Determining the Effects of Phytosterol Enriched Margarine on Cholesterol in Normal Subjects

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Introduction

The Centers for Disease Control estimates that 1 in 6 Americans have high cholesterol—a dangerous and alarming statistic considering that high cholesterol is one of the leading factors in developing cardiovascular disease (1). Not surprisingly, the American Heart Association describes heart disease as the number one killer in the United States for both men and women. The American Heart Association estimates that nearly 2,300 Americans die of cardiovascular disease every day, making it a frightening and serious problem for the entire country (2). Several factors can lead to cardiovascular including obesity, lack of exercise, high blood pressure, and high cholesterol (2). Fortunately, lowering cholesterol is a feasible solution to reducing the risk of cardiovascular disease, and there are several ways to bring down levels of total cholesterol and Low Density Lipoprotein Cholesterol (LDL-C). Much discussion and research has been conducted about the noninvasive and relatively inexpensive use of phytosterols in lowering both Total Cholesterol (TC) levels and LDL-C levels (1). The purpose of this research proposal is to review some of the literature involving the use of phytosterols to lower cholesterol in human subjects, both those with hypercholesterolemia and with normal values. More specifically, this research proposal will explain the methods and procedures of an in class study that was conducted on college students to show the effects of consuming phytosterols on cholesterol levels.

Review of Literature

Seki et al. studied the relationship between phytosterols and cholesterol in 22 healthy normocholesterolemic men in a double blind research study involving phytosterol enriched vegetables oil (3). Twenty two men were given a control diet for the first two weeks of the study to ensure that they all got used to the experimental product (bread with vegetable oil)
and so it would give researchers a baseline standard to analyze the post-study results from. After the two week period, the men were split evenly into two groups— one that consumed bread with oil containing 1.336 g of phytosterols daily and one that consumed bread with oil containing only 0.117 g of phytosterols daily. These experimental diets lasted for 4 weeks, and blood samples were collected after fasting on weeks -2, 0, 2, and 4 (3).

Researchers found that the subjects consuming the phytosterol enriched vegetable oil (1.336 g phytosterol daily) saw reductions in serum total cholesterol by 3.3% and in LDL-C by 4.4%. Levels of Very Low Density Lipoprotein-Cholesterol (VLDL-C) and High Density Lipoprotein-Cholesterol (HDL-C) did not significantly differ among the experimental and control groups. Only data with p values less than 0.05 was considered significant and relevant information to the study (3).

Other than the bread with the phytosterol enriched or control oil, the men could eat their regular diets. The results of this study might have been more reliable if the men were given other standard dietary guidelines to follow so that the researchers could have been sure that it was in fact the phytosterol enriched oil that lowered the participants’ LDL-C and not other factors. Seki et al. (3) concluded that 1.336 g of phytosterols a day is indeed enough to lower humans’ total cholesterol and LDL-C even in a relatively short period of time and also that phytosterols are safe for humans to consume in these amounts.

Since bread with phytosterol enriched oil is not a common snack food item among Americans, researchers have explored phytosterol supplementation with other, more popular foods. Polagruto et al. (4) and his team of researchers studied the effects of phytosterol enriched cocoa snack bars on seventy hypercholesterolemic adult individuals. Thirty-five of
the participants (12 men and 23 women) were randomly assigned to a diet containing the phytosterol enriched snack bars with 1.5 grams of sterol esters per bar, and thirty-five of the participants (10 men and 25 women) were assigned to a control snack bar diet, which lacked the plant sterol ester enrichments. Although the study took place over 6 months, a rolling-enrollment style was implemented for starting subjects on the different diets, and each subject’s intervention diet actually only lasted 6 weeks. Subjects maintained their usual diet but were asked to consume the cocoa snack bar twice a day within 30 minutes of eating a meal. During weeks 0, 3 and 6, subjects participated in fasting blood draws. To determine compliance levels among subjects, researchers collected and counted the number of empty snack bar wrappers and uneaten snack bars during follow-up visits with subjects (4).

