Effects of soy isoflavone consumption following a high fat meal on the oxidative resistance of healthy young men
by Danielle Ann Dufner

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Health and Human Development
Montana State University

Abstract:
Coronary heart disease (CHD), also referred to as coronary artery disease (CAD) or atherosclerosis, is the leading cause of death and disability in today's society. The statistics of this progressive disease are astounding; of the 13.9 million Americans who currently have CHD approximately 450,000 die annually, consequently supporting the need for research leading to the prevention of atherogenesis. It has been proposed that the oxidation of low-density lipoproteins (DDL) promotes this process of atherosclerotic development. Soy foods and their components have become of particular interest in impeding the atherogenic process through their ability to reduce oxidative stress. In 1979, Zilversfnit hypothesized that atherosclerosis is a postprandial occurrence. Therefore, studies conducted on soy and its ability to reduce oxidative stress should not only be performed in chronic settings but also performed in the postprandial state. The purpose of this study was to determine if a difference exists between 39 g soy (85 mg isoflavones) and 39.9 g milk protein (0 mg isoflavones) in combination with a high-fat meal in relation to their protection against postprandial oxidation. Fifteen healthy nonvegetarian men 20-47 years of age reported to the Nutrition Research Lab on the campus of Montana State University on two nonconsecutive days to participate in a double blind crossover study. Upon reporting to the lab the subjects had height and weight measurements completed, blood drawn via venipunture, then were presented with their challenge meal in which they had 20 minutes to consume. The challenge meal consisted of 2 apple muffins and a soy or milk shake (956 calories, 41% fat, 41% carbohydrate, and 18% protein). Plasma samples were obtained at baseline (fasted) and hours 2, 4, and 6 postprandially. Isolated LDL were subjected to ex vivo copper-induced LDL oxidation. Lag time, propagation rate, and initial absorbance were calculated for each time point analyzed. Results showed no significant difference ($p>0.05$) between the main effects (protein type, time points) or their interaction on LDL oxidation (lag time, propagation rate, or initial absorbance). Future studies are needed to clearly define the role of soy in its ability to reduce postprandial LDL oxidation..

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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TABLE OF CONTENTS

TABLE OF CONTENTS – CONTINUED

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

LIST OF FIGURES – CONTINUED

ABSTRACT

Coronary heart disease (CHD), also referred to as coronary artery disease (CAD) or atherosclerosis, is the leading cause of death and disability in today's society. The statistics of this progressive disease are astounding; of the 13.9 million Americans who currently have CHD approximately 450,000 die annually, consequently supporting the need for research leading to the prevention of atherogenesis. It has been proposed that the oxidation of low-density lipoproteins (LDL) promotes this process of atherosclerotic development. Soy foods and their components have become of particular interest in impeding the atherogenic process through their ability to reduce oxidative stress. In 1979, Zilversmit hypothesized that atherosclerosis is a postprandial occurrence. Therefore, studies conducted on soy and its ability to reduce oxidative stress should not only be performed in chronic settings but also performed in the postprandial state. The purpose of this study was to determine if a difference exists between 39 g soy (85 mg isoflavones) and 39.9 g milk protein (0 mg isoflavones) in combination with a high-fat meal in relation to their protection against postprandial oxidation. Fifteen healthy nonvegetarian men 20-47 years of age reported to the Nutrition Research Lab on the campus of Montana State University on two nonconsecutive days to participate in a double blind crossover study. Upon reporting to the lab the subjects had height and weight measurements completed, blood drawn via venipunture, then were presented with their challenge meal in which they had 20 minutes to consume. The challenge meal consisted of 2 apple muffins and a soy or milk shake (956 calories, 41% fat, 41% carbohydrate, and 18% protein). Plasma samples were obtained at baseline (fasted) and hours 2, 4, and 6 postprandially. Isolated LDL were subjected to *ex vivo* copper-induced LDL oxidation. Lag time, propagation rate, and initial absorbance were calculated for each time point analyzed. Results showed no significant difference ($p>0.05$) between the main effects (protein type, time points) or their interaction on LDL oxidation (lag time, propagation rate, or initial absorbance). Future studies are needed to clearly define the role of soy in its ability to reduce postprandial LDL oxidation.

