EFFECT OF CONSUMING FORTIFIED BREAKFAST CEREAL ON VITAMIN E AND CALCIUM STATUS

A DISSERTATION

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BY

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To the Dean of the Graduate School:

I am submitting herewith a dissertation written by Jill Killough entitled "Effect of Consuming Fortified Breakfast Cereal on Vitamin E and Calcium Status". I have examined this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Nutrition.

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We have read this dissertation and recommend its acceptance:

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Accepted:

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DEDICATION

In loving memory of my father, John E. Chesser, who at the young age of 44 passed away from a cerebrovascular accident. Thank you for raising me to have faith in God, the drive to succeed, teaching me the value of education, and giving me unconditional love. To my mother, Debra Chesser Paulsen, thank you for providing me with all of these things as well as your continuous encouragement and support. Without my parents, I would not be where I am today.

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ABSTRACT

JILL KILLOUGH

EFFECT OF CONSUMING FORTIFIED BREAKFAST CEREAL
ON VITAMIN E AND CALCIUM STATUS

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Vitamin E and calcium are two shortfall nutrients that are required in optimal amounts from the diet to perform necessary functions in the body. Fortified breakfast cereal can provide up to 100 % of the daily value for several vitamins and minerals, including vitamin E and calcium. This study investigated the consumption of a 1-ounce serving of fortified breakfast cereal on vitamin E and calcium dietary intake among adults, using a six-week randomized, controlled, parallel-arm, open-label design, with a two-week pre-intervention period and a four-week post-intervention period. In addition, serum tocopherol and lipid-corrected tocopherol concentrations were evaluated. Forty-three participants enrolled in the study with forty-two participants (10= males; 32=females) completing the study. Participants were in self-reported good health; 18 years of age and older; and not currently taking a vitamin-mineral supplement, lipid altering medication, or hormone replacement therapy. Participants were randomized to either the control or intervention group, which received fortified breakfast cereal. Participants recorded six-weeks (two non-consecutive week days and one weekend day per week) of dietary intake and had two blood draws (pre- and post-intervention). Dietary intakes were analyzed using the Nutrient Data System for Research version 2010. Mean compliance for fortified breakfast cereal consumption in the intervention group

was 100% for men and ranged from 95.6 to 97.3% for women. There were significant between-group differences in total vitamin E (α-tocopherol), synthetic vitamin E (all-rac-α-tocopherol), and calcium intakes at weeks 3 & 4 and at weeks 5 & 6 ($p<.001$). Additionally, total vitamin E (α-tocopherol), synthetic vitamin E (all-rac-α-tocopherol), and calcium intakes were all significantly higher at weeks 3 & 4 and weeks 5 & 6 as compared to weeks 1 & 2 in the intervention group ($p<.001$). There were no significant between-group differences for either serum alpha-tocopherol or lipid-corrected alpha-tocopherol concentrations. There were no between-group differences in serum total cholesterol, triglycerides, LDL-C, or HDL-C concentrations. Fortified breakfast cereal can be easily incorporated into a normal diet among healthy adults and is a feasible option for increasing dietary intakes for two shortfall nutrients in the United States.

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CHAPTER I

INTRODUCTION

As a nation, we suffer from chronic diseases such as cardiovascular disease and osteoporosis which can be debilitating. Heart disease and stroke have afflicted roughly three million people as the cause of their disability (Centers for Disease Control and Prevention [CDC], 2010). It is estimated that one in three people (81 million) in the United States (U.S.) currently lives with one or more types of cardiovascular disease. Additionally, heart disease is the leading cause of death in the U.S. for both men and women, with stroke ranking as the third leading cause of death. In 2010, the American Heart Association (AHA) Strategic Planning Task Force and Statistics Committee developed the organization's impact goal which aims "to improve the cardiovascular health of all Americans while reducing deaths from cardiovascular disease and stroke by 20 percent" by the year 2020 (Lloyd-Jones et al., 2010). The focus of the goal is prevention and education for lifestyle modification. To assist in this effort a new definition of cardiovascular health was categorized into ideal, intermediate, or poor.

Ideal cardiovascular health is defined by seven modifiable health behaviors and factors, which are not smoking, body mass index < 25 kg/m2, physical activity, diet consistent with current recommendations, untreated total cholesterol < 200 mg/dL, untreated blood pressure <120/<80 mm Hg, and fasting blood glucose < 100 mg/dL. Intermediate and poor cardiovascular health is defined by varying ranges of the above seven behaviors and factors. Most Americans fall into

these two categories. Less than 5% of America's population would be qualified as having ideal cardiovascular health (Kones, 2011).

Osteoporosis, another condition that contributes to a high level of disability in the U.S., is characterized by porous bone due to low bone mass and loss of bone structure. Osteoporosis can lead to an increased risk of fracture, particularly in the hip, wrist, and spine (National Osteoporosis Foundation, n.d.). The number of individuals diagnosed with osteoporosis in the U.S. is estimated to be approximately 10 million, with an additional 34 million individuals at risk for developing osteoporosis due to low bone mass. Individuals with osteoporosis are at risk for developing problems due to physical deterioration and diminished quality of life such as limitations in performing activities of daily living (Kotz, Deleger, Cohen, Kamigaki, & Kurata, 2004).

Prevention is the key in combating chronic disease in our nation. It is important to recognize that the risk for developing cardiovascular disease and osteoporosis can be modified by proper nutrition. Adequate dietary intakes of vital nutrients can lower risk. Decreased cardiovascular disease risk has been associated with higher intakes of vitamin E in both men and women (Rimm et al., 1993; Stampfer, Hennekens, Manson, Colditiz, & Willett, 1993). Currently, the AHA advises supplementation of antioxidants such as vitamin E and C and beta-carotene not be used for prevention of cardiovascular disease due to adverse implications observed in cardiovascular disease events and mortality (Lichtenstein et al., 2006). Conversely, the AHA does recommend consuming food sources that contain antioxidant nutrients such as fruits, vegetables, whole grains, and oils. Adequate intake (AI) of calcium throughout the lifespan is also necessary to ensure proper bone health (National Osteoporosis Foundation, n.d.). However, although both

vitamin E and calcium are available in many foods and easily obtained from the diet, national nutritional surveys consistently demonstrate below optimal intakes (Moshfegh, Goldman, Ahuja, Rhodes, & LaCombe, 2009; Gao, Martin, Lin, Bermudez, & Tucker, 2006).

Intakes of vitamin E in the U.S. are sufficient to prevent deficiency symptoms, such as peripheral neuropathy. However, dietary intakes of vitamin E are well below the recommended amounts for both men and women, despite the vitamin being present in a wide variety of foods (Gao et al., 2006; Talegawkar et al., 2007). The majority of men and women in the U.S. fail to meet current recommendations for vitamin E intake, with only 8.0% of men and 2.4% of women meeting the Estimated Average Requirement (EAR) from dietary sources alone (Mars et al., 2004).

Low calcium intakes have also been documented since the major findings from the first National Health and Nutrition Examination Survey (NHANES), 1971-1974 (Lowenstein, 1981). In What We Eat in America, NHANES, 2005-2006, women aged 19-70 years consumed calcium amounts below recommended intakes for calcium, and men aged 51-70 years were also below recommended intakes, with only 44% of adult males and 21% of adult females meeting the AI for calcium (Moshfegh et al., 2009).

In 2010, the Dietary Guidelines Advisory Committee (DGAC) recognized vitamin D, calcium, potassium, and dietary fiber as shortfall nutrients for both children and adults (Dietary Guidelines Advisory Committee [DGAC]; United States Department of Agriculture [USDA], 2010). Recommendations promoted increased intakes of these nutrients regardless of age. Additionally, the DGAC reported solid fats and saturated fatty acids are being consumed in excess. Solid fats include those that are solid at room temperature, typically from animal sources, or vegetable oils

that have undergone hydrogenation. These types of fats are often found in commonly consumed food products and are considered to be indirect sources of fat or hidden sources. Examples include food products such as cheese, creams, ice cream, and many baked goods. Lauric, myristic, palmitic, and stearic fatty acids are the largest contributors to saturated fatty acid intakes in the American diet with palmitic and stearic acids accounting for the major constituents of animal fats. Food items that are high in saturated fatty acids and contribute to the highest percent intakes in the U.S. include regular cheese (8.5% of intake), pizza (5.9% of intake), grain-based desserts (5.8% of intakes), and dairy desserts (5.6%) for a cumulative contribution percentage of 25.8. While saturated fatty acid intake continues to increase in the diet, oils such as sunflower, safflower, corn, and canola are falling below dietary targets. These types of oils provide essential fatty acids such as linoleic and alpha-linoleic and also provide a source of vitamin E, thus leading to shortfalls.

The 2005 DGAC also recognized vitamin E, calcium, potassium, and magnesium among the shortfall nutrients for both children and adults (DGAC; USDA, 2005). The DGAC recommended increasing dietary intakes of vitamin E, potassium, and fiber regardless of age; increasing intakes of vitamins A and C, calcium, and magnesium by adults; and increasing intakes of calcium and magnesium by children age 9 years or older.

Foods that are recommended as excellent dietary sources of vitamin E and calcium include fortified breakfast cereals. Thus, consumption of fortified breakfast cereals may facilitate the intake of two shortfall nutrients. To date there is limited knowledge on the influence of the consumption of fortified breakfast cereal on vitamin E or calcium intake and plasma/serum levels of vitamin E in either humans or animals.

Statement of Purpose

The purpose of this study was to determine the effect of consuming a fortified breakfast cereal on vitamin E and calcium status among adults using a randomized, controlled trial, parallel arm, open-label design.

Null Hypotheses

1. There will be no difference in vitamin E (total α-tocopherol) or calcium intake between those who consume a fortified breakfast cereal (the Intervention Group) and those who do not (the Control Group).
2. There will be no difference in vitamin E (total α-tocopherol) or calcium intake over time between those who consume a fortified breakfast cereal (the Intervention Group) and those who do not (the Control Group).
3. There will be no interaction between vitamin E (total α-tocopherol) or calcium intake and time in those who consume a fortified breakfast cereal (the Intervention Group) and those who do not (the Control Group).
4. There will be no difference in the lipid-corrected serum level of vitamin E (α-tocopherol) between those who consume a fortified breakfast cereal (the Intervention Group) and those who do not (the Control Group).

Delimitations

This study had the following delimitations:

1. Participants were recruited from Lamar University in Beaumont, Texas or through individuals who worked at Lamar University.
2. Recruitment of study participants was limited to those who were able to complete a series of visits to Lamar University.
3. Study participants were required to speak, read, and understand English.
4. Study participants were not taking lipid-lowering medications, hormone-replacement therapy, or vitamin supplements (multi-vitamin, vitamin E, or calcium).
5. Study participants were adults, age $\geq$ 18 years.

Limitations

This study had the following limitations:

1. Those who participated may represent a more motivated set of individuals interested in the effect of fortified breakfast cereal intake on vitamin E and calcium status.
2. Study participants may not have accurately recorded their diets.
3. The values reported in the Nutrition Data System for Research (NDSR) software database may not appropriately reflect the nutrient profile of foods consumed by participants.
4. The amount of fortified breakfast cereal provided to study participants may be greater than would be eaten in a non-experimental setting (one serving per day for four weeks).

Significance of the Study

In summary, it is important to consider strategies aimed at improving dietary vitamin E and calcium intake, as well as determining the feasibility of these strategies. The consumption of breakfast cereals fortified with vitamin E may be a feasible way to increase intake of the vitamin, resulting in greater serum concentrations. Additionally, consumption of a breakfast cereal fortified with vitamin E can be an easily planned way to improve vitamin E status and result in a concomitant improvement in calcium status. A 1-ounce serving of a fortified breakfast cereal, which contains synthetic vitamin E, can provide 13.5 mg of 2-R isomers of α-tocopherol, equivalent to 112% of the EAR, 90% of the Recommended Dietary Allowance (RDA), and 100% of the Daily Value (DV). Fortified breakfast cereals can also provide 1000 mg per serving, or 100% of the DV of calcium. In addition, breakfast cereal is usually consumed with milk, which can provide approximately 150 mg of calcium per ½ cup (United States Department of Agriculture [USDA], 2008).

Definitions

alpha-tocopherol = biologically active form of vitamin E, RRR-alpha tocopherol

all-rac-alpha-tocopherol= synthetic form of vitamin E, manufactured as all-racemic alpha tocopheryl acetate

antioxidant = inhibits oxidation or inhibits reactions promoted by oxygen or peroxides

calcium = a mineral found in the bone, teeth, and the blood; important for muscle contraction, heart action, nervous system maintenance, and normal blood clotting

cardiovascular disease = also known as heart disease, a broad term used to describe a range of conditions which includes diseases of the vessels, arrhythmias, and congenital heart defects

fortified = foods that have vitamins and/or minerals added to increase nutritional value

free radical= an atom or group of atoms with at least one unpaired electron and a proton that has no electrical charge; in the body it is often an oxygen molecule that has lost an electron and will stabilize itself by stealing an electron from a nearby molecule

free radical scavenger= a compound that reacts with free radicals in a biological system such as vitamin E, vitamin C, and beta-carotene, reducing free-radical induced damage by breaking the chain reaction

osteoporosis = a condition characterized by decrease in bone mass and density, occurring especially in post-menopausal women, resulting in predisposition to fractures and bone deformities

shortfall nutrient = a nutrient consumed below optimal levels

stereoisomer = molecules that have the same molecular formulas and sequence of bonded atoms, but that differ only in the three-dimensional orientations of their atoms in space

vitamin E = a fat-soluble vitamin, essential for normal reproduction; an important antioxidant that neutralizes free radicals in the body

CHAPTER II

REVIEW OF LITERATURE

Metabolism of Vitamin E

Vitamin E is a group of lipid-soluble, antioxidants (Brigelius-Flohe & Traber, 1999). Molecular structures having vitamin E antioxidant activity include four tocopherols: alpha (α), beta (β), gamma (γ), and delta (δ), and four tocotrienols (α, β, γ, δ) (see Figure 1) (Institute of Medicine [IOM], Food and Nutrition Board, 2000). Tocopherols are more abundant than tocotrienols in the U.S. diet. Tocopherols have a saturated phytyl tail attached to the two position of the chromanol ring. Each tocopherol has three stereocenters with four asymmetrical carbon atoms each having two possible configurations (R or S). These two stereoismoners are mirror images of each other, meaning they are not identical or non-superposable to each. Alpha (α)-tocopherol contains eight stereoisomers (RRR, RRS, RSR, RSS, SRR, SRS, SSR, and SSS). Natural, 2R',4R',8R'-alpha tocopherol or simply RRR-alpha tocopherol has methyl groups at the 2', 4', and 8' position on the phytyl tail in the R configuration. Synthetic alpha-tocopherol has methyl groups in the 2', 4', and 8' position on the tail that can be in the R or the S configuration, only the 2-R configurations are considered as biologically active.

Biological activity of natural RRR-α-tocopherol is greater than other natural forms of vitamin E and all-rac-α-tocopherol. All-rac-α-tocopherol contains all eight stereoisomers in equal proportions; therefore, it has approximately half of the biological activity of the natural RRR-α-

tocopherol form. Simple differences in absorption and metabolism do not satisfactorily explain the differences in isomer biological activity. Dietary vitamin E is passively absorbed with dietary lipids in the upper intestine. Vitamin E is incorporated into the mixed micelles composed of monoacylglycerols, fatty acids, bile acids, and phospholipids for transport to the enterocyte (Kayden & Traber, 1993). After uptake, vitamin E is secreted into chylomicrons, which are transported via the lymph to the liver and then distributed into the different lipoproteins to be delivered to tissues in the body. This process is similar for all forms of vitamin E and only after passage into the liver does it differ (Traber & Sies, 1996).

Despite the fact that various forms of vitamin E have similar antioxidant properties, forms of vitamin E other than α-tocopherol are not readily recognized by the hepatic α-tocopherol transfer protein (α-TTP) (Hosomi et al., 1997). Alpha (α)-TTP transfers liver α-tocopherol to extrahepatic tissues (Qian et al., 2005); α-TTP selectively combines with RRR-α-tocopherol rather than other tocopherols present in the liver, indicating specific preference for this stereoisomer (Hosomi et al., 1997; Brigelius-Flohe & Traber, 1999). Alpha (α)-TTP also binds more strongly with the other 2-R stereoisomers as compared to the 2-S stereoisomers. Alpha (α)-tocopherol is the prevalent form found in serum as a result of the action of hepatic α-TTP in exporting α-tocopherol from the liver.

