THE DOSE-DEPENDENT EFFECTS OF SOY ISOFLAVONES ON CHOLESTEROL METABOLISM IN OVARIECTOMIZED HAMSTERS

By

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CHAPTER I

INTRODUCTION

The deaths caused by coronary heart disease (CHD) have increased in the United States as the percentage of individuals over 65 years of age has risen (Center for Disease Control, 2002). Additionally, the economic impact of CHD is a big concern. The American Heart Association (AHA) estimates that the total cost of the disease currently is about $286.5 billion (AHA, 2002).

The age specific risk of CHD is lower in women than in men. The CHD mortality risk in women is approximately equal to that of men 10 years younger (Bush, 1996). However as women age they seem to lose their cardio-protection and this loss is attributed to the lowered levels of circulating estrogens that occurs following menopause (Senoz et al., 1996). The lowered levels of estrogen result in altered lipid metabolism as well as other physiological changes causing an increase in CHD risk (Pasquali et al., 1999).

Initially it was thought that the use of hormone replacement therapy (HRT) by postmenopausal women could prevent or at least minimize these changes. A meta-analysis by Anderson et al. (1995) showed that HRT was associated with a reduction in CHD risk by 35% to 50% in postmenopausal women. However the results of these studies were based on the intermediate markers that indicated beneficial effects. The recent findings from the secondary prevention trials and observational studies using HRT showed an increase risk of CHD in the first year of hormone use (Fletcher & Colditz, 2002). Additionally HRT is seen to be associated with an increased risk of

breast, endometrial and uterine cancers (Teede, 2002). In some women HRT may cause the resumption of menses, tenderness of breast and abdominal bloating (Sullivan & Fowlkes, 1996).

These side effects caused by HRT raise the need to find safer alternative therapies that will reduce the risk of CHD without any harmful side effects. Phytoestrogens are diphenolic compounds that are found in a wide variety of plant foods and have been reported to exert estrogenic effects in the absence of endogenous estrogens. Phytoestrogens and their metabolites exert their estrogenic effects by binding to estrogen receptors (Wade and Zucker, 1970). There are three main classes of phytoestrogens namely isoflavones, lignans and coumestans. Soy and flaxseed are rich in isoflavones and lignans, respectively (Murky et al., 1998). The main sources of dietary coumestans are broccoli, peas and beans (Boker et al., 2002).

Epidemiological data shows that higher intake of soy containing foods is associated with a lower risk of coronary heart disease (Lissin & Cooke, 2000). Terpstra et al., (1991) showed that consumption of soy protein exhibited hypocholesterolemic effects when compared to casein in hypercholesterolemic male hamsters. Soy is a rich source of the phytoestrogens, isoflavones mainly genistin and diadzin. A meta-analysis has indicated that isoflavones lower cholesterol in people with hyperlipidemia (Tikkanen & Adlercreutz, 2000). However, the effective dose and the cholesterol lowering mechanisms of soy isoflavones have not been fully elucidated.

To elucidate the hypocholesterolemic mechanisms of soy isoflavones the selection of an appropriate animal model is important. The lipoprotein profiles of the hamster more closely resemble that of humans than that of rats and mice (Foxall et al.,

1992). Sohn et al., (1999) showed that the ovariectomized hamster is a better model to study the pattern of lipid changes exhibited by postmenopausal women. Therefore, ovariectomized hamster model was used in this study to test the following hypothesis.

The **hypothesis** of this study was that soy isoflavones, irrespective of protein source dose-dependently improve plasma lipid profiles in an ovariectomized hamster model of postmenopausal hypercholesterolemia. This hypothesis was tested using the following specific aims:

Specific aim 1: To confirm that ovariectomy alters the lipid profiles, e.g. increases total cholesterol and free- cholesterol concentrations, and decreases high-density lipoprotein cholesterol (HDL) in hamsters.

Specific aim 2: To study the dose-dependent effect of soy isoflavones on lipid profiles of ovariectomized hamsters.

Specific aim 3: To examine the cholesterol-lowering mechanisms of soy isoflavones in ovariectomized hamster model of hypercholesterolemia by determining the *in vitro* intestinal and hepatic rates of sterol synthesis and measuring the fecal bile acid excretion.

CHAPTER II

REVIEW OF LITERATURE

Coronary Heart Disease in Women

Coronary heart disease (CHD) is the number one cause of mortality for both men and women in the United States (CDC, 2002). The number of new and recurrent coronary attacks every year are over a million, of which 40% die (AHA, 2002). The total cost of CHD has been estimated to be $286.5 billion, which includes the health care costs and the loss of productivity due to premature mortality (AHA, 2002). Every year more than a 500,000 women, age 35 years and older, die from CHD (CDC, 2001). Menopause is found to be a major risk factor for this increased risk of CHD in women (Tsang et al., 1996). As life expectancy increases, the number of women who will live half their adult lives after menopause will increase (WHO, 1996). The World Health Organization (WHO) also estimates that by 2010, postmenopausal women will comprise about 10.5% of the world's population. Therefore, it is clearly seen that the number of women who will be suffering from CHD will also increase as our population ages.

Although heart disease manifests 10 years later in women than in men, CHD mortality rates are virtually the same for both (Bush, 1996) and this ten-year lag time is due to the cardio-protective effects of circulating estrogens. This gender difference in the development of heart disease is also seen in other primates. Hamm and colleagues (1983) showed that the male primates developed significantly more coronary artery stenosis in comparison to their female counterparts, which was not due to the differences in lipid levels between the sexes. They concluded that gender had an important role to play in the

development of heart disease and male gender had a increased risk of developing CHD (Hamm et al., 1983).

This lowered risk of CHD between genders is seen only in only younger (premenopausal) women. CHD incidence is increased in postmenopausal women and is almost equal to the incidence of heart disease in men. A high correlation between CHD and menopause was detected in the Framingham Study where 2873 women were monitored for 24 years (Gordon et al., 1977). This increased risk of CHD in postmenopausal women may be due to genetic differences, absence of estrogens, or the combination of various other factors (Tsang et al., 1996). Therefore steps should be taken to prevent or lower the incidence of CHD in this high-risk population.

Risk Factors for Coronary Heart Disease

The risk factors for CHD include obesity, cigarette smoking, sedentary life style, and hyperlipidemia. Since the main emphasize of this thesis is to investigate the relationship between dietary soy isoflavones and lipid metabolism, this topic will be elaborated.

Hyperlipidemia

Hyperlipidemia is considered one of the major but reversible risk factor for CHD affecting approximately 90 million Americans (AHA, 2002). Lipids are transported in the blood in the form of lipoproteins and hyperlipidemia is characterized by an increase in the levels of lipoproteins in plasma. Increased dietary fat, sedentary lifestyle and obesity

are some of the factors that cause a raise in the plasma lipoproteins (NCEP, 2001). This increase may also be caused by certain genetic defects. Lowering cholesterol levels, namely total cholesterol (TC), low-density lipoproteins-cholesterol (LDL) and increasing high-density lipoproteins-cholesterol (HDL) can reduce the prevalence of CHD (Kannel, 1987).

Lipoproteins and atherosclerosis

Lipoproteins are lipids that are combined with proteins to form water-soluble lipoprotein complexes. The four major physiologically important groups of lipoproteins are chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). This classification of lipoproteins is based on their separation properties upon ultracentifugation (Murray, 1993). Lipoproteins also differ in their source, composition and physiological action.

Atherosclerosis

Atherosclerosis is the progressive narrowing and hardening of the arteries over time. It involves the cellular infiltration of several cell types, including monocytes, T lymphocytes, and perhaps even mast cells. Monocytes interact with the endothelial layer, attach firmly to the endothelium, and migrate into the subendothelial space, where the monocytes differentiate into macrophages. Macrophages release a variety of chemicals, including cytokines, and also take up lipids, becoming foam cells. Macrophages and foam cells secrete growth factors, which lead to cell proliferation and matrix production, as well as metalloproteinases, which lead to matrix degeneration. Thus, macrophages and

foam cells both contribute to lesion growth and may contribute to instability and thrombotic events (Ross, 1999).

