AN ABSTRACT OF THE THESIS OF

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Abstract approved: _____

Maret G. Traber

The present study was undertaken to analyze the vitamin E, total lipids, and cholesterol in regular and fat-reduced milk to assess whether decreases in fat result in lower vitamin E contents. Milk samples of varying fat contents (11%, 3.3%, 2%, 1% and 0.5% fat) were obtained from a local dairy on six different occasions. α-tocopherol was the major form of vitamin E found in different types of milk. γ-tocopherols and α-tocotrienol were found to a lesser extent in different milks. As the fat content of milk decreased from 11% to 0.3%, vitamin E content also decreased steadily. For example, raw milk as compared to non-fat milk had both a higher α–tocopherol contents (45.5 ± 4.59 μg/100 ml vs. 4.46 ± 0.54; $p \leq 0.0001$) and a higher total lipids (3.46 ± 0.49 μg/100 ml vs. 0.30 ± 0.07 g/100 ml, ($p \leq 0.0001$). The other detected forms of vitamin E, γ-tocopherol and α-tocotrienol, also varied as the total lipids. Vitamin E, cholesterol and total lipids increased as cream was added back to non-fat milk. For every 10 mg of cholesterol there was an increase of approximately 4 μg of α-tocopherol. For every 1 g increase in total lipid content, the

α-tocopherol content increased by 17 μg. This study indicates that vitamin E; especially exotic forms of this vitamin are present in the dairy products analyzed. Also, vitamin E content varies with the total lipid and the cholesterol content. We suggest that vitamin E fortification of milk might be a reasonable approach to restore α-tocopherol intakes to those seen with whole milk.

Vitamin E, Total Lipids and Cholesterol in Cow's Milk of Varying Fat Contents

by

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I understand that my thesis will become part of the permanent collection of Oregon State University Libraries. My signature below authorizes release of my thesis to any reader upon request.

Supriya Kaushik

CONTRIBUTIONS OF THE AUTHORS

Dr. Maret G. Traber was involved in the design, analysis and writing of the manuscript. Dr. Rosemary C. Wander involved in the study design and is a co-author of the manuscript. Dr. Bruce German and Mr. Scott Leonard were involved in the study design.

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1 INTRODUCTION

Guidelines for Americans have recommended that total dietary fat constitute 30% or less of total energy intake and saturated fat constitute less than 10%. Excessive consumption of dietary fat is associated with an increased incidence of obesity, cardiovascular disease, hypertension and certain types of cancer. In an effort to assist the American population in reducing fat intake, Healthy People 2000 called upon the food industry to increase the availability of fat-modified foods by the year 2000. By 1992, the food industry introduced 5600 fat-modified food products. By 1995, this number increased to 8500. In 1996, 2076 new reduced or low-fat products were introduced. These fat-modified food products can help Americans reduce their fat intake. Fat modified food products comprise of a range of food products in which the fat content of the traditional full-fat food product has been modified either by replacement of fat with a reduced fat or non-fat ingredient or removal of fat from the full-fat food product. For example, skim milk produced by removing fat from full-fat milk represents a fat-modified-food by omission of fat.

Vitamin E is a fat-soluble vitamin. Hence, it is expected that removal or replacement of fat in fat-modified foods will decrease the amounts of vitamin E in the fat-modified food product.

Both vitamin E and fat are important in human health, but may have opposite effects. The American public has focused on decreasing dietary fat to produce health benefits. However, modifying dairy-product fat either by reducing total fat or altering the kind of fat may decrease its vitamin E contents. Is fat removal detrimental because it reduces vitamin E contents? The present study was undertaken to analyze the vitamin E, total lipids, and cholesterol in regular and fat-modified dairy products to assess whether decreases in fat result in lower vitamin E contents.

1.1 Hypothesis

The hypothesis of this work is that "Vitamin E content is lower in fat-modified food products as compared to the corresponding full fat food products". The experiment was conducted with the following specific aims.

1.2 Specific Aims

To analyze in regular and reduced-fat dairy products:

- α-Tocopherol and non-α-tocopherol forms
- Cholesterol
- Total lipids

2 LITERATURE REVIEW

2.1 Health Risks from High Fat Diets

The Third National Health and Nutrition Examination Survey (1988-91) reported that among adults, approximately 33 percent of men and 36 percent of women were overweight. Moreover, the prevalence of overweight in United States has continued to increase (1997). Recommendations from various scientific bodies, including the U.S. Surgeon General's Report on Nutrition and Health (1988), the National Academy of Sciences Diet and Health Report (1989), Healthy People 2010: National Health Promotion and Disease Prevention Objectives (2000) and the USDA Dietary Guidelines for Americans (1995), have suggested for individuals over the age of two years that total dietary fat constitute 30% or less of total energy intake and that saturated fat constitute less than 10%.

The above recommendations were based on the evidence that excessive consumption of dietary fat is associated not only with obesity but also with increased incidence of cardiovascular disease, hypertension and certain types of cancer (Rolls, 1995, Krauss, *et al.*, 1996, Wynder, *et al.*, 1997). Epidemiological studies show a positive relationship between dietary cholesterol and saturated fat, serum cholesterol and an increased risk of mortality from coronary heart disease. There is substantial evidence that increased blood levels of low density

lipoproteins (LDL) cholesterol are causally related to increased risk of coronary heart disease and lowering total and LDL cholesterol will reduce the incidence of coronary heart disease (National Cholesterol Education Program, 1990). Decreasing the total fat intake could result in lowering energy consumption and weight loss and thereby reduce the problems of hypertension and cardiovascular disease.

2.2 Dairy Products as a Source of Dietary Fat

Diet therapy is the first approach for lowering elevated blood cholesterol (National Cholesterol Education Program, 1990). Dietary recommendations to lower elevated levels of serum cholesterol emphasize a reduced intake of total fat, saturated fat and cholesterol. Since milk fat contains primarily saturated fats and small amounts of cholesterol, both butter and full fat dairy products are usually eliminated in cholesterol reducing diets. The hypercholesterolemic effect of milk fat is attributed not only to its cholesterol content but also to its saturated fatty acid content, especially 12, 14 and 16 carbon chain length saturated fatty acids (lauric acid C 12:0, myristic acid C 14:0, and palmitic C 16:0). Experimental evidence suggests that this hypercholesterolemic effect is greatest for myristic acid, intermediate for palmitic acid and least for lauric acid (Hegsted, *et al*, 1965, Denke and Grundy, 1992, Zock, *et al*, 1994). The effects of skim milk on circulating cholesterol concentrations have been compared with those of whole milk using a crossover design in which subjects consumed a diet

consistent with American Heart Association recommendations (Steinmetz, *et al*, 1994). Each subject consumed 660 ml milk/d as part of the 2 experimental diets for 6 wk, each separated by 10–16 wk. Blood samples were taken at baseline, 3 wk, and 6 wk for analysis of total cholesterol, LDL-cholesterol, HDL-cholesterol, and triacylglycerol concentrations. A significant decrease in total cholesterol was observed in the skim milk group at 6 wk as compared with the whole milk group. Total cholesterol and LDL-cholesterol concentrations decreased by 0.40 and 0.19 mmol/L, respectively, with skim milk consumption after 6 wk compared with baseline. Another study comparing the effects of whole milk and skim milk on serum total cholesterol levels within isoenergetic diets demonstrated that whole milk resulted in a 7-13% elevation in total cholesterol (Roberts, *et al.*, 1982).

2.3 Does Vitamin E Alleviate the Risk of Dietary Fat?

Several prospective studies suggest an inverse association between dietary vitamin E intakes or plasma α–tocopherol concentrations and coronary heart disease. Large scale prospective studies involving 40, 000 men (4 yr. follow-up) (Rimm, *et al.*, 1993) 80,000 women (8 yr. follow-up) (Stampfer, *et al.*, 1993) revealed that large doses of vitamin E supplements (>100 IU/d) were associated with a significantly decreased risks of coronary heart disease. The Nurses' Health Study (NHS) found that the reduction in relative risk of coronary heart disease was only apparent when middle-aged women who had consumed

supplemental vitamin E intake for more than 2 yr. (Stampfer, *et al.*, 1993). Although these data suggest that high plasma α–tocopherol concentrations resulting from high intakes of supplemental and dietary vitamin E are associated with a reduced risk of cardiovascular disease, they do not prove a causal relationship. Also, noted in the NHS, women who took supplements exhibited greater use of post-menopausal hormones, were vigorous exercisers, and likely to be nonsmokers.

In the above studies, the relationship between vitamin E and coronary heart disease risk was seen only in persons taking high dose vitamin E supplements, but in other studies, the association has only been observed for dietary vitamin E. The Iowa Women's Health Study found a relationship between dietary vitamin E intakes and the risk of death from coronary heart disease in a population of 34,486 post-menopausal women.(Kushi, *et al.*, 1996). In the study population as whole, intakes of vitamin A, vitamin C, and supplementary vitamin E did not appear to be associated with the risk of death from coronary disease. Among women who did not take vitamin supplements, dietary vitamin E intake showed a significant inverse association with coronary heart disease risk. Foods associated with reduced risk included margarine, nuts and seeds, and mayonnaise and creamy salad dressings.

There have been three major intervention studies using vitamin E to test its effects on coronary heart disease risk. The Cambridge Heart Antioxidant

Study (CHAOS), a double-blind placebo controlled study looked at the effect of vitamin E (α-tocopherol) in the secondary prevention of coronary heart disease. 2002 patients with angiographically confirmed coronary artery disease were prestratified by planned therapy and by risk factors for coronary disease and then randomly assigned to a pharmacological dose of vitamin E or placebo (soybean oil). The study demonstrated a 76% reduction in second non-fatal heart attacks in patients, who previously had one heart attack (Stephens, *et al.*, 1996).