CHAPTER 1

INTRODUCTION

Coronary heart disease (CHD), also referred to as coronary artery disease (CAD) or atherosclerosis, is the leading cause of death and disability in today's society. The statistics of this progressive disease are astounding; of the 13.9 million Americans who currently have CHD approximately 450,000 die annually, supporting the need for research leading to the prevention of atherogenesis (1). The process of athlerosclerotic development can be explained in part by, but not limited to, heredity, aging, obesity, hypertension, hypercholesterolemia, or diabetes (1). Lifestyle factors also play a major role in the incidence of CHD: smoking, minimal exercise, and the consumption of a high fat diet have also been identified as key contributors to this disease (1-5).

Recently, research in the area of CHD has focused on performing feeding studies in the postprandial state, since the majority of humans exist primarily in this fed condition throughout an average day (1). Americans are an extremely convenience-oriented population, typically choosing foods very high in fat (predominantly saturated fat). The correlation between a high fat intake and atherosclerotic development has thus been well documented (2-5, 6, 7). A diet rich in polyunsaturated fatty acids (PUFA) has been shown to reduce postprandial triglyceride (TG) response to fatty meals, while diets rich in saturated fatty acids (SFA) increase the response (2, 3, 4). This is increasingly important due to the fact that a high postprandial plasma TG concentration is considered a risk marker of the athlerosclerotic event (4).

Postprandial low-density lipoprotein (LDL) particles are more easily oxidized than fasting LDL (3). This may be partially due to the fact that as LDL stays in the blood for a longer period of time (as occurs with consumption of a high fat meal) the LDL is at an increased risk of becoming oxidized. This oxidative event potentially leads to the attachment of the now oxidized LDL to the arterial wall, and ultimately to foam cell formation and blockage of the artery. This deposition of oxidatively-modified LDL in the arterial intima has been identified as an important initial event in atherogenesis (3).

Soy foods and their components have become of particular interest in impeding the atherogenic process; this has lead to the 1999 FDA health claim for the incorporation of soy protein in relation to CHD reduction through its hypocholesterolemic effects (8). Soy foods and their components have been recognized as containing potential antioxidants known as isoflavones. A number of studies have demonstrated the antioxidant properties of soy protein isoflavones both *in vivo* and *in vitro* (9, 10, 11). This antioxidant property of soy in reducing oxidative stress provides a possible mechanism by which consumption of soy foods may decrease the risk of CHD (9). It is possible that the antioxidant property of soy isoflavones could play a role in prohibiting the progression of CHD.

Epidemiological studies have consistently demonstrated a strong positive correlation between the intake of SFA with the prevalence of CHD in various populations (1). The combination of the LDL-lowering effects and antioxidant effects of soy protein and their isoflavones has been shown to contribute to lower rates of CHD among Asian

individuals who consume generous amounts of soy foods, compared to the rates among Westernized societies who consume minor amounts (12, 13).

The purpose of this study was to determine if a difference exists between 39 g soy protein (85 mg isoflavones) and 39.9 g milk protein (0 mg isoflavones). The soy or milk protein was consumed in combination with a high saturated fat diet to determine their relative protection against postprandial LDL oxidation. A high-fat meal (2 apple crumb muffins, soy or milk shake, and a banana) was implemented containing 956 calories, 44 g fat (41%), 26 g saturated fat (21% of total fat), 100 g carbohydrate (41%), 45 g protein (18%), 39 mg cholesterol, and 895 mg sodium.

Following a high saturated fat meal, soy protein (85 mg aglycone isoflavones) consumption compared to milk protein (0 mg aglycone isoflavones) will significantly enhance the oxidative resistance of postprandial LDL as measured by copper-induced LDL oxidation.

