EFFECTS OF SOY PROTEIN ON LIPIDS AND LIPOPROTEIN(a) IN MEN AND WOMEN

By

SONNY BRANDON HODGES

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1998

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2000

EFFECTS OF SOY PROTEIN ON LIPIDS

AND LIPOPROTEIN(a) IN MEN AND WOMEN

Thesis Approved:

Thesis Adviser

Dean of Graduate College

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my advisor, Dr. Bahram H. Arjmandi for his invaluable contribution, vigilant supervision, caring guidance, and true fellowship. Sincere appreciation also goes to my committee members Dr. Barbara Stoecker and Dr. Janice Hermann whose guidance, input, and encouragement are also invaluable.

I would also like to express sincere gratitude to fellow graduate students and research staff Nasrin Sinichi, Lisa Hammond, Latha Devareddy, Dr. Dania Khalil, Dr. Edralin Lucas, and Shanil Juma for their valuable time, tireless effort, and unending support in this study and throughout my graduate education. Sincere gratitude also goes to the entire Nutritional Sciences Department for their assistance and support in carrying out the many tasks involved in completing this study. Deep thanks are also extended to Dr. Mark Payton for his invaluable assistance in the statistical analyses of data for this study, and Dr. Mark Munson for his valuable time and input.

Moreover, I would like to give my special appreciation to my grandparents, parents, and brother for their unending love, encouragement, and support throughout my life and education. I truly would not have come this far without you.

I thank God for blessing me with the ability to achieve my goals, and the family and friends who have been there with me along the way.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Cardiovascular disease is the leading cause of death in the United States (1). Although the death rate from cardiovascular disease has declined slightly over the last two decades, the incredible economic and physical burden of survived cardiovascular events, and the risk for developing these conditions, continue to rise (2).

The burden of cardiovascular disease is the major public health problem, costing Americans an estimated $326.6 billion in 2000 (2). Though most deaths from coronary heart disease (CHD) occur in those over 65, over 59 million Americans have at least one form of cardiovascular disease, including stroke, hypertension, complications from rheumatic fever, and congenital heart disease (2). From age 45 to 64, one in nine women and one in six men have some form of heart disease. After age 65, those numbers take on a dramatic change, with one in eight men and *one in three women* being afflicted (2).

Mortality rates from all forms of CHD increase with age in all races, with African Americans having the highest rate. However, this rate is only about 5% higher than Whites (2). American Indians, Asians, and Hispanics follow in decreasing numbers, respectively (2).

A great achievement in the fight against CHD was the identification of its risk factors. Only after these findings was the power of prevention, not just treatment,

realized. In all population, clinical, and pathologic studies, high serum cholesterol levels were found to be at the forefront of risks associated with CHD, with other factors such as smoking, obesity, and physical activity following close behind (1).

Any patient diagnosed with hypercholesterolemia is first placed on a cholesterol-lowering diet. The American Heart Association recommends the Step I and II diets, which reduce dietary intake of cholesterol, fat, and saturated fat (1). The Step I and II diets are designed to reduce low-density lipoprotein- (LDL-) cholesterol by about 8 and 15%, respectively (3). If no successful lowering of cholesterol is observed, the patient is usually prescribed a cholesterol-lowering drug (1). Until recently, these were the only available alternatives for those with hypercholesterolemia.

There is increasing evidence that vegetable proteins may provide a new alternative in the prevention and lowering of elevated cholesterol levels (4). Recently, the Food and Drug Administration approved the use of soy protein in lowering cholesterol (5). Vegetable proteins, specifically soy proteins, reduce plasma cholesterol, especially when cholesterol is elevated by dietary sources (4). The first study to show a significant decrease in cholesterol by soy protein was conducted in 1967 (4). Though there were only six hypercholesterolemic subjects, results showed a mean decrease in cholesterol from 7.6 mmol/L to 5 mmol/L after only 4 weeks on a diet in which the only source of protein was textured soy protein.

This study was seemingly ignored for almost ten years. In 1977, Sirtori et al. began a series of clinical trials with soy protein (4). An 8-week study of 127 outpatients on a low-lipid, high polyunsaturated to saturated fat ratio diet resulted in a mean

reduction of cholesterol by about 23% in the 67 men and about 25% in the 60 women (4). Many similar studies have been conducted yielding similar results.

However, there have been several referenced studies examining soy that have not produced positive results (6-7). In these studies, failure may have been due to patients with very mild hypercholesterolemia, low protein content in the diet, patients with hypertriglyceridemia, or any combinations of these factors (4). Other possible explanations include failure to follow diets, or patients who were already on some kind of drug therapy (4).

Today, the consensus is that, despite any "negative" findings, soy protein-based diets are effective in lowering serum cholesterol concentrations. The question then arises as to the properties of soy that are responsible for its hypocholesterolemic effects. Initial studies were conducted under the hypothesis that cholesterol absorption/elimination and steroid excretion were possible mechanisms (8). Though there were reductions in total and LDL-cholesterol in these studies, no difference in fecal steroid excretion (neutral steroid or bile acid) could be found (8).

It was not until 1989 that the attention turned to LDL-receptor activity as another likely explanation for the hypocholesterolemic effects of soy. When additional cholesterol was given, textured soy protein intake stimulated clearance, most likely due to increased LDL-receptor activity (9). Various studies, from 1989 to the present have strengthened this hypothesis (10-11).

A possible mechanism in the hypocholesterolemic effect of soy centers on its content of isoflavones. Isoflavones are estrogen-like compounds found in soy protein. Estrogen has been shown to reduce or prevent the occurrence of CHD in women (12), by

improving lipid and lipoprotein metabolism (13). Though much less potent than estrogen, isoflavones have the ability to interact with both known types of estrogen receptors in humans (8).

Other theories behind the hypocholesterolemic effect of soy protein center on its amino acid content, specifically the ratio of arginine to lysine, as well as the presence of saponins and trypsin inhibitors (8, 14).

Also, the ability of soy to reduce cholesterol has prompted a recent focus on how soy alters serum levels of lipoprotein(a) (15). One recent study found that soy protein may increase lipoprotein(a) (15).

In order to understand soy's benefits to cardiovascular health, we must conclusively identify its effects on blood lipids and markers for cardiovascular health. Currently, there is minimal data available regarding the effects of soy consumption in mixed populations of normo- and mildly hypercholesterolemic men and women. This study was initiated to examine the effects of 40 grams (g) soy protein daily for three months on blood lipids and markers for coronary heart disease. The ***hypothesis*** of this study is that soy protein consumption by humans reduces the risk of coronary heart disease by improving serum lipid profiles and body composition. To test our hypothesis, we have two specific aims as follows:

Specific Aim 1: To determine if 40 g of soy protein daily for three months lowers total and LDL-cholesterol, and increases HDL-cholesterol.

Specific Aim 2: To determine if 40 g of soy protein daily for three months lowers lipoprotein(a) concentration.

Specific Aim 3: To determine if 40 g of soy protein daily for three months affects anthropometric parameters associated with increased risk of coronary heart disease.