Low–Density Lipoprotein (LDL)

Increase in the LDL level greater than 3.36 mmol/L is associated with an increase in heart disease risk (The Expert Panel, 1993). The National Cholesterol Education Program has suggested that the increase in the LDL level is the most preventable risk factor that is associated with CHD (Sempos et al., 1993).

LDL consists of a surface monolayer of phospholipids and free cholesterol and a single molecule of apolipoprotein (apo) B, which encircles the lipoprotein. This surface monolayer surrounds a hydrophobic core of mainly cholesteryl esters but also some triglycerides. In itself, LDL is almost certainly not proinflammatory, but the particle can become modified in many ways. It is the modified LDL particle that is proinflammatory and proatherogenic

Of all of the plasma lipoproteins, LDL has been most investigated in terms of its role in inflammation. LDL readily enters the arterial wall by crossing the endothelial membrane. Once in the arterial wall, if LDL accumulates, it is subject to a variety of modifications. The best known of these is oxidation, both of the lipids and of the apo B. LDL is also subject to aggregation, and its phospholipids are subject to hydrolysis by phospholipases to form lysophosphatidylcholine. Several other chemical modifications have also been reported. The net effect of these changes is the production of a variety of modified LDL particles, and the evidence is very strong that these modified LDL particles are proinflammatory (Steinberg et al, 1989).

High-Density Lipoprotein (HDL)

HDL has the same essential structure as LDL, with a surface monolayer of phospholipids and free cholesterol and a hydrophobic core consisting mainly of cholesteryl esters but also some triglyceride. However, HDL particles are smaller and contain different apolipoproteins, mainly apo A-I and apo A-II. Both these apolipoproteins have properties that protect the lipids against oxidative modification. Additionally, some of the other proteins transported by HDL have antioxidant properties. Whereas LDL is very susceptible to oxidative modification, HDL is relatively resistant to it, and this is one of the reasons underlying the anti-inflammatory properties of HDL (Rye et al., 1999).

Data from the Framingham Heart Study indicate that low HDL level is an independent risk factor for CHD. Even in individuals whose LDL levels were approximately 100 mg/dL, low HDL remained a very strong risk factor, and individuals with low HDL were still at considerably elevated risk (Gordan et al., 1977). The National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines, issued in 2001, redefined low HDL as <40 mg/dL, increasing this cut point from the previous value of 35 mg/dL. This change resulted in millions of additional people who now are considered to have low HDL level (NCEP, 2001). Reduced HDL level is also seen to increase the incidence of myocardial infarction (Abbot et al., 2002). The Writing Group for the postmenopausal estrogen/progestin interventions (PEPI) trial (1995) has reported that HDL was a better predictor of CHD in women than LDL. Studies have also indicated

that coronary risk is increased by 2-3% for every 1% reduction in HDL level in humans (Zhang et al., 2000).

HDL is believed to protect against atherosclerosis at least in part through the process of reverse cholesterol transport, whereby excess free cholesterol (FC) is removed from cells in peripheral tissues, such as macrophages within the arterial wall, and returned to the liver for excretion in the bile. Even small increases in HDL may confer substantial benefit. Intervention to raise HDL levels should be considered in high-risk individuals.

Efflux of cholesterol from foam cells leads to a reduction in foam cell formation, although the macrophages may accumulate, they are not converted into foam cells. As a result, the inflammatory process is arrested to a certain extent. Therefore, HDL is anti-inflammatory and also protects against the development of atherosclerosis (Miyazaki et al., 1992). HDL has protective effects in addition to promoting cholesterol efflux. One of the best known of these is the ability to inhibit the oxidation of LDL. To the extent that LDL oxidation is an important step in the development of the inflammatory process, this property of HDL is clearly anti-inflammatory (Mackness et al., 1993)

Chylomicrons and Very-Low Density Lipoproteins (VLDL)

Chylomicrons are small lipid droplets, which contain cholesterol and triglycerides. They are manufactured by the epithelial cells in the small intestine and function as a transport vehicle from the gut. VLDL carries endogenous triglycerides secreted by the liver. The metabolism of VLDL is similar to that of chylomicrons (Murray, 1993). VLDL is also a circulating precursor of LDL.

Lipoprotein lipase acts on the chylomicrons and VLDLs and removes the triglyceride molecule resulting in remnant chylomicrons and VLDLs. The total amount of cholesterol carried in these remnant molecules is about 30 times higher than in LDL particles and it is thought that their deposition in the arterial wall is more harmful than that occurring with LDL (Seman et al., 1999)

VLDL remnants and chylomicron remnants behave in much the same way as LDL. They enter the subendothelial space, where they become modified, and the modified remnants stimulate MCP-1, promote the differentiation of monocytes into macrophages, and are taken up by the macrophages to form foam cells. Like LDL, the remnant lipoproteins are proinflammatory and proatherogenic (Doi et al., 2000)

Lipoprotein (a) [Lp(a)]

Plasma levels of Lp(a) are usually predictive of atherosclerotic risk, and there is increasing evidence that the Lp(a) particle is involved in atherosclerosis as well as in thrombosis (Sandkemp et al., 1990). Increased accumulation of Lp(a) in atherosclerotic plaque has been seen in postmortem studies and in fresh human arterial wall tissue (Reblin et al., 1995).

Lp(a) contains a large protein molecule, Apo(a) which is attached to LDL–like particle by a disulfide bond. The molecular weight of apo(a) can vary widely among individuals, depending on the number of its repeats. Lp(a) is accumulated in the atherosclerotic plaque and it is taken up by foam cell precursors leading to increased production of foam cells (Sandkemp et al., 1990). When oxidized Lp(a) particles are

engulfed by macrophages, they are transformed into foam cells within the arterial wall thus leading to the progression of atherosclerosis (Haijer et al.,1989).

Lp(a) is also seen to interfere with thrombolysis. Several in vitro studies have shown that Lp(a) competes with plasminogen for binding sites on endothelial cell surfaces (Ranby M,1982, Harpel et al., 1989). Through these mechanisms Lp(a) is seen to enhance the risks of atherosclerosis.

Apo A

Apolipoprotein A-I (apoA-I) alone or as part of HDL is considered to have antiatherogenic properties (Harpel et al., 1989). Apo A-I is the primary protein constituent of HDL, defining its size and shape, solubilizing its lipid components, removing cholesterol from peripheral cells, activating the lecithin cholesterol acyl transferase (LCAT) enzyme, and delivering the resulting cholesterol esters to the liver (Philips et al., 1997). Lecithin:cholesterol acyltransferase is an extracellular enzyme that is synthesized and secreted by the liver, circulates in the plasma, and acts on plasma HDL and it also promotes non-enzymatic transfer of cholesteryl esters from HDL to VLDL and LDL Thus apo A helps in clearing the cholesterol from circulation.

Some of the factors that are seen to increase the levels of apo A-I are exercise, moderate alcohol consumption, female gender and sex steroids like estrogen (Hargrove at al., 1999). The results of a clinical trial show that estrogen increases the concentrations of apo A by increasing the synthesis of the protein and by suppressing the activity of hepatic triglyceride lipase enzyme (Quintao et al., 1991). This explains another cardio protective role of estrogen.

Apo B

Apolipoprotein (apo) B is a single molecule that encircles the LDL surface. Increased triglyceride levels stimulate the assembly and secretion of apolipoprotein (apo) B and VLDL. The result is an increased number of VLDL particles and increased level of triglycerides in the plasma, which lead to the increased risk of atherosclerosis (Murphy et al., 2000). Apo B also promotes cholesterol accumulation in the arterial tissue through being modified by oxidation and specific binding to extracellular matrix proteoglycans (Sakata et al., 2001).

