1998

The Correlation Between the Dietary Intake of the Fat-soluble, Antioxidant Vitamins Alphatocopherol and Retinol and Their Fasting Plasma Concentrations in Postmenopausal Women

Michelle C. Ethun
College of Saint Benedict/Saint John's University

Follow this and additional works at: http://digitalcommons.csbsju.edu/honors_theses

Part of the Nutrition Commons

Recommended Citation
http://digitalcommons.csbsju.edu/honors_theses/669

Available by permission of the author. Reproduction or retransmission of this material in any form is prohibited without expressed written permission of the author.
THE CORRELATION BETWEEN THE DIETARY INTAKE OF THE FAT-SOLUBLE, ANTIOXIDANT VITAMINS ALPHA-TOCOPHEROL AND RETINOL AND THEIR FASTING PLASMA CONCENTRATIONS IN POSTMENOPAUSAL WOMEN

A THESIS

College of St. Benedict / St. John’s University

In Partial Fulfillment

of the Requirements for the Distinction

and the Degree Bachelor of Arts

In the Department of Nutrition

by

Michelle Ethun

May, 1998
Introduction

CHD is the leading cause of death in older women with 500,000 dying annually (1-3). Mortality due to this disease is now greater in women than in men (1). To date very little research has been done on women. Women were excluded from clinical trials because it was assumed that men and women of all ages have similar risk factors which contribute to coronary heart disease (CHD) in a similar manner (1, 4,5). Other reasons for excluding women from research were the possible risks to fetuses, the complications of fluctuating hormone levels associated with menstrual cycles, and the cost and sample size required for sex specific research questions (4,5). However, the initial studies on women reveal a different relative risk profile for CHD compared to men which supports their need to be studied further (4,5).

The incidence of CHD increases significantly in women after menopause (6). The decrease in estrogen levels associated with the loss of ovarian function contribute to a reduced insulin sensitivity and an unfavorable change in lipoprotein profile (2,3,6,7). There is an increase in low density lipoproteins (LDL), total cholesterol, and triglycerides and there may be a decrease in high density lipoproteins (HDL) (2,3,6,7). Also associated with the increased incidence of CHD is the cessation of menses which results in an increase in serum ferritin levels (8). Serum ferritin is a reflection of body iron stores and iron is a known catalyst for the production of free radicals which can initiate LDL oxidation and accelerate atherogenesis (9,10). When postmenopausal women are compared to pre-menopausal women they have more LDL in an oxidized form (7).

Antioxidant vitamin intake and their plasma concentrations are important in the prevention of CHD. Recent epidemiological studies indicate that 1) an increase in antioxidant vitamin intake is associated with a decrease in the incidence of CHD in both men and women and 2) an increase in the plasma concentrations of antioxidant vitamins, particularly alphatocopherol, is associated with a decreased susceptibility of LDL to oxidative modification (11). When LDLs are oxidatively modified they become much more atherogenic. Oxidized LDLs are recognized and taken up by the scavenger receptor which operates independently of the classical LDL receptor. The scavenger receptor is not regulated and takes up modified LDL at a rate 3-10 times that of the classical receptor allowing for substantial cholesterol accumulation and greatly
enhancing the progression of atherosclerosis (12). Antioxidants scavenge free radicals which protects the LDL from initial oxidation and terminates oxidation once it has started.

For antioxidants to be useful in preventing CHD, it is necessary that they are present in the plasma; therefore, the best measure of protection is the plasma concentration. However, taking blood samples and performing plasma analysis is impractical with a large number of people on a regular basis. If plasma levels can be estimated from diet records then blood tests would not be necessary and diet records could be used to screen a larger number of people. This study looked at how well the intake of the fat-soluble, antioxidant vitamins alpha-tocopherol and retinol correlate to their fasting plasma concentrations in a group of postmenopausal women. If dietary intake can accurately predict plasma concentrations then diet records could serve as a screening tool which could target a population that has largely been ignored and identify those needing appropriate nutrition intervention. This could have significant implications for the prevention and treatment of CHD in postmenopausal women. We could also screen younger patients with risk factors (i.e. family history) and implement preventive measures before CHD develops and decrease the likelihood of it occurring.

Literature Review

Atherosclerosis is the main contributor to cardiovascular disease and is the leading cause of death for people in Western populations (12,13). More than five million people are diagnosed with CHD each year (14). Atherosclerosis is a slow, progressive disease characterized by a hardening and narrowing of arteries due to plaque build-up on inner walls (15). The development of atherosclerosis has three characteristic stages: foam cell formation, plaque formation, and complex lesion development (12). One theory is that atherosclerosis starts with endothelial injury. Monocytes adhere to damaged areas, become macrophages, and travel to subendothelium. Macrophages have the ability to bind lipids, predominantly low density lipoproteins (LDL), and develop into foam cells which become evident as fatty streak formations. Circulating platelets adhere to the damaged areas and release platelet derived growth factors causing smooth muscle cell proliferation and fibrous plaque formation. Then through calcification, necrosis, hemorrhage, ulceration, or thrombosis, a complex lesion is formed that is
generally considered clinical atherosclerosis (12).