CHAPTER II

REVIEW OF LITERATURE

Incidence of Coronary Heart Disease in Humans

According to the American Heart Association (AHA), the number of deaths from coronary heart disease has been on the rise as the percentage of people over the age of 65 has increased (2). This recent finding conflicts with expectations, especially since the mortality rate from CHD was on the decline over the last decade (16). The discrepancy in thinking seems to be due to the update of the U.S. population projection. As part of the AHA's 1999 Heart and Stroke Statistical Update, the U.S. population projection for 2000 consists of many more older people than the last figure, calculated in 1940 (2). Cardiovascular disease (CVD) is the leading cause of death in American men and women (17). It causes 1.6 times as many deaths as cancer in men, and twice as many deaths as cancer in women (17). Of the 59.7 million Americans who have some form of cardiovascular disease, 12.2 million have coronary heart disease (17). In 1997, CVD claimed the lives of 953,110 Americans, which accounts for 41.2 percent of all deaths, and CHD was responsible for *half* of all CVD deaths (17). From 1900 to 1965, deaths from CHD have risen from almost 0 to over 700,000 and have not fallen below that mark to date (17).

The identification of risk factors for CHD has made the goal of prevention seem much more attainable. However, the tremendous effort put forth to inform the public about modifiable risk factors has been surprisingly ineffective. It appears that people are resistant to changing old habits in ways that could seriously reduce their risk for CHD (1).

Risk Factors for Coronary Heart Disease

Obesity

The relationship between obesity and CHD has long been established (18). The definition of obesity varies because of the difference in criteria used in its assessment. One classification of obesity is based on the body mass index (BMI) (19). Weight is measured in kilograms and divided by height measured in meters squared (19). Low risk of health complications is associated with a BMI of $\leq$ 25-30 (20). A BMI of 30-35 is correlated with a moderate risk of health complications (20). A BMI of 35-40 or greater is correlated with a high risk of health complications (20).

According to the Center for Disease Control, 105.7 million Americans age 20 and older are considered overweight (using a BMI of 25 or higher), and 43.1 million are considered obese (using a BMI of 30 or higher) (17).

As important as the amount of fat weight in assessing CHD risk, the distribution of fat plays a key role as well. Studies have shown that upper body fat, particularly abdominal fat, has been linked to a greater increase in risk for CHD than lower body fat (21). Upper body fat, also termed android fat, is particularly common in men. This may also explain the increased risk for CHD in men than women of similar age (21).

Physical activity

Lack of physical activity is a risk factor for development of CHD (1). About 25% of all Americans age 18 or older have no leisure-time physical activity, and at least 60% of adults do not achieve the recommended 30 minutes of vigorous physical activity at least 3-4 days per week (17). Benefits of physical activity in the prevention of CHD include, but are not limited to: improved vascular integrity, decreased resting blood pressure, decreased resting heart rate, increased heart efficiency, as well as a significant improvement of blood lipid profiles (22). Exercise has been shown to significantly decrease total cholesterol, LDL-cholesterol and triglycerides, and increase HDL-cholesterol (23-24).

Smoking

There are more preventable deaths from cardiovascular disease caused by smoking than any other modifiable risk factor (25). Smoking causes about 1 in 5 deaths from cardiovascular disease (17). The main cause of death related to smoking is myocardial infarction. Smoking accelerates the rate of atherosclerosis and is implicated in cardiac complications such as hypercoagulability, increased cardiac work, reduced oxygen transport, catecholamine liberation, and vasoconstriction (26).

Hyperlipidemia

About 99.5 million Americans have a total blood cholesterol level $\geq$ 200 mg/dL, which is considered borderline-high (17). Nearly 40 million Americans have a total blood cholesterol $\geq$ 240 mg/dL, which is considered high (17). These numbers indicate the extent to which Americans are battling high blood lipids.

Triglycerides, non-esterified fatty acids, phospholipids, and cholesterol are the major lipids found in the blood. Cholesterol and phospholipids make up hormones and membranes of cells. Because triglycerides and non-esterified fatty acids are insoluble in water, their transport in the aqueous environment of the blood is very difficult. In order to travel in the blood, they must be bound to the abundant serum protein, albumin, or inside the core of special transport proteins called lipoproteins. Transport with albumin is relatively inefficient, therefore, the majority of lipids are transported via lipoproteins. Lipoproteins are composed of a triglyceride and cholesterol core surrounded by a hydrophilic layer of phospholipids and cholesterol, as well as apolipoproteins, which are dispersed throughout the lipoprotein. Apolipoproteins can function in structure stabilization, receptor recognition, enzyme activation, or any combination of these.

The four primary classes of lipoproteins, in order of lowest to highest density, include chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). Chylomicrons make up the major portion of lipoprotein from dietary fat and are usually not present in the blood stream during the fasting state. VLDL's presence in the blood is very short lived, as it is quickly converted to LDL.

Low-density lipoprotein transports about 60% of the cholesterol found in the blood. Its main function is to carry cholesterol to tissues where it is used in making cell membranes and hormones. LDL binds to receptors in cell walls, and is then taken in by the cell and degraded. This process is especially important in the endothelium of the vascular system, where the process of atherosclerosis, and ultimately coronary heart disease originates.

Atherosclerosis is a disease of the vascular endothelium in which lipid material accumulates in the vessel walls, eventually causing swelling, or even damage to the endothelium, which accelerates the collection of lipids and proteins. This deposited lipid combined with clotting factors and proteins is known as plaque. An excess of LDL in the endothelium promotes the formation of plaques, which can expand inner vessel walls, and ultimately cause blockage (27).

High-density lipoprotein, the other major carrier of cholesterol, opposes LDL by removing cholesterol from the artery wall, and by preventing the oxidation of lipids in LDL. The role of HDL in atherosclerosis is significant, as it can prevent the formation of plaques by removing deposited cholesterol in the vascular endothelium (27).

A lipoprotein often overlooked when considering risk factors for CHD is lipoprotein(a) (Lp(a)). High lipoprotein(a) levels are correlated with premature myocardial infarctions. Lp(a) may contribute to clot formation through several mechanisms including inhibited fibrinolysis, increased oxidation of LDL-cholesterol, and increased deposition of cholesterol in arterial walls (28). Lp(a) consists of low-density lipoprotein and apolipoprotein(a), yet its quantity in the blood seems to be independent of LDL-cholesterol levels (28).

Cholesterol-lowering Drugs

From our understanding of cholesterol, it is clear that reducing LDL-cholesterol can prevent formation and progression of plaques that may block vessels. Many of today's lipid- lowering drugs are engineered to reduce LDL-cholesterol levels. However, these drugs must be taken continuously in order to achieve continued results (29).

One of today's most widely used lipid-lowering drugs is the classification known as hydroxymethyglutaryl coenzyme A (HMG-CoA) reductase inhibitors ("statins"). Such drugs include: fluvastatin (Lescol™), pravastatin (Pravachol™), simvastatin (Zocor™), Lovastatin (Mevacor™), atorvastatin (Lipitor™), and cerivastatin (Baycol™). As the name implies, these drugs work by inhibiting HMG-CoA reductase, the rate-limiting enzyme that catalyzes the conversion of HMG-CoA to mevalonate, in the synthesis of cholesterol. It has been reported that these drugs are most effective in lowering LDL-cholesterol and triglycerides, and they may increase HDL levels as well (29).