The results of the Quebec study showed that CHD risk was highest in men with elevations in apo B (Lamarche et al., 1999). In another analysis of the Quebec Cardiovascular Study in which men were stratified by apo B levels and LDL particle size, high apo B concentration was associated with increased riskof CHD, and the cumulative effect of high apo B and small, dense LDL was associated with a marked increase in CHD risk. The study concluded that if apo B is reduced to less than 120 mg/dL, the LDL particle size no longer has an effect, because lower apo B levels to linked with lower LDL oxidation. Thus high levels of apo B are considered as a predictor of CHD.

Triglycerides

Elevated triglyceride levels represent an independent risk factor for coronary heart disease. The National Cholesterol Education Program revised the acceptable level of fasting triglycerides from less than 200 mg/dL to below 150 mg/dL (Miller et al., 2002). Numerous factors suggest that increased serum triglyceride levels are associated with

increased atherosis risk. Hypertriglyceridemia leads to the accumulation of chlyomicron and VLDL remnants that are atherogenic. Increase in triglycerides also leads to the generation of small and dense LDLs and causes the lowering of HDL. Hypertriglyceridemia is also associated with increased coagulability and decreased fibrinolysis, as it causes an increase in the levels of plasminogen activator inhibitor and thereby activating of clot formation.

Thus these risk factors and their mechanisms that are associated with hyperlipidemia lead to the development of atheroaclerosis and our goal should be to lower the levels of biomarkers that may increase the risk of atherosclerosis and increase the biomarkers that prevent the disease.

Diet

Diet plays an important role in primary and secondary CHD prevention (Wasling, 1999). Reducing fat and cholesterol intake has been the main focus of the National Guidelines in the prevention of CHD risk. Replacing saturated with unsaturated fat has been shown to be more effective in lowering heart disease than merely reducing total fat intake (Hu et al., 2001). Studies that have examined dietary intervention have found that restricting saturated fat and increasing the intake of essential fatty acids, especially omega - 3 fatty acids, reduces CHD risk (Schaefer, 2002).

Along with the modifications in fat intake, higher intake of cereals, vegetables, legumes and fruits, fish, cheese and yogurt as dairy products, rapeseed and olive oils as edible fats have been shown to decrease the incidence of CHD (Renaud and Lanzmann-Petithory, 2001).

In addition to these risks women are seen to suffer from an increased risk of heart disease caused by ovarian hormone deficiency following menopause.

Menopause and Coronary Heart Disease

A high correlation between CHD and menopause was detected in the Framingham Study where 2873 women were monitored for 24 years (Gordon et al., 1977). Menopause marks the end of a woman's reproductive cycle. It is characterized by an acute change in hormonal balance. Menopause results in the permanent cessation of ovarian function, this typically occurs around the age of 51 years (McKinlay et al., 1992). The relationship between menopause and heart disease is very complex, it is influenced by many factors, including changes in glucose tolerance, body weight, blood pressure, and most importantly changes in lipid profiles (Bonithon et al., 1990). Thus, the health needs of postmenopausal women are different from those of younger women or men of the same age group.

The changes in lipid profiles that occur after menopause may be an important factor in determining the development of CHD (Jenner et al., 1993). Kannel et al., (1987) in the Framingham study, clearly showed that there was an increase in TC and LDL after menopause. A slight decrease in HDL level was also seen (Kannel et al., 1987). High levels of circulating LDL lead to an increase in the formation of the atherosclerotic plaque (Pearson, 2002).

Wakatsuki et al. (1995) determined that the rise in LDL following menopause is due to the increased activity of an enzyme, lipoprotein lipase caused by estrogen deficiency. Additionally, the impairment of LDL receptors that occurs after menopause

results in hypercholesterolemia (Arca et al., 1994). The atherogenic potential of LDL depends on their size and density. The smaller and denser particles cause a greater risk for atherosclerosis (Lamarche et al., 1999). The size and density of LDL particles decrease after menopause. The cumulative effects of increase in LDL levels and reduction in size and density of LDL fractions add to the risk of atherosclerosis in postmenopausal women (Ikenoue et al., 1999).

Lp(a) levels in circulation are closely related to the circulating levels of female hormones (Genest et al., 1992). The levels of Lp(a) are seen to be increased in postmenopausal women when compared to premenopausal women (Brown et al., 1993) and high levels of Lp(a) cause an increase in clot formation, oxidation of LDL and deposition of cholesterol in arterial walls leading, to premature myocardial infarctions (Seman et al., 1999).

Earlier studies have shown that the increase in CHD risk, caused by low levels of circulating female sex hormones can be prevented by administering exogenous estrogens. Alexandersen et al., (2001), showed that increase in aortic cholesterol concentrations caused by ovariectomy in rabbits, was lowered by administering estrogens. 17β estradiol improved the lipid profiles of ovariectomized cynomolgus monkeys (Greaves et al., 2000). However estrogen by itself when given to women is seen to increase the incidence of ovarian cancer.

Estrogen in combination with progestins given to postmenopausal women is referred to as hormone replacement therapy (HRT). But recent findings from two clinical trials have disproved the cardio-protective effects of HRT (Grady et al., 2002, Hulley et al., 2002).

Hormone Replacement Therapy and Heart Disease

In the early 1960's it was shown that women with premature ovariectomy were at an increased risk of developing CHD (Sznajderman & Oliver, 1963). Previous observational studies have shown that women who use HRT have a 30 to 50% reduction in mortality rate when compared to those who do not (Greendale et al., 1999). HRT is prescribed for the alleviation of the symptoms of menopause like hot flashes, urogenital atrophy and osteoporosis (Jones, 1980). Estrogen is believed to improve lipid profiles and lower the risk of CHD.

Estrogen and Lipid Metabolism

Earlier HRT has been shown to improve lipid profiles by decreasing TC, LDL and increasing HDL levels (Barnet-Conor & Bush, 1991). Estrogens have a beneficial effect on cholesterol metabolism and deposition, which prevents the formation of atheroclerotic plaque (Sarrel, 1990). Estrogen stimulates the synthesis of LDL receptors in the liver thus lowering the concentration LDL in the plasma. The level of HDL in the plasma is increased by decreased hepatic triglyceride lipase activity (Wakatsuki et al., 1998). HRT also inhibits plaque formation by reducing fibrinogen (Rosenberg 1993). Sanada and co-workers (2000) found that HRT reduces the levels of atherogenic remnant lipoprotein cholesterol, which are increased after menopause.

Estrogen is also seen to prevent LDL oxidation, as oxidized LDL particles are more atherogenic than unoxidized LDL. Estrogens may act in a similar fashion like vitamin E and help in scavenging lipid peroxyl radicals that cause lipid peroxidation, thus help prevent atherosclerosis (Subbaiah et al., 1993). Another mechanism by which

randomized controlled trial (WHI) by the writing group for the women's health initiative investigators (2002) clearly showed the harmful effects of HRT.

The HERS II trial investigated the cardio-protective effects of HRT regimen in older women with an average age of 71 years with pre-existing CHD. The investigators concluded that postmenopausal hormone replacement therapy should not be used to reduce the risk of CHD events in women with CHD (Hulley et al., 2002, Grady et al., 2002)

The WHI trial looked at the cardio-protective effects of HRT on healthy women (aged 50-79 years). At about five years after the follow up, the trial was terminated earlier than planned as the breast cancer risk in women using HRT increased and outweighed the benefits. The conclusion of the study was that HRT regimen should not be initiated or continued for the primary prevention of CHD (Writing group for the women's health initiative investigators, 2002).