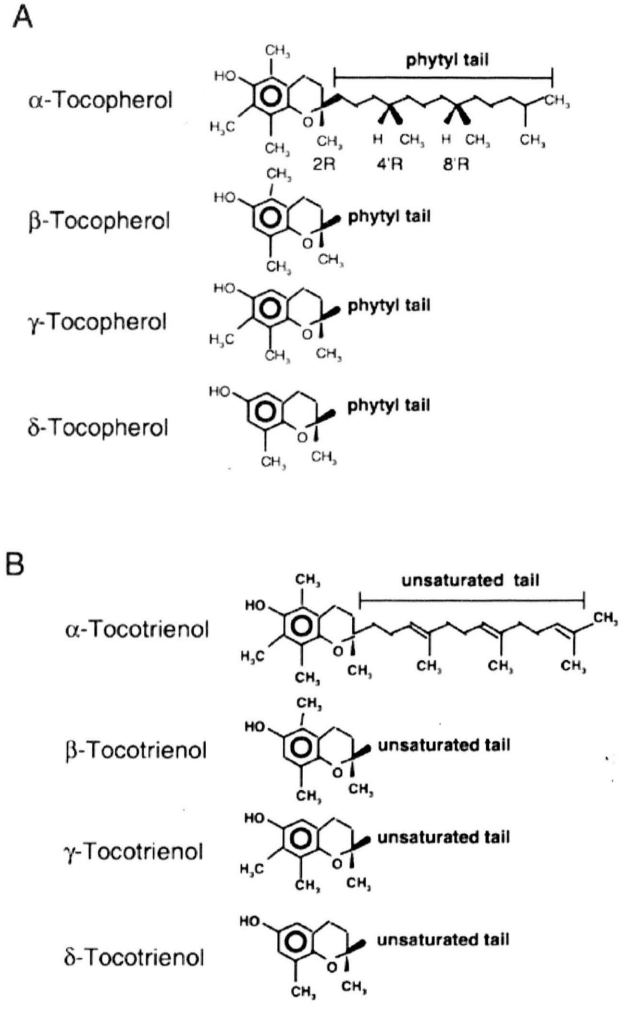

Figure 1. Tocopherol and Tocotrienol Structures

From the DRI Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids A Report of the Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Food and Nutrition, Washington, D.C.: National Academy of Press. Copyright 2000 by the National Academy of Sciences. Adapted from Traber, M. (1999). Vitamin E. In: Shils M, Olson, J, Shike, M, Ross, A (eds.), *Modern Nutrition in Health and Disease (pp372-362).* Baltimore, MD: Williams & Wilkins.

Vitamin E Recommendations

In the U.S., vitamin E intake is defined as 2-R isomers of α-tocopherol. The 2000 Dietary Reference Intakes (DRIs) increased the 1989 RDA for vitamin E from 10 and 8 mg of α-tocopherol equivalents (α-TE) per day for adult men and women, respectively to 15 mg of 2-R isomers of α-tocopherol for both men and women (IOM, Food and Nutrition Board, 2000). The EAR was set at 12 mg of 2-R isomers α-tocopherol per day for adult men and women. The recommendations set in the DRI represent a substantial increase in the recommended intakes for vitamin E and are now based on the α-tocopherol form only. Previously, the recommendations were based on α-TE, which included the biological activity of γ-tocopherol resulting in approximately 20% of the dietary intake of vitamin E provided as γ-tocopherol. The RDA limits the vitamin E intake recommendation to include only the natural form (RRR-) as well as the other three synthetic 2-R stereoisomer forms (RSR-, RRS-, and RSS-). The other naturally occurring 2-S forms of vitamin E are not considered in the latest recommendations for vitamin E intake due to lack of conversion to α-tocopherol and due to reduced hepatic binding to α-TTP for export (Hosomi et al.,1997). Gamma tocopherol is the most abundantly consumed tocopherol in the U.S. due to the consumption of vegetable oils, such as soybean oil, canola oil, and cottonseed oil (IOM, Food and Nutrition Board, 2000). However, achieving the RDA for vitamin E must be based solely on the consumption of α-tocopherol in the natural or synthetic form.

Function of Vitamin E

Vitamin E is a chain-breaking antioxidant that inhibits the peroxidation of lipids (Burton & Ingold, 1986; Packer, 1991). It has been hypothesized that α-tocopherol's major function is as a

free radical scavenger, with the primary importance of reacting with free radicals to reduce damage (Traber & Atkinson, 2007). All atoms, in their basic state, have an equal number of electrons and protons; therefore, there is no net charge. A free radical has an unpaired electron but does not have any net electrical charge due to being balanced by a proton (Eitenmiller & Lee, 2004). In the body, it is often an oxygen molecule that has lost an electron and will stabilize itself by stealing an electron from a nearby molecule. This process is autoxidation and is a chain process that is composed of three stages: initiation, propagation, and termination. Initiation of autoxidation occurs when an unsaturated fatty acid has a hydrogen molecule that is lost due to exposure of the lipid to light, heat, or radiation. When this lipid free radical is formed, it will then steal an electron from another molecule generating another lipid radical making this a self-perpetuating process. Antioxidants, such as vitamin E, protect against the development of damage from oxidation by donating a hydrogen atom to react with the free radical generated from either a fatty acid or a peroxide compound thereby breaking the chain reaction. This addition of the hydrogen to the free radical results in the original fatty acid or a stable hydrogen peroxide. Free radicals and lipid peroxidation have been implicated as causes for various chronic diseases, with polyunsaturated fatty acids serving a key part in this process (Traber & Atkinson, 2007). It is thought that alpha-tocopherol specifically functions to protect and maintain the integrity of long-chain polyunsaturated fatty acids in the membranes of cells. In addition to vitamin E's antioxidant capabilities, there is epidemiological evidence suggesting that high vitamin E intakes correlate with reduced risk of cardiovascular disease (Rimm et al., 1993; Stampfer et al., 1993). However, not all studies have found a protective benefit of vitamin E supplementation on reducing risk of developing major

cardiovascular events or cancer (Lee et al., 2005). Nonetheless, vitamin E is considered to be a nutrient with significant health benefits associated with antioxidant function.

Intake in the United States

Reports of overt deficiency in the U.S. are rare and typically seen only in individuals who are unable to absorb vitamin E or in those who have genetic alterations in their ability to maintain serum concentrations (IOM, Food and Nutrition Board, 2000). Therefore, dietary intakes among individuals in the U.S. are considered to be sufficient to prevent deficiency symptoms, such as peripheral neuropathy. At this time, there have been few experimental studies that have examined vitamin E deficiency in humans, but these studies have provided support for the current RDA. However, some studies indicate inadequate dietary intakes for both men and women. Maras et al. (2004) found that the majority of men and women in the U.S. failed to meet the current recommendations for vitamin E intake, with only 8.0% of men and 2.4% of women meeting the EAR. Furthermore, only 5.8% of men and 2.1% of women in the Southern region of the U.S. met the EAR as compared to 9.0% and 2.6% in the Northeastern region of the U.S.

Similar results were observed in data obtained from the NHANES (2001-2002), with only 5% of men and 4% of women meeting the RDA for vitamin E and only 10-11% and 7-8% meeting the EAR, respectively (Gao, Wilde, Lichtenstein, Bermudez, & Tucker, 2006). Another study examined a population subset, which included Hispanic and non-Hispanic elderly. Only 4.7% of women and 7.9% of men met the EAR for vitamin E from diet alone. Furthermore, 94% of Hispanics and 95% of non-Hispanics did not meet the vitamin E recommendation from diet alone (Gao, Martin et al., 2006). When intake from supplements was included in the calculation of vitamin

E intake, 81% of Hispanics and 65% of non-Hispanics still did not meet the EAR. Overall, non-Hispanic whites had higher total α-tocopherol intake than Hispanics (9.9 mg/d vs. 7.5 mg/d, P=0.008); however, these intakes were below the RDA. The differences in intake were due to higher vitamin E containing supplement use in non-Hispanic whites. However, plasma α-tocopherol concentrations were not significantly different between the two groups. The use of vitamin E containing supplements did not directly influence plasma concentration levels. Overall, a greater total α-tocopherol intake was positively associated with higher plasma α-tocopherol concentrations, even after the exclusion of supplement users.

Talegawkar et al. (2007) investigated α-tocopherol intakes of African-American adults participating in the Jackson Heart Study. Similar to other study findings, only 5.8% of men and 4.5% of women met the EAR for vitamin E from food alone. However, 44.2% of men and 49.2% of women met the recommendation from foods and supplement use. Therefore, despite prevalent vitamin E supplement use, more than half of the population did not meet the EAR for α-tocopherol. Snack chips, oils, salad dressings, and fish preparations contributed the most α-tocopherol to the participant's diet in this study.

Murphy, Subar, and Block (1990) utilized the data from 11,658 adults in the NHANES II to estimate intakes of vitamin E and polyunsaturated fatty acids in the U.S. At that time, the RDA was based on the 1989 recommendations of 10 and 8 mg of TE/day for men and women, respectively. Median intakes were considerably lower than recommended levels (7.3 and 5.4 mg/day) for men and women, respectively. Furthermore, the fruits-and-vegetables groups and the fats-and-oils group each provided 20% of the vitamin E in the diet, with breakfast cereals providing

approximately 9.3% of the total vitamin E intake. However, the food item providing the greatest amount of vitamin E per serving, when divided by race, was different. For whites, the food group contributing the greatest amount was fortified breakfast cereals providing 33.5 mg α-TE or 26.8 mg α-tocopherol per serving, approximately 8.2% of total vitamin E. For blacks, margarine ranked as the greatest contributor, with fortified cereals contributing 6.9% of total vitamin E. However, fortified breakfast cereals were not ranked in the top 25 contributors for blacks as compared to whites. Interestingly, Maras et al. (2004) reported that vitamin E consumption patterns had changed in recent years and noted a decrease in the consumption of the fats-and-oils group which contribute to lower amounts of vitamin E. Additionally, ready-to-eat cereal, sweet baked products, white breads, beef, oils, and salad dressings were the top contributors of α-tocopherol for men and women. On average, women consumed less than half of the EAR and men consumed just over half, with only a small fraction of men and women meeting the EAR. These findings raise the question as to whether the RDA of 15 mg/day and the EAR of 12 mg/day are achievable from the diet.

To answer this question, studies have examined the maximal amounts of α-tocopherol intake possible from foods alone. Utilizing data from the 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII), linear programming was used to determine the maximal amount of α-tocopherol intake (Gao, Wilde, Maras, Bermudez, & Tucker, 2004). A diet set at a total energy intake of 2000 kilocalories (kcals), along with a saturated fat intake limit of $\leq$ 10% of total energy as well as a total fat intake limit of $\leq$ 30% of total energy, was used to develop the maximal α-tocopherol diet. The maximal α-tocopherol diet contained six servings of breads and cereals,

eleven servings of fruits and vegetables, two servings of meat, one serving of milk and dairy products, zero fats and oils, and two servings of nuts and seeds. The current RDA could be met from foods for all age-sex groups, except women > 50 years, with a total fat restriction to ≤ 30% of total energy intake. Conversely, a fat intake of ≤ 30% of energy offered a limited amount of food group choices that could provide adequate α-tocopherol. Additionally, the diet had to contain nuts and seeds, which are not widely consumed in the U.S.

In a similar study, linear programming was also used to formulate diets with maximal amounts of α-tocopherol intake for four sex and age strata of participants of the NHANES 2001-2002 (Gao, Wilde et al., 2006). Five diet models were formulated to include a number of constraints. Diet model 1 included the constraints of food consumption and energy intake, model 2 added a sodium constraint (< 2300 mg/day), model 3 added a DRI constraint (set to meet each age-sex strata's RDA or AI for 15 essential nutrients), model 4 added a saturated fat constraint (≤ 10%), and model 5 added a fat constraint (between 20-35% of total energy). Diet model 1 provided 24.9 and 24.0 mg of α-tocopherol for men 19-50 years of age and > 50 years; 18.7 and 19.3 mg of α-tocopherol for women 19-50 years and > 50 years of age. When all nutrient constraints were added to the model, the amounts decreased to 19.9 and 19.5 mg of α-tocopherol for men 19-50 years of age and > 50 years; 15.8 and 15.4 mg of α-tocopherol for women 19-50 years and > 50 years of age. At least 25% of total energy must come from fat, to meet the RDA for vitamin E. The current RDA could be met from food alone for all age-sex strata but this could only be done with dietary choices that are typically not consistent with the American diet.

Sources of Vitamin E

Food sources that are rich in vitamin E include fats and oils used in cooking, with the primary source of vitamin E from edible vegetable oils (Murphy et al., 1990; Sheppard, Pennington, & Weihrauch, 1993). Many vegetable oils are consumed directly, and indirectly as ingredients in foods (DGAC, 2010). The major cooking oils in the U.S. include soybean and canola. Palm oil, soybean oil, and canola oil are the top three contributors for total worldwide consumption of vegetable oils (USDA, 2009). However, these commonly consumed oils contain a larger proportion of the tocopherol content as γ-tocopherol (Eitenmiller & Lee, 2004). Therefore, soybean and canola oils will not contribute as effectively as other oils in meeting the vitamin E DRI requirements. Conversely, there are several oils which have a higher α-tocopherol concentration, with approximately half of the total tocopherol present provided as α-tocopherol. Examples of these include wheat germ oil, sunflower oil, almond oil, cottonseed oil, safflower oil, and olive oil (Eitenmiller & Lee, 2004). However, not all of these oils are processed for use as direct oils in cooking in the U.S. Furthermore, processing of these oils results in decreasing amounts of tocopherol as compared to the amounts found in the crude oil state. Refining of oils allows for the removal of impurities in the crude oil to make it an edible product and more palatable. The final step of this process is deodorization which allows for any remaining undesirable byproducts to be removed resulting in two products, distillate and refined oil. This process results in a substantial loss of tocopherol (Eintenmiller & Lee).In addition to oils contributing to vitamin E intake, there are several food sources that contain high amounts of vitamin E as well as contribute to intake.

Food groups commonly considered to be rich in vitamin E include unprocessed cereal grains, nuts, fruits, vegetables, and meats. Foods high in vitamin E content are fortified, ready-to-eat cereals (1.6-13.4 mg/ounce [oz.]), sunflower seeds (7.4 mg/oz.), almonds (7.3 mg/oz.), sunflower oil (5.6 mg/tablespoon), and almond oil (5.33 mg/tablespoon) (USDA, 2008). Among fruits and vegetables, sources of α-tocopherol include avocados, mangos, soybeans, broccoli, asparagus, pumpkin, and dark leafy greens such as Swiss chard and mustard greens, although these rich food sources are consumed by relatively small numbers of adults (Maras et al., 2004).

In a cross-sectional study using the NHANES 2001-2002 data, the major dietary α-tocopherol contributors by food groups in the U.S. population were dark green vegetables, orange vegetables, and legumes (Bachman, Reedy, Subar, & Krebs-Smith, 2008). However, these foods were consumed below the recommended intakes, with approximately 5-6% of total vitamin E intake coming from each group. Major contributors of oil-and-fat sources were potato chips (16.3% of total vitamin E intake), salad dressing (13% of total vitamin E intake), and nuts/seeds (10.2% of total vitamin E intake). In general, Americans are not consuming the most nutrient-dense forms of basic food groups such as dark green and orange vegetables and are consuming more oil and fat products. However, ready-to-eat cereal (28.7% of total vitamin E intake) was the top contributor for the whole grain food group.

Vitamin-E fortified, ready-to-eat cereals are major contributors of α-tocopherol intake in the U.S. diet (Sheppard et al., 1993; Maras et al., 2004). In 2004, ready-to-eat cereals were the major contributors of α-tocopherol for both men and women (Maras et al.; 2004). Regionally, ready-to-eat cereal was the major contributor for people living in the Northeast and West, the second highest

contributor for people in the Midwest and the third highest contributor for people living in the South. The authors emphasized the need for education to the U.S. population to improve α-tocopherol intakes focusing on the importance to include more vitamin E rich foods such as nuts, seeds, and whole grains. Additionally, use of cooking oils that contain greater amounts of α-tocopherol, such as sunflower oil and cottonseed oil are also recommended.