The GISSI-Prevenzione Trail (Investigators, 1999) was a study of more than 11,000 individuals with a recent myocardial infarction (MI)) who were randomly assigned to fish oil, vitamin E, or both, in 2 x 2 factorial design study. Supplements included n-3 polyunsaturated fatty acids (PUFA) 1 g daily, vitamin E 300 mg, the combination of the above and neither. The study was open label and was carried out for an average of 3.5 years. The results were favorable for the fish oil supplement and neutral for vitamin E. However, this study had some shortcomings. Patients in this study were primarily on Mediterranean diet, which could have confounded benefit of vitamin E due to presence of other antioxidants in the diet. Also, a four-way analysis was carried out which suggested that α-tocopherol supplementation resulted in statistically significant 20% reduction in cardiovascular deaths, a 23% reduction in cardiac death, a 25% reduction in coronary death, and a 35% reduction in sudden death (Jialal, *et al.*, 1999). In the accompanying editorial (Brown, 1999) pointed out that larger

beneficial effects seen in the subjects in the CHAOS study may have been a result of their genetically susceptibility to vitamin E intervention.

However, the recently released Heart Outcomes Prevention Evaluation (HOPE) Trial of subjects with vascular disease and diabetes also did not show a benefit for vitamin E, although the ACE inhibitor ramipril was shown to be beneficial in reducing cardiovascular risk (Yusuf, *et al.*, 2000). Thus, the role of vitamin E in decreasing coronary heart disease risk remains controversial.

2.4 Vitamin E Structures and Nomenclature

2.4.1 Naturally Occurring Vitamin E Forms

Vitamin E is an essential, fat-soluble vitamin that occurs naturally in eight different compounds as shown in Figure 2-1 and Figure 2-2. These compounds fall into two classes: the tocopherols, characterized by a phytyl side chain and the tocotrienols, characterized by an unsaturated side chain. Each class is composed of four homologs (vitamers). Homologs in each class are designated as α, β, γ, and δ (Machlin, 1991). The most abundant and active isomer of vitamin E is α-tocopherol. α-tocopherol is also referred to as (5,7,8-trimethyl tocol).

2.4.2 Synthetic Compared With Natural α-Tocopherol

2.4.2 Synthetic Compared With Natural α-Tocopherol

Synthetic α-tocopherol contains 8 stereoisomers as a result of the three chiral centers in the phytyl tail. According to the National Research Council, natural α-tocopherol should be designated as RRR-α-tocopherol (formerly d-α-tocopherol) and the synthetic compound should be designated as all-rac-α-tocopherol (formerly dl-α-tocopherol) (National Research Council, 1989).

The eight stereoisomeric forms that comprise synthetic vitamin E are RRR, SRR, RRS, SRS, RSS, SSR, RSR and SSS.

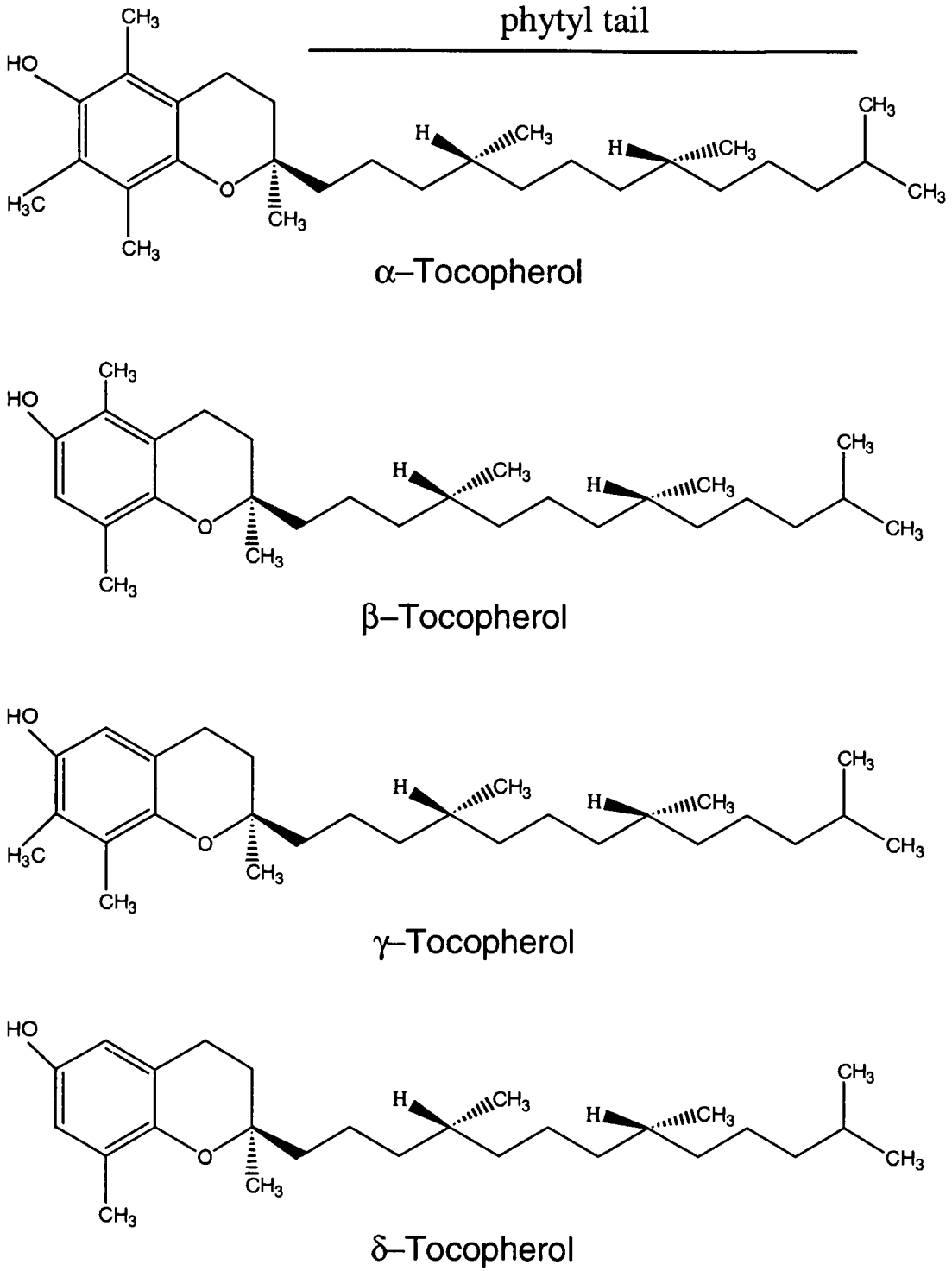


Figure 2-1 Structures of Tocopherols

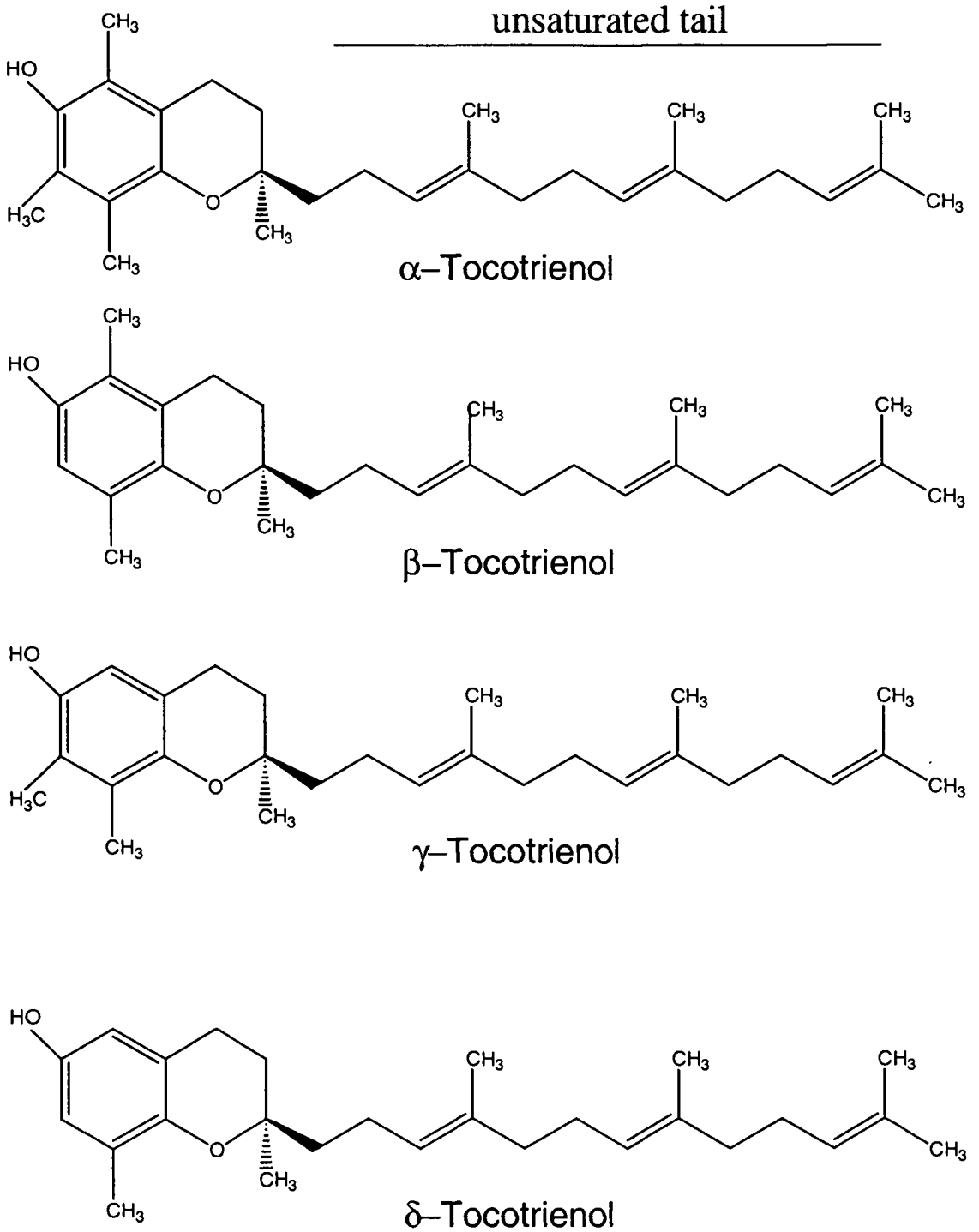


Figure 2-2 Structures of Tocotrienols

2.5 Biological Activity

Biological activity of the eight naturally occurring homologs in rats varies according to the number and the position of the methyl groups on the chroman ring and by the configuration of asymmetric carbons in the side chain. Synthetic and natural α-tocopherol have different biologic activities in rats. The relative biological values for vitamin E analogs are in Table 2-1.