For every year of use of HRT there is an increase in relative risk of breast cancer (Lobo et al., 1995). There is an increased risk of occurrence of breast cancer with prolonged use of HRT, however short-term use may not increase the risk. This may be due to the changes in the composition of breast tissue that is associated with prolonged hormone use.

Other harmful side effects of HRT include vaginal bleeding, somatic discomforts like breast tenderness, thrombosis, allergic manifestations (Harlap, 1992).

The whole purpose of HRT in healthy women is to preserve health and prevent disease. But the results of the earlier mentioned clinical trials showed other wise. Even if

the absolute risk is low it can affect substantial number of women (Fletcher & Colditz, 2002).

All these factors suggest that women must be provided with alternative therapies to address the major issues related to menopause namely, reducing the risk of developing cardiovascular disease, osteoporosis and other health problems that increase as women age (Kass & Annese 2000).

Therefore, there is a need for studies to develop therapies that can prevent the incidence of CHD without any harmful effects. Epidemiological data suggest that the incidence of heart disease is lower in Asian population and is attributed to high phytoestrogen consumption (Messina et al., 1994). Phytoestrogens, a class of compounds that are known to exhibit estrogen-like properties, have been extensively studied to provide a solution to alleviate the problems faced by postmenopausal women.

Plant Estrogen-Like Compounds (Phytoestrogens)

As the name suggests phytoestrogens are plant estrogen-like compounds that may have beneficial effects on the cardiovascular system and may help in alleviating some of the symptoms that are attributed to menopause such as osteoporosis and breast cancer (Wroblewski & Cooke 2000).

Phytoestrogens were discovered in the 1940's. Isoflavones, lignans and coumestans are the major types of phytoestrogens. Common and significant source of isoflavones are soybeans. Cereals and oilseeds like flaxseed are rich sources of lignans. Broccoli and alfalfa sprouts are good sources of dietary coumestans. Phytoestrogens are

heterocyclic compounds that have structural similarities to estrogenic steroids (Murky et al., 1998).

Plant isoflavones and lignans are converted to heterocyclic phenols in the human gut and these compounds are similar in structure to estrogen and they exhibit weak estrogenic properties (Murkey et al., 1998). Phytoestrogens have varying effects on different tissues and various types of phytoestrogens have varying affinities to estrogen receptors (Anderson et al., 1995).

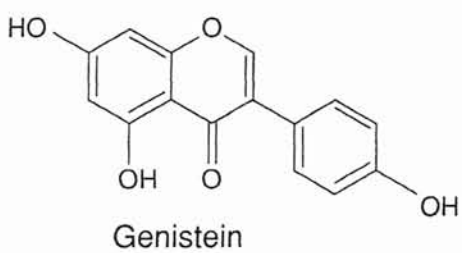
Phytoestrogens have shown both estrogenic and anti-estrogenic properties. They are estrogenic because they have a tendency to bind to estrogen receptors (Wang et al., 1996). They are anti estrogenic because, unlike estrogen they inhibit the activity of aromatase and the proliferation of the breast cells (Lee et al., 1987). The cloning and description of estrogen receptor β (ER-β) has helped to better understand the effects of closely related estrogenic substances. Phytoestrogens may also act as anti-oxidants. This conclusion is based on the oxidative resistance of the LDL obtained from participants consuming high levels of phytoestrogens (Tikkannen et al., 1998).

The incidence of chronic diseases like cardiovascular disease, cancer and stroke is much less in countries consuming high amounts of soy when compared with countries that do not traditionally consume soy (Messina et al., 1994). Japanese women are found to have significantly lower rates of deaths due to cardiovascular disease and cancers (Boring et al., 1995). Of the foods consumed by humans, the level of isoflavones is the highest in soybeans.

Soy Isoflavones

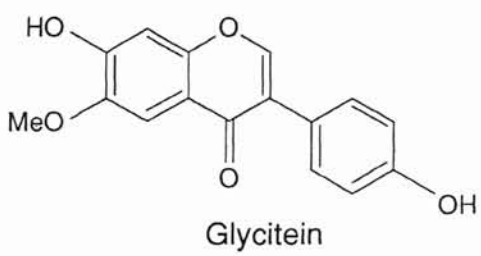
As mentioned earlier, soy is the richest source of isoflavones. Genistin, daidzin and glycetin are the three major isoflavones that present in soy. One gram of soy contains about 1-3 mg of diadzin, genistin, glycetin and their corresponding glucosides (Barnes & Messina, 1991). Genistein and diadzein comprise the major portion of isoflavones in soy and they have been shown to bind to estrogen receptors, a property explained by their structural similarity with estrogens (Verdeal et al., 1980). The finding by Kuiper et al. (1998) showed that the binding affinity of genistein to ER-β was about 20 times greater than ER-α. ER-β is expressed in non-reproductive tissue, such as bone and the vascular system (Enmark & Gustafsson, 1998). This may explain some of the cardio-protective effects of soy.

Structures of Three Major Soy Isoflavones



Genistein

Daidzein



Glycitein

A number of studies have been conducted to investigate the cardio protective effects of soy in the context of its protein and its isoflavone content. The exact mechanism of the hypocholesterolemic effect of soy remains elusive and is multi-factorial. One proposed mechanism is that soy may increase the fecal bile acid excretion and alter the rate of bile acid synthesis, thereby maintaining cholesterol homeostasis. Duane (1997) has shown that soy increased the hepatic cholesterol secretion.

Arjmandi et al., (1997) conducted a randomized study using 72 female rats. The animals were divided into six groups of 12 rats each and placed under different treatment diets: sham-operated + control, ovarectomized (ovx) + control, ovx + soy with isoflavones (soy), ovx + isoflavones depleted soy protein (soy-), and ovx + E_2 (17β-estradiol). The results of this study showed that the serum total cholesterol concentrations were significantly higher in ovx control animals in comparison with sham. This ovarictomy-induced hypercholesterolemia was reduced by soy, and E_2 treatments. These findings show us that soy, similar to estrogen can reduce the postmenopausal rise in serum cholesterol.

Anthony et.al., (1996) showed that in male rhesus monkeys, soy protein with isoflavones lowered TC and LDL and increased HDL, whereas neither casein and lactalbumin mixture nor an alcohol extracted soy protein with low isoflavones lowered affected the lipid profiles. Anthony (2000) reported that soy protein, isoflavones or both are seen to exert beneficial effects on blood pressure, vascular and endothelial function, aggregation and serotonin, LDL oxidation, smooth muscle cell proliferation and migration and inhibit atherogenesis.

However, there are some studies that do not support these findings and they have shown that soy protein does not have any beneficial effects on hypercholesterolemia (Gooderham et al, 1996). In the study investigating atherosclerosis in cynomolgus monkeys, Anthony et al., (1996) found less coronary artery consuming soy protein with isoflavones(+) compared to soy protein with trace amounts of isoflavones(–) or with casein. The discrepancies in the findings of the studies may have been due to the isoflavone content of the soy protein used or the study design. Potter et al., (1998) suggested that isoflavones present in soy may have an independent effect on plasma lipid profiles.

Isoflavones are conjugated substances, when hydrolysed by β-glucosidases in the jejunum, release bioactive aglycones, daidzein and genistein. These aglycones have show affinity for estrogen receptors and have other non-hormonal effects on the cellular mechanisms (Setchell et al., 1997).

Beneficial Effects of Soy Isoflavones

A linear relationship is seen between the isoflavone content of soy and cholesterol reduction. In a study by Crouse et al. (1999) it was seen that the hypocholesterolemic effect of soy was lost when the isoflavones were removed from the soy protein by alcohol extraction.