CHAPTER 2

REVIEW OF LITERATURE

According to the most widely accepted theory of atherogenesis, oxidatively modified LDL activates a series of cellular events in the arterial wall ultimately leading to plaque formation (13, 14). Generally, it is believed that the formation of a lesion in the arterial wall is initiated by a response to some endothelial injury brought on by hyperlipidemia, or by toxic or infectious agents (15). This oxidative event is thought to occur once the LDL particle has become isolated from circulating water-soluble and fat-soluble (as in the α-tocopherol component of LDL) antioxidants (16). From this process the formation of fatty streaks within the arterial wall is initiated due to the oxidation of LDL occurring predominantly within the subendothelial space of the intima (16). The LDL fraction is the principal carrier of cholesterol in the plasma. It is hypothesized that LDL may play a role at several steps in atherogenesis; however, the precise mechanisms by which LDL promotes the development of lipid laden foam cell in the fatty streak lesion remains to be fully established (17).

Oxidative stress is a term used to describe any challenge in which pro-oxidants (e.g. copper, hydrogen peroxide) predominate over antioxidants (e.g. soy isoflavones, vitamin E). Pro-oxidants are compounds that interfere with normal metabolism by oxidizing (removing electrons) normal cellular macromolecules (18). Oxidative stress is a key factor in two processes leading to atherosclerosis: (1) LDL modification and (2) endothelial dysfunction both caused by hypercholesterolemia and hypertriglyceridemia.

Because oxidized LDL appears to be such an important component of the atherogenic process, it is valuable to examine the lipoprotein itself, and how it undergoes this oxidative process (17).

One of the initial events in LDL oxidation is the free radical peroxidation of PUFA and to lesser extents monounsaturated fatty acids (MUFA) in LDL. Initially, there is conjugated diene formation due to hydrogen abstraction and molecular rearrangement. After oxygen uptake, peroxyl radicals form, which in turn extracts hydrogen atoms from fatty acids, initiating a reaction that leads to the formation of hydroperoxides (Figure 1). This event prevents the recognition of LDL by the LDL receptor; thus oxidized LDL is processed by the scavenger receptor pathway, leading to cholesterol accumulation and foam cell formation (17). Modified LDL (predominately oxidized) is taken up via the scavenger receptors on macrophages creating the metabolically active foam cells found in the early stages of atherosclerosis (5, 15, 17). This results in substantial accumulation of cholesterol in the macrophages and smooth muscle cells in the arterial wall (4, 17, 19).

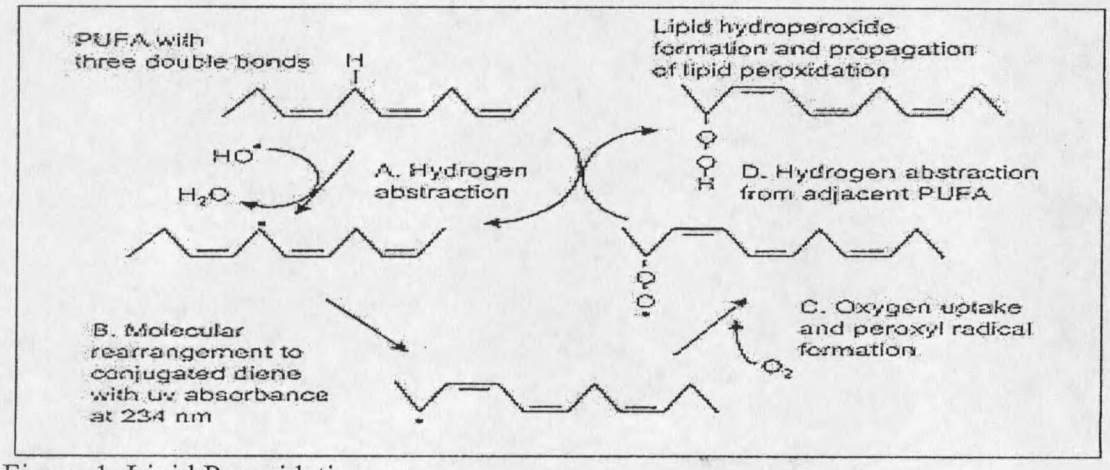

Figure 1. Lipid Peroxidation

The Postprandial State

Over twenty years ago, Zilversmit stated that atherosclerosis is a postprandial phenomenon with lipoprotein particles in the fed state contributing to atherosclerosis (1); this concept has since been confirmed by others (20, 21). Because of our western society's consumption of regular meals throughout a day, we exist primarily in a postprandial state. Zilversmit also supported the view that arterial lipid accumulated as the result not only of abnormally high concentrations of LDL in the blood plasma, but also as a consequence of the normal process of lipid absorption and transport. This process has the potential to be pathogenic in persons who consume a diet rich in fat and cholesterol (1).