Table 2-1 Relative Biological Values for Vitamin E Analogs

Vitamin E Homolog	Relative Potency IU/mg	Relative Potency Compared to d-alpha
α-tocopherol	1.49	100%
β-tocopherol	0.75	50%
γ-tocopherol	0.15	10%
δ-tocopherol	0.05	3%
α-tocotrienol	0.75	30%
β-tocotrienol	0.08	5%

The biological activity of vitamin E has been determined by using rat fetal resorption assays and applied to humans with no species adjustment. As defined by Machlin, the biologic activity of vitamin E is based on its ability to prevent or reverse specific vitamin E deficiency symptoms in animals (Machlin, 1991).

The biologic activities of different forms of vitamin E are apparently dependent on the function of α-tocopherol transfer protein (α-TTP). This protein binds the vitamin and enhances its transfer between membranes and is found in the liver cytosol of humans (Arita, *et al.*, 1995). α-TTP selectively recognizes α-tocopherol, the most biologically active form of vitamin E. The relative affinities of α-TTP towards various forms of vitamin E, were calculated from the degree of competition with RRR-tocopherol. The degrees of competition were 100% RRR-tocopherol, 38% β-tocopherol, 9% γ-tocopherol, 2% δ-tocopherol, 2% α--tocopherol acetate, 2% α-tocopherol quinone, 11% SRR α-tocopherol, α-tocotrienol 12% and 9% trolox (Hosomi, *et al.*, 1997).

2.6 Vitamin E Functions

2.6.1 Antioxidant

Vitamin E functions as a biological antioxidant. It protects polyunsaturated fatty acids (PUFA) within the phospholipid of the cell membrane and in plasma lipoproteins (Burton and Ingold, 1986) (Niki, 1987, Tappel, 1962). In the absence of vitamin E, a peroxyl radical ($ROO^{\bullet}$) reacts with PUFA to form hydroperoxide and another peroxyl radical causing a chain reaction.

$$ROO^{\bullet} + PUFA \rightarrow ROOH + ROO^{\bullet}$$

Vitamin E inhibits lipid peroxidation by scavenging the lipid peroxyl radicals. The hydroxy (-OH) group of the chromanol group gives up its hydrogen atom to peroxyl radical ($ROO^{\bullet}$) to form hydroperoxide and tocopheroxyl radical (Vit E-$O^{\bullet}$):

$$ROO^{\bullet} + \text{Vit E-OH} \rightarrow ROOH + \text{Vit E-}O^{\bullet}$$

Vitamin E acts as a chain breaking antioxidant. It breaks the chain reaction of lipid peroxidation. However, the tocopheroxyl radical could then act as a radical.

2.6.2 Interaction With Other Antioxidants

The tocopheroxyl radical can be reduced to regenerate tocopherol by interactions with reductants, which serve as hydrogen donors (AH):

$$\text{Vit E-}O^{\bullet} + AH \rightarrow \text{Vit E-OH} + A^{\bullet}$$

Glutathione and ascorbic acid have been shown to regenerate α-tocopherol from the tocopheroxyl radical in chemical oxidant systems (McCay, 1985, Sies, Stahl, *et al.*, 1992, Wefers and Sies, 1988).

2.6.3 Molecular Functions of α-Tocopherol

Protein kinase C (PKC) belongs to family of isoenyzmes that are extremely important in cellular signal transduction. Diacylglycerol (DAG) a product of phospholipase is involved in activation of PKC. PKC is required for

smooth muscle cell proliferation *in vitro* and also *ex vivo*. α-Tocopherol inhibits smooth muscle cell proliferation via inhibition of PKC. Also, protein kinase C is inhibited by physiologic concentrations of α-tocopherol in the cells (Boscoboinik, *et al.*, 1991). However, subsequent studies have been contradictory. α-tocopherol concentrations up to 400 μg/ml had no direct effect on the activity of PKC isoforms α and β_{II} activity (Kunisaki, *et al.*, 1995). The exact mechanism of α-tocopherol mediated inhibition of protein kinase C is still unclear. α-tocopherol, but not β-tocopherol, prevents the phosphorylation of protein kinase Cα (PKCα) and this could be a mechanism by which α-tocopherol decreases PKCα activity (Clement, *et al.*, 1997).

Vitamin E may also play a role in preventing activation of intracellular signal transduction pathway such as nuclear transcription factor κB (NF-κB). Through these mechanisms α-tocopherol may influence cellular functions such as cell proliferation, platelet aggregation, cellular superoxide and cytokine production.

2.7 Absorption and Plasma Transport

Simultaneous intake and digestion of dietary fat facilitates absorption of vitamin E from the intestinal lumen. Bile salts and pancreatic enzymes are essential components for the formation lipid–bile micelles, together with free fatty acids and other fat-soluble vitamins. Absorbed vitamin E is incorporated

into chylomicrons in the enterocyte and is transported through the lymph into circulation. During catabolism of chylomicrons, various forms of vitamin E are transferred to circulating lipoproteins (Bjorneboe A, *et al.*, 1987). This occurs during the metabolism of triglyceride rich lipoproteins. After the hydrolysis of chylomicrons by lipoprotein lipase, excess surface is created and transferred to high-density lipoprotein (HDL). HDL can transfer vitamin E to other lipoproteins and to tissues (Granot, *et al.*, 1988, Traber, *et al.*, 1992).

The liver is the central site of vitamin E regulation (Yoshida, *et al.*, 1992). It exhibits a distinct preference for RRR-α-tocopherol. The hepatic RRR-α-tocopherol is then secreted in very low-density lipoprotein (VLDL) (Traber, *et al.*, 1990). Unlike other fat-soluble vitamins, vitamin E is distributed to the tissues primarily by lipoproteins and does not have a transport protein. Of the vitamin E compounds that have been studied only γ- and α-tocopherols present in foods are retained in human tissue to any extent. Increasing α-tocopherol intake decreases plasma and tissue concentrations of γ-tocopherol (Handelmann, *et al.*, 1994).

2.8 Vitamin E Metabolism and Excretion

α-Tocopheryl quinone is a result of the 2-electron oxidation of α-tocopherol. α-tocopheryl quinone can then be reduced to hydroquinone and subsequently conjugated to glucuronic acid. The glucuronic acid-hydroquinone

conjugate can be either excreted into bile or degraded in the kidneys to α-tocopheronic acid as shown in Figure 2-3 (Drevon, 1991).

Unoxidized vitamin E metabolites found in human urine include both the metabolite of α-tocopherol (2,5,7,8-tetramethyl-2 (2'carboxyethyl)-6-hydroxychroman, α-CEHC) and γ- tocopherol (2,7,8-trimethyl-2-(2'carboxyethyl)-6-hydroxychroman, LLUα) as shown in Figure 2-4 (Schultz, *et al.*, 1995) (Wechter, *et al.*, 1996). The major route of excretion of ingested vitamin E is via the fecal elimination (Machlin, 1991).