Researchers analyzed the subjects’ fasting blood samples for a number of components, all of which are discussed in the article (4). However, the most relevant lab values were the changes in serum phytosterol levels that occurred over the 6 week intervention period. Subjects on the phytosterol-enriched diet saw a 4.7% reduction in total cholesterol levels ($P=0.0002$) and a 6% reduction in LDL levels ($P<0.001$) at the end of their dietary intervention. Although HDL levels were not affected by the phytosterol enriched diet, the total cholesterol to HDL level ratio decreased by 7.4% ($P<0.001$). Researchers also determined that total cholesterol, LDL, and the total cholesterol to HDL ratio decreased as soon as 3 weeks into the dietary intervention. Subjects on the control diet without phytosterol enriched cocoa bars did not see a change in total cholesterol levels, LDL cholesterol levels, or the total cholesterol to HDL ratio (4).

This experiment would have been more reliable if researchers had conducted the entire study during the same 6 weeks and also placed all subjects on a control diet for 2
weeks before splitting the groups into the experimental and control groups. Determining a more accurate way to assess compliance with the diets would have also been helpful because simply counting empty wrappers, etc. was not very dependable. Researchers concluded that phytosterol enrichment of popular snack products such as chocolate is a safe and effective method for lowering both total cholesterol and LDL levels (4).

Both of the previously mentioned studies involved phytosterol enrichment of food products that contained some amount of fat. To determine the effects of phytosterol enrichment in little to no fat containing products, Devaraj et al. (5) conducted a study using orange juice as a means of phytosterol enrichment. Seventy-two subjects with normal blood counts ranging in age from 20 to 73 years participated in the study. This 10 week study consisted of a 2 week baseline period in which subjects all received unfortified orange juice and then an 8 week dietary intervention period in which subjects were randomly placed on either a plant sterol-fortified orange juice diet with 2 grams of plant sterols each day or a placebo orange juice diet without any added plant sterols. Subjects participated in a baseline blood draw at the beginning of the study, another blood draw after the 2 week baseline diet, and then a final blood draw at the end of the 8 week dietary intervention period. Subjects were instructed to record a 3-day diet record at the beginning and end of the study (5).

Researchers found no change in any cholesterol levels during the first 2 weeks of the study (the baseline period) (5). However, there was a 7.2% decrease in total cholesterol and a 12.4% decrease in LDL-C for those subjects on the sterol-fortified orange juice diet (P<0.0001). Furthermore, non-HDL cholesterol levels also decreased by 7.8% for those on the sterol-fortified diet (P<0.01). No change occurred in total cholesterol, LDL-C or non-HDL for subjects in the placebo orange juice diet group (5).
More information about several aspects of the study including the breakdown and demographics of the subjects and how compliance and adherence to the diet was monitored would have been helpful to better understand the experiment and its results. Devaraj and his team of researchers concluded that a significant total cholesterol and LDL-C decrease can be achieved by fortification of a non-fat food or drink such as orange juice (5). They point out that not only is this an effective means of lowering cholesterol, but that it also follows along with guidelines for heart healthy eating because it is low in fat, full of nutrients, and appeals to a wide variety of populations (5).

Racette et al. (6) also researched the connection between dietary phytosterols and cholesterol levels, but this study compared the change in cholesterol with three different amounts of phytosterols administered to all participants at different times during the timeframe of the study. A 5 day menu cycle was established for a baseline diet containing ≤60 mg phytosterols, a diet containing ≈ 400mg of phytosterols, and a diet containing ≈2000mg of phytosterols. Participants were randomly assigned to each of these diets in different orders for 4 weeks each with one week between starting each different diet. All menu items for each different diet were prepared in the metabolic kitchen, and participants ate breakfast and dinner at the research facility. The twenty subjects who completed the study adhered to the diet very well for several reasons - the main ones being that their meals were prepared for them and that the participants ate 2 of their meals a day at the research center. Fasting blood samples were taken twice during each feeding period, and fecal excretion of cholesterol was determined at the end of each feeding period through fecal collection (6).