Serum α-Tocopherol Concentrations

Despite vitamin E's importance and low intakes relative to the DRIs, few population-based studies have investigated dietary intake and effect on serum levels of the vitamin. Utilizing the NHANES III data, Ford and Sowell (1999) examined serum α-tocopherol concentrations among 16,295 U.S. adults. Approximately 27% of the U.S. population had a low serum α-tocopherol concentration (defined as < 20 μmol/L). Serum concentrations by sex were similar, with 29% of men and 28% of women having low concentrations. However, there were ethnic differences, with 41% of African-Americans, 28% of Mexican Americans, 26% of Whites, and 32% of Other participants all falling below < 20 μmol/L. Reasons for the racial differences in serum concentrations were unclear; however, dietary intake could possibly account for the difference. Sinha et al. (1993) examined external determinants of α-tocopherol status in non-supplement and supplement users. Vitamin E intake, total fat intake, and body mass index were the major determinants of α-tocopherol plasma concentrations in non-supplement users. Vitamin E intake was the only determinant of plasma α-tocopherol concentrations in supplement users. Additionally, a positive correlation between vitamin E intake and α-tocopherol status and a negative correlation between intake and γ-tocopherol status were reported.

The effect of consumption of a vitamin-E-rich diet as compared to taking a vitamin E supplement on vitamin E status was reported by McGavin, Mann, Skeaff, and Chisholm (2001). Participants were provided with ground hazelnuts (25 mg vitamin E/100 g), a canola-based margarine (15 mg vitamin E/100 g), and wheat germ oil (127 mg vitamin E/100 g). The supplement group was provided with a 200 International Unit (IU) RRR-α-tocopherol capsule. On a daily basis, participants in the vitamin E-rich diet were asked to consume 70 g of hazelnuts, 30 g of margarine, and 5 ml of wheat germ oil, which provided approximately 28 mg of vitamin E per day. By week 8, the vitamin-E-rich diet group increased consumption from 11 mg/day to 21 mg/day. Increased intake was achieved through consumption of the dietary sources containing vitamin E, which provided replacement of saturated fat foods in the diet by unsaturated fats rich in vitamin E, such as nuts and vegetables. The additional vitamin E intake resulted in a 3.4 μmol/L increase in plasma α-tocopherol at week 6 to 29.6 μmol/L (95% Confidence Interval (CI) 1.6-5.3) when compared to 24.5 μmol/L in the control group. The supplement group consumed the 200 IU RRR-α-tocopherol capsule daily for 8 weeks and had a significant increase in plasma α-tocopherol at week 2 to 44.3 μmol/L (95% CI 14.9), which continued through week 8. Therefore, both dietary vitamin E and supplemental vitamin E can contribute to increases in serum concentration. However, the extent of dietary modification required to achieve these increases may be difficult in practice. Supplementation appears to be the best means of bringing about a substantial increase in plasma α-tocopherol concentration.

Bioavailability of Vitamin E

Vegetable fats and oils are the major naturally occurring sources of vitamin E in the diet, demonstrating that the current trend to reduce fat consumption may be accompanied by a reduced consumption of vitamin E. It is known that vitamin E absorption requires the presence of fat; however, there is limited information on the influence of the fat quantity needed for optimal absorption and bioavailability. Additionally, to date there is limited knowledge on the influence of fortified breakfast cereal on α-tocopherol serum concentrations in either humans or animals. In an animal study, Mitchell, Grundel, and Jenkins (1996) investigated the bioavailability of all-rac-α-tocopherol in rats consuming fortified breakfast cereals. Mean plasma α-tocopherol concentrations in rats fed the vitamin E from cereal sources were not different from those consuming the tocopheryl acetate standard. However, lipid adjusted α-tocopherol levels in the cereal-fed rats were generally lower than those of the corresponding groups fed a standard of 35 mg or 70 mg/kilogram (kg) diet (see Table 1). In addition to plasma mean levels, the bioavailability of the cereals in tissue and plasma response was examined as compared to the standard acetate. The cereal which had the lowest bioavailability as compared to the standard was bran flakes, with low levels indicated in the heart and adipose tissue as well as in the plasma and the plasma to cholesterol ratio ($p < .05$). Corn flakes and whole grain did not differ as compared to the standard. Bioavailability of vitamin E from whole grain cereal and corn flakes was comparable to that of a RRR-α-tocopheryl acetate standard; however, extrapolation to humans may be limited.

In a study conducted by Brink, Haddeman, and Tijburg (1996), the absorption of vitamin E in the form of cold-water-soluble deuterium labeled (DL)-α-tocopheryl acetate by rats fed a very-

low-fat evening meal (7 g/kg), a low-fat evening meal (52 g/kg), or a high-fat evening meal (190 g/kg) was determined. Vitamin E concentrations were measured in rats consuming a meal without vitamin E or supplemented with 660 or 2700 mg DL- α-tocopheryl acetate/kg of product, producing final vitamin E concentrations of 0, 41.5, or 169.9 α-TE/kg. Absorption of vitamin E from a very-low-fat evening meal varied from 73-83% and depended on the vitamin E concentration.

Vitamin E absorption was decreased in rats consuming the very-low-fat evening meal with 41.5 α-TE as compared to the low-fat evening meal and the high-fat evening meal containing 41.5 α-TE. Absorption, when expressed as percent of intake, was decreased at higher vitamin E concentrations with lower amounts of fat, indicating a decreased efficiency of vitamin E absorption. Therefore, the addition of vitamin E, as DL-α-tocopheryl acetate, to very-low-fat and low-fat products may be helpful in meeting the vitamin E intake requirements.

In 2004, the effect of varying amounts of fat in a meal on absorption of vitamin E in humans was investigated (Jeanes, Hall, Ellard, Lee, & Lodge, 2004). Participants consumed 150 mg of a ^{2}H-labeled RRR-α-tocopheryl acetate capsule following a test meal, which included toast with butter (17.5 g of fat), cereal with full-fat milk (17.5 g of fat), cereal with semi-skimmed milk (2.7 g of fat), and water (0 g of fat). There was a significantly greater amount of ^{2}H-labeled α-tocopherol concentration in the plasma from the high-fat cereal meal as compared to the low-fat cereal meal, demonstrating that the amount of fat consumed at the time of vitamin E consumption influences absorption.

Table 1

Effect of Dietary Treatment on Plasma Lipid Adjusted α-Tocopherol Levels (μg/mg of cholesterol)

Dietary Treatment	α-tocopherol (mg/kg) diet	*α-tocopherol (μg/mg of cholesterol)*
Control	35	10.7 ± 1.0^{b}
Standard	35	$9.9 \pm 0.4^{b,c}$
	70	14.2 ± 1.1^{a}
Corn	35	$7.6 \pm 0.4^{c,d}$
	70	11.9 ± 0.4^{b}
Whole Grain	35	7.5 ± 1.2^{d}
	70	11.8 ± 0.8^{b}
Bran Cereal	35	8.0 ± 0.5^{d}
	70	10.8 ± 0.7^{b}

Note: Values with a different superscript are significantly different, $p < 0.05$. From Mitchell, G., Grundel, E., & Jenkins, M. (1996). Bioavailability for rats of vitamin E from fortified breakfast cereals. *Journal of Food Science,* 61, 1257-1260.

In a study conducted by Bruno, Leonard, Park, Zhao, and Traber (2006), human vitamin E requirements were assessed with the use of apples fortified with DL-α-tocopheryl acetate. The apples were consumed with a breakfast meal containing 0%, 6%, or 21% kcal from fat during three separate trials. Mean estimated percentage of labeled α-tocopherol absorbed increased as fat increased, with 33 ± 5% absorption occurring during the 21% fat trial. Given the 33% absorption rate associated with the 21% fat consumption, the amount of dietary vitamin E that would be needed to replace irreversible losses daily is ≤ 15 mg, which is supportive of the current DRI.

Vitamin E bioavailability from fortified breakfast cereal was compared to encapsulated supplements (Leonard, Good, Gugger, & Traber, 2004). The bioavailability of vitamin E after

consumption of 30-IU fortified cereal was 6 ± 2 times the 400-IU provided as a capsule. Bioavailability of vitamin E increased to 26 ± 8 times after consumption of the 400-IU fortified cereal as compared to the 400-IU provided as a capsule, which indicated a greater bioavailability from fortified breakfast cereal over the encapsulated supplement. The decrease in bioavailability of the 400-IU provided as a capsule may be due to a decrease in α-tocopherol absorption which could be, in part, due to simultaneous fat-free milk consumption. However, the breakfast cereals were also consumed with fat-free milk and an unexpected increase in vitamin E bioavailability occurred. Fine dispersal of α-tocopherol on the cereal flakes as compared to the capsule, which is a globular form, may have also caused the difference.

Consumption of fortified-breakfast cereals can be an important contributor to α-tocopherol intake and plasma concentrations of the vitamin. Inclusion of vitamin E fortified foods in planning for future intervention trials should be done. Additionally, the use of vitamin E fortified foods would be important to consider in attempts to increase the vitamin E intake of Americans, especially those who are consuming a low-fat diet as their vitamin E intake may be less than optimal.

Metabolism of Calcium

Calcium accounts for 1-2% of body weight in adult humans, with greater than 99% of total body calcium found in bones and teeth (IOM, Food and Nutrition Board, 1997). The remainder is found in the blood, extracellular fluid, and other tissues. Calcium combines with phosphate to form hydroxyapatite, the mineral portion of bones and teeth; therefore, calcium is essential for bone health. Calcium is absorbed by active transport and passive diffusion across the intestinal mucosa and facilitated by 1,25-dihydroxyvitamin D (1,25 $(OH)_2D$), the active form of vitamin D (Weaver &

Heaney, 1999). This mechanism of action accounts for the greatest absorption of calcium at moderate and even low calcium intakes. Passive diffusion occurs when individuals consume high calcium intakes. Fractional calcium absorption has long been recognized as inversely associated with calcium intake. It also varies throughout the lifecycle, with greatest needs found in infancy and decreasing as we age. Additionally, racial differences in calcium metabolism have been noted in both children and adults (Weaver & Heaney).

Once in the blood, calcium plays a vital role in vascular contraction, vasodilation, muscle contraction, and nerve transmission. Blood calcium levels are tightly regulated through the actions of parathyroid hormone (PTH), vitamin D, and calcitonin. If blood calcium concentrations decrease, PTH synthesis is triggered and released to act in three main areas. First, PTH promotes reabsorption of calcium in the distal kidney tubule. Next, production of 1,25 $(OH)_2D$ indirectly affects gastrointestinal absorption of calcium. Lastly, bone resorption of calcium is induced by PTH due to the low levels of calcium in the blood. Therefore, there is no direct biochemical assay which reflects calcium nutritional status as blood calcium is tightly controlled. Indirect indicators of calcium status have been used to determine calcium adequacy and include calcium intake in relation to fracture rates; calcium intake and bone mineral density; and calcium intake and risk for chronic disease. These types of indirect indicators were used to develop the AI for calcium (IOM, Food and Nutrition Board, 1997).

Calcium Recommendations

Recommendations for dietary calcium intake are based on the AI provided by the DRI (IOM, Food and Nutrition Board, 1997; IOM, Food and Nutrition Board, 2011). The recommended

level for men and women 19-50 years of age is 1000 mg/day, and for men and women 51 years of age and older, 1200 mg/day is recommended.

Sources of calcium include a variety of foods; however, 73% of the calcium in the American diet is supplied from milk products, followed by fruits, vegetables, and grain products. Dairy sources of calcium include, but are not limited to, non-fat yogurt (452 mg/8 oz.), fat-free milk (306 mg/8 oz.), and whole milk (276 mg/8 oz.) (USDA, 2008). Additionally, fortified breakfast cereals can provide a large amount of calcium per 1-ounce serving, ranging from 236-1043 mg. Other foods high in calcium include calcium fortified soy beverages (368 mg/cup), sardines (325 mg/3 oz.), and turnip greens (124 mg/1/2 cup).

Calcium Intake in the United States

Calcium intakes have long been below optimal levels based on sex, race, and age category. Data from the USDA 1987-88 Nationwide Food Consumption Survey were utilized to determine populations at most risk for below optimal calcium intake along with sources of calcium (Fleming & Heimbach, 1994). Mean per capita calcium consumption for the U.S. population was 737 mg daily. Intakes generally decreased with age for both males and females. Furthermore, calcium consumption varied by region, ethnicity, as well as income. The lowest per capita consumption rates were reported in the South, for non-Hispanic blacks with lowest incomes as compared to the highest per capita consumption in the West, for non-Hispanic whites with the highest incomes. Overall, a large percentage of the U.S. population was consuming less calcium than the current AI. Food sources of calcium by major food groups were also determined, with milk and milk products contributing 53% of calcium intakes, followed by milk as an ingredient (19%), and grains and grain

products (12%). Since the major contributor of calcium in the diet is from milk and milk products, some researchers have examined whether the current dairy recommendations are adequate.

In 2004, Fulgoni, Huth, DiRienzo, & Miller examined calcium intake of various age groups that met or exceeded the intake of dairy products and determined the number of dairy servings needed to lower the prevalence of inadequate calcium intake. Utilizing data from the CSFII 1994-1996, 1998, and the NHANES 1999-2000, calcium intake was determined for individuals who met the recommended daily dairy servings based on the Food Guide Pyramid (FGP). Consuming the recommended number of FGP servings for dairy met calcium intake recommendations; however, there were few people who actually met or exceeded the FGP dairy recommendations. For adults aged 19-50 years (n= 4913 subjects), only 1124 subjects met the dairy recommendations (3.2 servings/day; 1420.2 mg calcium/day), approximately 22.9% met the FGP recommendations. A substantial number of subjects, 3789, did not meet the FGP recommendations thus contributing to low calcium intakes. The average serving and calcium amounts consumed were 0.87 servings/day and 596.4 mg calcium/day. For adults 51 years of age and older, there were only 5.3% who met the recommendation for daily calcium intake. Furthermore, three dairy servings per day were required to meet or exceed the calcium intake recommendations (19+ yrs, 1241 $\pm$ 53 mg/day and 51+ yrs, 1217 $\pm$ 53 mg/day).

In a more recent study, older black and white women were matched according to socioeconomic status and age to determine daily dietary calcium intake as well as sources (Mojtahedi, Plawecki, Chapman-Novakofski, McAuley, & Evans, 2006). There were no significant differences in age for each group (black women 66.9 $\pm$ 6.2 years vs. white women 67.1 $\pm$ 5.5

years, p = .85). Dietary calcium intake between black and white women were not significantly different (974 ± 524 vs. 1070 ± 600 mg/day; p = .65). Additionally, there were no differences in total calcium intake between black and white women (1485 ± 979 vs. 1791 ± 887 mg/day; p = .15). The food sources that contributed the most to dietary calcium intake was dairy foods with intakes differing by race (black women, 402 ± 269 mg/day; white women, 603 ± 376 mg/day; p = .02). Fortified cereals were a major source of calcium for black women but not for white women. Calcium supplementation did not contribute to total calcium intake in both groups; however, more white women (n=23, 70%) than black women used supplements (n=19, 58%). There were no significant racial differences in the calcium intake from supplements (black women, n=19; 889 ± 605 vs. white women, n=23; 1034 ± 460 mg/day; p = .20). Both groups fell short of the recommendations for calcium intake, even with supplementation.

Plaweckit, Evans, Mojtahedi, McAuley, and Chapman-Novakofski (2009) further investigated calcium sources and adequacy of intake in black and white postmenopausal women. Calcium intakes were determined using a 24-hour recall and the calcium-focused food frequency questionnaire (CFFFQ). Calcium intakes, as determined from the CFFFQ, significantly correlated with the 24-hour recall for all food groups (p <.001) except vegetables (p = .08). However, the CFFFQ estimate for mean calcium intake, as compared to the 24-hour recall, was higher (221 mg/day [p <.001]). Inadequate intake, < 800 mg/day, was found in 56% of participants, using the 24-hour recall and 45% of participants using the CFFFQ. Specific group findings indicated that regardless of dietary assessment method used, white women had higher calcium intakes than black women. For the CFFFQ, white women consumed 1104 (632) mg/day as compared to black

women consuming only 768 (531) mg/day, p <.001. When utilizing the 24-hour recall, white women consumed 875 (429) mg/day as compared to black women who consumed 573 (365) mg/day, p <.001. The primary source for calcium in both groups was dairy products (55% for black women and 57% for white women), followed by grains (13% for both racial groups).