Anti-thrombotic Effects of Soy Isoflavones

Platelet aggregation plays a major role in the progression of cardiovascular disease. Soy isoflavones are found to reduce platelet aggregation induced by seratonin

and thrombin in animals (Williams & Clarkson, 1998). The positive results using the free form isoflavones have so far have not been reproduced in humans consuming average amounts of soy protein. Studies are currently being done to further examine soy's effect on platelet aggregation.

Of all the soy isoflavones, the cardio protective effects of genistein have been investigated the most. In vitro, studies have shown that genistein, can alter specific cellular processes in the coagulation system associated with the development of atherosclerotic plaque (Raines & Ross, 1995). As discussed earlier one of the essential steps in the atherosclerotic process is the adherence of platelets to foam cells and the subsequent increase in growth factors released from platelets. The up-regulation of growth factors appears to be an integral step in the development of atherosclerotic plaque (Kanazaw et al., 1995). Genistein has been shown to prevent the development of atherosclerosis by: 1) interfering with the activation and accumulation of platelets; 2) reducing the production of platelet-derived growth factors, which are believed to play an important part in the proliferation of smooth muscle cells in the atherosclerotic plaque; and 3) inhibiting the action of thrombin, an enzyme that converts fibrinogen into fibrin to form a blood clot (Wilcox & Blumenthal 1995). Studies are now being done to evaluate the ant- thrombotic effects of isoflavones on humans.

Genistein may also inhibit atherosclerosis is by inhibiting the migration and proliferation of the smooth muscle cells that are important in the promotion and the progression of plaque formation (Schonherr, 1997).

Antioxidant Effects of Soy Isoflavones

In-vitro studies suggest that genistein and daidzein inhibit LDL oxidation in a manner similar to that of vitamin E (Hodgson et al., 1996). Feeding isoflavone-rich soy protein is seen to inhibit oxidation of LDL isolated from rats (Anderson et al., 2002). In an a clinical trial it was seen that LDL oxidation was also suppressed when a soy isoflavone rich beverage was administered to both healthy subjects and patients who had suffered a stroke (Kanazawa et al., 1995)

Favorable Blood Vessel Effects of Soy Isoflavones

The results of a study done by Honore et al., (1997) indicate that isoflavones like estrogens promote vasodilation. In this study using matched soy diets, one rich in isoflavone and one with low levels, investigators examined coronary vascular reactivity on atherosclerotic monkeys. The high isoflavone diet resulted in less constriction in males, and vasodilation in female monkeys. An intravenous administration of genistein caused dilation in the previously constricted arteries of the females fed the low isoflavone diet. Another study by Nestel et al., (1997) showed similar results in women who were post-menopausal or experiencing menopause. The results of this study showed that when compared to a placebo, daily administration of a pure isoflavone preparation for five to 10 weeks improved arterial elasticity by 26 percent. Arterial compliance was improved to about the same extent as is achieved with conventional hormone replacement therapy. These studies suggest another beneficial effect of isoflavone on the circulatory system and prevent the incidence of atherosclerosis.

All the three soy isoflavones namely genistein, daidzein and glycetein inhibit the proliferation of smooth muscle cells by inhibiting DNA synthesis in the smooth muscle

cell. Genistein acts as a protein tyrosine kinase inhibitor that prevents the cell migration into the intima of the artery (Pan et al., 2001). Another mechanism by which isoflavones exert a cardio protective is by up regulating LDL-receptor activity (Kirk et al., 1998).

By looking at the pharmacokinetics of soy isoflavones it is seen that maintaining high steady state of plasma concentrations can be maintained only by the daily intake of phytoestrogens throughout the day (Setchell, 2000).

The consumption of 34 –165 mg/d of isoflavones in foods like isolated soy protein, soy flour and other foods that contain soy showed an increase in sex hormone binding globulin (Duncan et al., 1999) and decreased the occurrence and the severity of hot flashes and vaginal dryness (Baird et al., 1995). In October 1999, FDA approved a health claim that can be used on labels of soy-based foods to advertise their heart-healthy benefits. The agency reviewed research from 27 studies that showed soy protein's value in lowering levels of TC and LDL or the "bad" cholesterol. The claim states that "Diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease".

These studies suggest that soy isoflavones may play an important role in lowering cholesterol. However the optimal dose of isoflavone and the exact mechanism of cholesterol lowering action need to be elucidated. This study was designed to examine the dose-dependent effect of soy isoflavones on ovariectomy-induced hypercholesterolemia and to elucidate the mechanism of action.

Hamster as a model for hypercholesterolemia

Pig is an acceptable model for studying hypercholesterolemia and atherosclerosis as they are similar to humans in this aspect. (Van Tol et al.,1991). Pigs develop hyperlipidemia and atherosclerosis when fed high fat diets like humans (Beakey et al., 1988). However pigs are difficult to handle and are expensive and this raises the need for least expensive and are easy to handle animals used in scientific research and the selection of the appropriate model is important.

Rats are not considered as a good model to study the cholesterol metabolism and atherosclerosis because their lipid profiles are quite different from humans. Rats carry a major portion of their plasma cholesterol as HDL and they are resistant to the development of plaque (Shefer et al., 1992).

Rabbits are seen to develop aortic lesions in response to a high fat diet. Although rabbit has been used as model of human atherosclerosis, it is not the best model as they carry most of the cholesterol in the VLDL fraction unlike humans (Badimon et al., 1990).

Hamsters are considered good model for studying hypercholesterolemia because of various reasons. The LDL/ HDL ration in the hamsters is increased with an increase in the total cholesterol concentration. This pattern is similar to that seen in humans. Additionally, serum triglycerides are increased in hamsters fed a high fat. Hamsters, when fed high fat diet develop atherosclerosis (Otto et al., 1995). Hamsters are easy to handle and are not very expensive. Therefore female Golden Syrian hamster was selected in this study, to study the effect dose dependent effects of isoflavones and also to elucidate the hypocholesterolemic effect so soy isoflavones.

CHAPTER III

Materials and Methods

Seventy-two 6-month old female Golden Syrian hamsters (Harlan Sprague-Dawley, Indianapolis, IN) were housed three in a cage and kept in an environmentally controlled laboratory. Guidelines for the ethical care and treatment of animals from the Animal Care and Use Committee at Oklahoma State University were strictly followed. After three days of acclimation, hamsters were randomized by weight into six groups of 12 hamsters each and either sham-operated (sham) or ovariectomized (ovx). Treatment groups were sham, ovx control, ovx + E_2 (10 μg E_2/kg body weight), ovx + Iso2 (0.0095g isoflavones/kg diet) , Iso4 (0.018g isoflavones/kg diet), or Iso8 (0.038g isoflavones/kg diet). A twelve-hour light: twelve-hour dark cycle was followed and hamsters were fed a semi-purified casein-based and cholesterol-free powdered diet with or without isoflavones (Table 1) for 120 days. E_2 was dissolved in a small volume of absolute ethanol and the concentration was adjusted with sesame oil. Hamsters were injected subcutaneously with either E_2 or solvent vehicle daily. Hamsters were pair-fed to the mean food intake of the estrogen-administered group and had free access to deionized water. Food intake and body weight were monitored routinely.

Table I

Grouping and Diets

Name of the group	Diet Fed
Sham + control	Control
Ovx + control	Control
Ovx + E2	Control
Ovx + Iso2	Control + 0.0095g Isoflavones /kg diet.
Ovx + Iso4	Control + 0.019g Isoflavones /kg diet
Ovx + Iso8	Control + 0.038g Isoflavones /kg diet

Table II

Composition of Diet

Ingredient	Amount (g/kg diet)
Carbohydrates (total)	395
Rice flour[1]	395
Fiber (total)	144
Wheat bran[2]	72
Cellulose[3]	72
Protein (total)	240
Casein[3]	240
Fat (total)	154
Hydrogenated coconut oil[3]	96
Safflower oil[3]	19
Soybean oil[3]	39
Choline chloride[3]	3
Potassium bicarbonate[4]	20
Vitamin mix[5]	10
Mineral mixture[6]	34
Isoflavones[7]	0.0, 0.0095, 0.019, or 0.038

[1]California Natural Products (Lathrop, CA).