It has been shown in studies with both experimental animals and humans that LDL becomes elevated when atherogenic (e.g. high fat) diets are incorporated (1). A proposed mechanism by which a high-fat (especially saturated fat) diet leads to atherosclerosis is by the meal's influence in elevating serum triglycerides (TG). A high postprandial plasma TG concentration is considered a risk marker of coronary heart disease (CHD). It is known that a diet rich in PUFA (predominantly n-3 PUFA) can reduce the postprandial TG response to fatty meals while diets rich in SFA has the potential to increase the response (4).

In a recent study completed by Ursini et al. (5), a test meal was adopted mirroring a typical English/American breakfast including bacon, eggs, bread with butter and coffee. The nutrient composition was 11% protein, 34% carbohydrate, and 55% fat, comprising approximately 1200 kcal. Nine male volunteers participated in this high fat feeding study

in which the primary endpoint was the accumulation of plasma peroxides, reflective of lipid peroxidation (oxidation). The results demonstrated an increase in the plasma peroxide level from baseline (207 ± 96.7 pmol/ml) vs. those obtained two hours after breakfast (412 ± 195.8 pmol/ml) in all subjects (153% average change). The results of this study highlights that a high saturated fat diet is a direct source of lipid hydroperoxides in lipoproteins, and thus this diet could become atherogenic (5).

Despite past evidence for the significance of postprandial studies, most research has been completed on subjects in the fasted state because it is thought to be more reproducible for research studies. The level of TG in the LDL fraction is increased in the postprandial state, which increases the susceptibility of LDL to oxidative modification (2). Since the absorption and transport of dietary fat is mediated by plasma lipoproteins and that intestinally derived lipoproteins have been implicated in the development of atherogenesis, investigations into lipoprotein metabolism need to be conducted in the fed state (3).

Soy Isoflavones

Isoflavones are naturally occurring plant chemicals belonging to the phytoestrogen class. Recent epidemiological evidence and experimental data from both human and animal studies are highly suggestive of beneficial effects of isoflavones on human health. Specific claims have been made for the physiological properties of isoflavones to aide in reducing the incidence of atherosclerosis that ultimately leads to CHD (26, 27).

Isoflavones are found almost exclusively in legumes; the soybean in particular provides an abundant source. The major isoflavones, daidzein and genistein are found in four chemical forms: aglycone (genistein and daidzein), glycoside (daidzin and genistin), acetylglucoside and malonylglucoside (27). Isoflavones occur predominantly as glycosides in plants and consequently are highly polar (water-soluble) compounds. The chemical form in which isoflavones occur is an important consideration because it may influence the biological activity, such that the aglycone form has increased activity and absorption over other forms (26).

The two primary soy phytoestrogens, genistein and daidzein, bind to estrogen receptors (Ers) with low affinity (28). Genistein and daidzein both bind to Ers, explaining their structural similarity with estrogens (Figure 2). Setchell et al. (29) suggested that soy protein foods could lower cholesterol since they exhibit weak estrogenic activity due to the phytoestrogens genistein and daidzein binding to Ers.

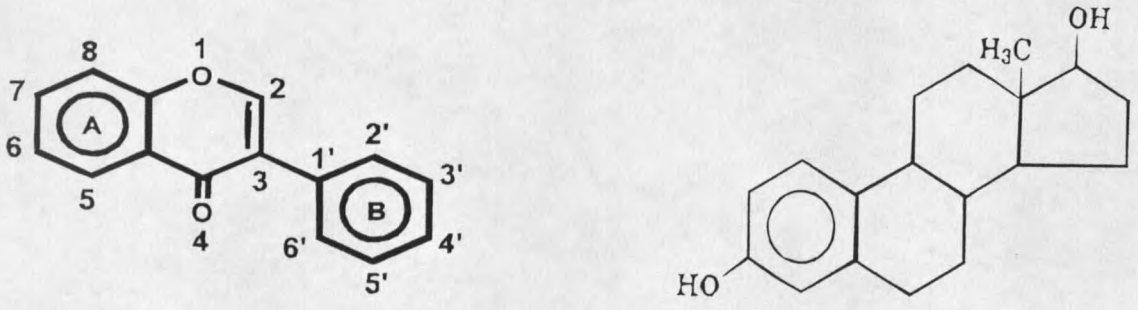

Figure 2. Comparison of Isoflavone Structural Similarity to Estrogen

Isoflavone structure*:

*Genistein = OH at positions 4', 5, 7

*Daidzein = OH at positions 4', 7

Estrogen structure

Anthony et al. (25) fed a diet containing casein, intact soy protein isolates (SP+) and soy protein isolates from which the phytoestrogens were removed (SP-) to 160 cynomolgus monkeys. All monkeys were fed the diets for 14 months, during which time cardiovascular disease risk factors, including plasma lipids were measured. The diets were identical in the percentages of energy from protein (18.5%), fat (40.6%), and carbohydrate (40.9%), and had the same amount of cholesterol (0.31 mg/kcal). There were no isoflavones in the casein diet, low amounts (equivalent to 16 mg per individual per day) in the SP- diet, and approximately a 10-fold higher amount of isoflavones (equivalent to 143 mg per individual per day) in the SP+ diet. Serum lipoproteins (VLDL and LDL) in the SP+ animals were significantly reduced (426 ± 23.94 mg/dL casein, 394 ± 22.00 mg/dL SP-, and 276 ± 22.39 mg/dL SP+; $p<0.05$) and high density lipoprotein (HDL) cholesterol levels were significantly increased as compared to SP- and control animals (40.2 ± 3.47 mg/dL casein, 47.9 ± 3.09 mg/dL SP-, and 59.8 ± 3.08 mg/dL SP+; $p<0.05$). The mean atherosclerotic plaque size was reduced in SP+ animals, suggesting the possibility of antiatherogenic effects caused by alteration in the serum lipoprotein profile (25). This study shows that the incorporation of soy isoflavones in conjunction with soy protein as compared to soy protein alone has the ability to reduce serum lipoprotein (very low-density lipoproteins or VLDL and LDL) levels while simultaneously increasing HDL cholesterol levels.

Analyses of the isoflavone content of numerous soy foods generally indicate that most contain 0.1 – 3.0 mg/g of total isoflavone (26). Although a high proportion of foods contain soy products, these are mostly soy oils and soy lecithin; these soy products are

devoid of isoflavones, so the average daily dietary intake of isoflavones in Western populations is typically negligible (<1 mg/d). Isoflavones migrate with the protein fraction of the soybean during its processing, and because soy protein is rarely a normal component of the average Western diet, this accounts for the low daily intake reported in the American population (26). The typical Asian diet contains approximately 50-100 mg of isoflavones per day (30, 31). This is typical of the Asian diet excluding metropolitan areas that have adopted westernized eating patterns, in which isoflavone consumption drops dramatically to 25 mg per day. In westernized societies, such as our own, the typical consumption is noted to be less than 5 mg per day. Tofu and tempeh have 38.3 and 60.5 mg isoflavones per serving respectively vs. second generation products such as soy hot dogs or soy-based ice creams which have substantially lower amounts of isoflavones (approximately 6 mg per serving) because they frequently contain considerable amounts of non-soy ingredients (30, 31).

The serum cholesterol and LDL cholesterol lowering effects of soy protein-containing foods have been investigated in a large number of studies since the 1940s. In 1995, Anderson et al. (32) conducted a meta-analysis; including 38 clinical studies reported in 29 articles. The mean daily intake of soy protein in the meta-analysis was 47g (17 – 124 g/d), however 14 of the studies (37%) reported an intake of ≤31 g per day. The meta-analysis showed an overall net reduction in serum LDL cholesterol of 12.9%. The decrease was said to be related to the initial serum cholesterol level ($p<0.001$), such that insignificant changes were found in individuals with low initial serum cholesterol and the greatest reductions occurred in those with the highest levels of initial cholesterol

(32). Additionally, soy protein consumption was associated with significant reductions in total cholesterol (23.2 mg/dL, 9.3%); and triglycerides (13.3 mg/dL, 10.5%), whereas a nonsignificant increase of 2.4% ($p>0.05$) in serum concentrations of HDL-cholesterol was apparent. The results of the meta-analysis supported the theory that the consumption of soy protein (average of 47 g/d) when compared to animal protein significantly decreases serum concentrations of total cholesterol, LDL cholesterol, and TG (22, 32).