Many factors contribute to atherosclerosis including smoking, hypertension, high serum total cholesterol, high serum LDL cholesterol, obesity, diabetes, lack of exercise, stress, heredity, and being male under age 55 (9). However, these identified risk factors account for only half of all cases of CHD (9,16,17). Because only half of the cases of CHD can be linked to classical risk factors then it is necessary to determine what else is causing this disease if it is going to be prevented. Elevated levels of LDLs are responsible for a greater risk than elevated total cholesterol in accelerating atherogenesis and have emerged as a primary risk factor for atherosclerosis (18,19).

LDLs are responsible for transporting cholesterol to cells. They are composed of a central hydrophobic core of cholesterol esters, triglycerides, beta carotene, and other carotenoids (Figure 1). The core is surrounded by a hydrophilic monolayer of phospholipids [mainly lecithin and little sphingomyelin and lysolecithin], free cholesterol, and the protein apolipoprotein B-100 (12,19). Alpha-tocopherol is also located within the LDL molecule although the exact location has yet to be identified.

**Surface:** apo B, phospholipids, free cholesterol, alpha-tocopherol*

**Core:** triglycerides, cholesterol esters, alpha-tocopherol*, beta-carotene, other carotenoids

*Exact location of alpha-tocopherol in not known. (From Jialal, Ishwarlal; Fuller, Cindy. Oxidatively Modified LDL and Atherosclerosis: An Evolving Plausible Scenario. Critical Reviews in Food Science and Nutrition. 36: 341-353. 1996.)

**Figure 1.** The composition of the LDL molecule. 

LDLs increase the risk of atherosclerosis but an even greater risk occurs when LDLs are oxidatively modified (18). The reason this risk factor was previously missed is because you do
not need high amounts of LDL in order for them to be harmful if they are in this oxidized form. LDL receptors on endothelial cells take up the LDL molecule which contributes to foam cell accumulation within arteries (18). A second type of receptor, the scavenger receptor, recognizes modified forms of LDLs. When polyunsaturated fatty acids (PUFAs) within the LDL molecule are oxidized, the modified LDL molecule is taken up by scavenger receptors on macrophages, smooth muscle cells, and/or endothelial cells (9). This pathway is 3-10 times faster than the classical LDL receptor pathway and has no negative feedback mechanism like the classical receptor which greatly accelerates atherosclerosis (12).

The oxidation of LDL occurs through a cascade of events that starts with the generation of free radicals in the body (18). Free radicals have one or more unpaired electrons causing them to be highly reactive (9). The most significant radicals are superoxide, peroxyl, and lipid oxyl and hydroxyl radicals (18). The steps directly leading to oxidized LDL formation are outlined below.

The first step is the reduction of molecular oxygen to superoxide and peroxy anion. This occurs in normal bodily processes by molecular oxygen gaining one or more free electrons from the body.

Reaction #1: \( \text{O}_2 + e^- \rightarrow \text{O}_2^- \) (superoxide) + e- \( \rightarrow \text{O}_2^{2-} \) (peroxyl anion)

The peroxyl anion can then gain two hydrogen ions from its surrounding molecules and form hydrogen peroxide. Hydrogen peroxide can also be formed during normal cellular metabolism (ex: phagocytosis).

Reaction #2: \( \text{O}_2^{2-} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 \) (hydrogen peroxide)

The superoxide and hydrogen peroxide, if not removed, can continue to react with iron or copper to produce an even more reactive hydroxy radical, \( \text{OH}^- \). The reaction of the ferrous ion (\( \text{Fe}^{2+} \)) and hydrogen peroxide to form a hydroxy radical is referred to as the superoxide driven Fenton reaction and can be summarized as follows.

Reaction #3:  
(1) \( \text{O}_2^- + \text{Fe}^{3+} \rightarrow \text{O}_2 + \text{Fe}^{2+} \)
(2) \( \text{H}_2\text{O}_2 + \text{H}^+ + \text{Fe}^{2+} \rightarrow \text{HO}^- \) (hydroxy radical) + \( \text{H}_2\text{O} + \text{Fe}^{3+} \) (Fenton Reaction)

These preliminary steps of radical formation directly lead to the peroxidation of PUFAs within the LDL molecule. There are two stages to peroxidation including initiation and
propagation (Figure 2) (19). The initiation step starts when the hydroxy radical abstracts a hydrogen atom from a methylene group located between two of the double bonds within PUFAs. A carbon centered allylic radical is produced that rearranges into a conjugated diene. With conjugation starting on the atom adjacent to the free radical, the electron is able to delocalize through all of the conjugated $p$ orbitals allowing for some stabilization of the free electron. However, the species is still unstable and will react with molecular oxygen to form a lipid peroxyl radical. The propagation step involves the reaction of the lipid peroxyl radical with other PUFAs within the LDL molecule producing more lipid peroxyl radicals at an increasing rate (9).