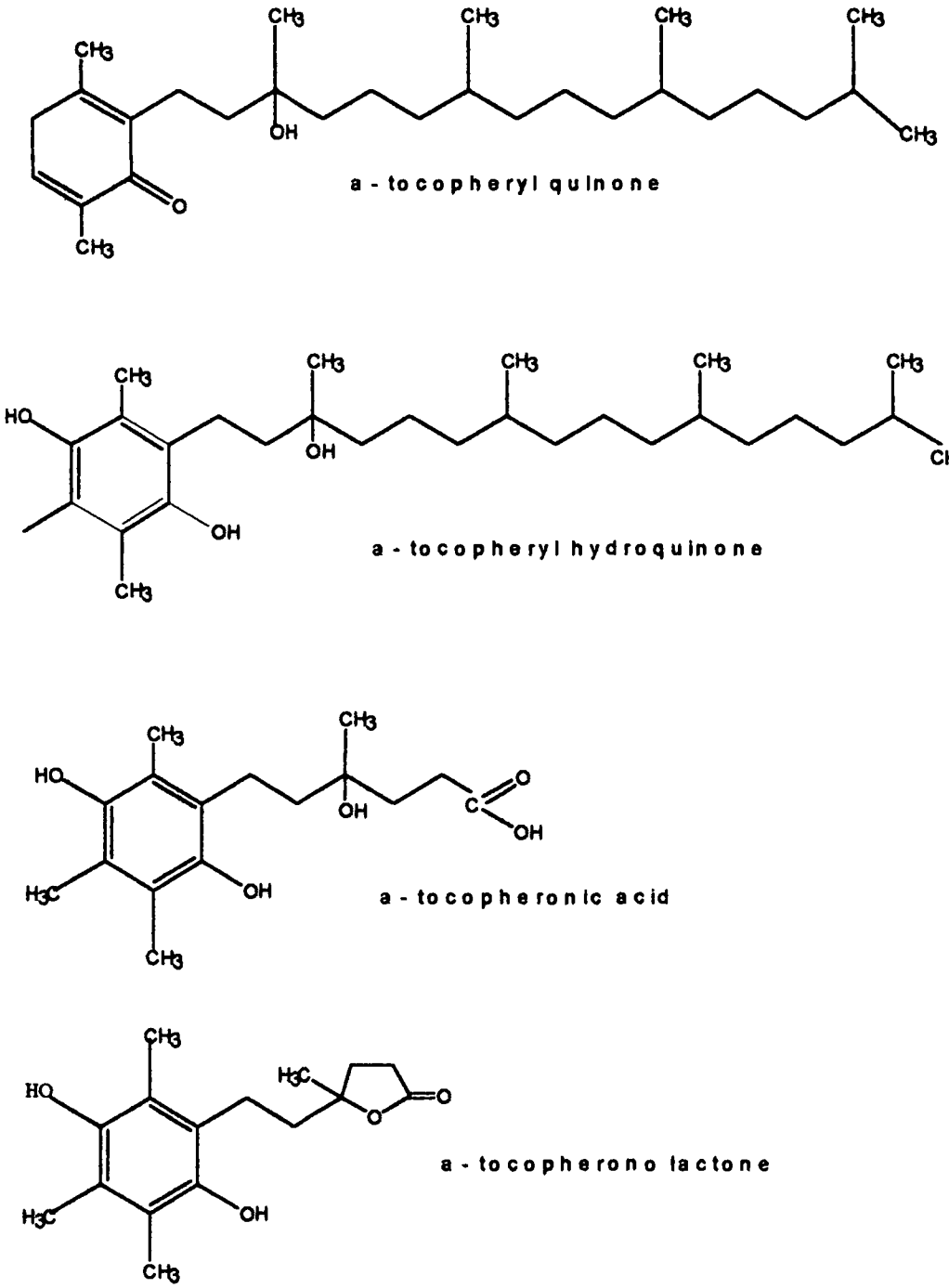
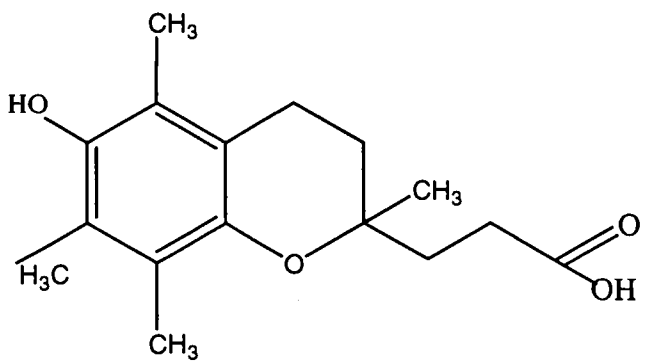
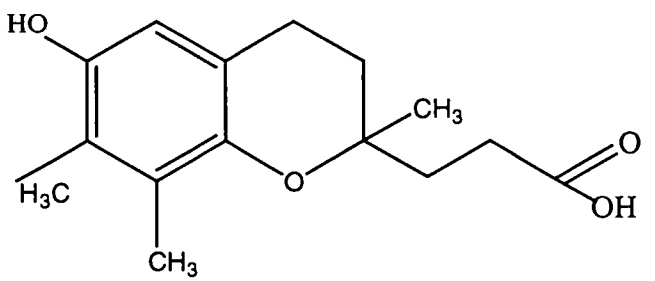


Figure 2-3 Oxidation Products of α-Tocopherol



α–CEHC



γ–CEHC

Figure 2-4 Urinary Metabolites

2.9 Dietary Vitamin E

2.9.1 Recommended Intakes

The current vitamin E Recommended Dietary Allowance (RDA) for both men and women is 15 mg of α-tocopherol (Institute of Medicine, 2000). The 1989 Recommended Dietary Allowance (RDA) for vitamin E was 10 mg for

men and 8 mg for women of RRR-α-tocopherol or RRR-α-TE per day. One α-TE equivalent is defined as the activity of 1 mg of RRR α-tocopherol. To quantify total α-TE in the natural forms of vitamin E from mixed diets, the following conversion factors are used: mg β-tocopherol × 0.5; mg γ-tocopherol × 0.1; and mg tocotrienols × 0.3. In the presence of synthetic all-rac-α-tocopherol, milligrams of the compound present should be multiplied by 0.74 (National Research Council, 1989).

2.9.2 Estimated Intakes from National Surveys

Serum α-tocopherol concentrations in the normal U.S. population were estimated in the Third National Health and Nutrition Examination Survey (NHANES III). The mean α-tocopherol concentration was 26.8 μmol/L with a range of 0.65-232.18 μmol/L. The 25^{th}, 50^{th}, 75^{th} percentiles were 19.6, 24.1 and 30.4 μmols/liter, respectively. The mean α-tocopherol/cholesterol ratio was 5.1. The 25^{th}, 50^{th}, 75^{th} percentiles were 4.1, 4.7 and 5.5, respectively. Approximately, 27 percent of the US population had low serum α-tocopherol (Ford and Sowell, 1999).

According to the National Research Council, the current estimated average daily intake of α-TE's ranged from 7-11 mg. According to the 1985 Continuing Survey of Food Intakes by Individuals (CSFII) data, vitamin E intakes among men 19 to 50 years of age in the U.S. averaged 9.8 mg of α-TEs

(1986). This data was based on a one-day diet recall. The corresponding figures for women 19 to 50 years of age and their children 1 to 5 years of age (collected from over 4 non-consecutive days of dietary records) were 7.1 and 5.5 mg of α-TEs, respectively (1987).

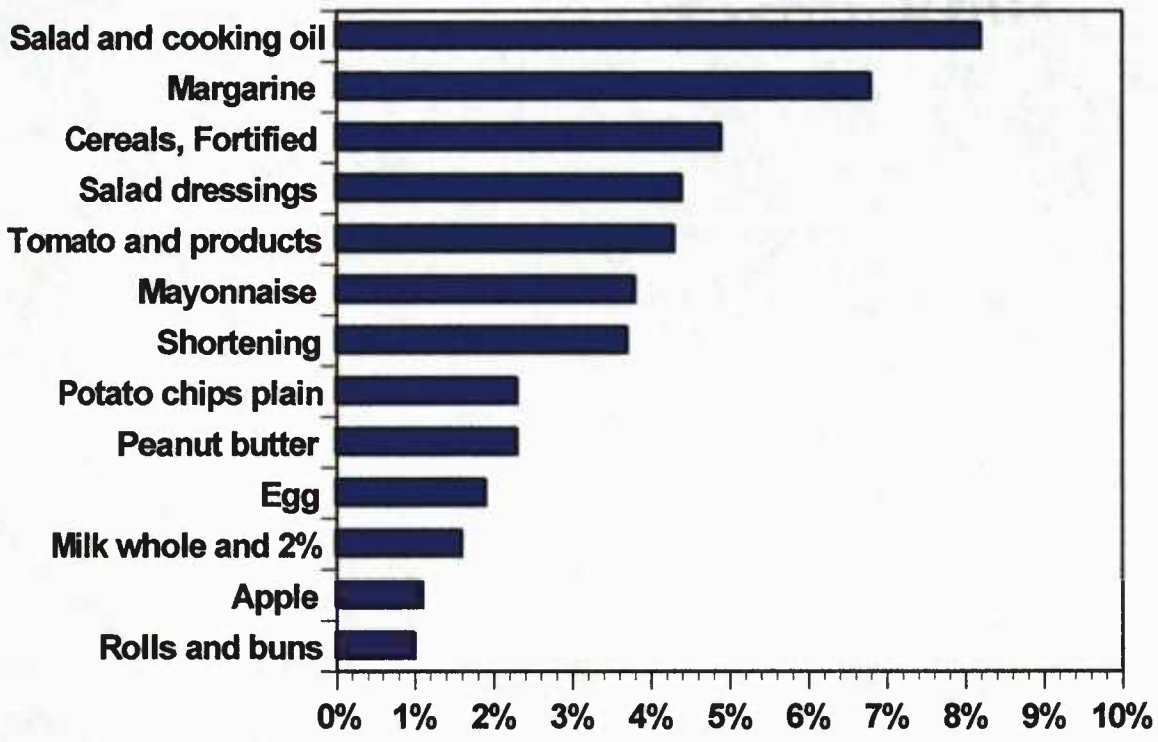


Figure 2-5 Percent of Total Vitamin E from Different Foods

Data from 1987-1988 Nationwide Food Consumption Survey provides a information about vitamin E sources in the U.S. diet (Sheppard, *et al.*, 1993). This report indicated that 12 foods provided approximately 50% of vitamin E in the US diet on an α-TE basis. Salad dressings and margarine contribute to significant amount of vitamin E. Milk, apples and eggs cannot be considered as a good source of vitamin E; however, because large amounts of these foods are consumed, they are significant dietary vitamin E sources. Data from Continuing Survey of Food Intakes by Individuals (CSFII, 1994) (Haytowitz, 1997) provides a more recent compilation of vitamin E in the US diet. Most dietary vitamin E comes from vegetable oil. Only about 46.3% of the sources are shown in Figure 2-5; remaining sources contribute less than 1% each. The latest CSFII (1994-1996) data on antioxidant intakes and smoking status was conducted to identify major food contributors of antioxidants in men and women in different smoking categories (nonsmokers, former smokers and current smokers). Desserts (7-9%) were major sources of total vitamin E in all 3 categories. Other foods contributing to considerable amounts of vitamin E were poultry, eggs, breads, condiments, and salad dressings, fish, and salty snacks. Some differences were observed among the 3 smoking groups; nuts and seeds was listed as one of the major contributors to vitamin E intakes in current and former smokers but not in nonsmokers (Ma, *et al.*, 2000).