Another classification of lipid-lowering drugs is known as fibric acid derivatives ("fibrates"). This class of drugs includes gemfibrozil (Lopid™), fenofibrate (Triclor™), clofibrate (Atromid-S™), and bezafibrate (Bezalip™). Fibrates are most often effective in lowering triglycerides, but may also decrease LDL-cholesterol and increase HDL-cholesterol, by involving the activation of peroxisome proliferator-activated receptor-alpha-1 in the liver, thereby improving transport rates of several lipoproteins (30, 31, 32).

Niacin (nicotinic acid) is used to decrease triglycerides, LDL-cholesterol, and lipoprotein(a), but most effectively increases HDL-cholesterol (33, 34). The use of niacin

to improve lipid profiles has been limited due to the side effects experienced from the high amounts and inconvenient dosing schedules required (7, 33).

Bile-acid binding resins, cholestyramine (Questran) and colestipol (Colestid), are most effective in lowering LDL-cholesterol in people without hypertriglyceridemia (35). They work by up-regulating LDL-receptor, thereby decreasing intrahepatic cholesterol through interruption of enterohepatic circulation of cholesterol-rich bile acids (36). The most common complaints from users of resins include GI upset and constipation (29). Resins were among the very first drugs developed for hyperlipidemia, but their use has diminished over time with the development of other more effective drugs with fewer side effects.

The Hypocholesterolemic Property of Soy

Recently the Food and Drug Administration (FDA) approved soy as a food source that can lower cholesterol and therefore lowers the risk of CHD. The FDA's approval is based on numerous animal and clinical studies that have shown the efficacy of soy in lowering cholesterol. The hypocholesterolemic effects of soy protein have been extensively studied in men, premenopausal women, postmenopausal women, and various animal models (8, 9, 11, 37-38). However, its cholesterol lowering effects in mixed populations have not been studied. Postmenopausal women, in particular, can benefit from consumption of soy products since their risk for cardiovascular disease nearly doubles as they enter menopause (5, 39).

Isoflavones are constituents of soy that has been extensively investigated. Isoflavones belong to a class of phytochemicals called phytoestrogens, named for their

similarity in function to estrogen. Genistein, the most predominate isoflavone, has been shown in vitro and in vivo to have an estrogenic effect about 1×10^{-4} that of estradiol (12). Isoflavones are readily converted by intestinal bacteria into equol, which is rapidly absorbed in the gut, conjugated in the liver, and excreted in the urine. Elevated urinary isoflavone levels (as much as 1,000-fold) have been reported in humans following soy consumption (21).

Soy protein also contains several trypsin inhibitors, which work by stimulating the secretion of cholecystokinin (40). Cholecystokinin stimulates contraction of the gall bladder and bile acid secretion, which binds cholesterol in the intestine for excretion. Soy protein contains considerable amounts of the Bowman-Birk inhibitor, a trypsin inhibitor found in some grains and beans, which may partially explain the hypocholesterolemic effect of soy protein. However, studies conducted with soy protein, casein, and varying amounts of added trypsin inhibitor suggest that its role is negligible (40).

Vegetable proteins, specifically soy protein, contain compounds known as saponins (8). Saponins are complex structures consisting of carbohydrate moities attached to an aglycon, a steroidal molecule. It has been suggested by animal studies that saponins decrease cholesterol by increasing bile acid excretion (41). However, when saponins are added to soy protein, no effects are observed (42), giving the idea that saponins are not responsible for the cholesterol-lowering effect of soy protein.

Also present in soy protein are phytates, or phytic acid, which chelate calcium, iron, magnesium, and zinc, thus decreasing their absorption. Diets with high zinc to copper ratios are associated with hypercholesterolemia (8). Animal studies have shown

that addition of phytates to the diet lowered serum total cholesterol (43). Therefore, it is theorized that by improving the zinc to copper ratio, soy protein lowers cholesterol.

The amino acid content of soy protein has also been considered as a basis for its hypocholesterolemic effect. Animal studies have reported that arginine decreases blood cholesterol levels, while lysine increases blood cholesterol levels (44). Soy protein has a more suitable ratio of arginine to lysine than other proteins, such as casein (8). One animal study showed that adding lysine to soy protein caused an increase in total cholesterol by about 50% (14).

Other animal studies have reported that when soy protein is exposed to pepsin, two different fractions, high-molecular-weight fraction (HMF), and lower-molecular-weight fraction (LMF), are formed (45). When HMF was fed to hypercholesterolemic women, steroid excretion increased and cholesterol levels decreased (46).

The exact mechanism, or mechanisms, underlying soy protein's ability to lower cholesterol is not completely understood. There are many nutritive and non-nutritive constituents of soy protein that have been, and continue to be investigated. It is most likely that soy protein's hypocholesterolemic effect is due to a combination of its many beneficial components.

CHAPTER III

RESEARCH DESIGN AND METHODS

Subject Characteristics

Men and women with diverse ethnic backgrounds, who live in metropolitan and rural areas within reasonably commutable distances, were recruited for this study from Oklahoma State University faculty and staff, health-care clinics, independent living facilities, churches, and through advertisements at large in Stillwater, Oklahoma, and the surrounding area. A total of 135 mobile individuals (65 men and 70 women), ranging in age from 27 to 87 years were included in the study. Subjects were pre-screened via a phone interview, which included a short medical history questionnaire to identify qualified potential participants. Subjects were excluded if they had rheumatoid arthritis, joint pain due to injury, cancer or a history of cancer, insulin dependent diabetes mellitus, kidney disease, gastrointestinal or chronic digestive disorders, and allergy to milk, eggs, or soy. There were no special selection criteria in regard to cholesterol levels. A total of ninety subjects (44 men and 46 women) completed the study.

Study Overview

The study was a double-blind design. Eligible men and women were randomly assigned to consume 40 g of protein per day in the form of a powdered supplement containing either soy protein or casein (control). Of the 135 initial study participants, 64 consumed soy and 71 consumed casein. It was made clear to the study participants that they had an equal chance of being placed on either the soy or the casein regimen. The treatment period was three months. Regimens were provided to the subjects monthly for daily consumption of two packets containing a powdered drink mix, donated by Protein Technologies International (St. Louis, MO). Both regimens supplied equal amounts of protein (40 g/day), carbohydrate (18 g/day), fat (0 g/day), and total calories (240 kcal/day). The rationale for choosing the soy dose (40 g/day) was based on the effective amount previously used in clinical studies (11). Compliance with the study protocol was monitored via the following means: 1) subjects were provided with a monthly calendar for recording consumption of the contents of the provided packets on a daily basis; 2) subjects returned any unconsumed packets to the investigators on their monthly visits, unused supplies were counted and recorded; and 3) body weight was monitored monthly to ensure the treatment regimens were not promoting excess caloric intake. Subjects were given access to an RD/LD for advice on how to incorporate the supplement into their diets. A total of ninety subjects (44 men and 46 women) completed the study with 45 subjects on each of the two treatments.