1) hydroxy radical abstracts a hydrogen atom from methylene group of a PUFAs  
2) lipid carbon centered allylic radical is produced  
3) rearrangement to more stable conjugated diene and attack by molecular oxygen  
4) lipid peroxyl radical is formed which attacks another PUFA within the LDL molecule

Figure 2. The initiation and propagation stages of the oxidation of PUFAs within the LDL molecule.
Oxidation alters the composition of the LDL molecule. The cholesterol is oxidized to oxysterols (the primary being 7-ketocholesterol); the predominant phospholipid, lecithin, decreases while the amount of lysolecithin increases; there is a decrease in the PUFAs content and lipid hydroperoxides decompose into the aldehydes 4-hydroxynonenal, malondialdehyde, and 2,4-heptadenal and ketones (14,20). The aldehydes and ketones react with the ε-amino group of lysine residues in apo B and form a Schiff base (14). This increases the negative charge of the LDL molecule and it is no longer recognized by the classical LDL receptor, but is recognized and taken up by scavenger receptors (9,19,20).

In addition to being taken up at an increased rate, oxidized LDL has other properties that contribute to atherosclerosis development. In general, oxidized LDL increases foam cell formation by 1) attracting additional monocytes to damaged areas, 2) trapping macrophages, and 3) inhibiting mobilization from subendothelium (13,19). In addition, oxidized LDL is cytotoxic causing cell injury which can lead to complete cell death (13,20). The mechanisms by which it exhibits these effects include: are decreasing platelet derived growth factor (PDGF) in macrophages, increasing prostacyclin production by human endothelial cells, and decreasing fibrinolysis in blood vessels by increasing plasminogen activator inhibitor-1 (PAI-1) (19). Oxidized LDL also reduces nitric oxide (NO) production in a dose and time dependent manner which further increases the progression of atherosclerosis (21). Inducible NO synthase and NO in macrophages and foam cells may have the ability to regulate vascular tone by promoting vasodilation, inhibiting smooth muscle proliferation by decreasing platelet adhesion, decreasing platelet aggregation, and decreasing leukocyte adhesion (13,21). NO may even have the power to destroy foam cells (21).

Antioxidants can play a very important role in preventing the development of atherosclerosis by blocking the oxidation of LDL. The fat-soluble antioxidant vitamin, alphatocopherol, can act as a chain-breaking antioxidant (22). Alpha-tocopherol is the most important form of tocopherol because its concentration is 15 times that of all other lipid soluble antioxidants (17,23). Alpha-tocopherol reacts with peroxyl radicals at a faster rate than PUFAs. The alcohol (OH) substituent at the 6 position on alpha-tocopherol has the ability to trap lipid peroxyl radicals by transferring its hydrogen atom with 1 electron forming an unreactive lipid
hydroperoxide (Figure 3) (24). The chain reaction stops because the tocopheroxyl radical is stable and does not propagate further radical reactions with PUFAs (24).

![Figure 3. Termination of the oxidation of PUFAs within the LDL molecule by the transfer of a hydrogen atom from alpha-tocopherol.]

Oxidation of PUFAs within the LDL molecule begins only after all the alpha-tocopherol is depleted (17). It is suggested that one molecule of alpha-tocopherol protects 3000 PUFA (9). On average, one LDL molecule contains six molecules of alpha-tocopherol and about 2700 molecules of fatty acids, most of which are PUFAs (9,23). Ideally alpha-tocopherol has the potential to completely protect PUFAs from oxidation. However, alpha-tocopherol only protects PUFAs for a short period of time if it does not have reducing molecules available to regenerate it from its radical form (9).

Both plasma levels and dietary intake levels of alpha-tocopherol are inversely associated with CHD (17). For men, higher plasma levels of alpha-tocopherol are associated with a lower mortality rate from CHD. There was no correlation between total cholesterol or blood pressure and CHD in this study which are usually considered significant risk factors; this suggests that alpha-tocopherol may play a more significant role in heart disease than once thought (17). The Nurse’s Study and Physician’s Study show that supplemental intake of 100 IU (67 mg TE) of alpha-tocopherol for two years is associated with a reduced risk of CHD in both women and men, 40% decreased risk for women and 41% for men (25,26).