2.9.3 Food Vitamin E Contents

Vegetable Oil: α-tocopherol is found in safflower oil, sunflower oil and wheat germ oils whereas corn oil and soybean oil predominantly contain γ-tocopherol as well as some tocotrienols. Cottonseed oil, as well as palm oil, contain both α and γ tocopherol in equal proportion. Also, palm oil contains large amounts of α and γ tocotrienols (Sheppard, *et al.*, 1993). The tocopherol content of margarine is highly variable depending on the oils used in manufacture. Higher values of γ-tocopherol are seen if soybean oil is used.

Apart from vegetable oils, nuts and unprocessed cereals also are good vitamin E sources. Meat, fish, animal fats and most fruit and vegetables have little vitamin E (Bauernfield, 1980).

Dairy Products: α-tocopherol is the primary form of vitamin E in dairy products. There is a seasonal variation in vitamin E content of cow's milk. During periods of fresh grass consumption the α-tocopherol content of cows' milk can rise to 0.12 mg /100 g milk compared to an average content of 0.07 – 0.09 mg/100g (Bauernfield, 1980). Pasteurization and skimming reduces the milk vitamin E content. There is great interest in the non-α-tocopherol forms of vitamin E in milk, but these have not been previously reported.

Table 2-2 Tocopherol Contents of Dairy Foods

Foods	Serving size	α-tocopherol (mg/serving)	α-tocopherol (mg/100 g)
Cream, fluid, light	1 Tbsp	0.02	0.15
Milk whole	1 C	0.24	0.10
Milk 2%	1 C	0.17	0.07
Non fat milk	1 C	0	0
Processed cheese	1 1/2 oz.	0.18	0.40
Cheddar cheese	1 1/2 oz	0.14	0.30

Vitamin E intake estimates from the NFCS 1977-1978 and the CSFII 1989-1991 were compared with estimates from the first two years of the CSFII 1994-96. The percentage of adults drinking whole milk declined progressively for both women and men, while the percentage drinking skim milk drinkers has increased (Enns, *et al.*, 1997). This suggests that vitamin E intakes from milk have also decreased.

2.10 Milk Composition

Fat is the major source of energy in milk. Bovine milk consists of 3-5% total lipid. About 98% or more of lipid is in the form of triglycerides. Sterols account for 0.2-0.5 % (10-20 mg/100 g) (Jensen, *et al.*, 1990)

2.10.1 Fatty Acids

More than 400 different fatty acids have been identified in bovine milk (Jensen, Ferris, *et al*, 1991). About 70% of total fatty acids in milk are saturated which include fatty acids like lauric, myristic, palmitic, and stearic acids. Palmitic acid (16:0) is the most abundant of the saturated fatty acid (20-25 % of total fatty acids). Oleic acid (18:1) is most abundant unsaturated fatty acid. Around 2% of fatty acid are polyunsaturated. Approximately 5% of all unsaturated bonds are in the *trans* position as a result of ruminal biohydrogenation, although the *cis* forms of geometric isomers are commonly seen.

2.10.2 Triglycerides

Triglycerides account for 98 % of milk lipids. The triglyceride molecule is made up of glycerol backbone with fatty acid molecules attached at upper sn-1 position (which generally binds to longer carbon length fatty acids) and middle sn-2 position and bottom sn-3 position (which are mainly shorter carbon length and unsaturated fatty acids). Other classes of lipids include phospholipids, which are mainly associated with the fat globule membrane, and cholesterol (Jensen and Ferris, 1990).

2.10.3 Milk Fat Globule and Membrane

2.10.3 Milk Fat Globule and Membrane

Milk fat exists in milk as small globules and are protected and separated from each other by a membrane called the milk fat globule membrane (MFGM) (Walstra, *et al.*, 1984). The fat globules are formed in the mammary secretory epithelial cell, at move towards the apical cell membrane as they increase in size and are finally extruded into alveolar lumen wherein the globules are enveloped by the globular membrane. The core of globule consists mainly of triglycerides surrounded by MFGM. Approximately 60% milk phospholipids, and 85% milk cholesterol are located within the membrane, which also contains high concentrations of milk enzymes (Jensen and Ferris, 1990)

2.10.4 Changes Caused by Milk Processing

Milk is an oil- in-water emulsion. If raw milk is left to stand, the cream will separate. This is due to differences in densities between the fat and plasma phases of milk. Stabilization of the fat emulsion in milk can be achieved by homogenization. This process involves mechanical treatment of fat globules in milk by passing milk through narrow slit, which results in decrease in average diameter of fat globule and significantly increases the surface area. This process prevents the cream from rising to the top by decreasing the diameter and size distribution of the fat globules (Walstra and Jenness, 1984).

3 REMOVAL OF MILK FAT FROM COW'S MILK DECREASES THE VITAMIN E CONTENTS OF THE RESULTING DAIRY PRODUCTS

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3.1 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 & half, whole milk, reduced-fat milk, low-fat milk and non-fat milk) were obtained from a local dairy on six different occasions. α-tocopherol was the major form of vitamin E (>85%); γ-tocopherols and α-tocotrienol were present to a lesser extent. As the fat content of milk products decreased from 11% to 0.3%, the vitamin E contents decreased. For example, raw milk as compared to non-fat milk had both higher α-tocopherol contents (45.5 ± 4.59 μg/100 g vs. 4.46 ± 0.54; $p \leq 0.0001$) and higher total lipids (3.46 ± 0.49 μg/100 g vs. 0.30 ± 0.07 g/100 g; $p \leq 0.0001$). Vitamin E, cholesterol and total lipids increased as cream was added back to non-fat milk. For every 1 mg cholesterol increase, there was an increase of approximately 4 μg of α-tocopherol. For every 1 g increase in total lipids, the α-tocopherol content increased by 17 μg. These data demonstrate that removal of milk fat remarkably changes the vitamin E content of various milk products.

3.2 Introduction

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 (8). 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 (13, 16).

There have been three major intervention studies using vitamin E to test its effects on coronary heart disease risk. The Cambridge Heart Antioxidant Study (CHAOS), 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 non-fatal heart attacks in patients, who previously had one heart attack (17). The GISSI-Prevenzione trail (5) was a study of more than 11,000 individuals with a recent myocardial infarction (MI) who were randomly assigned to fish oil, vitamin E, or both, in 2 x 2 factorial design study. The results were favorable for the fish oil supplement and neutral for vitamin E. However, the recently released Heart Outcomes Prevention Evaluation (HOPE) Trial of subjects with vascular disease and

diabetes also did not show a benefit for vitamin E, although the ACE inhibitor ramipril was shown to be beneficial in reducing cardiovascular risk (21). 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 fat to benefit health. However, modifying dairy product fat contents either by 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 the 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.

3.3 Methodology

3.3.1 Selection of Analytical Samples

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

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 laboratory on ice and analyzed within 24 hours from the time of collection. Six different batches were analyzed.

3.3.2 Laboratory Analyses

Vitamin E: The distribution of α- and γ tocopherols α- and γ-tocotrienols in raw, whole, 2%, 1%, non-fat milk and half-and-half was determined as described (11). Approximately 0.1-2.5 g of the sample was weighed into a screw top test-tube. The milk vitamin E was extracted following saponification with ethanolic potassium hydroxide (KOH). Briefly, the milk samples were mixed

with 2 ml of 1% ascorbic acid in ethanol. After addition of 0.3 ml of saturated KOH, samples were heated at 70 ° C for 30 min. (The amount of KOH was increased if droplets of triglycerides were seen after initial saponification). After cooling the samples on ice, 25 µL of butylated hydroxy toluene (BHT) and 1 ml of 1 % ascorbic acid in water was added to the samples. These samples were then extracted with 2 ml of hexane. 1.5 ml of upper layer of hexane was aliquoted into conical tubes, evaporated under nitrogen and the residue was resuspended in 100 µL of 1:1 ethanol: methanol. An appropriate aliquot was then injected using the SIL-10A autoinjector with sample cooler into the HPLC system (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, Lafayette, IN). The mobile phase consisted of a mixture of methanol-water (99:1 % v/v) and 0.1%(w/v) lithium perchlorate. The total run time for the assay was approximately 12 minutes. Data was integrated using Shimadzu Class-VP automated software program (Columbia, MD). Authentic α- and γ tocopherols α- and γ-tocotrienols were used as external standards for quantification of vitamin E (Cognis Corporation, La Grange). No γ-tocotrienol was detected in the samples.

Cholesterol: The cholesterol content of milk sample was measured in an aliquot of hexane used for vitamin E analysis using a cholesterol kit from Sigma Diagnostics (St. Louis, MI, Procedure No.352). Briefly, 200μL of hexane was aliquoted into conical tube, evaporated under nitrogen and the residue was resuspended in 200 μL of isopropanol. Samples were incubated at 37° C for 5 minutes after addition of the cholesterol reagent. The amount of cholesterol in the sample was determined spectrophotometrically by measurement of the absorbance of pink/red color at 500 nm.

Total lipids: Total lipids were extracted from milk samples using methanol and chloroform (2). A known amount (1-3.5 g) of the sample was weighed into a screw top test-tube and extracted with a mixture of methanol, chloroform and water in the ratio of 2:1:0.8 for one hour at room temperature. A known amount of chloroform-containing lipid was transferred then to a pre-weighed screw-top tube and chloroform was evaporated under nitrogen in 50°C water bath, then completely dried overnight in 70° C oven. The total lipids were calculated from the weight of the aliquot and expressed as weight in grams per 100 g milk.