Subjects met with the investigators for a total of five visits. Visit 1 included a verbal and a written explanation of the project, signing a consent form, a detailed medical

history questionnaire to confirm prescreening findings and to insure that subjects did not have any conditions violating the inclusion/exclusion criteria. Subjects were scheduled to come back to the study site and instructed regarding blood and urine collection for their next visit. Visit 2 occurred between the hours of 8-10 a.m. for the collection of over-night fasted blood samples (20 ml) of venous blood drawn by a registered and licensed nurse), a 24-hour urine specimen collected during the day prior to this visit, and anthropometric data. Anthropometric measurements included: height, weight, and waist to hip ratio. Participants were given their food supplies at this visit. Visits 3 and 4 were monthly visits for the purposes of replenishing the subject's food supply, monitoring intake of the supplies as well as body weight. The final visit, visit 5, occurred three months from initiation of the study, and included all measures and assessments performed on Visit 2.

Subject Confidentiality

Upon entrance into the study, each subject was assigned an identification number. This number was used for tracking the subject's records throughout the study. The principal investigator kept confidential data such as medical history, nutrition questionnaire and assigned numbers in a secured cabinet with restricted access. Thereafter, the samples from each study participant carried a number with no personal information available to the laboratory or data entry personnel.

Blood Collection and Serum Analyses

Fasting venous blood for plasma and serum analyses were collected at a designated time from each subject in Vacutainer (Franklin Lakes, NJ) tubes with appropriate anticoagulants or without anticoagulants at baseline and at the end of the study. Serum and plasma were separated from the blood (centrifuged at 1500 x g for 20 minutes) within 2 hours of collection and immediately aliquoted into small volumes and stored at -80°C until required for analysis. In this study, we measured serum total cholesterol, HDL-cholesterol, triglycerides, and lipoprotein(a). All the measurements were reported in Standard International (SI) Units.

Serum triglycerides (TG) and total-cholesterol (TC) were determined enzymatically using kits from Roche Diagnostic Systems (Somerville, NJ). The method for total cholesterol is based on the procedure described by Allain et al (47). Cholesterol is released enzymatically from its esters by cholesterol esterase. Total free cholesterol is oxidized by cholesterol oxidase producing hydrogen peroxide. Hydrogen peroxide, when combined with 4-aminoantipyrine and phenol, forms a quinoneimine dye that absorbs at 500 nm. The absorbance is directly proportional to the cholesterol concentration in the sample.

In the assessment of triglycerides, they are hydrolyzed by lipoprotein lipase to glycerol and fatty acids. Glycerol then reacts with adenosine triphosphate (ATP) and oxygen to produce hydrogen peroxide. The hydrogen peroxide reacts with 4-chlorphenol and 4-aminophenazone, and forms a quinoneimine dye that absorbs at 500 nm. The absorbance is directly proportional to the tryglyceride concentration in the sample.

Serum HDL-cholesterol was determined by a direct method (Unimate HDL Direct, Roche Diagnostic Systems, Somerville, NJ) utilizing synthetic polymers, polyanions and detergent. These compounds solubilize cholesterol from VLDL, LDL and chylomicrons but not HDL. The cholesterol in HDL is then determined enzymatically using the method described by Allain et al (48). LDL cholesterol was calculated using the Friedewald equation: (48).

$$LDL\text{-}C = (Total\text{-}C) - (HDL\text{-}C) - (TG/5)$$

Lipoprotein(a) was determined by an immunoprecipitation technique (DiaSorin, Stillwater, MN). The sample (antigen) is reacted with an antibody producing turbidity. The amount of turbidity is directly proportional to sample concentration and is measured at a wavelength of 340 nm.

Each of these tests were performed on a Cobas-Fara II Clinical Analyzer (Montclair, NJ) following the manufacturer's instructions and using commercially available calibrators and quality control samples.

Anthropometric Measurements

Height, weight, and waist-to-hip ratio were collected at baseline. Waist-to-hip ratio was measured during the final visit. Weight was monitored during each monthly follow-up visit. If weight gain was apparent, counseling was available to make adjustments in the diet to prevent further gain. Instructions were also provided for inclusion of the supplement into the diet. The protocol for assessing anthropometric measurements was adopted from the NHANES III survey (49).

Body weight was measured on a medical scale (Health-O-Meter, Continental Scale Corp., Chicago, IL) and subjects dressed in light clothing and without shoes or jewelry. The height was taken on an Acustat Genetech Stadiometer (San Francisco, CA). Subjects were asked to take a deep breath, stand with their heels together and touching the wall, and their shoulders and head touching the back of the stadiometer. Heights were measured to the nearest 0.1-inch.

Circumferences were measured with measuring tape while subjects were wearing light clothing, were relaxed, were standing erect, and had their arms at their sides and feet together. Waist circumference was measured midway between the lower rib and iliac crest whereas hip circumference was measured at the outermost points of the greater trochanters (50). The value obtained was the ratio of the waist circumference to the hip circumference.

Data Management and Statistical Analyses

A graduate student trained for permanent storage of data compiled the data from subjects on a weekly basis and entered it into the central database filing system. The laboratory-generated raw data and printouts were recorded/kept in a secured storage area. All of the original data were stored in a locked cabinet with restricted access.

Descriptive statistics were calculated for all variables and included means and standard deviations. The data were analyzed using PC SAS version 6.12 (SAS Inst., Carry, NC). The primary outcome variables were serum lipid parameters and anthropometric parmeters. Treatment (soy vs. casein) effects were assessed using

analysis of variance. Statistical significance level was set at $p<0.05$ for all statistical analyses.

CHAPTER IV

RESULTS

Subject Participation

Subjects participating in this study included one hundred thirty-five healthy men and women with a mean (±SD) age of 57.6 ± 1.1 years. Subject dropouts included a total of forty-five over the entire course of the study, who were also excluded from statistical analyses. Reasons for discontinuing included taste aversion, gastrointestinal disturbance, inconvenience of powdered-protein consumption, or starting a new drug therapy that could affect the outcome of the study.

Anthropometric Measurements in All Participants

Consumption of 40 g soy protein or casein daily for a three-month period did not significantly increase body weight. In contrast, subjects on soy protein had somewhat (-2.44 lbs) lower final body weights. There were no observed significant differences in anthropometric measurements between the treatment groups **(Table I)**.

Serum Lipid Levels in All Participants

The changes in the serum levels of cholesterol, triglycerides, and Lp(a) before and after both treatments are reported in **Table II**. Soy protein did not lower serum total- and LDL-cholesterol or Lp(a) in comparison with casein. Additionally, as expected, soy regimen somewhat ($p<0.1$) increased serum HDL cholesterol concentrations in comparison with casein.

Serum Lipid Data for All Participants with Total Cholesterol $\geq$200 mg/dl

Soy protein also did not lower serum total cholesterol in comparison with casein after subjects with total cholesterol measurements <200 mg/dl were eliminated from the data set.