Recently, the association of calcium intake and physical activity to bone mass was measured by quantitative ultrasound (QUS) in both pre- and post-menopausal women (Dionyssiotis, Paspati, Trovas, Galanos, & Lyritis, 2010). Calcium intakes were assessed using a questionnaire and participants were then divided in two groups based on daily calcium consumption of either, greater than 800 mg/day or less than 800 mg/day. Additionally, participants were further divided into three groups based on activity level (sedentary, moderately active, and systematically active). Calcium consumption was inadequate in most groups with less than one-third of the pre- and post-menopausal women consuming more than 800 mg of calcium per day. QUS scores were determined according to daily calcium consumption in both pre- and post-menopausal women. The group with the highest QUS score was pre-menopausal women who consumed more than 800 mg per day and who were also physically active ($p < .05$). However, the QUS score did not differ in post-menopausal women regardless of the amount of daily calcium intake or physical activity level. Overall, QUS scores were substantially decreased and were associated with aging and menopausal status.

A major concern is how best to meet calcium needs in groups that are falling below levels of optimal dietary intake. Professional organizations agree that food should be the first priority in meeting calcium needs (National Institute of Health Consensus Panel on Optimal Calcium Intake,

1994; American Medical Association, Council on Scientific Affairs, 1997). For those who avoid or limit foods naturally rich in calcium, such as milk and other dairy foods, calcium-fortified foods can be consumed to assist in achieving recommended amounts (Bryant, Cadogan, & Weaver, 1999; Weaver, Proulux, & Heaney, 1999). However, the bioavailability of calcium that is consumed from calcium-fortified products may differ from that of milk (Gueguen & Pointillart, 2000).

Bioavailability of Calcium

Studies examining fortified food product absorption rates have reported mixed results. Calcium absorption from fortified ice cream was compared to calcium absorption from milk in adults, age 25 to 45 years (Van Der Hee et al., 2009). Participants consumed a standard portion of ice cream (60 g) fortified with milk minerals and contained a low level (3%) of butter fat (227 mg calcium); ice cream (60 g) fortified with milk minerals and contained a typical level (9%) of coconut oil (224 mg calcium); and a reduced-fat milk (1.7%) (223 mg calcium) for calcium comparison. Absorption of calcium was determined using dual-labeled, stable isotopes. The mean fractional absorption of calcium from the two ice cream products (3% butter fat ice cream, 26% $\pm$ 8%; 9% coconut oil ice cream, 28% $\pm$ 5%) was not significantly different from that of reduced-fat milk (31% $\pm$ 8%), with all products containing equal amounts of calcium. Overall, fortified ice cream can be a useful dietary source for calcium.

Another study found that a calcium-fortified soy beverage had decreased bioavailability as compared to cow's milk in 16 healthy men, with the calcium from the soy beverage absorbed at only 75% the efficiency of the milk (Heaney, Dowell, Rafferty, & Bierman, 2000). Additionally, it was noted that 60% more calcium (500 mg) would need to be added in the soy beverage to have a

comparable absorption to milk. In another study by Zhao, Martin, and Weaver (2005), calcium bioavailability of a calcium carbonate fortified soymilk was found to be comparable to cow's milk in young women (0.211 $\pm$ 0.057 vs. 0.217 $\pm$ 0.040). Fractional calcium absorption did not differ between the two sources. However, both calcium carbonate fortified soymilk and cow's milk fractional calcium absorption were higher in comparison to soymilk fortified with tricalcium phosphate (0.181 $\pm$ 0.039). Therefore, the type of calcium used in fortified food items must be considered.

In one study, the influence of calcium-fortified breakfast cereal in children on calcium absorption rates and bioavailability was examined. Participants were provided two servings of either a low-calcium cereal (39 mg/serving) or a calcium-fortified cereal (156 mg/serving) for 14 days (Abrams, Griffin, Davila, & Liang, 2001). Calcium absorption was determined using a stable, labeled isotope added to the milk or the calcium-fortified cereal. The fractional absorption rates of the milk and the fortified cereal were not significantly different. Additionally, milk fractional absorption did not differ when given along with the low-calcium cereal. There was a significant increase in calcium absorption from 215 $\pm$ 45 mg/day to 269 $\pm$ 45 mg/day with the addition of the calcium-fortified cereal ($p < .001$), indicating that a calcium-fortified cereal was beneficial in increasing calcium absorption (See Table 2).

Overall, breakfast cereal fortified with calcium can provide a practical approach to increasing calcium intakes in children and possibly adults. However, fortified breakfast cereals can vary in the amounts that calcium is provided, ranging from 246 mg -1033 mg per serving (USDA,

2008). Conversely, there are no data at this time on fortified breakfast cereal absorption rates when calcium is provided at 100% of the DV or 1000 mg.

Table 2

Calcium Status in Children Fed Low-Calcium Cereal as Compared to Fortified-Calcium Cereal

Variable	Cereal-Low	Cereal-Fortified	P value
Calcium intake (mg/day)	699 ± 58	912 ± 55	<.001
Total Calcium absorption (mg/day)	215 ± 45	269 ± 45	<.001
Calcium retention (mg/day)	116 ± 54	172 ± 52	<.001

Note: Significance set at $p<0.05$. From Abrams, S., Griffin, I., Davila, P., & Liang, L. (2001). Calcium fortification of breakfast cereal enhances calcium absorption in children without affecting iron absorption. *Journal of Pediatrics, 139*, 522-526.

Fortification of Breakfast Cereals

Food fortification is the addition of micronutrients (vitamins and minerals) to foods to ensure that minimum dietary requirements are met (World Health Organization, Food and Agriculture Organization of the United Nations, 2006). Additions are often made to commonly consumed food items, such as staples in the diet or condiments. Benefits of fortification include the prevention or decreasing the risk of below optimal levels of micronutrients in a population or a specific population group; the contribution of a micronutrient to correct a known deficiency in a population or a specific population group; the potential influence on dietary intakes and nutritional status; and the favorable influences of micronutrients on improving health status. Mass fortification

is the practice of fortifying foods that are commonly consumed such as cereals, condiments, and milk. Market driven fortification is also used in efforts to target public health efforts in meeting nutrient requirements. Fortified cereals available in the U.S. that provide a high amount of vitamin E (100 % of the DV) include Total® cereal, General Mills; Product 19®, Kellogg's; Smart Start Antioxidant®, Kellogg's; and Fiber Plus Antioxidant's®, Kellogg's. Fortified cereals available in the U.S. that provide a high amount of calcium (100% of the DV) include Total® cereal, General Mills (USDA, 2008; Kellogg's Product Information, 2011).

The most commonly utilized type of vitamin-E fortificant used is all-rac-alpha tocopheryl acetate (Bieri, Corash, & Hubbard, 1983).Tocopherol esters are stable and readily available for use in supplements and fortified foods. Calcium carbonate is the most commonly used form of calcium fortificant in food products such as cereals and bars (Rafferty, Walters, & Heaney, 2007). This is due to the relative low cost and the dry, chalky mouthfeel is less likely to be noticed in these types of products.

CHAPTER III

METHODOLOGY

Study Design

The effect of consuming a fortified breakfast cereal on vitamin E dietary intake and serum concentrations as well the concomitant influence on calcium intake among adults was examined. The number of participants needed to achieve adequate power for a repeated measures analysis of variance (ANOVA) design was determined to be 36 with alpha set at 0.05, power at 0.80, and an effect size of 0.40, utilizing G*Power 3.1.0 (Faul, Erdfelder, Lang, & Buchner, 2007). The study design included a 2-week pre-intervention period of normal diet consumption, followed by a 4-week intervention period. A provision of fourteen 1-ounce servings of General Mills Total Whole Grain® and fourteen 8-ounce servings of Horizon Organic® 2% milk in shelf stable cartons (optional) was provided for participants in the intervention group on a bi-weekly basis. Participants in the control group continued with their normal diet. See Appendix A for a flow diagram of the study design.

Participants

The participants were both men and women, 18 years or older, who were in self-reported good health. The number of participants who enrolled in the study was 43. Participants were randomly assigned (by a toss of a coin) to either the intervention or control group. Of the participants who enrolled, 21 were randomized to the intervention group (16 women and 5 men) and 22 were randomized to the control group (17 women and 5 men). One participant withdrew

from the study after the first two weeks which reduced the number to 21 participants in the control group (16 women and 5 men).

Protection of Human Participants

Permission to conduct this study was obtained from the Lamar University Institutional Review Board and the Texas Woman's University Institutional Review Board for the Houston Center. All participants were informed that participation was voluntary and there would be no penalty for refusing to take part or discontinuing participation at any time. Participants were made aware of any potential risks and benefits. All participants were given a $35 gift card at the conclusion of the study.

Data Collection Procedures

Participants were recruited through the distribution of fliers in the Department of Family & Consumer Sciences, Lamar University. Participants were provided with a verbal description of the study and a written informed consent. Participants were allowed time to ask questions, to read the informed consent, as well as adequate time to decide on study participation. Participants were then asked to sign and initial each page of the informed consent if they were electing to enroll in the study. A copy of the signed informed consent was provided to the participant. Inclusion criteria included participants of at least 18 years of age, in self-reported good health, and willing to consume fortified breakfast cereal. Exclusion criteria included females who were pregnant or planning to become pregnant during the 6-week study period. Participants could not have a food allergy or intolerance that would cause an inability to consume Total Whole Grain® cereal or a condition that would prevent them from undergoing an overnight, 8-12 hour fast. Participants could

not be taking a single or multi-vitamin supplement or medication that would influence serum lipid levels, including hormone replacement therapy. Neither race nor ethnicity was used as an inclusion or exclusion criteria.

Participants made a total of four visits to the Department of Family & Consumer Sciences at Lamar University. At the time of the first visit, participants were randomized by a toss of a coin to either the control group or the intervention group and were assigned a subject code. Participants also provided their age reported as their date of birth. The primary researcher measured weight and height in all participants without shoes using an electronic scale (Taylor (5553) Products Inc., Las Cruces) and a stadiometer (SECA Products Inc., Hanover). Height was measured and recorded to the nearest 0.1 centimeter and weight was measured and recorded to the nearest 0.1 pound. The primary researcher provided instructions to the participants regarding a 3-day diet (three days per week; two non-consecutive weekdays and one weekend day) as well as instructions on how to complete a diet diary. The instructions included how to best describe food items or nature of the food, the amount or the serving estimation, the time eaten, and where the food was consumed (home, car, work, or restaurant). Handouts were provided to participants regarding appropriate serving size and participants were offered measuring spoons and cups if needed. Participants were instructed to record their normal diet over the next two weeks, recording a total of six days. Blank diet diaries were provided for the two 3-day time periods and pre-marked with the participant's code number. Additionally, participants were given a study schedule outlining the dates for subsequent visits as well as the fasting blood draw dates (see Appendix B). Verbal

and written instruction was provided for fasting 8-12 hours after the last meal prior to the morning blood draw scheduled for the second visit date.

At the second visit, two weeks after the initial visit, from 7:00a.m. to 9:00a.m.the primary researcher weighed the participants without shoes and recorded to the nearest 0.1 pound; 10 ml of blood was drawn from the median cubital vein under fasting conditions (8-12 hours after last meal) by a registered nurse at the Lamar University health center; and diet diaries were returned to the primary researcher. A light breakfast that included fruit juice, fruit, and a granola bar was provided for the participants after completing the blood draw. Returned diet diaries were reviewed for completeness with clarifications made as necessary. For example, a diet diary entry of milk could be clarified to be milk, 2% fat, fortified with vitamin A based on participant feedback regarding the food item consumed. Participants were questioned as to the completeness and thoroughness in the recording of their intake, in an effort to avoid underreporting of intakes. Participants in the control group were provided blank diet diaries for the next two weeks and were instructed to continue their usual diet, recording for two 3-day time periods as previously instructed. Participants were reminded to not begin taking any multi-vitamin supplement or a single vitamin E or calcium supplement during the remainder of the study. Participants in the intervention group were provided blank diet diaries with an added compliance check-off sheet on the back of each diary (see Appendix C). Participants were instructed to continue documenting as previously instructed for the next two 3-day periods with the addition of the provided cereal recorded in the diary when consumed. Participants were provided with 14 individually labeled servings of Total Whole Grain® cereal and instructed to consume one serving per day. Participants were offered Horizon Organic®

shelf-stable 2% milk but were not required to take the milk or consume the cereal with milk. Participants were then instructed to use the check-off sheet on the back of the diet diaries to mark when the cereal was consumed and to return any uneaten cereal and/or milk. Blood samples were collected without use of an anticoagulant and allowed to clot for 30 minutes at 25°C. The blood was then centrifuged at 2500 revolutions per minute for 15 minutes. Using a transfer pipette, the top yellow serum layer was then placed into a tube coded with the time, date, and participant identification code for storage. Serum samples were stored at -80°C for three months.

At the third visit, diet diaries were returned for the previous two weeks and reviewed for completeness with clarifications made as necessary. Participants were questioned as to the completeness and thoroughness in the recording of their intake, in an effort to avoid underreporting of intakes. Participants assigned to the control group were provided blank diaries and instructed to record their usual diet for the final two 3-day periods as previously instructed. Participants assigned to the intervention group returned any uneaten cereal and/or milk and were provided with 14 individually labeled servings of Total Whole Grain® cereal and milk (if desired). Participants assigned to the intervention group were provided blank diaries with the compliance check-off list on the back and instructed to record their usual diet for the final two 3-day periods as previously instructed. Participants were instructed to return any uneaten cereal and/or milk at the final visit.

At the final visit, two weeks after the third visit, from 7:00a.m. to 9:00a.m the primary researcher weighed the participants without shoes and recorded to the nearest 0.1 pound; 10 ml of blood was drawn from the median cubital vein under fasting conditions (8-12 hours after last meal) by a registered nurse employed by the Lamar University health center; and diet diaries were

returned to the primary researcher. A light breakfast that included fruit juice, fruit, and a granola bar was provided to the participants after completing the blood draw. Returned diet diaries were reviewed for completeness with clarifications made as necessary which were obtained through a follow-up phone call as needed. Blood samples were collected without use of an anticoagulant and allowed to clot for 30 minutes at 25°C. The blood was then centrifuged at 2500 revolutions per minute for 15 minutes. Using a transfer pipette, the top yellow serum layer was then placed into a tube coded with the time, date, and participant identification code for storage. Serum samples were stored at -80°C for three months.

Analysis

Nutrient Intake Analyses

Intakes from the collected diet diaries were analyzed for total energy (calories), macronutrients, and micronutrients using the Nutrient Data System for Research (NDSR) software version 2010, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN. In addition, intakes of energy, macronutrients, as well as micronutrients provided by Total Whole Grain® cereal, were determined. The values provided by NDSR for Total Whole Grain® cereal that were of interest to this study were 12.8 mg of total α-tocopherol, 28.2 mg of all-rac-α-tocopherol, and 945 mg of calcium per 1 oz. serving. The values provided by the USDA National Nutrient Database for Standard Reference, Release 23 were 13.5 mg of total α-tocopherol and 1000 mg of calcium per 1 oz. serving (USDA, 2008). Therefore, no adjustments were made to the NDSR reported values due to the values not being significantly different between the two databases. A protocol was determined and utilized for food items that were not found in the

database which followed the NDSR guidelines for resolving missing food items. If a food item was missing and was a generic item, database review and selection of a similar food item was employed. If the food item was a brand name product, nutrient information from the product label or manufacturer's website was obtained for database review and selection of a similar food item was employed. Additionally, NDSR outlines data entry rules in the software manual which were utilized to maintain and ensure consistency in data entry. All diet diaries were reviewed and clarified as appropriate by the primary investigator, who is also a registered dietitian. Dietary data for each participant was aggregated into three 2-week blocks and was input in this format. Therefore, there were a pre-intervention period (weeks 1 & 2); a 2-week post-intervention period (weeks 3 & 4); and a 4-week post-intervention period (weeks 5 & 6) used for diet entry and analysis. Compliance for cereal consumption was assessed through the use of the compliance check-off sheet on the back of each of the diet diaries used by participants in the intervention group, diet diary review by the investigator, and the return of any uneaten cereal. Compliance for milk consumption was not assessed due milk consumption with the fortified cereal not being a required component of the study.