[2]Natural Ovens of Manitowoc (Manitowoc, WI).

[3]Harlan-Teklad (Madison, WI).

[4]Sigma Chemicals (St. Louis, MO).

[5]Vitamin mixture, (TD #40060, Harlan Teklad, Madison, WI).

[6]Mineral Mixture (TD #170911, Harlan Teklad, Madison, WI).

[7]Isoflavone (Protein Technologies Inc., St. Louis, MO) contents of control, LD, MD, and HD diets, respectively. This amount includes all forms of isoflavones such as aglycones, glycosides and glycoside esters (*mg/g product*): genistein-containing compounds, 11.84; daidzein-containing compounds, 6.38; glycitein-containing compounds, 0.74. Aglycone components of the isoflavones (*mg/g product*): genistein, 7.09; daidzein, 3.81; glycitein, 0.47.

Autopsy and Sample Collection.

One hundred days after treatment the animals were euthanized. At the mid point of the dark cycle the animals were anesthetized with a mixture of ketamine hydrochloride (100 mg/kg body weight) and xylazine (5 mg/kg body weight). For assessing the rate of sterol synthesis one hour prior to sacrifice, tritiated water was rapidly injected into the femoral vein and the animal was placed under the hood (Arjmandi et al., 1997). One hour after the injection the animals were exsanguinated via the abdominal aorta.

Blood samples were collected and plasma was separated by centrifugation at 1500 x g for 20 minutes at 4°C. Aliquots of plasma were frozen and kept at -20°C for later analyses. The liver was removed immediately and rinsed with ice-cold saline solution. The total weight of the liver was recorded. A portion of liver was weighed and was stored in 85% alcoholic KOH to be used for measuring the hepatic rate of sterol synthesis. The remaining portion was stored at -20°C for liver lipid analyses.

The small intestine was flushed of its contents using saline. The flushed intestine was blotted and weighed. It was then stored in 85% KOH solution for measuring the rate of sterol synthesis.

Uterus was removed and weighed to confirm the success of ovariectomy. Spleen was weighed and discarded.

Measurement of Intestinal and Hepatic Rates of Sterol Synthesis.

The hepatic and intestinal rates of sterol synthesis were measured to determine whether the rates of sterol synthesis were affected by ovariectomy and different doses of isoflavones. The *in vivo* rates of sterol synthesis were measured as previously described (Arjmandi et al., 1992, 1997) in liver and small intestine. The specific activity of water was calculated using the formula by Jeske and Dietschy (1980).

$$(kBq\ ^{3}H/L\ serum) \times (1.09) / (\mu mol\ water\ /L\ water)$$

The term 1.09 was used to correct the specific activity of plasma water determined at 1 hour after injection of ^{3}H water to the mean specific activity of body water present throughout the 1-hour period of time.

Small intestine was placed in 30 ml of 5% alcoholic KOH until completely dissolved. It was saponified on a steam bath. aliquots of 10 ml were made in duplicates. Petroleum ether (15 ml) was added to the samples thrice to extract the sterols. The extracted sterols were evaporated to dryness under the fume hood. The residue was dissolved in 3x2 ml ethanol: acetone (1:1) and transferred to centrifuge tubes. The contents were acidified with a drop of 1 mol /L HCl and sterols were precipitated with 2 ml of 0.5% digitonin in 50% ethanol. The digitonin precipitate was washed twice with 5 ml acetone and then 5 ml diethyl ether. The precipitate was spread on the sides of the tube and was allowed to dry and then vacuum dried in an oven at 80°C for 15 minutes. The precipitate was then dissolved in pyridine and free sterol was extracted using 3 ml of

diethyl ether twice and was transferred to a counting vial and air dried. The vials were placed in the vacuum oven at 80°C for 1 hour. Methanol (1 ml) was added to dissolve the sterols and 10 ml of Scinti Safe scintillation solution was added and the radioactivity of the mixture was analyzed in a liquid scintillation counter (Arjmandi et al., 1992, 1997).

Plasma Total Cholesterol, Free Cholesterol, HDL-C and Triglycerides.

Plasma total cholesterol, free cholesterol, HDL-C and triglycerides were measured using the enzymatic kits from Roche laboratories. These tests were performed using a Cobas Fara II clinical analyzer (Montclair, NJ) following the manufacture's instructions and using commercially available calibrators and quality control samples.

The total cholesterol concentration was measured using a procedure described by Allain et al. (1974). In this procedure cholesterol was released enzymatically from its esters by cholesterol esterase and was oxidized by cholesterol oxidase producing hydrogen peroxide. Hydrogen peroxide when combined with 4-aminoantipyrine and phenol forms a quinone dye that absorbs light at 500 nm. The absorbance is directly proportional to the cholesterol concentration in the sample.

Plasma triglycerides (TG) concentrations were determined enzymatically using commercially available kits from Roche Diagnostics. In this procedure TG 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- chlorophenol and 4- aminophenazone and forms a quinoemine dye that has an absorbance of 500 nm. The absorbance is directly proportional to the triglyceride concentration in the sample.

Serum HDL-cholesterol was measured by a direct method 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 (1974). HDL-cholesterol concentrations were determined enzymatically using commercially available kits from Roche Diagnostics.

Liver Lipids And Liver Total Cholesterol.

Liver lipids and total cholesterol were measured. Portions of the liver were homogenized and extracted with chloroform methanol mixture (2:1 v/v). NaCl solution (0.13 mol/ L) was added and the phases were separated and aliquots of the organic phase were analyzed for liver cholesterol. Liver total cholesterol was determined using a color reagent of glacial acetic acid $FeSO_4$- H_2SO_4 (Searcy & Bergquist, 1960). Total lipids were determined using Folch gravimeteric method. The remainder of the organic phase was evaporated, dried and was weighed to measure the total lipids (Folch et al., 1957). The intra- and inter-assay coefficients of variation were 3.1% and 4.2%, 4.1% and 5.4 % for liver total lipid and cholesterol, respectively.

Measurement of Fecal excretion of neutral sterols

For the measurement of fecal excretion of neutral sterols, three day fecal collection was done and the collected feces were desiccated and stored until further analysis. Neutral sterols were measured using the modified method of Arjmandi et al., (1992) using gas chromatography. 0.05g of finely powdered fecal sample was digested along with an internal standard, 0.25 mg of 5-alpha cholestane, using acetic acid at 110°C

for 1.5 hrs. After the samples were cooled, the total lipids were extracted using toluene. This extract was evaporated using nitrogen at 60°C. The fractions obtained by evaporation were resuspended using cholylglycine hydrolase in acetate buffer. To this mixture 1.0 ml of deionized water, 0.25 ml of 7.5 mol/L NaOH and 4 ml of methanol were added, mixed thoroughly and the pH was adjusted to 8-9. Neutral sterols from this mixture were extracted four times using petroleum ether and dried under nitrogen at 40°C. The residues were resuspended in petroleum ether and derivatized using silylating reagent. After the process of derivitization the neutral sterols were dried under nitrogen again and redissolved in 100 µl of ethylacetate and 1 µl of this sample was injected into the Hewlett Packard gas chromatograph which was set up at the following conditions: Column (J& W Scientific #122-4732), Temperature injector 250°C, detector 280°C, oven temperature - Initial 250°C for 20 minutes , and elevated to 310°C by increasing 10°C/minute to final temperature and holding for 2 minutes, using helium carrier gas.