Table I. Anthropometric Data for All Participants

	Soy Protein		Casein		Effect Of Treatment*Gender	Effect Of Treatment
Physical Parameters	Baseline	Final	Baseline	Final	*p*-value	*p*-value
Age (yrs)	57.30 ± 1.62 n=44	----	57.78 ± 1.53 n=46	----	----	----
Body Weight (lbs)	209.70 ± 62.79 n=43	206.26 ± 64.61	197.46 ± 40.83 n=43	199.11 ± 41.87	<0.41	<0.22
Height (in)	67.93 ± 0.45 n=43	----	67.79 ± 0.42 n=43	----	----	----
Body Fat %	36.1 ± 7.20 n=34	35.10 ± 9.87	35.87 ± 7.48 n=34	35.01 ± 7.05	<0.56	<0.85
BMI	31.64 ± 8.41 n=42	31.19 ± 8.80	30.48 ± 6.68 n=40	30.75 ± 6.90	<0.41	<0.25
Waist (in)	40.32 ± 6.88 n=43	40.88 ± 6.94	39.17 ± 5.67 n=43	39.95 ± 5.95	<0.26	<0.68
Hip (in)	44.98 ± 7.13 n=43	44.99 ± 7.50	43.87 ± 5.09 n=43	43.86 ± 5.6	<0.74	<0.94
Waist:Hip Ratio (in)	0.89 ± 0.06 n=43	0.91 ± 0.07	0.89 ± 0.07 n=43	0.91 ± 0.06	<0.24	<0.83

Values presented are mean ± SD.

Table II. Serum Lipid Data for all Participants

	Soy Protein		Casein		Effect of Treatment*Gender	Effect of Treatment
Serum Parameters	Baseline	Final	Baseline	Final	*p*-value	*p*-value
Total Cholesterol (mg/dl)	221.15 ± 41.09 n=44	221.50 ± 37.21	229.20 ± 46.28 n=44	229.70 ± 45.98	<0.57	<0.98
HDL-cholesterol (mg/dl)	56.16 ± 20.75 n=44	58.02 ± 16.42	58.73 ± 18.41 n=44	57.43 ± 2.56	<0.90	<0.10
Triglyceride (mg/dl)	207.64 ± 112.47 n=44	203.64 ± 151.21	169.34 ± 86.59 n=44	200.85 ± 133.07	<0.73	<0.03
LDL-cholesterol (mg/dl)	123.47 ± 35.99 n=44	123.09 ± 36.15	136.60 ± 38.01 n=44	132.10 ± 33.89	<0.60	<0.31
Lipoprotein(a) (mg/dl)	25.55 ± 26.81 n=42	29.93 ± 27.68	33.65 ± 32.95 n=38	36.01 ± 35.84	<0.61	<0.35

Values presented are mean ± SD.

Table III. Serum Lipid Data for all Participants with Total Cholesterol < 200 mg/dl

	Soy Protein		Casein		Effect of Treatment*Gender	Effect of Treatment
Serum Parameters	Baseline	Final	Baseline	Final	*p*-value	*p*-value
Total Cholesterol for All Participants (mg/dl)	246.86 ± 34.14 n=28	237.32 ± 29.93	247.79 ± 33.43 n=32	250.69 ± 39.36	<0.50	<0.32
Total Cholesterol for All Men (mg/dl)	248.47 ± 28.19 n=14	230.57 ± 21.81	243.67 ± 32.54 n=11	246.36 ± 45.17	----	----
Total Cholesterol for All Women (mg/dl)	245.58 ± 31.79 n=14	244.07 ± 42.97	250.34 ± 34.28 n=21	252.95 ± 36.95	----	----

Values presented are mean ± SD.

CHAPTER V

DISCUSSION

The present study was designed to evaluate the cholesterol-lowering properties of soy protein in comparison with casein, as a part of an unmodified diet. Many clinical trials have demonstrated the hypocholesterolemic effects of soy protein in subjects with elevated serum cholesterol (6-8, 10). The results of this study neither confirm nor reject the potential benefits of soy consumption on lipid profiles in humans. The findings of this study indicate that consumption of soy protein in the amount of 40 g per day has little effect on total-, HDL-, and LDL-cholesterol concentrations in normolipidemic as well as mildly hypercholesterolemic men or women. For the present study, however, it should be noted that subjects were not recruited on the basis of baseline cholesterol values. In fact, the study participants were only mildly hypercholesterolemic, with average means of baseline total cholesterol ranging from 222 to 224 mg/dl, respectively for soy and casein regimens. Also, as part of the original study protocol, study participants were not required to modify their dietary intake, as has been the case in many clinical trials examining the hypocholesterolemic effects of soy protein (51-52).

One interesting effect of soy protein was its ability to lower serum triglyceride levels in comparison to casein. The lowering effect is accentuated by the large increase in triglyceride levels in those on the casein regimen. One explanation for this effect may be

the lower baseline triglyceride level in those on the casein regimen, especially if study participants were not truly fasting for their final blood draw.

It is also difficult to explain the lack of effect of soy protein on serum cholesterol, especially given the majority of studies demonstrating a cholesterol-lowering effect of soy protein, and the recent FDA approval of the use of soy protein to lower cholesterol. One possible explanation could be the near normal baseline cholesterol levels of the participants. It has been clearly established in several studies that the reduction of cholesterol with soy protein was inversely related to the baseline level of cholesterolemia (4). However, when the 60 subjects with total cholesterol levels below 200 mg/dl were excluded from analyses, there was no significant lowering of total cholesterol observed. Many studies demonstrating the hypocholesterolemic effect of soy protein required subjects to totally or partially replace animal protein with soy protein (11, 52), or to follow a conventional low-fat (39), or NCEP Step I diet (21). No dietary modifications other than consumption of the powdered supplement were required in this study.

Conclusions

The results of this 12-week study suggest that soy protein exhibits little effect on anthropometric parameters associated with coronary heart disease. Furthermore, soy protein may also be of little benefit in lowering blood lipids of normolipidemic men and women. However, it should be noted that soy protein exhibited much more favorable effects on blood lipid profiles after 24 weeks on a soy protein regimen (11). It should be also be noted that soy protein exhibited much more favorable effects on anthropometric

and lipid parameters in this study when using a paired t-test to compare the two treatments' (soy protein and casein) baseline and final measurements (**Appendix II**).

Additional studies are needed to confirm whether the beneficial effects of soy protein on blood lipid profiles and body composition found in other studies are due to the addition of soy protein, or are merely due to dietary modifications. It may be the case that soy protein has a synergistic effect on blood lipids when combined with a low-fat diet.

LITERATURE CITED

1. Guide to Primary Prevention of Cardiovascular Diseases: AHA recommendation. AHA Medical/Scientific Statement. American Heart Association. 2000.

2. Heart and Stroke Facts. Statistical Supplement. American Heart Association. National Health Center. 1999.

3. Stone, N.J., Nicolosi, R.J., Kris-Etherton, P., Ernst, N.D., Krauss, R.M., Winston, M. Summary of the scientific conference on the efficacy of hypocholesterolemic dietary interventions. AHA Conference Proceedings. Circulation. 94: 3388-3391. 1996.

4. Sirtori, C.R., Lovati, M.R., Manzoni, C., Monetti, M., Pazzucconi, F., Gatti, E. Soy and cholesterol reduction: clinical experience. J. Nutr. 125: 598S-605S. 1995.