Foods that contain significant amounts of alpha-tocopherol include; vegetable oils, nuts, whole grains, butter, liver, egg yolks, and some fruits and vegetables (27). However, in order to
obtain 100 IU (67 mg TE) from some of these sources, one would have to consume unrealistic amounts, ex: 25 T of vegetable oil, 10 oz of almonds, 13 T margarine, 200 servings of most vegetables, or 6 cups of wheat germ (28). Obviously this would be nearly impossible and, in most cases, would substantially increase calorie and total fat intake contributing additional risk. In order to decrease the risk of developing atherosclerosis, supplements are the best and the most logical option to increase intake to this level (10X the RDA). Toxicity effects are not a problem with intakes as high as 3200 IU (29). Excess appears to be excreted in the bile (30).

Ascorbic acid (vitamin C), retinol (vitamin A), beta-carotene, ubiquinol 10, lycopene, flavonoids, and selenium are other nutrients that have antioxidant characteristics. The antioxidant vitamins, ascorbic acid, beta-carotene, and ubiquinol, work in conjunction with alpha-tocopherol to inhibit oxidation. They can regenerate alpha-tocopherol from its radical form by transferring a hydrogen atom (12,31). Supplementation with 1 g of vitamin C increased plasma alpha-tocopherol levels (32). Alpha-tocopherol is then able to break more chain reactions. Ascorbic acid is the most important water soluble antioxidant and may act as LDLs first defense against oxidative modification (9,31). It can directly inhibit oxidation by providing reducing equivalents to peroxides thereby producing alcohols (22). Ascorbate also reacts with singlet oxygen, superoxide, and hydroxyl radicals and low levels in plasma and cells are associated with increased LDL oxidation and greater atherosclerosis development (22).

The carotenoids, retinol and beta-carotene, have the ability to react with lipid hydroperoxides and scavenge singlet oxygen (33). Both retinol and beta-carotene are inversely related to cardiovascular events (9,22). Increasing beta-carotene intake through supplements to 50 mg a day (10X average) decreases major coronary events by 44% (9). The concentration of retinol in the serum, however, is tightly regulated by homeostatic mechanisms and the majority of retinol is stored in the liver. Therefore, it is not likely that increased amounts of retinol would contribute significantly to a decrease in free radical production (33). Several flavonoids inhibit LDL oxidation in vitro and others decrease the adverse effects of oxidized LDL (27). Flavonoids are phenolic compounds found predominantly in fruits, vegetables, and red wine (16,27). Selenium is a trace mineral that may also inhibit the cytotoxic effects of oxidized LDL (27).
Preventing the oxidation of LDL by antioxidants has many benefits (11). By inhibiting oxidation; more NO is released, there is reduced expression of the adhesion molecule e-selectin, and protein kinase C is inhibited. The result is reduced clinical activity of CHD by inhibition of monocyte recruitment, plaque rupture, platelet adhesion, leukocyte adhesion, vasoconstriction, and thrombosis (11,21).

Materials and Methods

Subjects

A group of 32 postmenopausal women were actively recruited from the Monastery of the Sisters of the Order of St. Benedict in St. Joseph, MN. An informed consent was received from each subject and the study was approved by the Institutional Review Board: Human Subjects Committee at the University of Minnesota. Samples were obtained through a separate research project which examined the effects of flax seed on body hormone levels. Our samples were obtained during the control period of this study (0g flax). In other words, we are not looking at the effects of flax seed. The study took place in three, seven week sessions beginning in January 1997 and ending in October 1997. Subjects filled out a health history form and food frequency questionnaire. During the seventh week of each diet period a plasma sample and three day diet record were obtained.

Subjects eat most of their meals in the monastery dining room which has a seven week cycle menu. At each meal there are two to three entrees and dessert options available. In addition fresh fruit, salad, juices, milk, and coffee are always offered. The Sisters follow a cycle menu in which the recipes for the food items served are known; therefore, the measurements of their dietary intake is extremely accurate. The subjects are all non-smokers and on average consume less than two drinks of alcohol per month. Four subjects were on hormone replacement therapy. Other characteristics of the group are summarized in Table 1.
Table 1. Characteristics of Study Population

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
<th>Population Mean (34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.3</td>
<td>53-82</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5</td>
<td>17.0-34.0</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mg)</td>
<td>212</td>
<td>114-295</td>
<td>246</td>
</tr>
<tr>
<td>HDL Cholesterol (mg)</td>
<td>65.8</td>
<td>37-101</td>
<td>52.8</td>
</tr>
<tr>
<td>LDL Cholesterol (mg)</td>
<td>126</td>
<td>24-185</td>
<td>162</td>
</tr>
<tr>
<td>Multivitamin Use</td>
<td>3 subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-tocopherol Supplement Use</td>
<td>3 subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both Multivitamin and Alpha-tocopherol Supplement Use</td>
<td>2 Subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical Activity (20 minutes at least 3 days a week)</td>
<td>20 Subjects</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dietary Information

Semi-quantitative Willett food frequency questionnaire (FFQ) was given to subjects in November 1996 before the study started in January 1997. A dietitian explained the FFQ to the subjects and demonstrated typical portion sizes using food models and the bowls, glasses, and ladles used in the monastery dining room. The FFQ was originally modified for the Cancer Prevention Research Unit at the University of Minnesota to include additional vegetables, fruits, and low-fat foods. The FFQ contains 153 items with 32 vegetables, 18 fruits, and eight juices. Analysis of the FFQ was completed by Brian Randall at the University of Minnesota (Data Recognition).

