.53	47. \pm 1	193 \pm 1.7	12.1 \pm	7.00 \pm

^aFor both total lipid and α -tocopherol concentrations, pairwise comparisons between means for each of the products shown were significantly different ($P < 0.0001$) (except between raw and whole milk which was not significantly different).

^bFor cholesterol concentrations, pairwise comparisons between means were significantly different for all milk products ($P < 0.0001$); except $P < 0.009$ between raw and whole milk and $P < 0.004$ between reduced-fat and nonfat milk.

^cFor γ -tocopherol concentrations, pairwise comparisons between means were significantly different ($P < 0.0001$) only between ^{c,d,e,f,g}half and half and ^craw, ^dwhole, ^ereduced-fat, ^flow-fat, and ^gnonfat milks.

^dFor α -tocotrienol concentrations, pairwise comparisons between means were significantly different ($P < 0.0001$) only between ^{c,d,e,f,g}half and half and ^craw, ^dwhole, ^ereduced-fat, ^flow-fat, and ^gnonfat milks.

TABLE 2
Cholesterol and Vitamin E Contents of Milk Relative to Total Lipids

	Cholesterol per total lipids (mg/g) ^a	α -Tocopherol per total lipids	γ -Tocopherol per total lipids	α -Tocotrienol per total lipids
Raw	4.64 \pm 0.39 ^{b,d}	13.2 \pm 1.1	0.57 \pm 0.16 ^b	0.57 \pm 0.11
Whole	4.22 \pm 0.44 ^a	12.9 \pm 0.8	0.60 \pm 0.11 ^a	0.52 \pm 0.06
Reduced-fat	3.34 \pm 0.18 ^{c,d}	12.4 \pm 1.2	0.63 \pm 0.26	0.51 \pm 0.14
Low-fat	3.08 \pm 0.25	12.3 \pm 1.2 ^a	0.83 \pm 0.25	0.51 \pm 0.09
Nonfat	6.13 \pm	15.7 \pm 3.9	2.22 \pm 0.80 ^{a,b}	0.50 \pm 0.17
Half and half	4.11 \pm 0.12	16.7 \pm 0.6 ^a	1.04 \pm 0.19	0.60 \pm 0.24

^aFor cholesterol per total lipids, pairwise comparisons between means were significantly different between ^{a,b}nonfat and ^awhole or ^braw milks ($P < 0.0001$), or between ^{c,d}reduced-fat and ^cnonfat or ^draw milks ($P < 0.01$).

^bFor α -tocopherol per total lipids, pairwise comparisons between means were significantly different between ^alow-fat milk and ^ahalf and half ($P < 0.001$).

^cFor γ -tocopherol per total lipids, pairwise comparisons between means were significantly different between ^{a,b}nonfat milk and ^awhole or ^braw milks ($P < 0.0001$).

higher than reduced-fat milk (26.4 ± 3.6 [g/100 g]), low-fat milk (14.2 ± 1.7 [g/100 g]), or nonfat milk (4.5 ± 0.5 [g/100 g]).

Vitamin E and cholesterol concentrations relative to total lipids

Table 2 provides the vitamin E and cholesterol contents of milk relative to the total lipids for all the milk samples. The α -tocopherol per total lipids in nonfat milk was higher than in raw milk. Similarly, cholesterol per total lipids was higher in nonfat milk (6.13 ± 1.86 [g/g]) as compared with raw milk (4.64 ± 0.39 [g/g], $P < 0.0003$). The α -tocopherol and the total lipids in different dairy products were correlated (Fig. 1); for every 1 g increase in total lipids, the α -tocopherol content increased by 17 μ g.

Vitamin E content relative to cholesterol content

Table 3 provides the vitamin E content of milk relative to the cholesterol content for the milk samples analyzed. The α -tocopherol per cholesterol content was greatest in half and half (4.0 ± 0.1 μ g/mg) and lowest in nonfat milk (2.6 ± 0.3 μ g/mg, $P < 0.0001$). Interestingly, the γ -tocopherol per cholesterol content in nonfat milk was higher than that in raw milk

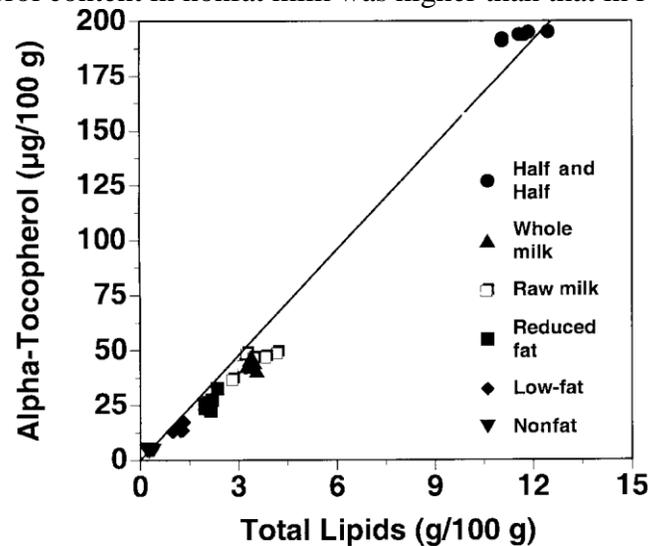


FIG. 1. Relationship between α -tocopherol and total lipids in milk samples of varying lipid contents. [$f(x) = 17.0x - 7.8$; $R^2 = 0.985$].