Total Fecal Bile Acids

Dried and pulverized feces sample (0.2g) was weighed and 4 ml of t-butanol/water (1:1 v/v) was added. The mixture was heated to 37°C for 15 minutes and mixed every 5 minutes when heating. The mixture was centrifuged at 1500 x g for 10 minutes and the supernatant was removed and analyzed using the Cobas Fara II clinical analyzer (Montclair, NJ) using the enzymatic kit (# 450-A) from Sigma Diagnostics (St.Louis, MO).

The principle of the kit is based on the property of bile acids to be oxidized in the presence of nicotinamide adenine dinucleotide (NAD) and the enzyme 3α-hydroxysteroid

dehydrogenase (3α-HSD) to form 3- oxo bile acids α-Hydroxybile Acids. The reduced form of NAD (NADH) released acts with nitro blue tetrazolium (NBT) to form formazan and the formazan has an absorbance of 530 nm and the color produced is directly proportional to the bile acid concentration in the sample (Sigma Product insert).

3α-HydroxybileAcids+NAD---(3α-HSD)-->3-OxoBileAcids+NADH

NADH + NBT -(Diaphorase)-> NAD + Formazan (Brown color)

Statistical Analyses

Data analyses involved computation of least square means and standard error (SE) of the means for each of the treatment groups using SAS version 8.2 (SAS Institute, Cary, NC). Analysis of variance and least square means were calculated using the general linear model procedure and the means were compared using Fisher's least significant difference for comparing groups. Differences were considered significant at $P<0.05$, unless otherwise stated.

CHAPTER IV

RESULTS

Food Intake, Body Weights and Organ Weights

The animals were divided in to different treatment groups based on their initial body weights, so there were no significant differences in the initial body weights among the groups (Table III). In spite of pair-feeding, a significant difference was seen in the final body weights of the treatment groups. The mean final body weight of the animals in the Iso2 group was significantly ($p<0.05$) higher than the sham, E_2, and Iso8 groups. No differences were seen among the sham, ovx, E_2, Iso4 and Iso8 groups. Weights of liver and small intestine were similar for all the groups. Ovariectomy caused a significant ($p<0.05$) atrophy of the uterus in all the ovx groups compared with the sham group ($p<0.05$). The E_2 administration significantly ($p<0.05$) increased the uterine weight in comparison with all the other ovx groups.

Plasma Lipid Profiles

Table IV shows the effects of ovariectomy and diet on lipid profiles of hamsters. Plasma total cholesterol was significantly ($p<0.05$) increased in the ovx control group when compared to the sham group. The cholesterol concentrations of Iso4 and Iso8 groups were significantly ($p<0.05$) lower than the ovx control group, thus showing the cholesterol lowering effects of soy isoflavones. There were no significant differences in the plasma total cholesterol among sham, E_2, Iso4 and Iso8 groups. No significant reduction in plasma total cholesterol was seen in Iso2 group when compared to the ovx

control group. No significant differences were seen in plasma HDL, Non-HDL and triglycerides levels for all the groups.

The mean free cholesterol concentration was significantly ($p<0.05$) lower in the sham animals when compared to the ovx control group. Iso4 group also had a significantly lower free cholesterol concentration when compared to the ovx control group. No significant differences were seen among the ovx control, E_2, Iso2 and Iso8 groups.

Liver Lipids and Rates of Hepatic and Intestinal Sterol Synthesis

As reported in Table V there were no significant differences in the liver total lipids among all the groups. Liver cholesterol was significantly ($p<0.05$) reduced in the ovx control group when compared with to sham. The liver cholesterol of E_2, Iso2 and Iso8 groups was significantly ($p<0.05$) lower when compared to ovx control.

There were no significant differences in either the hepatic or the intestinal rates of sterol synthesis among all groups (Table V).

Fecal Neutral Sterols and Bile Acid Excretion

There were no differences in the fecal excretion of neutral sterols among the groups. Neither ovariectomy nor different levels of soy isoflavones significantly affected the total bile acids excretion in hamsters (Table VII).

TABLE III

Effect of ovariectomy(ovx), three levels of isoflavones and 17β-estradiol (E_2) on body and organ weights in hamsters.

Parameter	Sham + Control	Ovx + Control	Ovx + E_2	Ovx + Iso 2	Ovx + Iso 4	Ovx + Iso 8
Body weight, (g)						
Initial	143 ± 3	144 ± 3	144 ± 3	145 ± 3	144 ± 3	142 ± 3
Final	153 ± 4^{bc}	156 ± 4^{ab}	143 ± 4^{c}	165 ± 4^{a}	162 ± 4^{ab}	154 ± 4^{bc}
Organ weights, (g)						
Uterus	0.72 ± 0.20^{a}	0.24 ± 0.04^{c}	0.51 ± 0.04^{b}	0.18 ± 0.03^{c}	0.19 ± 0.04^{c}	0.22 ± 0.20^{c}
Liver	4.70 ± 0.20	4.69 ± 0.20	4.29 ± 0.20	4.91 ± 0.19	4.63 ± 0.20	4.51 ± 0.20
Small Intestine	2.38 ± 0.07	2.42 ± 0.07	2.22 ± 0.07	2.38 ± 0.07	2.31 ± 0.07	2.28±0.07

Values are expressed as means ± SE (n = 12)

Values that do not share the same superscript letters are significantly ($p<0.05$) different from one another.

E_2 ; 17β-estradiol (10 μg E_2/kg body weight)

Iso 2; 0.0095g isoflavones/kg diet

Iso 4; 0.018g isoflavones/kg diet

Iso 8; 0.038g isoflavones/kg diet

TABLE IV

Effects of ovariectomy (ovx), three levels of isoflavones and 17β-estradiol (E_2) on plasma lipid profiles of hamsters.

Parameter (mmol/L)	Sham + Control	Ovx + Control	Ovx + E_2	Ovx + Iso 2	Ovx + Iso 4	Ovx + Iso 8
Total Cholesterol	3.7±0.3^{c}	5.1±0.3^{a}	4.1±0.3^{bc}	4.7±0.3^{ab}	4.5±0.3^{bc}	4.1±0.4^{bc}
HDL-Cholesterol	2.1±0.2	3.2±0.2	2.4±0.2	2.8±0.2	2.7±0.2	2.5±0.2
Free- Cholesterol	0.7±0.1^{b}	1.2±0.1^{a}	1.0±0.1^{a}	0.9±0.1^{ab}	0.8±0.1^{b}	1.0±0.1^{ab}
Triglycerides	1.2±0.1	1.1±0.1	1.3±0.1	1.4±0.1	1.3±0.1	1.2±0.1
Non- HDL Cholesterol[1]	1.6±0.1	1.9±0.1	1.7±0.1	1.9±0.1	1.8±0.1	1.6±0.1

Values are expressed as means ± SE. (n = 12)

Values that do not share the same superscript letters are significantly ($p<0.05$) different from one another.

[1]Non-HDL cholesterol = total cholesterol – HDL cholesterol

E_2 ; 17β-estradiol (10 μg E_2/kg body weight)

Iso 2; 0.0095g isoflavones/kg diet

Iso 4; 0.018g isoflavones/kg diet

Iso 8; 0.038g isoflavones/kg diet

TABLE V

Effect of ovariectomy(ovx), three levels of isoflavones and 17β-estradiol (E_2) on liver lipids and hepatic and intestinal rates of sterol synthesis in hamsters.