Serum Analyses

Serum samples were analyzed for concentrations of α-tocopherol and γ-tocopherol. Serum samples were deproteinized with ethanol, extracted with hexane, and quantified by high performance liquid chromatography as previously described by Siluk et al. (2007). See Appendix D for the full analysis details. The concentration of α-tocopherol in serum is influenced by the concentrations of lipids; therefore, serum lipid component concentrations were determined. The

lipid profile components included total cholesterol, high density lipoprotein (HDL-C), and triglycerides. All lipid serum analyses were conducted in at least duplicate with rechecks identified through the review of percent coefficient of variation (CV), which is the ratio of standard deviation to the mean along with identification of values outside normal ranges. Results with a CV higher than 7% and/or values outside the normal range were re-tested in duplicate. See Appendices E-G for a full description of the principle and procedure for each lipid component lab. The concentration of LDL-C was determined using the Friedewald's equation: LDL-C = total cholesterol - (HDL-C) - (Triglycerides/5) (Friedewald, Levy, & Fredrickson, 1972). Alpha-tocopherol levels are reported as both absolute and total cholesterol (lipid)-corrected values (Thurmham, Davies, Crump, Situnayake, & Davis, 1986).

Statistical Analyses

Data were checked for normal distribution through the review of the shape of the histograms and boxplots of the distribution scores. Student's *t*-test was used to determine differences in mean age, height, and weight. Gender differences between the intervention and control group were determined through the use of the Pearson's X^2.

Statistical analysis of dietary intake and serum data utilized repeated measures analysis of variance (ANOVA). A value of $p \leq .05$ was considered to be statistically significant. The main effects of treatment, time, and treatment by time interaction were determined. If the treatment by time interaction effect was significant, further all pair-wise comparison for post- hoc analyses were conducted. Both within-group and between-comparisons (control versus intervention) for dietary

intake, was carried out using the Least Significant Difference (LSD) *p* value of .05. Statistical analyses were conducted with SPSS version 17.0.0 (2008).

CHAPTER IV

RESULTS

The purpose of the study was to determine the effect of consuming fortified breakfast cereal on vitamin E and calcium status among adults using a randomized, controlled, parallel arm, open-label, trial design.

This study was designed to test the following null hypotheses:

1. There will be no difference in vitamin E (total α-tocopherol) or calcium intake between those who consume a fortified breakfast cereal (the Intervention Group) and those who do not (the Control Group).

   REJECT: There was a significant difference between the vitamin E (total α-tocopherol) and calcium intakes in the intervention group as compared to the control group.

2. There will be no differences in vitamin E (total α-tocopherol) or calcium intake over time in those who consume a fortified breakfast cereal (the Intervention Group) and those who do not (the Control Group).

   REJECT: There was a significant difference between the vitamin E (total α-tocopherol) and calcium intakes over time in the intervention group as compared to the control group for the time periods, weeks 3 & 4 and weeks 5 & 6.

3. There will be no interaction between vitamin E (total α-tocopherol) or calcium intake and time in those who consume a fortified breakfast cereal (the Intervention Group) and those who do not (the Control Group).

   REJECT: There was a significant interaction between the vitamin E (total α-tocopherol) and calcium intakes and time in the intervention group as compared to the control group for the time periods, weeks 3 & 4 and weeks 5 & 6.

4. There will be no differences in the lipid-corrected serum level of vitamin E (α-tocopherol) between those who consume a fortified breakfast cereal (the Intervention Group) and those who do not (the Control Group).

   FAIL TO REJECT: There was no significant difference between the intervention group and the control group for the lipid-corrected serum α-tocopherol levels.

Group Characteristics

Forty-three participants enrolled in the study with 42 participants completing the study. One female control participant withdrew from the study prior to the baseline blood draw. Of those completing the study, 10 were male and 32 were female. There were five males in the control group and five males in the intervention group with 16 females in the control group and 16 females in the intervention group. There were no significant between-group differences for sex, mean height, or mean initial weight (see Table 3). The mean age for the control group was 34.5 (10.5) and the intervention group was 33.1 (11.9) ($p = .684$); respectively. There were no between-group differences in the initial BMI scores, 26.8 (8.0) for the control group and 28.5 (7.0) for the intervention group ($p = .487$), (see Table 4). However, there was a significant within-group

difference in BMI over time for both the intervention and control group, p = .007, respectively. Further all pair-wise comparison indicated a significant within-group difference in BMI for both the intervention and control group, between the time frames of weeks 1 & 2 and weeks 3 & 4 (p = .003) (see Table 5). There was also a significant within-group difference for both the intervention and control group, between the times frames of weeks 1 & 2 and weeks 5 & 6 (p = .049). Additionally, there were no between-group differences for mean weight (p = .422).

Table 3

Group Characteristics

Variable	Control (*N*=21)	Intervention (*N*=21)	p-value
Sex, number (%)			
Male	5 (24)	5 (24)	.933
Female	16 (76)	16 (76)	
Age, mean years (SD)	34.5 (10.5)	33.1 (11.9)	.684
Height, mean cm (SD)	164.3 (6.7)	165.2 (9.5)	.729
Weight, mean kg (SD)	72.1 (21.0)	77.8 (20.3)	.372

Note: SD= Standard deviation. Pearson's X^2 was utilized for categorical variables. Student's t-test was utilized for continuous variables.

Table 4

BMI and Weight by Group Characteristic and Time

Variable	Control	Intervention	Time p-value	Treatment p-value	Treatment x Time p-value
BMI, mean (SD)					
Weeks 1 & 2	26.8 (8.0)[a]	28.5 (7.0)[a]	.007	.487	.758
Weeks 3 & 4	26.6 (7.9)[b]	28.3 (7.0)[b]			
Weeks 5 & 6	26.7 (8.2)[b]	28.3 (6.9)[b]			
Weight, mean kg (SD)					
Weeks 1 & 2	72.5 (21.0)[a]	77.8 (20.3)[a]	.005	.422	.829
Weeks 3 & 4	71.9 (20.7)[b]	77.2 (20.2)[b]			
Weeks 5 & 6	72.2 (21.2)[b]	77.3 (20.1)[b]			

Note: Significance level set at $P < 0.05$. For within-group time points, values are indicated by different superscripts if statistically significant, $P < 0.05$. Same letter superscripts indicate no statistical significance. For between-group differences, values are indicated by an asterisk if statistically significant, $P < 0.05$.

Table 5

BMI and Weight over Time

Variable	Time p-value
BMI, mean (SD)	
Between Weeks 1 & 2 and Weeks 3 & 4	.003*
Between Weeks 3 & 4 and Weeks 5 & 6	.299
Between Weeks 1 & 2 and Weeks 5 & 6	.049*
Weight, mean kg (SD)	
Between Weeks 1 & 2 and Weeks 3 & 4	.002*
Between Weeks 3 & 4 and Weeks 5 & 6	.283
Between Weeks 1 & 2 and Weeks 5 & 6	.041*

Note: Values indicated by an asterisk if statistically significant, $P < 0.05$, Least Significant Difference.

The intervention group, on average, weighed approximately 5 kg more at baseline as compared to the control group and that did not change over time. However, there were significant within-group differences for both the intervention and control groups, between the time frames of weeks 1 & 2 and weeks 3 & 4 (p =.002) as well as between weeks 1 & 2 and weeks 5 & 6 (p = .041), respectively (see Table 5).

Dietary Intake for Selected Macronutrients

Dietary intake of energy and selected macronutrients were examined using absolute values. There were no significant differences in energy intake or percent of selected macronutrients for the main effect of treatment, the main effect of time or the treatment by time interaction effect (see Table 6). However, there was a significant main effect of time for the monounsaturated fatty acid (MUFA) percentage (p = .046). There were no significant between-group differences (p = .614). Within the control and intervention groups, the MUFA percentage was significantly different between weeks 3 & 4 and weeks 5 & 6 (p = .009) (see Table 7).

Dietary intakes of selected macronutrients based on grams consumed per day were also examined. There was no significant between-group, within-group, or treatment by time interaction effect differences for grams of carbohydrate, protein, fat, or fiber (see Table 8). Dietary cholesterol (mg/day) was significantly different for the main effect of time (p = .04) with no between-group differences (see Table 9).

Table 6

Dietary Intake of Energy and Selected Percent Macronutrient by Group Characteristic and Time

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
Energy (kcal/day)					
Weeks 1 & 2	1751.0 (599.4)[a]	1526.0 (479.4)[a]	.282	.084	.912
Weeks 3 & 4	1732.6 (595.2)[a]	1466.2 (427.6)[a]			
Weeks 5 & 6	1658.6 (470.4)[a]	1416.7 (379.8)[a]			
% Carbohydrate					
Weeks 1 & 2	48.5 (8.5)[a]	48.3 (7.0)[a]	.066	.857	.768
Weeks 3 & 4	47.1 (10.4)[a]	48 2 (6.9)[a]			
Weeks 5 & 6	49.6 (8.4)[a]	49.8 (7.2)[a]			
% Protein					
Weeks 1 & 2	16.5 (4.9)[a]	17.7 (3.4)[a]	.226	.207	.999
Weeks 3 & 4	15.9 (3.1)[a]	17.1 (3.0)[a]			
Weeks 5 & 6	15.7 (3.6)[a]	16.9 (3.1)[a]			
% Fat					
Weeks 1 & 2	34.0 (6.3)[a]	32.7 (6.0)[a]	.225	.343	.957
Weeks 3 & 4	34.9 (8.0)[a]	33.1 (4.7)[a]			
Weeks 5 & 6	33.2 (6.2)[a]	31.9 (4.9)[a]			

Table 6 Continued

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
% MUFA					
Weeks 1 & 2	12.6 (3.1)[a]	12.4 (3.2)[a]	.046	.614	.612
Weeks 3 & 4	12.9 (3.1)[a]	12.7 (2.5)[a]			
Weeks 5 & 6	12.2 (3.0)[b]	11.4 (2.3)[b]			
% PUFA					
Weeks 1 & 2	7.4 (1.6)[a]	7.3 (2.6)[a]	.269	.904	.649
Weeks 3 & 4	8.2 (2.6)[a]	7.8 (2.2)[a]			
Weeks 5 & 6	7.8 (2.2)[a]	8.1 (1.9)[a]			
% SFA					
Weeks 1 & 2	11.4 (2.7)[a]	10.3 (7.8)[a]	.363	.164	.940
Weeks 3 & 4	11.1 (2.7)[a]	10.2 (2.2)[a]			
Weeks 5 & 6	10.7 (2.7)[a]	9.9 (2.4)[a]			

Note: MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; SFA = Saturated Fatty Acids. Significance level set at $P < 0.05$. For within-group time points, values are indicated by different superscripts if statistically significant, $P < 0.05$. Same letter superscripts indicate no statistical significance. For between-group differences, values are indicated by an asterisk if statistically significant, $P < 0.05$.

Table 7

Percent MUFA over Time

Variable	Time p-value
% MUFA	
Between Weeks 1 & 2 and Weeks 3 & 4	.522
Between Weeks 3 & 4 and Weeks 5 & 6	.009*
Between Weeks 1 & 2 and Weeks 5 & 6	.091

Note: Values indicated by an asterisk if statistically significant, $P < 0.05$, Least Significant Difference.

Table 8

Dietary Intake of Selected Macronutrients (g/day) by Group Characteristic and Time

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
Carbohydrate (g/day)					
Weeks 1 & 2	210.2 (70.4)[a]	192.0 (63.8)[a]	.383	.235	.959
Weeks 3 & 4	201.9 (69.2)[a]	182.7 (52.0)[a]			
Weeks 5 & 6	206.8 (61.0)[a]	184.2 (52.6)[a]			
Fat (g/day)					
Weeks 1 & 2	68.2 (33.2)[a]	58.0 (26.6)[a]	.138	.088	.807
Weeks 3 & 4	71.3 (32.8)[a]	56.2 (21.2)[a]			
Weeks 5 & 6	63.5 (23.6)[a]	52.3 (18.2)[a]			
Protein (g/day)					
Weeks 1 & 2	70.8 (27.0)[a]	67.1 (20.4)[a]	.140	.586	.973
Weeks 3 & 4	68.0 (27.3)[a]	63.8 (22.8)[a]			
Weeks 5 & 6	64.3 (20.4)[a]	61.5 (20.3)[a]			
Dietary Fiber (g/day)					
Weeks 1 & 2	16.9 (8.5)[a]	16.9 (8.5)[a]	.380	.155	.705
Weeks 3 & 4	15.6 (8.3)[a]	20.1 (10.7)[a]			
Weeks 5 & 6	15.7 (7.0)[a]	19.8 (8.8)[a]			

Note: Significance level set at $P<0.05$. For within-group time points, values are indicated by different superscripts if statistically significant, $P < .05$. Same letter superscripts indicate no statistical significance. For between-group differences, values are indicated by an asterisk if statistically significant, $P < .05$.

Table 9

Dietary Intake of Selected Lipids by Group Characteristic and Time

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
Cholesterol (mg/day)					
Weeks 1 & 2	209.8 (124.1)[a]	178.7 (108.3)[a]	.040	.262	.545
Weeks 3 & 4	220.8 (130.0)[a]	171.1 (105.9)[a]			
Weeks 5 & 6	178.2 (105.5)[b]	156.5 (80.0)[b]			
MUFA (g/day)					
Weeks 1 & 2	25.4 (14.2)[a]	22.3 (11.9)[a]	.119	.175	.937
Weeks 3 & 4	26.2 (12.3)[a]	21.7 (9.5)[a]			
Weeks 5 & 6	23.7 (9.6)[a]	19.2 (8.6)[a]			
PUFA (g/day)					
Weeks 1 & 2	14.8 (6.4)[a]	13.7 (8.5)[a]	.511	.269	.351
Weeks 3 & 4	16.9 (8.9)[a]	13.3 (5.7)[a]			
Weeks 5 & 6	14.8 (6.0)[a]	13.5 (5.3)[a]			
SFA (g/day)					
Weeks 1 & 2	22.6 (11.3)[a]	17.5 (6.3)[a*]	.143	.022	.920
Weeks 3 & 4	22.7 (10.2)[a]	17.1 (6.5)[a*]			
Weeks 5 & 6	20.4 (8.5)[a]	15.7 (5.1)[a*]			

Note: Significance level set at $P < 0.05$. For within-group time points, values are indicated by different superscripts if statistically significant, $P < 0.05$. Same letter superscripts indicate no statistical significance. For between-group differences, values are indicated by an asterisk if statistically significant, $P < 0.05$.

Dietary cholesterol intakes within both groups was significantly different between weeks 1 & 2 and 5 & 6 as well as between weeks 3 & 4 and 5 & 6 (p = .024; p = .015) (see Table 10).

Table 10

Cholesterol over Time

Variable	Time p-value
Cholesterol (mg/day)	
Between Weeks 1 & 2 and Weeks 3 & 4	.979
Between Weeks 3 & 4 and Weeks 5 & 6	.015*
Between Weeks 1 & 2 and Weeks 5 & 6	.024*

Note: Values indicated by an asterisk if statistically significant, $P < 0.05$, Least Significant Difference.

Between-group and within-group differences were not significantly different for grams of monounsaturated or polyunsaturated fat (see Table 9). Additionally, there was no significant treatment by time interaction effect. There was a significant difference between the groups for grams of saturated fat intake (p = .022). The control group consumed a mean higher level at weeks 1 & 2 and continued throughout the duration of the study as compared to the intervention group.

Intervention Group Compliance and Mean Intakes

Compliance for the intervention group was examined for males and females for each week during the intervention period. There were no significant differences between males and females for compliance of mean intakes for selected micronutrients (see Table11 and 12). Figure 2 shows the consistency of intake for total α-tocopherol and synthetic α-tocopherol (all-rac-α-tocopherol) throughout the intervention period.

Dietary Intake for Selected Micronutrients

Intakes of the selected micronutrients were examined using absolute values as well as energy-adjusted values (mg/1000 kcal). Total (natural + synthetic) dietary α-tocopherol levels were significantly different between the intervention and the control group at weeks 3 & 4 and weeks 5 & 6 as compared to weeks 1 & 2 ($p < .001$; $p < .001$) (see Table 13 and 14). There were no significant differences between weeks 3 & 4 and weeks 5 & 6 for the intervention group ($p = .485$). Additionally, there was a treatment by time interaction effect for total dietary α-tocopherol ($p < .001$).

Synthetic α-tocopherol (all-rac-α-tocopherol) intake was significantly higher in the intervention group as compared to the control group at weeks 3 & 4 and weeks 5 & 6 ($p<.001$) (see Table 13 & 14). There were no significant differences between weeks 3 & 4 and weeks 5 & 6 for the intervention group ($p = .204$). There were no within-group differences for the control group over time. An interaction of treatment by time effect for synthetic α-tocopherol intake was also found ($p < .001$), respectively. Conversely, there were no significant between-group or within-group differences for natural α-tocopherol intake ($p = .738$; $p = .519$).