($P < 0.0001$). The α -tocopherol and the cholesterol contents in the different dairy products were also correlated (Fig. 2); for every 1 mg of cholesterol there was an increase of approximately 4 μ g of α -tocopherol.

DISCUSSION

α -Tocopherol was the major vitamin E form found in this study of cow's milk products of varying fat contents; γ -tocopherol and α -tocopherol were found to lesser extent, while γ -tocotrienol was not detected. As the fat contents of the dairy products decreased from (11 to 0.3%), the vitamin E content also decreased. The α -tocopherol content of whole milk reported in this study was 44 μ g/100 g. Previously, whole milk (3.3%) was reported to contain 30 μ g α -

tocopherol/100 g, while milk containing 2% fat (protein-fortified) contained 40 µg α-tocopherol /100 g (12). Most dietary vitamin E is found in fats and oils. α-Tocopherol is found predominantly in canola, olive, and sunflower oils (14). Indeed, the major food source of vitamin E in the diet of Americans, as a result of its high fat contents, is desserts (15). Importantly, decreasing fat intake also decreases vitamin E intake (16).

The milk products examined in this study contained typical lipid levels. The fat contents ranged from 11.6% total lipid for half and half to 0.3% for nonfat milk. These total lipid values meet the FDA food labeling requirements for the products described. The total lipids in the raw milk that we obtained were typical for raw milk (3 to 5% total lipids) as previously reported (17).

Cholesterol is the major form of sterol found in dairy products and it, like vitamin E, varied with the total lipid contents of the dairy products. In our study, the cholesterol contents of different dairy products were: half and half 47.7 mg/100 g, whole milk 14.3 mg/100 g, reduced-fat milk 7.06 mg/100 g, low-fat milk 3.56 mg/100 g, and nonfat milk 1.74 mg/100 g. These values are similar to the values reported in the nutrition analysis software “The Food Processor” by ESHA Research (Salem, OR) (18).

The unique finding in our study was that dairy products

TABLE 3
Vitamin E Content of Milk Relative to Cholesterol Content

	α-Tocopherol per cholesterol	γ-Tocopherol per cholesterol	α-Tocotrienol per cholesterol
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Raw	2.8 ± 0.1 ^a	0.12 ± 0.03 ^{a,c}	0.12 ± 0.03
Whole	3.1 ± 0.2 ^{b,d}	0.15 ± 0.03 ^b	0.12 ± 0.02
Reduced-fat	3.7 ± 0.3 ^d	0.19 ± 0.09 ^c	0.15 ± 0.05 ^b
Low-fat	4.0 ± 0.2	0.27 ± 0.10 ^{a,b}	0.17 ± 0.03 ^a
Nonfat	2.6 ± 0.3 ^{c,d}	0.37 ± 0.10 ^{c,d}	0.08 ± 0.01
Half and half	4.0 ± 0.1	0.25 ± 0.05 ^{d,e}	0.15 ± 0.06 ^c

^aPairwise comparison for α-tocopherol per cholesterol were statistically significant (P < 0.0001) between ^{a,b,c}half and half and ^araw or ^bwhole and ^cnonfat milks; between ^dnonfat and ^dwhole milk (P < 0.0006).

^bPairwise comparisons for γ-tocopherol per cholesterol were statistically significant between ^{a,b}lowfat and ^araw (P < 0.001), and ^bwhole milk (P < 0.004); between ^{c,d}nonfat and ^creduced-fat (P < 0.0015) and ^dhalf and half (0.004); between ^ehalf and half and ^eraw milk (P < 0.01).

^cFor α-tocotrienol significant differences were observed between ^{a,b,c}nonfat and ^alow-fat (P < 0.01),

^breduced-fat milk (P < 0.01); or ^chalf and half (P < 0.005).

contain α-tocopherol. This particularly piqued our interest because of the potent antioxidant properties of α-tocopherol. Although α-tocopherol has just one-third the biological activity of α-tocopherol in rats (19), it has equal (20) or higher antioxidant activity compared with α-tocopherol (21,22). The α-tocopherol contents of dairy products ranged from 1.76 µg/100 g for

whole milk to 0.14 $\mu\text{g}/100\text{ g}$ for nonfat milk (Table 1). Other studies have not reported the presence of tocotrienols in dairy products. This may be because we used an extremely sensitive method for detection of tocopherols and tocotrienols (10). Nonetheless, γ -tocotrienol, a predominant vitamin E form in palm oil (14), was not found in milk. Alternatively, the presence of α -tocopherol in milk may be attributed to the tocotrienol content of the feed, specifically grasses consumed by the cows. Grasses of green pastures in the early stage of growth have an increased α -tocopherol content (23). Our milk samples were all obtained in late spring