5. Stein, K. FDA approves health claim labeling for foods containing soy protein. J. Am. Diet. Assoc. 100: 292. 2000.

6. Carroll, K.A. Review of clinical studies on cholesterol-lowering response to soy protein. J. Am. Diet. Assoc. 91: 820-827. 1991.

7. Grundy, S.M., Abrams, J.J. Comparison of actions of soy protein and casein on metabolism of plasma lipoproteins and cholesterol in humans. Am. J. Clin. Nutr. 38: 245-252. 1983.

8. Potter, S. M. Overview of proposed mechanisms for hypocholesterolemic effect of soy. J. Nutr. 125: 606S-611S. 1995.

9. Meinertz, H., Nilausen, K., Faergeman, O. Soy protein and casein in cholesterol-enriched diets: effects on plasma lipoproteins in normolipidemic subjects. Am. J. Clin. Nutr. 50: 786-793. 1989.

10. Lovati M.R., Manzoni C., Canavesi A., Sirtori M., Vaccarino V., Marchi M., Gaddi G. Soybean protein diet increases low density lipoprotein receptor activity in mononuclear cells from hypercholesterolemic patients. J. Clin. Invest. 80: 1498-1502. 1987.

11. Potter, S. M., Baum, J.A., Teng, H., Stillman, R.J., Shay, N.F., Erdman, J.W. soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am. J. Clin. Nutr. 68: 1375S-1379S. 1998.

12. Arjmandi, B.H., Khan, D.A., Juma, S.S., Svanborg, A.S. The ovarian hormone deficiency-induced hypercholesterolemia is reversed by soy proteins and the synthetic isoflavone, ipriflavone. Nutr. Res. 17: 885-894. 1997.

13. Walsh, B.W., Schiff, I., Rossner, B., Greenberg, I., Ravnikar, V., Sacks, F.M. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N. Engl. J. Med. 325: 1196-1204. 1991.

14. Kritchevsky, D. Dietary protein and experimental atherosclerosis. Ann. N.Y. Acad. Sci. 676: 180-187. 1993.

15. Nilausen, K., Meinertz, H. Lipoprotein(a) and dietary problems: Casein lowers lipoprotein(a) concentrations as compared with soy protein. Am. J. Clin. Nutr. 69: 419-425. 1999.

16. Heart and Stroke Facts. Statistical Supplement. American Heart Association. National Health Center. 1996.

17. Risk Factors; 2000 Statistical Supplement. American Heart Association. 2000.

18. Dawber, T.R. The Framingham Study. The epidemiology of atherosclerotic disease. Harvard University Press. 1980.

19. Welborn, T.A., Knuiman M.W., Vu H.T. Body mass index and alternative indices of obesity in relation to height, triceps skinfold and subsequent mortality: the Busselton health study. Int. J. Obes. Relat. Metab. Disord. 24: 108-115. 2000.

20. Bray, G.A. Pathophysiology of obesity. Am. J. Clin. Nutr. 55: 488S-494S. 1992.

21. Azevedo A., Ramos E., von Hafe P., Barros H. Upper-body adiposity and risk of myocardial infarction. J. Cardiovasc. Risk. 6: 321-5. 1999.

22. Laughlin, M.H. Cardiovascular response to exercise. J. Am. Physiol. 277: S244-259. 1999.

23. Halbert, J.A. Silagy, C.A. Finucane, P. Withers, R.T. Hamdorf, P.A. Exercise training and blood lipids in hyperlipidemic and normolipidemic adults: a meta-analysis of randomized, controlled trials. Eur. J. Clin. Nutr. 53: 514-522. 1999.

24. Kokkinos, P.F. Fernhall B. Physical activity and high density lipoprotein cholesterol levels: what is the relationship? Sports Med. 28: 307-314. 1999.

25. Ayanian, J.Z. Cleary, P.D. Perceived risks of heart disease and cancer among cigarette smokers. J. A. M. A. 281(17): 1019-1021. 1999.

26. Adnot, S. Tobacco: an atherogenic, thrombogenic or spasmogenic factor? Arch. Mal. Coeur. Vaiss. 5: 53-58. 1998.

27. Bonnefont-Rousselot, D., Therond, P., Beaudeux, J.L., Peynet, J., Legrand, A., Delattre, J. High-density lipoproteins (HDL) and the oxidative hypothesis of atherosclerosis. Clin. Chem. Lab. Med. 37: 939-48. 1999.

28. Seman, L.J., McNamera, J.R., Schaefer, E.J. Lipoprotein(a), homocysteine, and remnantlike particles: emerging risk factors. Curr. Opin. Cardiol. 14: 186-191. 1999.

29. Callister, T.C., Raggi, P., Cooil, B., Lippolis, N.J., Russo, D.J. Effect of HMG-CoA reductase inhibitors on coronary artery disease as assessed by electron-beam computed tomography. New Engl. J. Med. 339: 1972-1978. 1998.

30. Rader, D.J., Haffner, S.M. Role of fibrates in the management of hypertriglyceridemia. Am. J. Card. 83: 30F-35F. 1999.

31. University Department of Medicine, University of Western Austrailia. Fibrates, dyslipoproteinemia, and cardiovascular disease. Curr. Opin. Lipidol. 6: 561-574. 1999.

32. Staels, B., Dallongeville, J., Auwerx, J., Schoonjans, K., Leitersdorf, E., Fruchart, J.C. Mechanism of action of fibrates on lipid and lipoprotein metabolism. Circulation. 19: 2088-2093. 1998.

33. Goldberg, A.C. Clinical trial experience with extended-release niacin (Niaspan): dose-escalation study. Am. J. Cardiol. 82: 35U-38U. 1998.

34. McKenney, J.M., McCormick, L.S., Weis, S., Koren, M., Kafonek, S., Black, D.M. A randomized trial of the effects of atorvastatin and niacin in patients with combined hyperlipidemia or isolated hypertriglyceridemia. Am. J. Med. 104: 137-143. 1998.

35. Ast, M., Frishman, W.H. Bile acid sequestrants. J. Clin. Pharmacol. 30: 99-106. 1998.

36. Farmer, J.A., Gotto, A.M., Jr. Choosing the lipid-regulating agent: A guide to selection. Drugs. 53: 828-847. 1997.

37. Anderson, J.W., Johnstone, B.M., Cook-Newell M.E. Meta-analysis of the effects of soy protein intake on serum lipids. N. Engl. J. Med. 333:276-282. 1995.

38. Li, Z., McNamara, J.R., Fruchart, J.C., Luc, G., Bard, J.M., Ordovas, P., Wilson, W.F., Schaefer, E.J. Effects of gender and menopausal status on plasma lipoprotein subspecies and particle sizes. J. Lipid Res. 37: 1886-1896. 1996.

39. Messina M.J., Persky, V., Setchell, K.D.R., Barnes, S. Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr. Cancer. 21: 113-131. 1994.

40. Roy, D.M., Schneeman, B.O. Effect of soy protein, casein, and trypsin inhibitor on cholesterol, bile acids and pancreatic enzymes in mice. J. Nutr. 111: 878-885. 1981.