3.3.3 Statistical Analysis

Data were analyzed using a one-way analysis of variance model using StatView (SAS Institute Inc.). Differences between means were considered

statistically significant if ($p<0.05$). If significant differences were found, then Fishers post hoc tests were used for making pair-wise comparisons. Here differences between means were considered statistically significant if $p<0.01$.

3.4 Results

3.4.1 Milk Vitamin E, Cholesterol and Total Lipid Contents

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 non-fat milk were analyzed and results are shown in **Table 1**.

The total lipids content of milk increased from 0.30 ± 0.07 in non-fat to 11.6 ± 0.53 in half & half with the addition of increasing amounts of cream to non-fat 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-tocopherol and α-tocotrienol contents apparently varied in the different products depending on the fat content. Half-and-half contained the most fat and had the highest amount of α–tocopherol (193 ± 1.66 μg/100 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 ± 2.22 μg/100 g) was higher than reduced-fat milk (26.4 ± 3.58 μg/100 g), low-fat milk (14.2 ± 1.73 μg/100 g) and non-fat milk (4.5 ± 0.5 μg/100 g).

Table 3-1 Total Lipid, Cholesterol and Vitamin E Concentrations in Milk

	Total Lipids (g/100 g)[a]	Cholesterol (mg/100 g)[b]	α-tocopherol (μg/100 g)[a]	γ-tocopherol (μg/100 g)	α-tocotrienol (μg/100 g)[d]
Raw	3.46 ± 0.49	16.0 ± 1.5	45.5 ± 4.6	1.92 ± 0.44[c]	1.96 ± 0.51[c]
Whole	3.40 ± 0.11	14.3 ± 1.4	43.9 ± 2.2	2.06 ± 0.38[d]	1.76 ± 1.76[d]
Reduced-fat	2.12 ± 0.15	7.06 ± 0.5	26.4 ± 3.9	1.34 ± 0.58[e]	1.09 ± 0.31[e]
Low-fat	1.16 ± 0.15	3.56 ± 0.4	14.2 ± 1.7	0.96 ± 0.31[f]	0.59 ± 0.09[f]
Non-fat	0.30 ± 0.07	1.74 ± 0.3	4.5 ± 0.5	0.62 ± 0.11[g]	0.14 ± 0.03[g]
H & H	11.6 ± 0.53	47.7 ± 1	193 ± 1.7	12.1 ± 2.17[c,d,e,f,g]	7.00 ± 2.74[c,d,e,f,g]

[a]For 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).

[b]For 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

For γ-tocopherol concentrations, pairwise comparisons between means were significantly different ($P<0.0001$) only between [c]half & half and [c]raw, [d]whole, [e]reduced-fat, [f]low-fat, and [g]nonfat milks.

For α-tocotrienol concentrations, pairwise comparisons between means were significantly different ($P<0.0001$) only between [c,d,e,f,g]half & half and [c]raw, [d]whole, [e]reduced-fat, [f]low-fat, and [g]non-fat milks.

3.4.2 Cholesterol and Vitamin E Concentrations Relative to Total Lipids

Table 3-2 provides the vitamin E and cholesterol content of milk relative to the total lipid content for all the milk samples. The α-tocopherol per total lipids in non-fat milk was higher than in raw milk. Similarly, cholesterol per total lipids was higher in non-fat milk (6.13 ± 1.86 μg/g) as compared with raw milk (4.64 ± 0.39 μg/g, $p<0.0003$). The α-tocopherol and the total lipid contents in different dairy products were correlated (**Figure 3-1**). For every 1 g increase in total lipid content, the α-tocopherol content increased by 17 μg.

Table 3-2 Cholesterol and Vitamin E Contents of Milk Relative to Total Lipids

	Cholesterol per total lipids (mg/g)	α-tocopherol per total lipids (μg/g)	γ-tocopherol per total lipids (μg/g)	α-tocotrienol per total lipids (μg/g)
Raw	4.64 ± 0.39[b,d]	13.2 ± 1.1	0.57 ± 0.16[b]	0.57 ± 0.11
Whole	4.22 ± 0.44[a]	12.9 ± 0.8	0.60 ± 0.11[a]	0.52 ± 0.06
Reduced-fat	3.34 ± 0.18[c,d]	12.4 ± 1.2	0.63 ± 0.26	0.51 ± 0.14
Low-fat	3.08 ± 0.25	12.3 ± 1.2[a]	0.83 ± 0.25	0.51 ± 0.09
Non-fat	6.13 ± 1.86[a,b]	15.7 ± 3.9	2.22 ± 0.80[a,b]	0.50 ± 0.17
H & H	4.11 ± 0.12	16.7 ± 0.6[a]	1.04 ± 0.19	0.60 ± 0.24

For cholesterol per total lipids, pairwise comparisons between means were significantly different between [a,b] non-fat and [a] whole or [b] raw milks ($P<0.0001$), or between [c,d] reduced-fat and [c] non-fat or [d] raw milks ($P<0.01$).

For α-tocopherol per total lipids, pairwise comparisons between means were significantly different between [a] low-fat milk and [a] half and half ($p<0.001$).

For γ-tocopherol per total lipids, pairwise comparisons between means were significantly different between [a,b] non-fat milk and [a] whole or [b] raw milks ($P<0.0001$).

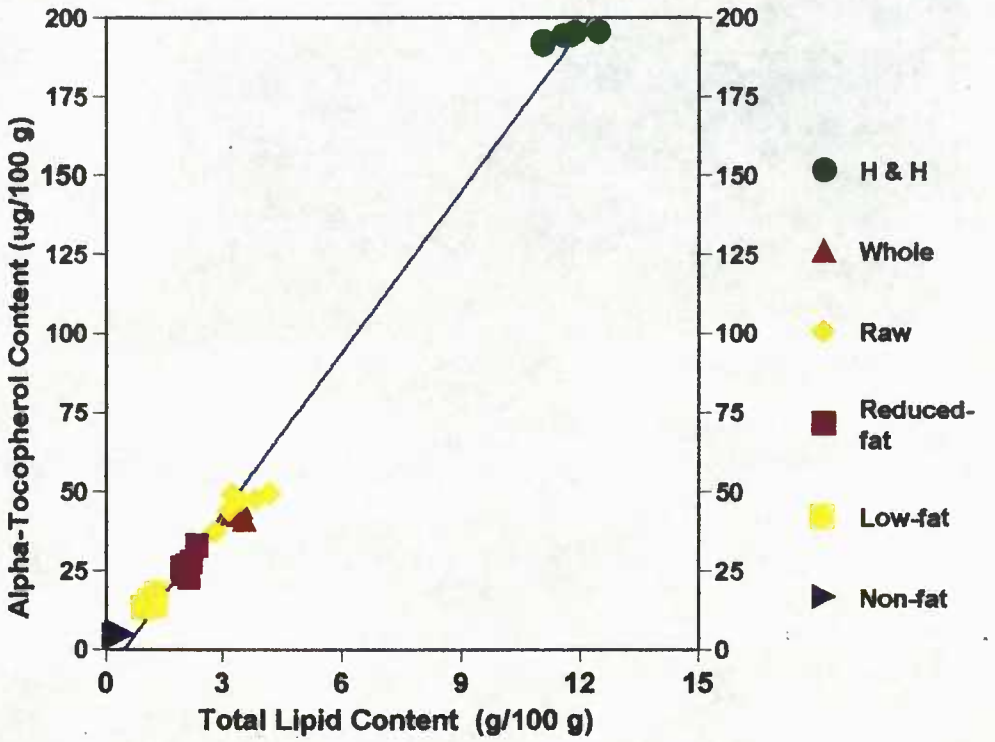

Figure 3-1 Relationship between α-Tocopherol and Total Lipid Content in Milk

3.4.3 Vitamin E content Relative to Cholesterol Content

Table 3-3 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 μg/mg) as compared with non-fat milk (2.6 ± 0.3 μg/mg, $p \leq 0.0001$). Interestingly, the γ-tocopherol per cholesterol content in non-fat milk was higher than that in raw milk ($p \leq 0.0001$). The α-tocopherol and the cholesterol contents in the different dairy products were also correlated **(Figure 3-2)**. For every 1 mg of cholesterol there was an increase of approximately 4 μg of α-tocopherol.

Table 3-3 Vitamin E Content of Milk Relative to Cholesterol Content

	α-tocopherol per cholesterol (μg/mg)	γ-tocopherol per cholesterol (μg/mg)	α-tocotrienol per cholesterol (μg/mg)
Raw	2.8 ± 0.1[a]	0.12 ± 0.03[a,e]	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]
Non-fat	2.6 ± 0.3[c,d]	0.37 ± 0.10[c,d]	0.08 ± 0.01[a,b,c]
H & H	4.0 ± 0.1[a,b,c,d,c]	0.25 ± 0.05[d,c]	0.15 ± 0.06[c]

Pairwise comparison for α-tocopherol per cholesterol were statistically significant (P<0.0001) between [a, b, c] half & half and [a]raw or [b]whole milks and [c]non-fat, except for reduced-fat and low-fat milk {p<0.01); between [d]nonfat and [e]whole milk (p<0.0006).

Pairwise comparisons for γ-tocopherol per cholesterol were statistically significant (P<0.0001) between [a,b]low-fat and [a]raw (p<0.001), and [b]whole milk (p<0.004); between [c]nonfat and [d]reduced-fat (p<0.0015) and [e]half & half (0.004); between [e]half and half and [a]raw milk (p<0.01).

For α-tocotrienol significant differences were observed between [ab]non-fat and [a]low-fat (p<0.01), and [b]reduced-fat milk; between [c]non-fat and [d]half-and-half (P<0.005).