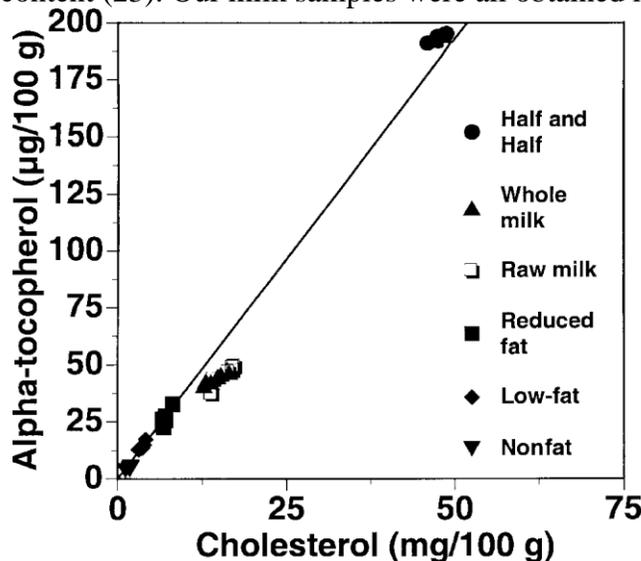


FIG. 2. Relationship between α -tocopherol and cholesterol in milk samples of varying lipid contents. [$f(x) = 4.1x - 6.8$; $R^2 = 0.985$].

from the neighborhood dairy where cows were allowed to eat grass.

It is clear that increased amounts of fat in the milk products result in increases in both cholesterol and vitamin E contents; therefore, the different forms of vitamin E were normalized to total lipid and cholesterol contents. The total lipids-adjusted cholesterol was highest in nonfat milk (Table 2). A possible explanation for this observation could be the disruption of milk fat globules during the centrifugation procedure, to isolate cream thereby contributing to residual fat globule membranes disproportionately rich in cholesterol, which accumulate in the nonfat milk (24). The cholesterol-adjusted α -tocopherol content was found to be lowest in nonfat milk (Table 3), suggesting association of α -tocopherol with the fat droplets rather than the membranes. Milk production typically involves separation of cream from raw milk and later adding the cream back to nonfat milk in varying amounts depending on the dairy product. Although statistically significant differences were found in the ratios between the vitamin E forms and lipids or cholesterol, in general, these differences were relatively minor and not likely to represent significant differences to the consumer.

Vitamin E was present in all the dairy products analyzed. Given in Table 4 is the vitamin E content of different milk products, when a quart is consumed, compared to rich sources of dietary vitamin E, almonds and frozen spinach (14). A single serving of almonds nearly provides the recommended daily allowance for vitamin E, while even a quart of whole milk does not provide an equivalent amount of α -tocopherol. For example, one serving of almonds provides 15

g fat and 12 mg α -tocopherol, while a cup of frozen spinach provides 1.5 g fat and 3.4 mg α -tocopherol. Even though spinach has one-tenth the fat content of almonds, it provides a significant amount of vitamin E as compared to quart of whole milk, which provides 33 g fat and 0.4 mg α -tocopherol. Although, milk cannot be considered a primary food source of vitamin E, it is the most commonly consumed dairy product and if large enough amounts of milk products are consumed they become a significant source of vi-

TABLE 4
Comparison of Milk Products, Almonds and Frozen Spinachs as Vitamin E Sources

	Amou nt	Calo ries	Total fat (g)	α - Tocopherol	γ - Tocopherol	α - Tocotrienol
Whole milk	1 quart	599	33	0.42	0.019	0.017
Reduced-fat	1 quart	485	19	0.25	0.013	0.010
Low-fat milk	1 quart	409	10	0.13	0.009	0.006
Nonfat milk	1 quart	342	2	0.04	0.006	0.001
Spinach.	1 cup	54	1.5	3.4	—	—
Almonds	2 Tbsp.	166	15	12	0.51	0.56

tamin E. However, according to the 1994–1995 Continuing Survey of Food Intakes by Individuals data, nonfat milk consumption is increasing, while whole milk intake is decreasing (25). Because the vitamin E content of nonfat milk is one-tenth that of whole milk, we suggest that vitamin E fortification of milk might be a reasonable approach to restore α -tocopherol intakes to those seen with whole milk. This is especially important because children consuming relatively low-fat diets have decreased vitamin E intakes (26).

In conclusion, this study indicates that vitamin E, especially α -tocopherol, γ -tocopherol, and α -tocopherol, is present in milk of various fat contents. Importantly, the α -tocopherol content of milk decreases along with cholesterol content as the fat content decreases.

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