A three day diet record was given during the seventh week of each diet period. Food records were distributed, collected, and reviewed for completeness by a nutrition student at each meal. Because the dining facility follows a seven week cycle menu, possible food choices were printed in advance. Subjects could easily identify the foods selected at the meal and estimate the portion size consumed. Most foods are served in standard portion sizes so little guessing is
involved. In addition, specific ingredients for all dishes are known in exact quantities which made analyzing the diet records extremely accurate. Diet records were analyzed at the University of Minnesota using the Minnesota Nutrition Data System (NDS) version 2.4 with Food Database version 6A and Nutrient Database version 21.

Sample Preparation and Procedure

Blood samples were collected in vacutainer tubes after an 8-10 hour fast on the morning following the three day diet records. The samples were centrifuged to separate the plasma layer from the red blood cells. For the first two diet periods the plasma was transferred in the absence of light to storage vials containing EDTA and stored under nitrogen -80°C until analyses were run. For the third diet period the samples were not transferred in the absence of light but were stored under the same conditions.

Vitamin A acetate (retinol internal standard), Vitamin A alcohol (retinol), +-alpha-tocopherol, alpha-tocopherol acetate were purchased from Fluka Chemical Corp. (Ronkonkoma, NY). Diethyl ether was purchased from Sigma-Aldrich (St. Louis, MO). Ethanol, methanol, and heptane were HPLC grade and obtained from the Chemistry stockroom at the College of St. Benedict (St. Joseph, MN). The HPLC column was Bio-Sil C-18 HL 90-5 S 150 X 4.6cm particle size 5μm from Bio-rad (Hercules, CA).

Internal standards of retinyl acetate and alpha-tocopheryl acetate were used to quantify the alpha-tocopherol and retinol present in each sample. First, a constant amount of the acetate was added to variable amounts of vitamin A alcohol (retinol) and +-alpha-tocopherol standards. Peak area ratios were determined for each of the concentrations of vitamin A alcohol and +-alpha-tocopherol standard. The concentration of the +-alpha-tocopherol standards ranged from 5 μmol/L to 80 μmol/L. The concentration of the vitamin A alcohol standards ranged from .25 μmol/L to 5.00 μmol/L. With this information it was possible to determine the amount of retinol and alpha-tocopherol in each subject’s plasma sample:

\[
\frac{\text{Concentration ratio of unknown}}{\text{Concentration ratio of standard}} = \frac{\text{Area ratio unknown}}{\text{Area ratio standard}}
\]

*Where the ratio is alpha-tocopherol/alpha-tocopherol acetate or retinol/retinyl acetate*
The concentration of retinol and alpha-tocopherol in the plasma samples were determined using High Pressure Liquid Chromatography (HPLC) according to a modified version of Bieri et al. 1979 (35). Volumes of the plasma samples and reagents were doubled from the original research in order to inject a full 90 μl of the sample into the column. Retinyl acetate solution in ethanol (1.0 μg/ml) and alpha-tocopheryl acetate solution in ethanol (45 μg/ml) was pipetted into a 1.5 ml bullet tube in the amount of 200 μl. Plasma was added in the amount of 400 μl and vortexed for 10 seconds. The lipid layer was extracted by adding 4 X 100 μl spectro grade heptane, vortexing after each addition for 45 seconds to ensure that the contents at the bottom of the bullet tube were thoroughly mixed. The bullet tubes were centrifuged for 7 minutes at 5400 rpm to separate the phases. As much of the top solvent layer as possible was drawn off with a 300μl pipet and transferred to a new 1.5 ml bullet tube. The solvent was evaporated under a stream of nitrogen while the tube was in a 60°C water bath. The lipid components were redissolved in 100 μl diethyl ether followed by 300 μl of methanol with gentle mixing by finger tapping. The contents were transferred to a small glass vial. A sample of 90 μl of the solution was injected into the column. The solvent was a 95:5 solution of methanol:acetonitrile. The current HPLC column suitable for separating fat soluble vitamins requires different column specifications than were used in the original research. In order to get good peak separation, the HPLC column was set with a flow rate of 1 ml/min, an attenuation of 4, an end time of 15 minutes, and UV detector set at 280 nm.