As shown in Table 13, there was a significant between-group difference for γ-tocopherol intake throughout the duration of the study. The control group consumed

Table 11

Total Whole Grain© Cereal Consumption Compliance and Mean Vitamin E Intakes (mg/day) by Sex

Variable	Week 3 Mean (SD)	Week 4 (Mean) SD	Week 5 Mean (SD)	Week 6 Mean (SD)
% Consumed				
Male (n=5)	100.0 (0.0)[a]	100.0 (0.0)[a]	100.0 (0.0)[a]	100.0 (0.0)[a]
Female (n=16)	96.4 (14.3)[a]	97.3 (10.8)[a]	95.6 (17.8)[a]	97.3 (7.8)[a]
Total α-tocopherol (mg/day)				
Male (n=5)	12.8 (0.0)[a]	12.8 (0.0)[a]	12.8 (0.0)[a]	12.8 (0.0)[a]
Female (n=16)	12.3 (1.8)[a]	12.5 (1.4)[a]	12.8 (2.3)[a]	12.5 (1.0)[a]
All-rac-α-tocopherol (mg/day)				
Male (n=5)	28.2 (0.0)[a]	28.2 (0.0)[a]	28.2 (0.0)[a]	28.2 (0.0)[a]
Female (n=16)	27.2 (4.0)[a]	27.4 (3.0)[a]	26.9 (5.0)[a]	27.4 (2.2)[a]

Note: SD = Standard Deviation. Significance level set at $P < 0.05$. Same letter superscripts indicate no statistically significant differences.

Table 12

Total Whole Grain© Cereal Mean Calcium Intakes (mg/day) by Sex

Variable	Week 3 Mean (SD)	Week 4 (Mean) SD	Week 5 Mean (SD)	Week 6 Mean (SD)
Calcium (mg/day)				
Male (n=5)	945.0 (0.0)[a]	945.0 (0.0)[a]	945.0 (0.0)[a]	945.0 (0.0)[a]
Female (n=16)	911.3 (135.0)[a]	919.7 (101.3)[a]	902.8 (168.8)[a]	919.7 (73.4)[a]

Note: SD = Standard Deviation. Significance level set at $P < 0.05$. Same letter superscripts indicate no statistically significant differences.

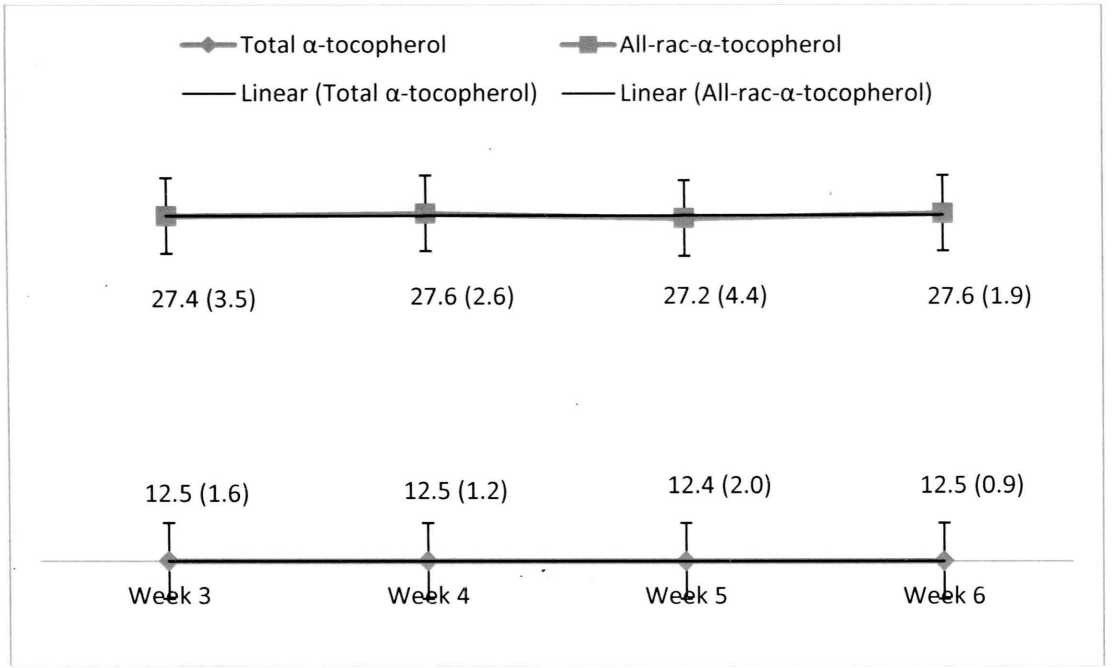

Figure 2

Mean Intake (mg/day) of Vitamin E Consumed from Total Whole Grain© Cereal per Week

Table 13

Dietary Intake of Selected Micronutrients by Group Characteristic and Time

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
Total α-tocopherol (mg/day)					
Weeks 1 & 2	8.0 (5.5)[a]	7.8 (4.8)[a]	<.001	<.001	<.001
Weeks 3 & 4	8.1 (7.0)[a]	19.5 (5.1)[b*]			
Weeks 5 & 6	8.7 (5.6)[a]	19.7 (4.3)[b*]			
All-rac-α-tocopherol (mg/day)					
Weeks 1 & 2	1.1 (2.4)[a]	2.0 (3.1)[a]	<.001	<.001	<.001
Weeks 3 & 4	1.6 (3.3)[a]	28. 9 (7.1)[b*]			
Weeks 5 & 6	3.1 (5.5)[a]	29.2 (14.4)[b*]			
Natural α-tocopherol (mg/day)					
Weeks 1 & 2	5.2 (9.8)[a]	5.1 (8.8)[a]	.738	.519	.935
Weeks 3 & 4	4.7 (10.1)[a]	5.2 (7.9)[a]			
Weeks 5 & 6	5.1 (9.5)[a]	5.1 (7.8)[a]			
Total γ-tocopherol (mg/day)					
Weeks 1 & 2	12.9 (5.7)[a]	10.4 (5.9)[a*]	.940	.015	.667
Weeks 3 & 4	14.3 (7.9)[a]	9.9 (4.4)[a*]			
Weeks 5 & 6	13.9 (6.3)[a]	10.2 (4.5)[a*]			

Table 13 Continued

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
Calcium (mg/day)					
Weeks 1 & 2	720.2 (315.7)[a]	751.0 (345.7)[a]	<.001	<.001	<.001
Weeks 3 & 4	662.6 (284.7)[a]	1618.0 (366.4)[b*]			
Weeks 5 & 6	652.1 (259.1)[a]	1655.9 (334.4)[b*]			

Note: Significance level set at $P < 0.05$. For within-group time points, values are indicated by different superscripts if statistically significant, $P < 0.05$. Same letter superscripts indicate no statistical significance. For between-group differences, values are indicated by an asterisk if statistically significant, $P < 0.05$

Table 14

Micronutrients over Time

Variable	Time p-value
Total α-tocopherol (mg/day)	
Between Weeks 1 & 2 and Weeks 3 & 4	<.001*
Between Weeks 3 & 4 and Weeks 5 & 6	.485
Between Weeks 1 & 2 and Weeks 5 & 6	<.001*
All-rac-α-tocopherol (mg/day)	
Between Weeks 1 & 2 and Weeks 3 & 4	<.001*
Between Weeks 3 & 4 and Weeks 5 & 6	.204
Between Weeks 1 & 2 and Weeks 5 & 6	<.001*
Calcium (mg/day)	
Between Weeks 1 & 2 and Weeks 3 & 4	<.001*
Between Weeks 3 & 4 and Weeks 5 & 6	.737
Between Weeks 1 & 2 and Weeks 5 & 6	<.001*

Note: Values indicated by an asterisk if statistically significant, $P < 0.05$, Least Significant Difference.

significantly higher amounts of γ-tocopherol at weeks 1 & 2, weeks 3 & 4, and at weeks 5 & 6 as compared to the intervention group (p = .015). This was due to the consumption of foods containing fats such as soybean oil. Dietary calcium intakes were significantly different between the control group, 662.6 (284.7) mg/day, and the intervention group, 1618.0 (366.4) mg/day, at weeks 3 & 4 (p <.001) (see Table 13 & 14). At weeks 5 & 6, dietary calcium intakes remained to be significantly different between the groups (p <.001). There were no significant differences between the control group and the intervention group at weeks 1 & 2 (p=.737) (see Table 13). An interaction of treatment by time effect for calcium intake was also found (p <.001), respectively.

Energy-Adjusted Micronutrients

Energy-adjusted values (mg/1000 kcal) for the selected micronutrients were significantly different between the intervention and the control group for dietary total α-tocopherol, synthetic α-tocopherol (all-rac-α-tocopherol), and calcium, $p < .001$ (see Table 15). Similarly, energy-adjusted values for γ-tocopherol remained significantly higher in the control group as compared to the intervention group ($p = .045$). There were no differences for energy-adjusted natural α-tocopherol intakes.

Serum Lipid Concentrations

As shown in Table 16, serum total cholesterol, triglyceride, HDL-C, and LDL-C concentrations were not significantly different between the intervention and control groups. Within the groups, significantly higher amounts were noted for post total cholesterol concentrations as compared to baseline concentrations ($p < .001$). Additionally, HDL-C levels for both the control and intervention groups were significantly higher for the post blood draw as compared to the pre blood draw ($p < .001$). LDL-C levels were also significantly higher for the post blood draw when compared to the baseline concentration ($p = .017$). There was a significant treatment by time interaction effect for LDL-C/HDL-C ratio ($p = .028$).

Serum Tocopherol Concentrations

Serum tocopherol concentrations were examined using absolute values as well as lipid-corrected values. There were no between-group, within-group, or treatment by time interaction effect for absolute serum α- and serum γ- tocopherol concentrations (see Table 17).

Table 15

Energy-Adjusted Dietary Intake of Selected Micronutrients by Group Characteristic and Time

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
Total α-tocopherol (mg/1000kcal)					
Weeks 1 & 2	4.4 (2.0)[a]	4.9 (1.9)[a]	<.001	<.001	<.001
Weeks 3 & 4	4.3 (2.6)[a]	14.0 (3.7)[b*]			
Weeks 5 & 6	5.4 (3.2)[a]	14.5 (3.2)[c*]			
All-rac-α-tocopherol (mg/1000kcal)					
Weeks 1 & 2	0.6 (1.2)[a]	1.2 (1.7)[a]	<.001	<.001	<.001
Weeks 3 & 4	0.9 (1.5)[a]	21.4 (7.7)[b*]			
Weeks 5 & 6	2.3 (4.2)[a]	22.4 (8.0)[b*]			
Natural α-tocopherol (mg/1000 kcal)					
Weeks 1 & 2	4.2 (1.5)[a]	4.4 (1.6)[a]	.701	.739	.597
Weeks 3 & 4	3.9 (2.2)[a]	4.4 (1.4)[a]			
Weeks 5 & 6	4.3 (2.4)[a]	4.4 (1.3)[a]			
Total γ-tocopherol (mg/1000kcal)					
Weeks 1 & 2	7.5 (2.3)[a]	6.6 (2.5)[a*]	.523	.045	.912
Weeks 3 & 4	8.0 (3.5)[a]	6.7 (2.4)[a*]			
Weeks 5 & 6	8.3 (3.1)[a]	7.0 (1.9)[a*]			

Table 15 Continued

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
Calcium (mg/1000 kcal)					
Weeks 1 & 2	423.5 (139.1)[a]	489.9 (191.3)[a]	<.001	<.001	<.001
Weeks 3 & 4	392.7 (135.2)[a]	1167.7 (306.4)[b*]			
Weeks 5 & 6	402.5 (144.0)[a]	1239.4 (363.3)[b*]			

Note: Significance level set at $P < 0.05$. For within-group time points, values are indicated by different superscripts if statistically significant, $P < 0.05$. Same letter superscripts indicate no statistical significance. For between-group differences, values are indicated by an asterisk if statistically significant, $P < 0.05$.

Table 16

Serum Concentrations of Lipid Components by Group Characteristic and Time

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
Total Cholesterol (mg/dL)					
Pre-intervention	143.5 (34.7)[a]	153.0 (44.0)[a]	<.001	.634	.072
Post-intervention	186.1 (37.7)[b]	167.7 (38.1)[b]			
Triglycerides (mg/dL)					
Pre-intervention	96.9 (89.9)[a]	85.1 (56.6)[a]	.458	.867	.077
Post-intervention	87.8 (58.9)[a]	107.1 (95.5)[a]			
HDL-C (mg/dL)					
Pre-intervention	46.2 (9.6)[a]	46.0 (12.4)[a]	<.001	.764	.474
Post-intervention	51.3 (9.8)[b]	53.2 (9.3)[b]			
LDL-C (mg/dL)					
Pre-intervention	81.3 (32.7)[a]	88.9 (46.1)[a]	.017	.397	.056
Post-intervention	117.2 (39.8)[b]	93.1 (38.6)[b]			
Total cholesterol/HDL-C ratio					
Pre-intervention	3.3 (1.3)[a]	3.6 (1.6)[a]	.874	.919	.326
Post-intervention	3.5 (1.5)[a]	3.3 (1.1)[a]			

Table 16 continued

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
LDL-C/HDL-C ratio					
Pre-intervention	1.8 (1.0)[a]	2.2 (1.4)[a]	.472	.615	.028
Post-intervention	2.5 (1.2)[a]	1.8 (0.8)[a]			
Triglyceride/HDL-C ratio					
Pre-intervention	2.5 (3.3)[a]	2.1 (1.8)[a]	.511	.934	.176
Post-intervention	1.8 (1.6)[a]	2.3 (2.8)[a]			

Note: Significance level set at $P < 0.05$. For within-group time points, values are indicated by different superscripts if statistically significant, $P < 0.05$. Same letter superscripts indicate no statistical significance. For between-group differences, values are indicated by an asterisk if statistically significant, $P < 0.05$.

Table 17

Serum Concentrations of Tocopherols and Lipid-Corrected Tocopherols by Group Characteristic and Time

Variable	Control Mean (SD)	Intervention Mean (SD)	Time p-value	Treatment p-value	Treatment x Time p-value
α-Tocopherol (μmol/L)					
Pre-intervention	9.7 (7.1)[a]	9.5 (3.6)[a]	.805	.657	.175
Post-intervention	8.7 (5.4)[a]	10.2 (3.9)[a]			
γ-Tocopherol (μmol/L)					
Pre-intervention	1.2 (0.7)[a]	1.5 (0.7)[a]	.315	.243	.725
Post-intervention	1.1 (0.8)[a]	1.3 (0.8)[a]			
α-Tocopherol / Total Cholesterol (μmol/millimoles)					
Pre-intervention	6.3 (4.0)[a]	5.8 (2.3)[a]	.006	.554	.036
Post-intervention	4.2 (2.2)[b]	5.5 (1.7)[b]			
γ-Tocopherol / Total Cholesterol (μmol/millimoles)					
Pre-intervention	0.8 (0.5)[a]	0.9 (0.5)[a]	.005	.238	.753
Post-intervention	0.6 (0.3)[b]	0.7 (0.4)[b]			

Note: Significance level set at $P < 0.05$. For within-group time points, values are indicated by different superscripts if statistically significant, $P < 0.05$. Same letter superscripts indicate no statistical significance. For between-group differences, values are indicated by an asterisk if statistically significant, $P < 0.05$

Between groups, lipid-corrected α-tocopherol and γ-tocopherol concentration values were not significantly different ($p = .554$) (see Table 17). Within the groups, the lipid-corrected α-tocopherol and γ-tocopherol concentrations significantly decreased for the post blood draw, ($p = .006$; $p = .005$). There was a significant treatment by time interaction effect for lipid-corrected α-tocopherol ($p = .036$).