41. Sidhu, G.S., Oakenfull, D.G. A mechanism for the hypocholesterolemic activity of saponins. Br. J. Nutr. 55: 643-649. 1986.

42. Potter, S.M., Jimenez-Florez, R., Pollack, J., Lone, T.A., Berber-Jimenez, M.D. Protein-saponin interaction and its influence on blood lipids. J. Agric. Food Chem. 41: 1287-1291. 1993.

43. Jariwalla, R.J., Sabin, R., Lawson, S., Herman, Z.S. Lowering of serum cholesterol and triglycerides and modulation of divalent cations by dietary phytate. J. Appl. Nutr. 42: 18-28. 1990.

44. Kurowska, E.M., Carroll, K.K. Hypercholesterolemic responses in rabbits to selected groups of dietary amino acids. J. Nutr. 124: 364-370. 1994.

45. Sugano, M., Goto, S., Yamada, Y. Cholesterol-lowering activity of various undigested fractions of soybean protein in rats. J. Nutr. 120: 977-985. 1990.

46. Wang, M.F., Yammamoto, S., Chung, H.M. Antihypercholesterolemic effect of undigested fraction of soybean protein in young female volunteers. J. Nutr. Sci. Vitaminol. 41: 187-195. 1995.

47. Allain, C.C., Poon L.S., Chan C.S.G., Richmond W., Fu P.C. Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470-475. 1974.

48. Hernandez, C., Chacon, P., Garcia-Pascual, L., Rossello, J., Simo, R. Lipoprotein (a) and the evaluation of low density cholesterol by the Friedewald formula: a new problem for an old equation Med. Clin. 113:290-291. 1999.

49. Kuczmarski, R.J., Flegal, K.M., Campbell, S.M., Johnson, CL. Increasing prevalence of overweight among U.S. adults: The National Health Examination Surveys, 1960-1991. J.A.M.A. 272: 205-211. 1994.

50. WHO Expert Committee on Physical Status. The use and interperetation of anthropometry. Report of a WHO Expert Committee. Geneva: World Health Organization. 1995.

51. Potter, S.M., Bakhit, R.M., Essex-Sorlie, D.L. Depression of plasma cholesterol in men by consumption of baked products containing soy protein. Am. J. Clin. Nutr. 58: 501-506. 1993.

52. Wong, W.W., Smith, E.O., Stuff, J.E. Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. Am. J. Clin. Nutr. 68: 1385S-1389S. 1998.

APPENDIX I

INSTITUTIONAL REVIEW BOARD APPROVAL FORM

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD

DATE: 10-28-98 **IRB #: HE-99-032**

Proposal Title: THE EFFECT OF SOY OR ITS ISOFLAVONES ON OSTEOARTHRITIS

Principal Investigator(s): Bahram H. Arjmandi, Mark E. Munson

Reviewed and Processed as: Modification

Approval Status Recommended by Reviewer(s): Approved

Signature: Carol Olson Date: January 27, 1999

Carol Olson, Director of University Research Compliance

Approvals are valid for one calendar year, after which time a request for continuation must be submitted. Any modification to the research project approved by the IRB must be submitted for approval. Approved projects are subject to monitoring by the IRB. Expedited and exempt projects may be reviewed by the full Institutional Review Board

APPENDIX II

ANTHROPOMETRIC AND SERUM LIPID DATA ASSESSED USING A T-TEST

Table I. Anthropometric Data for All Participants

	SOY PROTEIN			CASEIN		
Physical Parameters	Baseline	Final	*p*-value	Baseline	Final	*p*-value
Age (yrs)	57.30 ± 1.62 n=44	----	----	57.78 ± 1.53 n=46	----	----
Body Weight (lbs)	211.70 ± 6.51 n=43	206.26 ± 8.69	<0.24	195.74 ± 6.11 n=43	199.11 ± 8.71	<0.58
Height (in)	67.93 ± 0.45 n=43	----	----	67.79 ± 0.42 n=43	----	----
Body Fat %	36.10 ± 1.04 n=34	35.10 ± 1.15	<0.01	35.87 ± 0.96 n=37	35.01 ± 1.11	<0.02
BMI	32.38 ± 0.99 n=42	31.12 ± 1.19	<0.30	30.20 ± 0.95 n=40	30.61 ± 1.20	<0.55
Waist (in)	40.10 ± 0.80 n=43	40.88 ± 1.00	<0.15	38.77 ± 0.75 n=43	39.95 ± 1.00	<0.04
Hip (in)	45.34 ± 0.81 n=43	44.99 ± 1.03	<0.97	43.46 ± 0.77 n=43	43.85 ± 1.02	<0.95
Waist:Hip Ratio (in)	0.90 ± 0.01 n=43	0.91 ± 0.01	<0.06	0.89 ± 0.01 n=43	0.91 ± 0.01	<0.03

Values presented are mean ± SD.

Table II. Anthropometric Data for All Men

	SOY PROTEIN			CASEIN		
Physical Parameters	Baseline	Final	p-value	Baseline	Final	p-value
Age (yrs)	55.38 ± 2.40 n=22	----	----	56.86 ± 2.18 n=20	----	----
Body Weight (lbs)	233.74 ± 9.56 n=22	224.94 ± 12.15	P<0.09	199.82 ± 8.70 n=20	202.35 ± 12.74	P<0.70
Height (in)	71.01 ± 0.66 n=22	----	----	69.72 ± 0.60 n=20	----	----
Bodyfat %	32.79 ± 1.52 n=17	31.97 ± 1.63	P<0.17	31.70 ± 1.36 n=17	30.78 ± 1.63	P<0.08
BMI	32.80 ± 1.46 n=22	30.70 ± 1.67	P<0.11	29.23 ± 1.37 n=18	29.31 ± 1.75	P<0.69
Waist (in)	41.97 ± 1.18 n=22	42.85 ± 1.39	P<0.57	39.23 ± 1.07 n=20	40.65 ± 1.46	P<0.04
Hip (in)	45.90 ± 1.20 n=22	45.26 ± 1.46	P<0.92	42.76 ± 1.10 n=20	42.88 ± 1.50	P<0.62
Waist:Hip Ratio	0.94 ± 0.01 n=22	0.96 ± 0.01	P<0.24	0.92 ± 0.01 n=20	0.95 ± 0.02	P<0.01

Values presented are mean ± SD.

Table III. Anthropometric Data for All Women

	SOY PROTEIN			CASEIN		
Physical Parameters	Baseline	Final	*p*-value	Baseline	Final	*p*-value
Age (yrs)	59.23 ± 2.18 n=22	----	----	58.69 ± 2.15 n=24	----	----
Body Weight (lbs)	189.66 ± 8.83 n=21	187.58 ± 12.43	<0.99	191.65 ± 8.58 n=23	195.88 ± 11.88	<0.68
Height (in)	64.86 ± 0.61 n=21	----	----	65.87 ± 0.60 n=23	----	----
Bodyfat %	38.81 ± 1.43 n=17	38.23 ± 1.63	<0.02	39.71 ± 1.34 n=20	39.24 ± 1.50	<0.09
BMI	31.96 ± 1.35 n=20	31.53 ± 1.71	<0.92	31.18 ± 1.33 n=22	31.92 ± 1.63	<0.66
Waist (in)	38.24 ± 1.09 n=21	38.90 ± 1.42	<0.14	38.31 ± 1.06 n=23	39.24 ± 1.36	<0.43
Hip (in)	44.79 ± 1.10 n=21	44.71 ± 1.46	<0.88	44.16 ± 1.07 n=23	44.83 ± 1.40	<0.66
Waist:Hip Ratio	0.86 ± 0.01 n=21	0.87 ± 0.01	<0.15	0.87 ± 0.01 n=23	0.87 ± 0.01	<0.59

Values presented are mean ± SD.