Soy and Oxidation

Clinical and epidemiological studies indicate that lipoprotein oxidation may contribute to the development of inflammatory lesions that are typical of the first stages of atherosclerosis (24). Along with these studies it has also been shown that antioxidant supplementation with antioxidants such as vitamin E and soy may reduce the risk for atherosclerotic events (12).

Tikkanen et al. (16) hypothesized that isoflavone antioxidants derived from soy could be incorporated into lipoproteins and possibly protect them against oxidation. One way isoflavones can do this is by the binding of copper to apo B of LDL *in vivo*, thereby initiating lipid peroxidation. This type of mechanism has also been suggested for dehydroascorbic acid (also a water-soluble antioxidant) which, when coincubated *in vitro* with LDL, renders it resistant to oxidation (16). The possibility that isoflavone intake has an antiatherogenic effect has received support from the existence of plausible underlying mechanisms of protection, such as plasma lipid risk factor modification, and antioxidant protection of LDLs (11, 12, 22-25).

The oxidation of LDL results in a lipoprotein particle that is generally thought to be more atherogenic than the native LDL (33). Isoflavones, primarily genistein, have been reported to inhibit oxidative modification of LDL by macrophages, enhancing the resistance of LDL to oxidation and exhibiting antioxidant activities in the human by being incorporated in small quantities (~1%) in the LDL molecule itself (16, 23). Genistein is a naturally occurring polyphenolic compound known to have antioxidant properties. Genistein exhibits antioxidant effects *in vitro* including the inhibition of ADP and NADPH-dependent lipid peroxidation in rat liver microsomes and inhibition of the coupled oxidation of β-carotene and linoleic acid (34). This antioxidative activity has been related to the ability of isoflavones to scavenge free radicals. Recent work by Kapiotis (35) has documented the ability of genistein to inhibit LDL oxidation *in vitro* when challenged with copper ions or superoxide radicals as measured by thiobarbituric acid reactive substance (TBARS) formation, altered electrophoretic mobility and lipid hydroperoxides. The general endogenous antioxidants presumably present in LDL particles have not been fully characterized. It is hypothesized that dietary antioxidants such as soy derived isoflavones could play a role in protecting LDL against oxidation (23).

Anderson et al. (12) compared the effects of various diets containing antioxidant rich foods on serum lipids and lipoprotein oxidation in 60 male rats for 3 weeks. The purpose of this study was to determine if a diet containing isoflavone-rich soy protein could influence lipoprotein oxidation in rats. Comparison diets included a diet void of vitamin E (the control group), a diet high vitamin E (1000 units/kg), a diet low in soy

isoflavones (0.08 mg genistein/g of protein), a diet high in the soy isoflavone genistein (1.45 mg genistein/g protein), a diet containing 2% green tea, and a diet containing 50 mg/kg β carotene. Oxidative damage to lipoproteins was determined by measuring the lag phase and formation of conjugated dienes, lipid peroxides, and TBARS. A significant reduction in triglyceride values occurred ($p<0.001$) for the high-genistein group vs. the control group. A significant prolongation in the lag phase for the high and low genistein groups were observed with an increase of 83 min (49%, $p=0.002$) and 80 min ($p=0.002$), respectively. The high genistein group also had significantly lower ($p=0.01$) conjugated diene values (0.49 OD units) than the control group (0.68 OD units). High genistein soy protein intake also significantly decreased ($p=0.006$) lipid peroxides in lipoprotein fractions from control values from 982 nmoles/mg protein to 677 nmoles/mg protein, respectively. This study shows that the incorporation of soy isoflavones (particularly the isoflavone genistein) has a strong antioxidant characteristic to decrease the susceptibility of VLDL-LDL to oxidation (12).