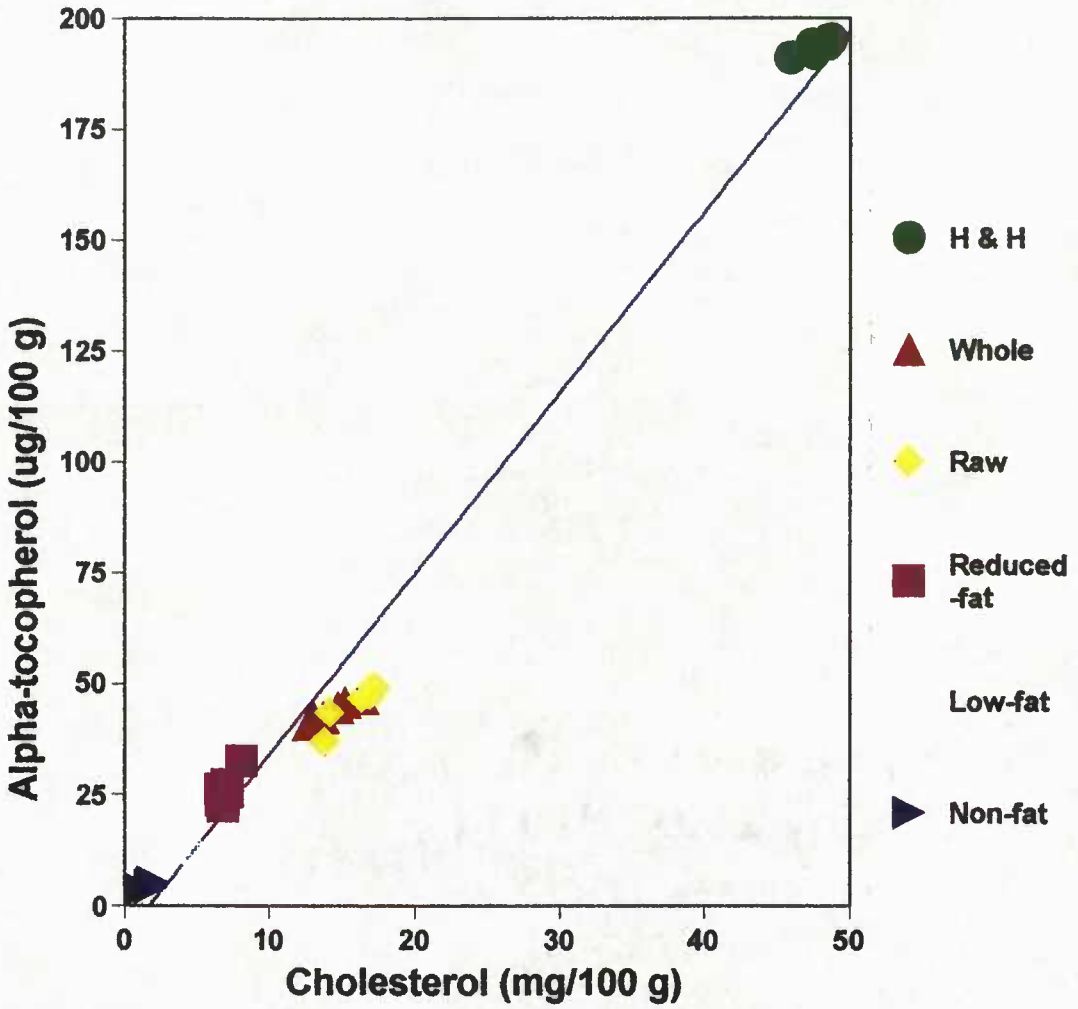

Figure 3-2 Relationship between α-Tocopherol and the Cholesterol Content in Milk

Figure 3-3 shows the distribution of different forms of vitamin E in each type of milk. 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%.

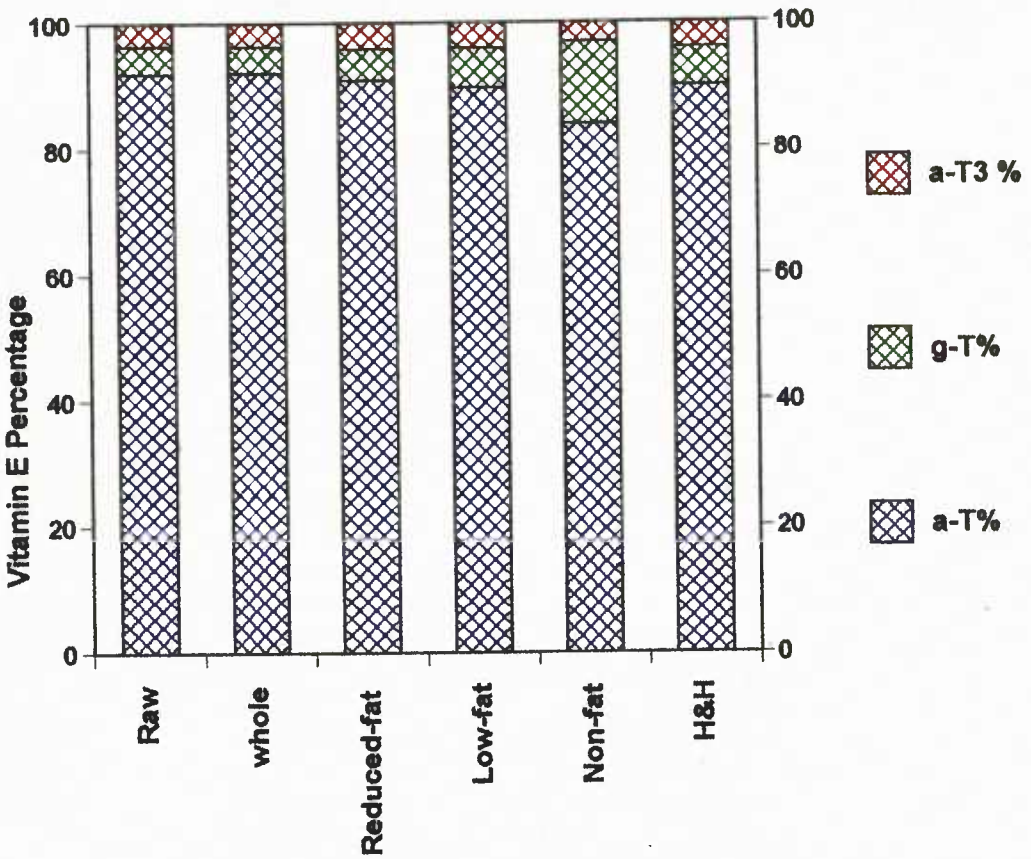

Figure 3-3 Vitamin E Percentages

3.5 Discussion

The milk products examined in this study contained typical lipid levels. The fat contents ranged from 11.6 percent total lipid for half-and–half to 0.3 percent for non-fat 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 percent total lipids) as previously reported (6).

Cholesterol is the major form of sterol found in dairy products (7) and it, like vitamin E, varied with the total lipid contents of the dairy products. In our study, the cholesterol contents for 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 non-fat 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) (12).

α-Tocopherol was the major vitamin E form found in this study of dairy products of varying fat contents; γ-tocopherol and α-tocotrienol were found to lesser extent, while γ-tocotrienol was not detected. As the fat contents of the dairy products decreased from (11 percent to 0.3 percent), the vitamin E content also decreased. The α-tocopherol content of whole milk reported in this study

was 44 μg/100g. Previously, whole milk (3.3 percent) was reported to contain 40 μg/100g of α-tocopherol (4, 9).

The unique finding in our study was that dairy products contain α-tocotrienol. This particularly piqued our interest because of the potent antioxidant properties of α-tocotrienol. Although α-tocotrienol has just one-third the biologically activity of α-tocopherol in rats (20) it has equal (18) or higher antioxidant activity than α-tocopherol (7, 14). The α-tocotrienol contents of dairy products ranged from 1.76 μg/100 g for whole milk to 0.14 μg/100 g for non-fat milk (Figure 3-3, 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 (11). Nonetheless, γ-tocotrienol, a predominant vitamin E form in palm oil (15) was not found in milk. Alternatively, the presence of α-tocotrienol 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 increased α-tocotrienol content (1). Our milk samples were all obtained in late spring from the neighborhood dairy where cows were allowed to eat grass.

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. It is clear that increased amounts of fat in 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 non-fat milk (Table 3-2). A possible explanation for this observation could be the disruption of milk fat globule during the centrifugation procedure, to isolate cream, thereby contributing to residual fat globule membranes disproportionately rich in cholesterol, which accumulates in non-fat milk(19). The cholesterol-adjusted α-tocopherol content was found to be lowest in non-fat milk (Table 3-3), suggesting association of α-tocopherol with the fat droplets rather than the membranes. 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 4-1 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 (15). A single serving of almonds nearly provides the RDA for vitamin E (10), 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 ten times lower fat content than almonds, it provides 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 good source of vitamin E, it is the most commonly consumed dairy product and if large enough amounts of milk products are they become a significant source of vitamin E for some individuals. However, according to 1994-1995 the CSFII data, non-fat milk consumption is increasing while whole milk intake is decreasing (3). Because the vitamin E content of non-fat milk is $1/10^{th}$ 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.

Table 4-2 Comparison among Milk Products, Almonds and Frozen Spinach

	Calories (kcal)	Total Fat (g)	α– Tocopherol (mg)	γ– Tocopherol (mg)	α– Tocotrienol (mg)
Whole milk *(1 quart)*	599	33	0.42	0.019	0.017
Reduced-fat milk *(1 quart)*	485	19	0.25	0.013	0.010
Low-fat milk *(1 quart)*	409	10	0.13	0.009	0.006
Non-fat milk *(1 quart)*	342	2	0.04	0.006	0.001
Spinach frozen *(1 cup)*	54	1.5	3.42	-	-
Almonds *(2 tbsp)*	166	15	12	0.51	0.56

In conclusion, this study indicates that vitamin E, especially α-tocopherol, γ-tocopherol and α-tocotrienol, 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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4 CONCLUSION

The present study was conducted to assess the vitamin E contents of milk with varying levels of fat with special emphasis on determination of various forms of vitamin E.