At the end of the study, the average intake of phytosterols during the different diet periods was collected and averaged 59, 459, and 2059 mg per day (6). Excretion of
cholesterol went up $36 \pm 6\%$ for the moderate phytosterol diet (459 mg per day) and increased to $74\pm 10\%$ for the high phytosterol diet (2059 mg per day). Absorption of cholesterol decreased, and cholesterol biosynthesis increased $31 \pm 6\%$ for the moderate phytosterol diet and $50 \pm 7\%$ for the high phytosterol diet. The high phytosterol diet lowered LDL-C values definitively by $-8.9 \pm 2.3\%$ and also decreased total cholesterol and non-HDL-C cholesterol. The moderate phytosterol diet’s lowering effect on LDL-C, total cholesterol, and non-HDL-C was not considered to be significant to this study because the p-value for this data was 0.077, which was not considered to be accurate enough to draw valid conclusions. The LDL/HDL ratio improved with both the moderate and high phytosterol diets (6).

This study would have been more significant if the researchers had divided the participants into groups which were assigned to one of the 3 different diets for the entire length of the study instead of alternating so quickly. This would have led to more definitive results, and the amount of phytosterols would have had a longer time frame to cause a more significant change in cholesterol levels. Authors of this article concluded that both the moderate and high phytosterol diets had a negative impact on cholesterol absorption and increased cholesterol excretion. The high phytosterol diet, however, caused more of a change in the total cholesterol and LDL-C levels than the moderate phytosterol diet. Therefore, the effects of phytosterols on LDL-C seem to be dose dependent (6).

**Summary/Conclusion of the Literature Review**

In the study conducted by Seki et al., researchers compared the impact of consuming either a trace amount of phytosterols or a moderate amount of phytosterols in 22 healthy
Researchers found that phytosterol enrichment is not only safe and healthy but also produced a LDL-C decrease in the participants (3). Similarly, in the study conducted by Racette et al., subjects consuming a moderate to high level of phytosterols excreted more cholesterol and absorbed less at the same time. This study points out, however, that only a high amount of phytosterols had a total cholesterol and LDL-C lowering ability (6). The previous study failed to mention this detail and implied that even a moderate amount of phytosterols would lower total cholesterol and LDL-C levels (3). Nonetheless, both of these studies produced evidence of a noninvasive method to lower blood cholesterol. These conclusions are further supported by the research of Polagruto et al. and Devaraj et al., who both determined that phytosterol fortification in popular foods such as chocolate snack bars and orange juice has a lowering effect on total cholesterol, LDL-C and non-HDL cholesterol (4, 5). Polagruto et al. and Devaraj et al. also proved that phytosterol enrichment is effective in both fatty and fat free foods, which will propel food scientists and manufacturers to experiment with phytosterol fortification in a wide variety of food and beverage products (4, 5). Given the large number of Americans with high cholesterol, the results of these studies have huge implications on the fight against cardiovascular disease, especially since they involve cheaper and less severe methods of fighting it, unlike surgery or expensive medications.

Methods and Procedures

Subjects:

To further investigate the relationship between phytosterol intake and cholesterol, the Fall 2010 class of Methods of Human Nutritional Assessment at Virginia Tech conducted an
informal study. Seventy-four subjects ranging in age from 20-24 years old and consisting of 62 females and 12 males who were enrolled in the class all participated in the dietary intervention set up by their professor. Each student’s body weight was measured and recorded at the beginning of the study, though it is important to note that the dietary interventions related to this study were not intended to induce weight loss or gain in any of the subjects. Subjects were instructed to maintain their physical activity level since this study was based on solely dietary intervention and not lifestyle factors that could influence cholesterol levels such as exercise. Keeping the subjects’ exercise levels the same helped to produce consistent results at the end of the study.