Lipid profile information was determined at Fairview University Medical Center lab.

Statistics

The mean and standard deviation was determined for alpha-tocopherol and retinol intake from the diet records and FFQ, and alpha-tocopherol and retinol plasma levels. Correlation coefficients were calculated for alpha-tocopherol and retinol intake from the diet records and FFQ and plasma levels.
Results

Analysis of the FFQ consistently suggests higher intakes for both alpha-tocopherol and retinol compared to the three day diet records. For example, the mean intake of alpha-tocopherol according to the three day diet records is 38.43 mg TE and is 84.56 mg TE from the FFQ. Three day diet records indicate that 66% of the population receive the RDA (8 mg) of alpha-tocopherol whereas the FFQ indicates that 100% are receiving the RDA (Table 2). The mean intake of retinol from the three day diet records is 1720 mcg RE and is 4720 mcg RE from the FFQ. Three day diet records indicate that 93% of the population meet the RDA (800 RE) for retinol whereas the FFQ indicate that 100% meet the RDA (Table 3). Supplements are providing eight subjects with additional alpha-tocopherol and two subjects with additional retinol (Table 1).

Table 2. Alpha-tocopherol intake from three day diet record and FFQ.

<table>
<thead>
<tr>
<th>Alpha-tocopherol</th>
<th>Dietary Intake</th>
<th>% subjects receiving RDA (8 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3-Day Diet Record</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (with supplement)</td>
<td>38.43 mg</td>
<td>66%</td>
</tr>
<tr>
<td>mean (no supplement)</td>
<td>11.98 mg</td>
<td></td>
</tr>
<tr>
<td>median</td>
<td>6.07 mg</td>
<td></td>
</tr>
<tr>
<td><strong>FFQ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (with supplement)</td>
<td>84.56 mg</td>
<td>100%</td>
</tr>
<tr>
<td>median</td>
<td>35.98 mg</td>
<td></td>
</tr>
<tr>
<td>Mean Intake NHANES III</td>
<td>7.72 mg</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Retinol intake from three day diet record and FFQ.

<table>
<thead>
<tr>
<th>Retinol</th>
<th>Dietary Intake</th>
<th>% subjects receiving RDA (800 RE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3-Day Diet Record</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (with supplement)</td>
<td>1720 RE</td>
<td>93%</td>
</tr>
<tr>
<td>mean (no supplement)</td>
<td>1670 RE</td>
<td></td>
</tr>
<tr>
<td>median</td>
<td>1709 RE</td>
<td></td>
</tr>
<tr>
<td><strong>FFQ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (with supplement)</td>
<td>4720 RE</td>
<td>100%</td>
</tr>
<tr>
<td>median</td>
<td>4944 RE</td>
<td></td>
</tr>
<tr>
<td>Mean Intake NHANES III</td>
<td>1067 RE</td>
<td></td>
</tr>
</tbody>
</table>

In general, this population consumes above average quantities of alpha-tocopherol and retinol when compared to the intake of women from similar age groups using NHANES III data. Eleven subjects, however, are still not meeting the RDA for alpha-tocopherol when using diet records to assess intake. Only five subjects are receiving 100 IU alpha-tocopherol which is considered the minimum necessary to demonstrate antioxidant benefits (25,26).

The mean plasma levels for alpha-tocopherol and retinol for our subjects are higher than levels reported in other studies. The mean plasma concentration of alpha-tocopherol for this population is 32.54 $\mu$mol/L with no supplement use and 52.76 $\mu$mol/L with supplement use. The plasma concentration of retinol for this population is 4.36 $\mu$mol/L.

Intake of alpha-tocopherol was positively correlated to plasma concentrations for both three day diet records and FFQ with correlations of $r=0.326$ and $r=0.327$ respectively. Intake of retinol was positively correlated to plasma concentrations for both three day diet records and FFQ with correlations of $r=0.204$ an $r=0.232$ respectively.
Discussion

As indicated from both the three day diet records and FFQ, this population of 32 postmenopausal women is receiving above average quantities of alpha-tocopherol and retinol when compared to NHANES III data. However, the diet records and FFQ gave very different values for both alpha-tocopherol and retinol intake. Alpha-tocopherol intake from the FFQ is more than two times greater than the intake from the diet records, 38.43 mg TE and 84.56 mg TE respectively. Retinol intake from the FFQ is almost three times as high as the intake reported from the diet records, 1720 mcg RE and 4720 mcg RE respectively. The amounts seen with the FFQ are unusually high and seem unrealistic when considering a typical diet, and appear to be over estimating alpha-tocopherol and retinol intake and suggests and error in processing the form. Therefore, greater emphasis is placed on the three day diet record.