According to this study the major form of vitamin E found in different types of milk was α-tocopherol. γ-tocopherols and α-tocotrienol were found to a lesser extent in various milks. It was observed that the vitamin E contents varied with the total lipid contents. Similar to the vitamin E contents, the cholesterol contents also varied with the total lipid contents with the highest concentrations in those products with the highest fat contents. Therefore, the different forms of vitamin E were normalized to cholesterol and total lipid contents. The total lipids-adjusted cholesterol was highest in non-fat milk. The cholesterol-adjusted α-tocopherol content was found to be lowest in non-fat milk. Routine milk production involves separation of cream from raw milk and later adding the cream back to nonfat milk in varying amounts depending on the dairy product. Statistically significant differences were observed 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.

In conclusion, vitamin E was present in all the dairy products. Although, milk cannot be considered as a good source of vitamin E, it is the most commonly consumed form of the dairy product and the larger amounts of these foods consumed makes them a significant source of vitamin E.

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APPENDICES

Appendix 1: Total Lipid and Cholesterol Contents of Various Milk Products

Total lipids (gm/100g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	11.7	3.25	4.20	2.14	1.00	0.27
Batch 2	12.4	3.41	3.48	2.19	1.22	0.20
Batch 3	11.5	3.39	3.84	1.97	1.29	0.34
Batch 4	11.0	3.48	2.83	2.35	1.33	0.40
Batch 5	11.8	3.34	3.16	1.96	1.15	0.26
Batch 6	11.0	3.55	3.26	2.09	0.97	0.31
Mean	**11.6**	**3.40**	**3.46**	**2.12**	**1.16**	**0.30**
Std	**0.53**	**0.11**	**0.49**	**0.15**	**0.15**	**0.07**

Cholesterol (mg/100 g)

	H&H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	48.4	13.0	17.3	6.79	3.14	1.16
Batch 2	48.7	16.5	16.4	7.08	3.34	1.81
Batch 3	47.4	13.8	17.0	6.67	3.69	1.80
Batch 4	45.9	14.7	13.8	8.11	4.14	1.77
Batch 5	48.5	15.3	14.2	7.10	3.95	1.94
Batch 6	47.5	12.7	17.0	6.62	3.08	1.95
Mean	**47.7**	**14.3**	**16.0**	**7.06**	**3.56**	**1.74**
Std	**1.06**	**1.44**	**1.54**	**0.55**	**0.44**	**0.29**

Appendix 2: Vitamin E Content of Various Milk Products

α-Tocopherol (μg/100 g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	194	42.9	49.3	22.5	12.9	3.69
Batch 2	195	46.7	46.8	27.4	13.4	4.11
Batch 3	194	42.4	47.4	23.7	13.6	4.55
Batch 4	191	44.8	37.1	32.7	17.4	4.38
Batch 5	195	45.9	43.5	25.7	15.0	5.26
Batch 6	192	40.9	48.8	26.3	13.0	4.77
Mean	**193**	**43.9**	**45.5**	**26.4**	**14.2**	**4.46**
Std	**1.66**	**2.22**	**4.59**	**3.58**	**1.73**	**0.54**

γ-Tocopherol (μg/100 g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	12.1	2.27	1.41	0.80	0.61	0.61
Batch 2	14.8	2.47	2.69	2.14	1.37	0.75
Batch 3	10.1	1.84	1.84	1.10	0.85	0.51
Batch 4	12.5	1.83	1.91	1.26	1.17	0.71
Batch 5	9.17	1.53	1.60	0.80	0.65	0.49
Batch 6	13.9	2.41	2.08	1.95	1.09	0.66
Mean	**12.1**	**2.06**	**1.92**	**1.34**	**0.96**	**0.62**
Std	**2.17**	**0.38**	**0.44**	**0.58**	**0.31**	**0.11**

α-Tocotrienol (μg/100 g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	9.30	1.92	2.03	0.64	0.42	0.10
Batch 2	7.96	1.81	1.52	1.27	0.61	0.15
Batch 3	1.73	1.63	2.91	1.15	0.65	0.18
Batch 4	8.09	1.78	1.66	1.27	0.61	0.15
Batch 5	6.46	1.44	1.63	0.78	0.58	0.16
Batch 6	8.46	1.97	2.03	1.42	0.65	0.11
Mean	**7.00**	**1.76**	**1.96**	**1.09**	**0.59**	**0.14**
Std	**2.74**	**0.20**	**0.51**	**0.31**	**0.09**	**0.03**

Appendix 3: Cholesterol and Vitamin E per Total lipids in Various Milk Products

Cholesterol per total lipid (mg/g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	4.13	4.01	4.12	3.17	3.14	4.30
Batch 2	3.91	4.84	4.72	3.23	2.74	9.03
Batch 3	4.09	4.06	4.43	3.38	2.86	5.30
Batch 4	4.15	4.24	4.89	3.45	3.11	4.42
Batch 5	4.09	4.57	4.50	3.62	3.43	7.47
Batch 6	4.29	3.59	5.23	3.17	3.18	6.28
Mean	**4.11**	**4.22**	**4.64**	**3.34**	**3.08**	**6.13**
Std	**0.12**	**0.44**	**0.39**	**0.18**	**0.25**	**1.86**

α-Tocopherol per total lipid (μg/g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	16.5	13.2	11.7	10.5	12.9	13.7
Batch 2	15.7	13.7	13.5	12.5	11.0	20.6
Batch 3	16.8	12.5	12.3	12.0	10.6	13.4
Batch 4	17.3	12.9	13.1	13.9	13.1	11.0
Batch 5	16.4	13.7	13.8	13.1	13.1	20.2
Batch 6	17.4	11.5	15.0	12.6	13.4	15.4
Mean	**16.7**	**12.9**	**13.2**	**12.4**	**12.3**	**15.7**
Std	**0.62**	**0.83**	**1.13**	**1.15**	**1.22**	**3.90**

γ-Tocopherol per total lipid (μg/g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	1.03	0.70	0.34	0.38	0.61	2.28
Batch 2	1.19	0.72	0.77	0.98	1.13	3.76
Batch 3	0.87	0.54	0.48	0.56	0.66	1.49
Batch 4	1.13	0.53	0.67	0.53	0.88	1.78
Batch 5	0.77	0.46	0.51	0.41	0.56	1.88
Batch 6	1.25	0.68	0.64	0.93	1.12	2.14
Mean	**1.04**	**0.60**	**0.57**	**0.63**	**0.83**	**2.22**
Std	**0.19**	**0.11**	**0.16**	**0.26**	**0.25**	**0.80**

α-Tocotrienol per total lipid (μg/g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	0.79	0.59	0.48	0.30	0.42	0.36
Batch 2	0.64	0.53	0.44	0.58	0.50	0.75
Batch 3	0.15	0.48	0.76	0.58	0.50	0.54
Batch 4	0.73	0.51	0.59	0.54	0.46	0.38
Batch 5	0.54	0.43	0.52	0.40	0.51	0.63
Batch 6	0.76	0.55	0.62	0.68	0.67	0.36
Mean	**0.60**	**0.52**	**0.57**	**0.51**	**0.51**	**0.50**
Std	**0.24**	**0.06**	**0.11**	**0.14**	**0.09**	**0.17**

Appendix 4: Vitamin E per Cholesterol in Various Milk Products

α-Tocopherol per cholesterol (μg/g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	4.00	3.29	2.85	3.31	4.12	3.18
Batch 2	4.00	2.83	2.85	3.87	4.02	2.27
Batch 3	4.09	3.08	2.79	3.55	3.69	2.52
Batch 4	4.16	3.04	2.68	4.03	4.20	2.48
Batch 5	4.02	3.01	3.06	3.61	3.80	2.71
Batch 6	4.04	3.21	2.86	3.97	4.21	2.45
Mean	**4.05**	**3.08**	**2.85**	**3.72**	**4.01**	**2.60**
Std	**0.06**	**0.16**	**0.12**	**0.28**	**0.22**	**0.31**

γ-Tocopherol per cholesterol (μg/g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	0.25	0.17	0.08	0.12	0.19	0.53
Batch 2	0.30	0.15	0.16	0.30	0.41	0.42
Batch 3	0.21	0.13	0.11	0.17	0.23	0.28
Batch 4	0.27	0.12	0.14	0.15	0.28	0.40
Batch 5	0.19	0.10	0.11	0.11	0.16	0.25
Batch 6	0.29	0.19	0.12	0.30	0.35	0.34
Mean	**0.25**	**0.15**	**0.12**	**0.19**	**0.27**	**0.37**
Std	**0.05**	**0.03**	**0.03**	**0.09**	**0.10**	**0.10**

α-Tocotrienol per cholesterol (μg/g)

	H & H	Whole	Raw	2 percent	1 percent	Non-fat
Batch 1	0.19	0.15	0.12	0.09	0.13	0.08
Batch 2	0.16	0.11	0.09	0.18	0.18	0.08
Batch 3	0.04	0.12	0.17	0.17	0.18	0.10
Batch 4	0.18	0.12	0.12	0.16	0.15	0.09
Batch 5	0.13	0.09	0.11	0.11	0.15	0.08
Batch 6	0.18	0.15	0.12	0.22	0.21	0.06
Mean	**0.15**	**0.12**	**0.12**	**0.15**	**0.17**	**0.08**
Std	**0.06**	**0.02**	**0.03**	**0.05**	**0.03**	**0.01**