CHAPTER V

DISCUSSION

The purpose of this study was to examine the effects of consuming a fortified breakfast cereal on dietary vitamin E and calcium intakes as well as the influence on serum α-tocopherol concentrations among adults. Vitamin E and calcium are two nutrients considered to be shortfall nutrients in the typical American diet. This intervention study demonstrated that consumption of a fortified breakfast cereal, in addition to a normal diet, increased dietary intakes of total α-tocopherol, in the form of all-rac-α-tocopherol, as well as calcium intake within the intervention group as compared to the control group. However, there were no significant between-group differences for serum concentration of α-tocopherol. Additionally, there were no significant between-group differences for the lipid-corrected α-tocopherol concentrations. Within-group differences, however, were demonstrated, with lipid-corrected α-tocopherol levels decreasing in both groups. The control group pre-intervention level was 6.3 (4.0) μmol/mmol and the post-intervention was 4.2 (2.2) μmol/mmol. The intervention group pre-intervention level was 5.8 (2.3) μmol/mmol and the post-intervention was 5.5 (1.7) μmol/mmol.

Dietary α-Tocopherol Intake

To date, this is the first study examining the influence of fortified breakfast cereal on vitamin E intake among adults in the U.S. In a study conducted by Leonard et al. (2004), fortified breakfast cereal bioavailability was found to be high in comparison to an encapsulated supplement

form. The findings support the ability of fortified foods, like fortified breakfast cereal, to act as a suitable carrier for vitamin E. Therefore, it was recommended future studies investigate the use of vitamin E-fortified foods as part of intervention trials in efforts to increase vitamin E intakes in Americans.

Dietary intake of total α-tocopherol as well as all-rac-α-tocopherol was significantly increased in the intervention group as compared to the control group at weeks 3 & 4 and weeks 5 & 6. Additionally, within the intervention group there were significant differences at weeks 3 & 4 and weeks 5 & 6 when compared to weeks 1 & 2. These differences continued when intakes were adjusted for energy (per 1000 kcals). There were no between-group differences for energy or macronutrients (CHO, fat, and protein) at weeks 1 & 2; weeks 3 & 4; or at weeks 5 & 6 demonstrating the consumption of fortified breakfast cereal was substituted for other food items usually consumed by the intervention group.

Secondary data analysis gathered from two national surveys conducted in the U.S., the 2001-2002 NHANES and the CSFII (1994-1996), have indicated below optimal intakes of vitamin E (Gao et al., 2006; Gao et al., 2004). Furthermore, the 2010 Dietary Guidelines Advisory Committee recommends increasing the consumption of oils as compared to the large amounts of solid fats that are currently consumed. Due to the dietary emphasis on these solid fats, the intakes of important fat-soluble vitamins and essential fatty acids may be consumed below optimal levels. Additionally, the IOM, Food and Nutrition Board (2000) cautions that low-fat diets might provide insufficient amounts of fat soluble nutrients unless Americans choose foods carefully and increase their

intakes of nuts, seeds, fruits, and vegetables. Other studies have made these same recommendations as well as indicated that a fat intake of ≤ 25 to 30% of energy intake offers only a small number of food options that provide adequate amounts of α-tocopherol (Maras et al., 2004; Gao, Wilde, et al., 2006). Food source options for consumers that are naturally high in α-tocopherol are shown in Table 18. However, these foods may not be readily consumed in the typical American diet.

In addition to the 2010 Dietary Guidelines for Americans, three food guides are commonly used in nutrition education efforts for the American population. These include the USDA's MyPyramid (2005), Harvard's Healthy Eating Pyramid (Willett & Skerrett, 2005), and the National Heart, Lung, and Blood Institute (NHLBI) Dietary Approaches to Stop Hypertension Healthy Eating Plan (2006). In a review of the three food guides, Reedy and Krebs-Smith (2008) examined the nutrient estimates provided by each of the plans as compared to the DRI recommendations.

Primary differences were found between the food guide recommendations for total oil consumption, amount of dairy products, types of vegetables, and protein sources. Overall, nutrient values were similar between all three plans excluding vitamin A, vitamin E, and calcium, which were due to differences in the plans' recommendations. The differences between the three plans for the amount of vitamin E and calcium recommended are outlined in Table 19.

There is a need for a consistent message with regards to vitamin E intake along with feasible ways such as commonly consumed food sources to improve intake. With the focus on low-fat consumption and the increased awareness regarding risk for developing heart disease, it is

Table 18

Food Sources Naturally High in Vitamin E (mg) per Serving

Food Source	Vitamin E (mg) per Serving
Wheat germ oil, 1 tablespoon	20.3
Almonds, dry roasted, 1 ounce	7.4
Sunflower seeds, dry roasted, 1 ounce	6.0
Sunflower oil, 1 tablespoon	5.6
Safflower oil, 1 tablespoon	4.6
Hazelnuts, dry roasted, 1 ounce	4.3
Peanut butter, 2 tablespoons	2.9
Peanuts, dry roasted, 1 ounce	2.2
Corn oil, 1 tablespoon	1.9
Spinach, boiled, ½ cup	1.9
Broccoli, chopped, boiled, ½ cup	1.2
Soybean oil, 1 tablespoon	1.1
Kiwifruit, 1 medium	1.1
Mango, sliced, ½ cup	0.9
Tomato, raw, 1 medium	0.8

Source: United States Department of Agriculture, Agricultural Research Service. (2008). USDA National Nutrient Database for Standard Reference, Release 23. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl

Table 19

Comparison of Vitamin E and Calcium Recommendations Taken From Three Food Guides

Nutrient	DRI	USDA MyPyramid		NHLBI Eating Plan		Harvard Eating Pyramid	
		Calculated Intake/Day	*% RDA or AI*	*Calculated Intake/Day*	*% RDA or AI*	*Calculated Intake/Day*	*% RDA or AI*
Vitamin E Males & Females >19 years (mg/day)	15	9.5	63	6.6	44	12.7	84
Calcium Males & Females 19-50 years	1000	1316	110	1335	111	552	46
Males & Females >51 years (mg/day)	1200						

From: Reddy & Krebs-Smith (2008). A comparison of food based recommendations and nutrient values of three food guides: USDA MyPyramid, NHLBI's Dietary Approaches to Stop Hypertension Eating Plan, and Harvard's Eating Pyramid. *Journal of the American Dietetic Association, 108*, 533-538.

important to offer consumers in the U.S. suitable options to reach nutrient recommendations. When examining compliance in this study, consumption of fortified breakfast cereal was high for all four weeks, for both males and females. Mean compliance for fortified breakfast cereal consumption in the intervention group was 100% for men for all four intervention weeks and for women, compliance ranged from 96.4%-week 3, 97.3%-week 4, 95.6%-week 5, and 97.3%-week 6, respectively. This demonstrated the ability for fortified breakfast cereal to be easily incorporated into a normal diet as well as provided a means to meet the DRI for vitamin E among adults.

Dietary Calcium Intake

Thus far, this is the first study to investigate the influence of fortified breakfast cereal consumption on calcium intakes among adults in the U.S. In a study conducted by Abrams et al. (2001), the influence of fortified breakfast cereal consumption on calcium intake and absorption was examined in children. The addition of a moderate amount of calcium to a breakfast cereal was beneficial, with both intake and fractional absorption rates increasing. The use of calcium-fortified breakfast cereal was recommended as a practical approach to increasing intake in children; however, extrapolation to adults may not be appropriate. Therefore, additional studies examining calcium intakes from fortified breakfast cereal were warranted.

In the current study, below optimal intakes of calcium were consumed at weeks 1 & 2; the control group consumed 720.2 (315.7) mg/day and the intervention group consumed 751.1 (345.7) mg/day. Increases in calcium intake in the intervention group at weeks 3 & 4 and at weeks 5 & 6 were seen due to the incorporation of the fortified breakfast cereal with intakes increasing to 1618.0 (366.4) mg/day at weeks 3 & 4 and to 1655.9 (334.4) mg/day at weeks 5 & 6. Calcium

intakes were significantly different in the control group, 662.6 (284.7) mg/day, as compared to the intervention group, 1618.0 (366.4) mg/day at weeks 3 & 4. Intakes remained significantly different at weeks 5 & 6, 652.1 (259.1) mg/day for the control group and 1655.9 mg/day (334.4) for the intervention group.

The 2005 and the 2010 DGAC recognized calcium as a shortfall nutrient and recommended that attempts be made to increase the nutrient across the life-cycle. In a study examining data from the NHANES (1999-2004), calcium intakes were found to be below optimal levels for approximately 70% of the population aged 2 and older (Nicklas, O'Neill, & Fulgoni, 2009). Daily dairy consumption to meet 100% of the AI for calcium for people aged 2 to 18 and 19 to 50 years was determined to be 2 servings. For people > 51 years, 3 servings are needed and 4 servings are needed for ages 9-18. A food group pattern was developed for individuals who did not consume dairy products to meet the calcium provided in one serving of milk from other foods items. The pattern demonstrates the number of servings needed by one food group to be equal to one serving of milk. A total of 5.3 servings of dark-green leafy vegetables, or 6.2 servings of legumes, or 53 servings from the meat and bean group, or 12 servings of whole grains would be need to be consumed to be equivalent to one serving of milk. Therefore, emphasis on dairy products is recommended to meet calcium intakes. Several foods are naturally high in calcium but are not high relative to the amount of calcium found in dairy products (see Table 20). Therefore, individuals who are unable to consume dairy products or who limit dairy need appropriate options, fortified breakfast cereal may provide one such option.

Table 20

Food Sources Naturally High in Calcium (mg per serving)

Food Source	Calcium (mg) per Serving
Yogurt, plain, low fat, 8 ounces	415
Sardines, canned in oil, with bones, 3 ounces	324
Cheddar cheese, 1.5 ounces	306
Milk, nonfat, 8 ounces	302
Milk, reduced-fat (2% milk fat), 8 ounces	297
Milk, lactose-reduced, 8 ounces	285-302
Milk, whole (3.25% milk fat), 8 ounces	291
Milk, buttermilk, 8 ounces	285
Mozzarella, part skim, 1.5 ounces	275
Yogurt, fruit, low fat, 8 ounces	245-384
Spinach, cooked, ½ cup	120
Turnip greens, boiled ½ cup	99
Kale, cooked, 1 cup	94
Cabbage, cooked, 1 cup	72
Kidney beans, cooked, 1 cup	50
Fish, catfish, cooked, 3 oz	37
Beets, cooked, 1 cup	27

Source: United States Department of Agriculture, Agricultural Research Service. (2008). USDA National Nutrient Database for Standard Reference, Release 23. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl

In this study, calcium consumption from fortified breakfast cereal was consistent for both men and women. Men consumed 945 (0.0) mg/day of calcium during each intervention week with 100% compliance per week. For women, due to varying compliance each week, a range of 902.8 (168.8) mg/day to 919.7 (73.4) mg/day of calcium was consumed. However, little is known regarding the absorption rates for calcium at this intake level from a fortified breakfast cereal source.

Calcium absorption depends on the amount of elemental calcium consumed at one time. As the calcium amount increases, the percent of calcium absorbed decreases (IOM, 2011). Absorption of calcium from supplements is highest in doses that are less than ≤ 500 mg. Absorption from food sources is considered to be approximately 30% but does vary by food source. It has been suggested that absorption of calcium from foods fortified with calcium would be most effective if no more than 30% of the DV (or 300 mg) of calcium per serving was provided (Rafferty et al., 2007). Smaller amounts of calcium are best absorbed if consumed throughout the day. Therefore, a single dose of 1000 mg of calcium in a fortified breakfast cereal is most likely to be absorbed at only 30%.

Serum α-Tocopherol Concentration

At this time, little is known regarding the consumption of a synthetic α-tocopherol added to a commonly consumed food item such as fortified breakfast cereal on serum α-tocopherol concentrations among adults. In a study conducted by Mitchell et al. (1996), the influence of a vitamin E-fortified breakfast cereal as compared to a tocopheryl acetate standard on serum tocopherol and lipid-corrected tochopherol concentrations was examined. Mean α-tocopherol concentrations were not significantly different in the rats fed the vitamin E fortified cereal sources as compared to the tocopheryl standard. However, lipid-corrected α-tocopherol concentrations were generally lower in the cereal fed rats as compared to the tocopheryl standard. Overall, bioavailability of vitamin E from a whole grain and corn flake cereal was considered to be comparable to the tocopheryl standard. Extrapolation to humans is unknown but does suggest that

the bioavailability of vitamin E from fortified breakfast cereal would be high. Therefore, future studies were warranted.

In this study, there were no between-group differences for either serum α-tocopherol or γ-tocopherol concentrations. However, both groups were considered to have low serum α-tocopherol concentrations, defined as < 20 μmol/L (Ford & Sowell, 1999). Additionally, it is possible that the amount of fat present at the time the fortified breakfast cereal was consumed may have influenced uptake. It is known that fat is necessary for the absorption of vitamin E; however, the amount of fat required to be present for maximal absorption is unknown (Lodge, Hall, Jeanes & Proteggente, 2006). In a study conducted by Jeanes et al. (2004), participants consuming 150 mg of deuterated RRR-α-tocopheryl acetate with toast and butter (17.5 grams of fat) as compared to cereal with full-fat milk (17.5 grams of fat) had significantly higher plasma uptake levels. Additionally, the high-fat meals (17.5 grams of fat) as compared to cereal with semi-skimmed milk (2.7 grams of fat) had significantly higher plasma concentrations of labeled α-tocopherol. Therefore, both fat amount and the type of food present at the time tocopherol is consumed influenced plasma tocopherol uptake.

Additionally, in this study there were no between-group differences in the lipid-corrected α-tocopherol or γ-tocopherol concentrations. There were, however, decreases within both the intervention and control groups for the lipid-corrected α-tocopherol post-intervention concentrations as compared to pre-intervention. This may be, in part, due to the significant increases seen within both groups in total cholesterol concentrations at post-intervention; therefore, decreasing the ratio of α-tocopherol to total cholesterol concentrations.

The type of synthetic α-tocopherol is also important to consider. Foods may be fortified with all-rac-α-tocopherol (all 8 stereoisomers) or RRR-α-tocopheryl acetate (1 stereoisomer) (Acuff, Thedford, Hidiroglou, Papas, & Odom, 1994). In humans, RRR-α-tocopheryl has more bioavailability than all-rac-α-tocopherol. RRR-α-tocopherol was shown to be almost twice as bioavailable as all-rac-α-tocopherol. In rats, all-rac-α-tocopheryl acetate has been shown to have lower apparent absorption rates as compared to RRR-α-tocopheryl acetate (Jensen, Norgaard, & Lauridsen, 2006; Horwitt, Elliot, Kanjananggulpan, & Fitch, 1982). In swine, RRR-α-tocopheryl acetate resulted in a two-fold increase in plasma tocopherol concentrations as compared to all-rac-α-tocopheryl acetate (Lauridsen, Engel, Craig, & Traber, 2002). However, extrapolation of animal studies to humans may be limited.

Serum Lipid Concentrations

Total cholesterol concentrations in both the control and intervention group were normal, below the recommended level of < 200 mg/dL, for both the pre- and post-intervention blood draws. There were no between-group differences for the pre- and post- intervention concentrations. However, there was a significant within-group increase in total cholesterol post-intervention concentration for both control and intervention groups, indicating an effect of time. Historical data indicates total cholesterol concentrations for an individual can fluctuate in repeated measurements of a week or more as much as 44.8 mg per 100 mL with an average of 29.4 mg per 100 mL (Gordon, 1959). Therefore, recommendations for serial determinations are made when studying the effects of dietary factors. Additionally, unexplained fluctuations in serum cholesterol levels may be partly associated with varying physical activity levels as well as the time of the blood collection.

There were no between-group or within-group differences for serum triglyceride concentrations. However, there were significant increases in HDL-C concentrations for both control and intervention groups over time but no treatment differences occurred. Physical activity was not monitored during this study; therefore, it is possible changes in physical activity levels may have influenced the concentrations.

Limitations

Limitations are associated with this study. First, this study did not assess bioavailability of either α-tocopherol or calcium consumed from the fortified breakfast cereal. Therefore, it is unknown whether the significant dietary calcium intakes would be absorbable and directly influence calcium status. Second, physical activity of the participants was not determined at baseline or recorded for the duration of the study. Therefore, it is unknown if fluctuations in serum lipid levels could have been influenced by exercise. Next, no data was collected on the menstrual cycles or on the pre- or post menopausal status of the women. Fluctuations in plasma α-tocopherol concentrations occur according to the phase of the menstrual cycle in premenopausal women (Lanza et al., 1998). Specifically, α-tocopherol concentrations have been found to be lower during the menses as compared to the luteal phase. Therefore, it is unknown if phases of the menstrual cycle in the female participants influenced α-tocopherol concentrations.