**Dietary Interventions:**

The two diet interventions used in this study were the Therapeutic Lifestyle Changes (TLC) Diet and the Phytosterols Supplemental Therapeutic Lifestyle Changes Diet (PS-TLC). Both of these diets instruct that no more than 30% of one’s calories should come from fat, with less than 7% of those fat calories coming from saturated fat, that less than 200 mg of cholesterol should be consumed per day, that 15-20% of calories should come from protein, that 50-65% of calories should come from carbohydrates, that 20-35 grams of dietary fiber should be consumed, and that alcohol should be eliminated from the diet. The difference in the two diets, however, is that the PS-TLC diet includes 1.5 g of phytosterols, consumed in the form of *Promise Active Light* spread (0.75 grams, twice a day). Students in this class developed 1 day menus for each of the diets before learning which diet they would be placed on to familiarize themselves with both of the diets and see the challenges presented in maintaining the restrictions of the diet interventions.
Subjects had the choice of which diet intervention they would like to be placed on, which was most likely done to increase willingness to participate and adhere to the diets. Forty-six students (7 males and 39 females) ended up picking the TLC diet, and 28 students (5 males and 23 females) chose the PS-TLC diet. The number of subjects is not split evenly because students were allowed to pick which diet they wanted to follow. Upon learning which diet they would be participating in, students recorded their regular diet for a week to use as a baseline diet for baseline lab values. After beginning the dietary interventions, subjects again recorded their diets each week for three weeks, this time with the restrictions of the TLC or the PS-TLC diets. Each week, students analyzed their diet records in a software called NutritionistPro©, which reports nutrient intakes based on the diet records that the students entered (7). Students were required to enter their weekly averages and percentages for specific nutrients on a Weekly Average Nutrient Form, which was to be used later by the professor when analyzing the results. Subjects also needed to burn a copy of each week’s diet record onto a CD to turn into the professor and graduate teaching assistants so that they could make sure the subjects were following their diets correctly. This also gave the professor and GTAs access to the nutrient analysis that the students performed on their diets each week.

**Blood Samples:**

To measure the cholesterol changes over the 3 week dietary intervention, fasting blood samples were taken at the end of the baseline period and post-dietary interventions. A certified phlebotomist, Ms. Rinehart, drew the blood samples and put them into vacutainers containing the anticoagulant EDTA. The blood samples were stored on ice after the blood draw until centrifugation at a speed of 3000 rpm for 20 minutes at 5°C to separate the plasma
from the red blood cells. After separation of the plasma from the red blood cells, the plasma was stored at 4°C until it was analyzed further in lab by the students.

**Data Analysis:**

Subjects determined their own plasma Total Cholesterol and HDL-C cholesterol levels during their lab time. Subjects used the method of Allain et al. (8) to determine both of these lab values. HDL-C, however, was determined after removing LDL-C and VLDL-C first by using the method of Finley et al (9). All determinations of unknown lab values were done in duplicates to ensure accuracy in the lab. Subjects had to obtain less than 10% error between the duplicates when finding plasma TC, and they had to obtain less than 5% error between the duplicates when determining HDL-C. Final HDL-C cholesterol levels were determined by using a standard curve to compare the absorbance of the HDL-C left in the supernatant with the corresponding HDL-C concentrations. The standard curve used standards of 50, 100, and 200 mg/dL for plasma TC and 25, 50, and 100 mg/dL for the HDL-C calculations. Once subjects plotted their own or other subjects’ data against the standard curves, they needed to multiply the HDL-C value by 1.1. This multiplication accounted for the 10% dilution of the plasma by the HDL-C precipitating reagent.

At the end of the dietary interventions, students once again received a blood draw, and these lab values were compared to their baseline lab values to see what difference, if any, the modified diets had on their cholesterol levels. The Student-t test enabled subjects, the professor, and GTAs to determine whether there was a significant change in the lab values and whether this change was statistically significant and relevant to the study.
References Cited

1. Website of the Centers for Disease Control: http://www.cdc.gov/cholesterol/
2. Website of the American Heart Association: www.americanheart.org