Many factors affect the dietary intake information. FFQ are designed to estimate the average intake of nutrients over a one year period. However, often FFQs are more reflective of recent intakes (36). It is difficult to estimate portion sizes, and weekly or yearly intakes of certain foods. The subjects filled out the FFQ in a room separate from the monastery dining room and had to estimate portion sizes without the actual food to use as a reference. References such as plastic foods models and the dining room bowls and glasses were available during the administration of the FFQ, however the subject had to translate their typical portion and these known serving sizes to the frequency of consuming a standard portion size of particular foods. Therefore, the FFQ relied heavily on the subject’s memory and ability to estimate portion sizes.

The problems associated with estimating portion sizes was minimized during the three days the subjects recorded their diet. Serving sizes are predetermined for most meats, desserts, and vegetables. Bowls, glasses and serving utensils are of known volumes for side dishes and beverages. Therefore, the subject only needed to indicate the size of bowl or glass used, or the number of ladles or spoon fulls of a particular food item they had and this serving was then accurately converted into grams or ounces. In addition, a nutrition student was present at each meal to help the subjects who were having difficulty describing their food choice or estimating the serving size. They also checked the food records for completeness and clarity as they were turned in. Therefore, the diet records did not have to rely heavily on the subject’s memory or
ability to estimate serving sizes like the FFQ did.

Despite the above average intakes seen with this population, 34% of the women are still not receiving the RDA for alpha-tocopherol according to their intakes from the diet records and only four are receiving 100 IU. On the other hand, all but 7% of the women are receiving the RDA for retinol. The discrepancy in antioxidant intake between alpha-tocopherol and retinol is due to the very different concentrations of nutrients in food sources providing these nutrients. Alpha-tocopherol is predominantly found in high fat foods such as vegetable oils, nuts, butter, and egg yolks; whole grains such as wheat germ; and small amounts in selected fruits and vegetables (28). A good source typically provides 4 mg TE per standard serving which is half of the RDA (8 mg). Preformed retinol is found in dairy products, organ meats such as liver, and high fat fish such as herring, sardines, and tuna (37). Retinol can also be obtained through the conversion of beta-carotene to retinol in the body. Sources of beta-carotene include orange fruits and vegetables and green leafy vegetables (37). A good source provides 2025 RE per standard serving which is more than double the RDA (800 RE). Therefore, if a person follows the food guide pyramid they will receive greater quantities of retinol and beta-carotene because these foods are found further down on the pyramid and more of these items are consumed. In our population 66% of the subjects receive less than 31% of their calories from fat each day and on average consume 4.5 servings of fruits and vegetables. Therefore, they are likely restricting sources of alpha-tocopherol but not beta-carotene.

Studies indicate that to see protective benefits a minimum of 100 IU of alpha-tocopherol must be consumed (25,26). Only five subjects received 100 IU alpha-tocopherol, and in all cases the additional amount was obtained through supplements. Even a diet with excellent sources of alpha-tocopherol cannot provide 100 IU and supplements are needed. Because of the tight plasma regulation of and the severe toxicity effects of retinol at intakes only 5X the RDA, it is not recommended that retinol be consumed at amounts above the RDA. On the other hand, beta-carotene is safe at amounts well above the RDA and benefits are observed at 50 mg per day. Beta-carotene, however, was not looked at in this study.

Plasma levels of alpha-tocopherol and retinol are higher with this population than reported in current literature which may reflect their above average intakes of alpha-tocopherol and
retinol. The mean plasma concentration of alpha-tocopherol for our population is 32.54 \mu\text{mol/L} with no supplement use in contrast to 26.2 \mu\text{mol/L} to 32.65 \mu\text{mol/L} found in the studies (38-41). With supplement use (>20 mg TE) our population had mean plasma levels of 52.76 \mu\text{mol/L} as opposed to 36.9 \mu\text{mol/L} in other studies (38-41). The plasma concentration of retinol for our population is 4.36 \mu\text{mol/L} compared to 1.63 \mu\text{mol/L} to 1.91 \mu\text{mol/L} from other studies (38-41).

In our study, alpha-tocopherol was positively correlated to plasma levels when either a FFQ or a diet record was used as the dietary assessment tool (Figure 4). While not strong, our correlation of \( r = 0.326 \) using the three day diet record and \( r = 0.327 \) using the FFQ is higher than the results from other studies looking at the diet and plasma relationship of alpha-tocopherol. Stryker et al. (1988) found a correlation between alpha-tocopherol intake and plasma alpha-tocopherol levels of \( r = 0.12 \) with no supplement use (42). Booth et al. (1997) found a correlation between of \( r = 0.21 \) with one blood sample and four diet days and \( r = 0.08 \) with three blood samples and twelve diet days with no intake of supplements at least four weeks prior to study (38). Ascherio et al. (1992) found a correlation of \( r = 0.12 \) for women with no supplement use and FFQ used as dietary tool (39).