Hwang et al. (37) conducted a study to determine if soy isoflavones could inhibit *in vitro* LDL oxidation. The LDL was incubated with genistein, daidzein, or equol at concentrations ranging from 0.5 to 10 μM. The LDL samples were isolated from one adult male volunteer; copper was then added to the cuvettes and formation of conjugated dienes was monitored at 234 nm for up to 16 hours. The results demonstrated a significant prolongation of lag time ($p<0.05$) at concentrations greater than 0.5 μM for equol, 1 μM for genistein, and 2.5 μM for daidzein. The researchers also noted that it was equol, a daidzein metabolite formed *in vivo* by gut microflora that proved to be the

most potent antioxidant tested. There has been speculation that interindividual variability in gut microflora may influence the conversion of daidzein to equol, leading to varying results among subjects. The use of samples from one individual is a limitation to this study (36, 37).

Six volunteers (three men, three women) participated in a study by Tikkanen et al. (16) to determine whether isoflavone antioxidants derived from soy could be incorporated into lipoproteins possibly protecting them against oxidation (16). Subjects received three soy bars (7.1 g soy protein, 12 mg genistein and 7 mg daidzein per bar) daily for two weeks. Fasting blood samples were collected following a 2 week run in, during the 2 weeks of the soy-feeding period as well as after a twelve day washout period. Following two weeks of soy feeding, a significant prolongation for lag time was observed for the soy feeding (147 ± 9 min) as compared to baseline to the soy feeding (173 ± 19 min) ($p<0.02$). Although large increases in plasma isoflavone levels occurred following two weeks of consumption, the incorporation of isoflavones into the LDL was determined to be less than 1% of total plasma isoflavones (16). These results indicate that following chronic consumption of soy, the LDL was resistant to oxidation for a longer period, however the mechanism for which soy isoflavones impacted the resistance of LDL to oxidation was not apparent.

Jenkins et al. (9) determined whether consumption of a moderate amount of soy protein (36 g/d) and associated isoflavones (168 mg/d) in breakfast cereal can favorably alter markers of increased cardiovascular disease risk in 25 hyperlipidemic subjects. Total conjugated dienes in the LDL fraction, a marker of oxidized LDL cholesterol was

significantly reduced on the soy diet compared with the control (9.2% ± 4.3%, p=0.42), and the ratio of conjugated dienes to cholesterol in the LDL fraction was also reduced (8.7% ± 4.2%, p=.050) at the end of the three-week treatment. This study indicates a beneficial effect of soy in reducing indices of LDL oxidation; supporting previous reports that soy isoflavone consumption may protect LDL cholesterol from oxidative damage (9).

In a similar study, Jenkins et al. (38) recruited 31 hyperlipidemic subjects (19 men; 12 postmenopausal women) in a randomized crossover study investigating the effects of soy protein foods on LDL oxidation. The subjects were asked to consume a test meal of 33g/d of soy protein (86 mg isoflavones/2000 kcal/d) for a month, followed by a control diet with no soy protein or isoflavones for a second month. The test diet decreased both oxidized LDL measured as conjugated dienes in the LDL fraction (56 ± 3 test vs. 63 ± 3 μmol/L control, p<0.001) and the ratio of conjugated dienes to LDL cholesterol (15.0 ± 1.0 test vs. 15.7 ± 0.9 control, p=.032), even in subjects who had already been using vitamin E supplements (400-800 mg/d). The consumption of high isoflavone foods appears to be associated with reduced levels of circulating oxidized LDL (38).

Wiseman et al. (39) also studied the use of isoflavones to increase the resistance of LDL to oxidation in humans. In this randomized crossover study, the effects of a textured soy protein high in isoflavones (HI, 21.2 mg daidzein, 34.8 mg genistein) were compared with a similar product yet low in soy isoflavones (LI, 0.9 mg daidzein, 1.0 mg genistein), both of which were consumed for 17 days. Plasma concentrations of F_2-isoprostanes, a biomarker of *in vivo* lipid peroxidation, as well as the resistance of LDL