Table IV. Serum Lipid Data for All Participants

	Soy Protein			Casein		
Serum Parameters	Baseline	Final	*p*-value	Baseline	Final	*p*-value
Total Cholesterol (mg/dl)	224.16 ± 5.45 n=44	221.84 ± 6.41	<0.86	222.27 ± 5.15 n=44	229.70 ± 6.43	<0.89
HDL-cholesterol (mg/dl)	57.03 ± 2.56 n=44	58.02 ± 2.55	<0.17	60.36 ± 2.42 n=44	57.43 ± 2.56	<0.34
Triglyceride (mg/dl)	197.50 ± 13.23 n=44	203.64 ± 7.51	<0.72	177.31 ± 2.55 n=44	200.85 ± 7.58	<0.01
LDL-cholesterol (mg/dl)	127.63 ± 4.83 n=44	123.09 ± 5.39	<0.88	126.44 ± 4.56 n=44	132.10 ± 5.41	<0.12
Lipoprotein(a) (mg/dl)	25.89 ± 3.67 n=42	30.08 ± 5.33	<0.005	26.02 ± 3.43 n=38	35.13 ± 5.55	<0.15

Values presented are mean ± SD.

Table V. Serum Lipid Data for All Men

	SOY PROTEIN			CASEIN		
Serum Parameters	Baseline	Final	p-value	Baseline	Final	p-value
Total Cholesterol (mg/dl)	226.03 ± 8.05 n=22	220.68 ± 9.06	<0.82	207.63 ± 7.33 n=20	214.40 ± 9.50	<0.63
HDL-cholesterol (mg/dl)	50.83 ± 3.78 n=22	53.55 ± 3.61	<0.46	55.26 ± 3.44 n=20	52.20 ± 3.79	<0.32
Triglycerides (mg/dl)	211.34 ± 19.64 n=22	194.41 ± 24.76	<0.38	178.57 ± 17.88 n=20	180.65 ± 25.97	<0.11
LDL-cholesterol (mg/dl)	132.94 ± 7.14 n=22	128.25 ± 7.62	<0.96	116.65 ± 6.50 n=20	126.07 ± 7.99	<0.68
Lipoprotein(a) (mg/dl)	22.67 ± 5.48 n=21	20.20 ± 7.45	<0.22	24.84 ± 4.81 n=18	37.47 ± 8.24	<0.45

Table VI. Serum Lipid Data for All Women

	SOY PROTEIN			CASEIN		
Serum Parameters	Baseline	Final	*p*-value	Baseline	Final	*p*-value
Total Cholesterol (mg/dl)	222.29 ± 7.33 n=22	223.00 ± 9.06	P<0.63	236.92 ± 7.23 n=24	245.00 ± 8.68	P<0.90
HDL-cholesterol (mg/dl)	63.23 ± 3.44 n=22	62.50 ± 3.61	P<0.23	65.47 ± 3.40 n=24	62.67 ± 3.46	P<0.74
Triglycerides (mg/dl)	183.66 ± 17.88 n=22	212.86 ± 24.76	P<0.71	176.06 ± 17.63 n=24	221.04 ± 23.71	P<0.02
LDL-cholesterol (mg/dl)	122.33 ± 6.50 n=22	117.93 ± 7.62	P<0.80	136.23 ± 6.41 n=24	138.13 ± 7.29	P<0.06
Lipoprotein(a) (mg/dl)	29.11 ± 4.88 n=21	39.96 ± 7.63	P<0.006	27.21 ± 4.88 n=20	32.79 ± 7.45	P<0.19

Values presented are mean ± SD.

DISCUSSION

Effects of Soy Protein Compared to Casein Using a T-Test

The previous tables contain the results of comparing the treatments' (soy protein and casein) baseline and final measurements using a t-test. The findings of this study according to these statistical analyses indicate that consumption of soy protein in the amount of 40 g per day has little effect on total-, HDL-, and LDL-cholesterol, or triglyceride levels. It is important to note that there was a trend of increasing LDL-cholesterol among women in the casein group. However, soy protein did not exhibit either of these effects in women.

An important effect of soy protein consumption was the significant increase ($p<0.006$) in lipoprotein(a) among women. This effect was not observed in men, as levels remained unchanged. High levels of lipoprotein(a) are now considered an independent risk factor for coronary heart disease, and have been reported to be a genetic factor that could affect a pathogenic trend in coronary heart disease. Since clinical reviews report that lipoprotein(a) levels do not respond to dietary intervention or some lipid-lowering drugs, it is interesting to observe this effect of soy protein. However, our data are not the first to report this effect of soy protein on Lp(a) (15). It is also interesting to note that Lp(a), a complex of LDL, increased independently of LDL-cholesterol levels among women on soy protein. This suggests that Lp(a) levels are controlled independently of LDL-cholesterol.

The findings of these statistical analyses suggest that soy protein may improve body composition by decreasing body fat percentage. In the soy protein group, a decrease in body fat percentage on average was observed in both men (-0.82%) ($p<0.17$) and women (-0.58%) ($p<0.02$). Subjects in the casein group tended to have decreased percent body fat, however, there were also significant increases in waist-to-hip ratio measurements in all subjects on the casein regimen. These findings suggest that soy protein may be beneficial in reducing abdominal body fat, especially in men, whose risk for CHD is higher than in women, particularly when considering high BMI and waist-to-hip ratio.

Conclusions

The results of these statistical analyses suggest that soy protein exhibits a favorable effect on % body fat and body fat distribution associated with coronary heart disease. Furthermore, soy protein may also be of benefit in maintaining healthy blood lipid profiles, in spite of it increasing serum levels of lipoprotein(a) in women.

Longer study duration, and dietary restrictions, such as low-fat, Step I, or Step II diets are needed to further elucidate the role of soy in the reduction of CHD risk. If other investigators confirm the findings of these analyses, further studies will be needed to determine if the previously reported beneficial effects of soy protein on blood lipid parameters and body composition are due to protein consumption alone, or the many nutritive constituents of soy protein.

VITA

Sonny Brandon Hodges

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF SOY PROTEIN ON LIPIDS AND LIPOPROTEIN(a) IN MEN AND WOMEN

Major Field: Nutritional Sciences

Biographical:

Education: Graduated from Guymon High School, Guymon, Oklahoma in May 1994; received Bachelor of Science degree in Nutritional Sciences from Oklahoma State University, Stillwater, Oklahoma in December, 1998. Completed the requirements for the Master of Science degree with a major in Nutritional Sciences at Oklahoma State University in May, 2000.