Retinol was also positively correlated to plasma levels when either a diet record or FFQ were used with correlations of \( r = 0.204 \) and \( r = 0.232 \) respectively (Figure 5). Again, the correlation is not strong but it is better than the results from other studies. Booth et al. (1997) found a correlation of \( r = -0.1 \) with one blood sample and four diet days and \( r = 0.17 \) with three blood samples and twelve diet days with no supplement intake at least four weeks prior to study (38). Ascherio et al. (1992) found a correlation of \( r = 0.018 \) for women where preformed retinol was the only measure of intake from FFQ (39).

The consistency of our subject’s diet may account for the higher correlations between diet and plasma than other studies observed. Our subject’s eat meals according to a seven week cycle menu with the same cereals, fruits, vegetables, beverages, and bag lunch options always available. Therefore, their diets may not be subject to the variations typical of most diets. The accuracy of estimating dietary intake improves with additional food records. Dixon reports that 30 days of dietary information is needed before accurate data is obtained that is correlated to plasma (36). The dietary intake of our subjects may have been more accurate with additional
Figure 4. Relationship between alpha-tocopherol intake and fasting plasma levels.
Retinol Intake vs. Plasma Levels

Three Day Diet Record

FFQ

Figure 5. Relationship between retinol intake and plasma levels.
days of food records which may result in even higher correlations between diet and plasma. A higher correlation may also be obtained if more than one plasma measurement was taken to minimize the effects of experimental error due to pipetting, incomplete mixing, or inaccurate HPLC reading.

Alpha-tocopherol intake and plasma level (Figure 4) showed a wide spread of plasma levels at intakes less than 50 mg TE. This result is consistent with other studies that indicate a low correlation without supplemental intake (43). When supplements were added at 25, 50, 100, 200, 400, and 800 IU a high correlation, r=0.91, was demonstrated (43). This suggests that a certain level of alpha-tocopherol intake may need to be achieved before plasma levels are significantly and proportionally affected. We do not see a linear trend with the subjects who took supplements in our study. We have three subjects with intakes greater than 100 mg TE and relatively low plasma levels and two subjects with intakes above 50 mg TE and less than 100 mg TE with the highest plasma levels. One explanation for this discrepancy is supplement intake was reported on a health history questionnaire given prior to the onset of this experiment. Intake of supplements may not be accurate due to failure to consume supplements in quantities reported on questionnaire or failure to report supplement use even if they are being used. In addition, the length of time the subject had been taking the supplements was not reported. Plasma levels may be affected differently depending on length of time they have been used.

An additional factors that may contribute to the low correlation are plasma regulation of alpha-tocopherol and retinol and plasma lipids. Plasma measurements may not be a good representation of intake because for both alpha-tocopherol and retinol plasma levels are regulated. Plasma retinol is tightly controlled and stored for extended periods of time in the liver (36). Plasma alpha-tocopherol may only be affected by extreme low or high amounts of intake (36). Machlin reports that a ten fold increase in alpha-tocopherol intake is needed to double plasma level (29). It is proposed that in order to obtain accurate plasma values for alphatocopherol, plasma lipid levels should be taken into consideration, however neither plasma alphatocopherol nor retinol was adjusted for plasma lipids (43). Plasma alpha-tocopherol increases in proportion to total plasma lipids (43). Our study revealed a positive correlation between alphatocopherol and total cholesterol and LDL cholesterol, r=0.422 and r=0.350 respectively. A
positive correlation was also seen for retinol and total cholesterol and LDL cholesterol, $r=0.315$ and $r=0.156$ respectively.

Although a positive correlation was seen between alpha-tocopherol and retinol intake and their plasma concentration and the correlations from this study were much better than other studies, the correlations were low enough to suggest that neither a three day diet record nor a FFQ can accurately predict plasma concentrations, nor do plasma levels reflect alpha-tocopherol intake for the levels consumed by these women. Therefore, it does not seem that an easy dietary assessment tool can be used as a screening tool for a large number of people that gives an accurate reflection of one’s antioxidant protection. However, it appears that a general recommendation of at least 100 IU of alpha-tocopherol for everyone is well supported to increase plasma levels and decrease the incidence of CHD.
References


Project Title: “The Correlation Between the Dietary Intake of the Fat-soluble, Antioxidant Vitamins Alpha-tocopherol and Retinol and Their Fasting Plasma Concentrations in Postmenopausal Women”

Approved by:

Amy Olson, Professor of Nutrition (Project Advisor)

Henry Jakubowski, Associate Professor of Chemistry

Ingrid Anderson, Professor of Nutrition

Chair, Department of Nutrition

Director, Honors Thesis Program

Director, Honors Program