There are also limitations associated with the use of a diet diary as a basis for estimation of nutrient intake. Participants may have not accurately recorded their intake. Participants may have not recorded all food and beverages consumed. Participants may have changed their eating habits as a result of the diet diary recording process. Furthermore, average participant calorie

consumption was low for both the control and intervention group throughout the duration of the study. This may be a result of the diet diary limitations. Lastly, compliance within the intervention group for the consumption of the fortified breakfast cereal may not accurately reflect the true consumption intakes of the participants due to the inaccurate recording of intake or the failure to return the uneaten cereal.

CHAPTER VI

CONCLUSIONS

In summary, this is the first study that has examined both dietary vitamin E and calcium intakes through the consumption of fortified breakfast cereal as well as examined serum tocopherol concentrations among adults. Favorable effects were seen on vitamin E and calcium intakes in the intervention group as compared to the control group due to the fortified breakfast cereal consumption compliance rates. Shelf-stable milk was available for the participants; however, this was an optional component and the cereal was not required to be consumed with milk. It is evident, based on the pre-intervention calcium intakes and the consistent calcium intakes during the intervention duration, the substantial increases in dietary calcium intakes was due to the consumption of the cereal. Additionally, positive effects were seen in the dietary vitamin E intakes as a result of high compliance rates from consumption of fortified breakfast cereal. However, no increases were observed in serum α-tocopherol or lipid-correct α-tocopherol concentrations. This may be, in part, due to the type of α-tocopheryl acetate used in the fortified breakfast cereal product as well as the amount of fat consumed with the fortified breakfast cereal. Other studies have shown significant increases in plasma α-tocopherol concentrations following the consumption of α-tocopherol combined with a higher fat meal or food component. The milk provided was a 2% reduced-fat milk. However, many participants did not consume milk with every serving of the cereal, instead consuming it as a dry snack item. Additionally, participants in both groups began

the study period with low serum tocopherol concentrations. Therefore, the intervention time frame may have not been long enough to produce a significant change.

Recommendations for future studies include determining the differences in serum α-tocopherol concentrations as a result of fortified breakfast cereal consumed without milk, fortified breakfast cereal consumed with 2% reduced-fat milk, and fortified breakfast cereal consumed with full-fat milk. The intervention time frame may also need to be extended beyond 4-weeks. Also, menstrual cycle for pre-menopausal women may need to be considered when examining serum tocopherol concentrations. Physical activity habits may also need to be recorded at the time of entry into the study and followed throughout the duration of the study. Additionally, further research is needed in the bioavailability of nutrients in fortified breakfast cereal. Therefore, studies examining the amounts of the nutrients absorbed as compared to the resulting plasma uptake are warranted. Serial determinations of lipid concentrations may be warranted in future feeding intervention studies. Food manufacturers may need to consider the absorbable amount of nutrients added to foods as compared to the total amounts added as part of the continuing effort to improve intakes of shortfall nutrients.

In conclusion, fortified breakfast cereal can easily be incorporated into the diet and should be considered by registered dietitians and other health professionals as a means to increase both vitamin E and calcium intakes among adults.

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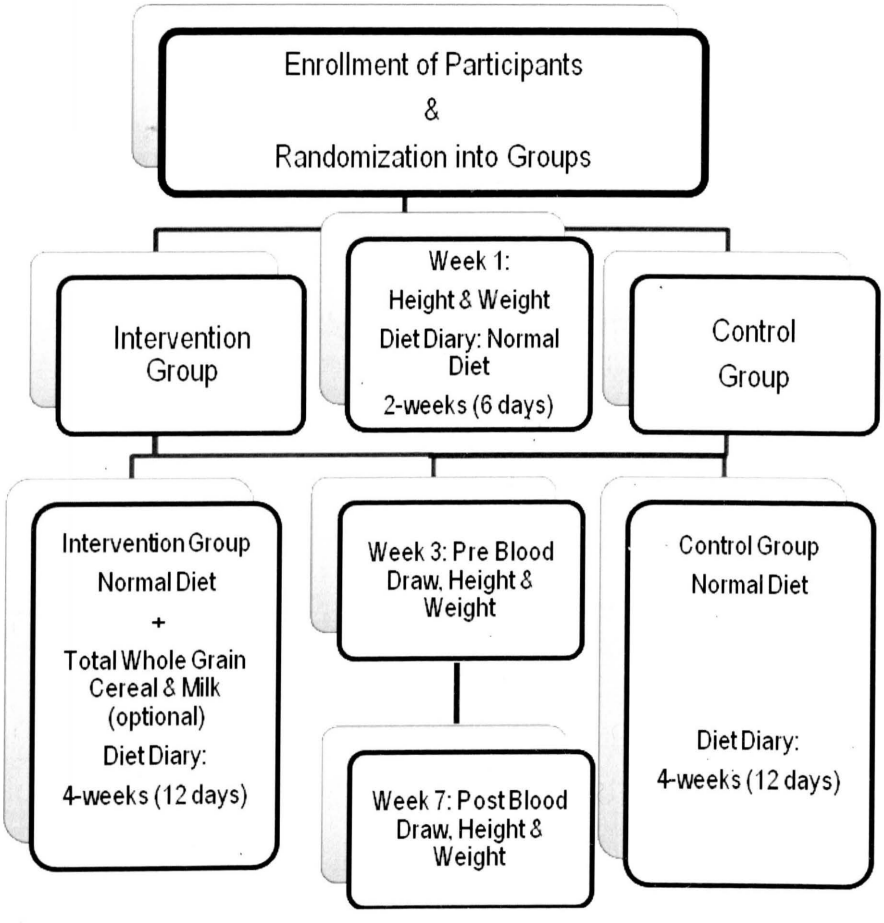
APPENDIX A

Study Design


Enrollment of Participants
&
Randomization into Groups
Intervention Group
Week 1:
Height & Weight
Diet Diary: Normal Diet
2-weeks (6 days)
Control Group
Intervention Group
Normal Diet
+
Total Whole Grain Cereal & Milk (optional)
Diet Diary:
4-weeks (12 days)
Week 3: Pre Blood Draw, Height & Weight
Week 7: Post Blood Draw, Height & Weight
Control Group
Normal Diet
Diet Diary:
4-weeks (12 days)

APPENDIX B

Study Schedule for Control and Intervention Group

Code:_____ Group: Control

Visit:	Weeks after 1st Visit:	Date	Group Assignment	Height Taken	Weight Taken	Blood Drawn	Pick-Up Blank Diaries	Return & Review Previous Diaries
1	0		✓	✓	✓		✓	
	1							
	2							
2	3				✓	✓	✓	✓
	4							
3	5						✓	✓
	6							
4	7				✓	✓		✓

Code:_____ Group: Intervention

Visit:	Study Week	Date	Group Assignment	Height Taken	Weight Taken	Blood Drawn	Pick-Up Blank Diaries	Pick-Up Food	Return of Uneaten Food	Return & Review Previous Diaries
1	1		✓	✓	✓		✓			
	2									
2	3				✓	✓	✓	✓		✓
	4									
3	5						✓	✓	✓	✓
	6									
4	7				✓	✓			✓	✓

APPENDIX C

Compliance Check-Off Sheet

Food Item	Mon	Tue	Wed	Thu	Fri	Sat	Sun	Not Eaten
Cereal #1								
Cereal #2								
Cereal #3								
Cereal #4								
Cereal #5								
Cereal #6								
Cereal #7								

APPENDIX D

Determination of Alpha and Gamma Tocopherol

Tocopherol Determination

Preparation:
Stock solution of alpha-vitamin E: 5 mg/ml in ethanol absolute
Stock solution of gamma-vitamin E: 5 mcg/ml in ethanol absolute
Stock solution of IS (Tocopherol Acetate) for vitamin E: 10 mg/ml in Methanol-ChCl3 24:1(V/V)
Diluent for standard working solution: ethanol absolute
Diluent for IS working solution: ethanol - ChCl3 3:1
Solution Mixture includes alpha tocopherol 2 mg/ml & gamma tocopherol 200 mcg/ml
For 1ml of the mixture:
400 mcl of alpha tocopherol 5 mg/ml + 400 mcl of gamma tocopherol 500 mcg/ml + 200 mcl of ethanol

Test Procedure:
Concentration of Standard Working Solution (mcg/ml)

alpha_gamma	Mixture (mcl)+ Diluent (mcl)	alpha_gamma Final in 50 mcl plasma
10_1	5+995	2_0.2
20_2	10+990	4_0.4
40_4	20+980	8_0.8
80_8	40+960	16_1.6
120_12	60+940	24_2.4
160_16	80+920	32_3.2
200_20	100+900	40_4

For standard samples: 40 mcl of blank human plasma (BHP) +10 mcl of ST working solution+ 200 mcl of extractant with 500 mcg/ml IS
For blank sample: 40 mcl of blank human plasma (BHP) +10 mcl of Ethanol + 200 mcl of extractant
For unknown samples: 50 mcl of blank human plasma (BHP) +200 mcl of extractant with 500mcg/ml IS
Samples vortexed, stand for 5 minutes, vortexed again, centrifuged for 10 minutes at 4^0 C, 13000rmp.Supernatant was taken to run.

The method for analysis of alpha and gamma-vitamin E:
1. MP: 93.5% MEOH in HPLC Water
2. Column: XTerra® MS C_{18}, 4.6 X 150 mm, 3.5 µm equipped with an Xterra® C18 guard column (3.9 X 20mm, 3.5 µm).
3. Detector: Waters 474 scanning Fluorescence Detector excitation: 295nm; emission: 330nm
4. 515 pump. The flow rate was set at 1.0 ml/min.
5. 717 plus autosampler. The injection volume was 30 µl.

APPENDIX E

Determination of Serum Cholesterol

Raichem Cliniqa Cholesterol Reagent, R80015

Test summary

In this method of serum cholesterol determination, cholesterol esters are hydrolyzed by an esterase to cholesterol and fatty acids.

$$\text{Cholesterol esters} \xrightarrow{\text{esterase}} \text{Cholesterol + fatty acids}$$

Cholesterol is oxidized by cholesterol oxidase to Δ4-Cholesterone with the simultaneous production of an equimolar amount of hydrogen peroxide:

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \Delta\text{Cholesterol} + H_2O_2$$

In the presence of a peroxidase, hydrogen peroxide oxidizes p-hydroxybenzenesulfonate and 4-aminoantipyrine to give a quinoneimine dye (colored red).

$$2\ H_2O_2 + \text{4-aminoantipyrine} + \text{p-hydroxybenzenesulfonate} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4\ H_2O_2$$

Reagent Composition

Reactive ingredients:

4-Aminoantipyrine	0.8 mmol/L
p-Hydroxybenzenesulfonate	20 mmol/L
Cholesterol Oxidase	≥ 200 U/L
Cholesterol Esterase	≥ 50 U/L
Peroxidase	≥ 25000 U/L

Non-reactive ingredients:

Buffers, stabilizers, and fillers

Test Procedure
Wavelength: 500 nm
Test*: 1mL reagent + 10 μL sample
Standard*: 1mL reagent + 10 μL sample
Blank*: 1mL reagent
Incubate: 10 minutes at 37°C
*For this study, the standard curve was established based on 250 μL reagent + 2.5μL sample= 300 mg/dL with 2.5μL utilized for each sample.

Standard 1: 250 μL + 2.5μL = 300 mg/dL
Standard 2: 250 μL + 1.25μL = 150 mg/dL
Standard 3: 250 μL + 0.625μL = 75 mg/dL
Standard 4: 250 μL + 0.3125μL = 37.5 mg/dL

Calculations: C_{st} = Value of standard in mg/dL

$$\frac{A_{sample}}{A_{standard}} \times C_{st} = \text{Cholesterol in sample in mg/dL}$$

APPENDIX F

Determination of HDL-C

Raichem Cliniqa HDL- Cholesterol Reagent, R82051

Test Summary

In this method of determination, very low density lipoproteins (VLD) and low density lipoproteins (LDL) are precipitated from serum by dextran sulfate and magnesium. After centrifugation, the HDL cholesterol fraction is determined in the clear supernatant by an enzymatic procedure.

Reagent Composition

Precipitating reagents (concentrations if dissolved per instructions)

Reactive ingredients:

Dextran sulfate (Mol. Wt. 500,000)	10 g/L
Magnesium sulfate	1 mol/L

Non-reactive ingredients:

Buffers, stabilizers, and fillers

Test Procedure

Pipette 1 mL each of water, controls and samples into the appropriately labeled centrifuge tubes adding 0.1 mL of Cliniqa precipitating reagent*. Mix immediately for approximately 5 seconds in a vortex type mixer then let stand for 5 minutes at room temperature. Centrifuge for 15 minutes at 2500 rpm. The supernatant should be clear. Carefully remove the supernatant immediately without distributing the precipitate and transfer to clean, dry tube. Wavelength: 500 nm

Blank*: 1mL Reagent; 50 μL Water

Standard*: 1mL Reagent; 50 μL Standard

Sample*: 1mL Reagent + 50 μL Sample

Incubate for 10 minutes at 37°C

*For this study, the standard curve was established based on 250 μL reagent + 12.5μL sample= 50 mg/dL with 2.5μL utilized for each sample.

Standard 1: 250 μL + 2.5μL = 300 mg/dL

Standard 2: 250 μL + 1.25μL = 150 mg/dL

Standard 3: 250 μL + 0.625μL = 75 mg/dL

Standard 4: 250 μL + 0.3125μL = 37.5 mg/dL

Calculations

$$\frac{A_s}{A_{std.}} \times \text{concentration of standard} \times \text{dilution factor} = \text{mg/dL HDL-C}$$

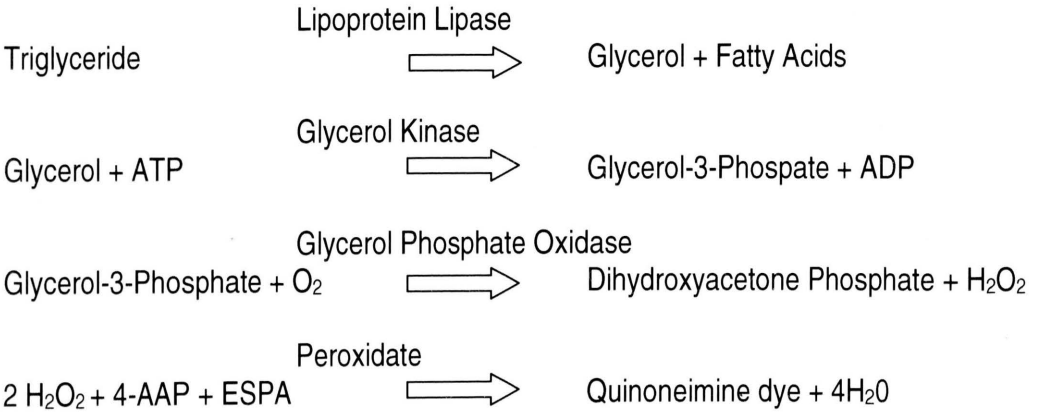
APPENDIX G

Determination of Triglycerides

Cayman Chemical Company Triglyceride Assay Kit, 10010303

Test Summary

In this method of determination, enzymatic hydrolysis of triglycerides by lipase to glycerol and free fatty acids is utilized then subsequently measured by a coupled enzymatic reaction system producing a brilliant purple color.



Reagent Composition

Triglyceride standard	1000 mg/dL
Triglyceride standard diluents	10 mL (salt solution)
Triglyceride assay buffer	15 mL of 50 mM sodium phosphate buffer
Triglyceride enzyme mixture	lyophilized enzyme mixture

Test Procedure

Standard Preparation: Add 200 µL of the triglyceride standard diluents to tubes 2-8. Add 400 µL to tube 1. Add 100 µL of the triglyceride standard to tube 1, mix thoroughly. The concentration of tube 1 is 200 mg/dL from which serial dilutions will be made.

Tube 1: 200 mg/dL
Tube 2: 100 mg/dL
Tube 3: 50 mg/dL
Tube 4: 25 mg/dL
Tube 5: 12.5 mg/dL
Tube 6: 6.25 mg/dL
Tube 7: 3.125 mg/dL
Tube 8: 0 mg/dL

Pipette 10 µL of each standard (tubes 1-8) per well (A1-A8) then pipette 10 µL of each sample to each well in duplicates. Initiate the reaction by adding 150 µL of diluted enzyme buffer solution to each well. Incubate for 15 minutes at room temperature.

Read absorbance at 540 nm.

Calculations:
Triglycerides (mg/dL) = [(Corrected absorbance) – (y-intercept)/ slope]