Parameter	Sham + Control	Ovx + Control	Ovx + E_2	Ovx + Iso 2	Ovx + Iso 4	Ovx + Iso 8
Liver total lipid(mg/g liver)	76.2 ± 4.5	67.3 ± 4.6	75.9 ± 4.8	80.3 ± 4.3	77.7 ± 4.6	72.2 ± 4.6
Liver Cholesterol(mg/g liver)	4.1 ± 0.5 [b]	2.2 ± 0.5 [c]	3.8 ± 0.5 [b]	4.6 ± 0.5 [ab]	3.3 ± 0.5 [bc]	3.8 ± 0.5 [b]
Liver (nmol[^{3}H]DPS/(g. liver. h)	93.2 ± 17.4	65.6 ± 17.4	117.7 ± 19.1	38.8 ± 16.1	93.2 ± 21.3	76.3 ± 21.3
Small Intestines (nmol[^{3}H]DPS/ (g.intestine.h)	33.7 ± 8.4	55.2 ± 9.2	43.2 ± 8.4	48.1 ± 9.2	49.3 ± 8.4	45.6 ± 8.4

Values are expressed as means ± SE. (n = 6)

Values that do not share the same superscript letters are significantly ($p<0.05$) different from one another.

E_2 ; 17β-estradiol (10 μg E_2/kg body weight)

Iso 2; 0.0095g isoflavones/kg diet

Iso 4; 0.018g isoflavones/kg diet

Iso 8; 0.038g isoflavones/kg diet

TABLE VI

Effect of ovariectomy(ovx), three levels of isoflavones and 17β-Estradiol (E_2) on neutral sterol and total bile acid excretion in hamsters.

Parameter (mg/g feces)	Sham + Control	Ovx + Control	Ovx + E_2	Ovx + Iso 2	Ovx + Iso 4	Ovx + Iso 8
Coprastanol/Cholesterol	57.2± 5.0	97.6 ± 45.5	42.7 ± 11.3	25.8 ± 5.1	124.4 ± 30.5	165.8± 55.5
Cholestanol	16.7 ± 4.1	27.4 ± 7.5	25.5 ± 11.9	17.1 ± 5.3	24.1 ± 5.3	30.4 ± 12.5
Coprastanone	11.6 ± 3.7	6.8 ± 1.7	22.4 ± 14.5	11.7± 5.9	16.8 ± 5.9	9.33± 2.8
Total bile acids	1.12 ± 0.03	0.14 ± 0.00	1.15 ± 0.09	0.90 ± 0.20	0.42 ± 0.28	0.96 ± 0.04

Values are expressed as means ± SE. (n = 4)

Values that do not share the same superscript letters are significantly ($p<0.05$) different from one another.

E_2 ; 17β-estradiol (10 μg E_2/kg body weight)

Iso 2; 0.0095g isoflavones/kg diet

Iso 4; 0.018g isoflavones/kg diet

Iso 8; 0.038g isoflavones/kg diet

CHAPTERVI

DISCUSSION

Food Intake, Body and Organ Weights

In spite of pair-feeding, ovariectomy caused an increase in the body weight. Sohn et al., (1999) and Arjmandi et al., (1997) reported significant increases in weight gain were caused due to ovariectomy in hamsters and rats, respectively.

E_2 administration reduced the weight and this reduction in weight was also seen in the study conducted by Wallen and co workers (2001), where the ovariectomy-induced weight gain in rats was reversed by estrogen. Though the mechanism behind this phenomenon is not clear, it is seen that weight gain is modulated by sex hormones suggesting that they exert a direct effect on overall body metabolism (Wallen et al., 2001). In the present study soy isoflavones at lower levels did not cause any significant changes in final body weight (Iso2 and Iso4). But a significant reduction in weight was seen in the mean final body weight of the animals in the Iso8 group when compared to the Iso2 group. This finding was in contrast to that of Uesugi et al (2001), who reported that oral administration of daidzin, genistin or glycitin prevented ovariectomy induced weight gain and uterine atrophy and that soy isoflavones may reverse the unfavorable changes caused by hormone deficiency.

No significant changes were seen in the liver weights among all the groups. As expected the weight of the uterus in all the ovx groups was significantly reduced when compared to the sham groups showing the success of ovariectomy. Estrogen is known to cause hypertrophy of uterine tissue (Dodge et al., 1996) and this is the reason for the higher mean uterine weight of the E_2 group when compared to the other ovx groups.

However, the dose of estrogen used (10 μg E_2/kg body weight) was not sufficient to bring the uterine weight up to those of the sham animals. In a study by Yamaguchi et al.(2001), they reported that the uterotrophic effect of dietary isoflavones was very weak when compared with that of estrogen. This explains the low uterine weight observed in all the Iso groups.

Plasma Lipid Profiles

As reported by earlier studies, ovariectomy caused a significant increase in the plasma total cholesterol concentrations, which is similar to the rise in blood lipids following menopause (Arjmandi et al., 1997, Sohn et al., 1999). This ovariectomy-induced rise in total cholesterol was prevented by higher doses of isoflavones (Iso4 and Iso8). Like estrogen, isoflavones prevented this rise in serum total cholesterol. There were no significant differences between the two higher doses of isoflavones groups suggesting that the doses of isoflavones reached a plateau. These lipid-lowering effects of isoflavones were consistent with the findings of Anthony et al., (1996). Greaves et al., (2000) also reported a reduction in total cholesterol in ovariectomized cynomolgus monkeys fed an isoflavone rich diet compared to a control group fed a casein-lactalbumin diet, however their results were not significant ($p<0.05$).

An increase in non-HDL cholesterol is seen after ovariectomy (Sohn et al., 1999, Lucas et al., 2001). This increase in Non-HDL cholesterol was reduced in the all the isoflavones groups when compared to the ovx control group. A higher reduction was seen in the groups as the concentration of isoflavones increased. But the reduction was not significantly different among all groups. Potter et al., (1998) showed a similar trend in

postmenopausal women. A significant reduction in non-HDL cholesterol was seen in women on an isolated soy protein with added isoflavones as compared to women who were on a diet with casein as their protein source.

Lucas et al., (2001) also reported that ovariectomy causes an increase in serum free cholesterol but the increase was not significant, however in this study it is seen that plasma free cholesterol is significantly increased after ovariectomy and soy isoflavones helped in bring down the level of free cholesterol significantly. In the study by Lucas et al., (2001) no significant changes was observed in the value of triglycerides among the groups. Soy isoflavones seem to have no major effect on plasma triglyceride levels. Gardner et al., (1998) also demonstrated that soy isoflavones lower plasma cholesterol but have no effect on triglycerides in hypercholesterolemic postmenopausal women.

Liver Lipids

No significant differences were seen in the mean value of liver total lipids. Ovariectomy does not seem to affect the liver lipid levels (Arjmandi et al., 1997, Lucas et al., 2001). However, liver cholesterol was significantly lower in the ovx control group contrary to the findings of these two studies. There were no differences in the liver cholesterol values among the sham E_2 and the Iso groups. This could be due to the decrease in the hepatic rate of sterol synthesis in these groups.

Hepatic and Intestinal Rates of Sterol Synthesis

There was no significant difference in the rate of sterol synthesis in the liver and in the small intestines. The sterol synthesis in the liver followed the same trend as shown

by Sohn et al., (1999). The lack of significant difference in the values may be due to the fact that the amount of radioactivity used in our study was much lower than the amount used in their study. They used 1.85 GBq whereas we used only 0.35 GBq of radioactivity which was almost one fifth of the amount used by Sohn et al., (1999).

Fecal Excretion of Neutral Sterols and Total Bile Acids

In 1992 Arjmandi et al. reported that, increased bile acids excretion causes a decrease in the cholesterol concentrations in the body. In a review by Potter (1998) it is reported that there is an increase in the fecal excretion of bile acids especially the neutral sterols in the soy group relative to casein. Lichtenstein (1998) also credited the hypocholesterolemic effect of soy to its ability to increase fecal excretion of bile acids.

The results presented in the table VII show the dose-dependent effect of soy isoflavones on fecal excretion of neutral sterols and total bile acids. Soy isoflavones did not have any significant effect on fecal excretion of neutral sterols. This could be due to a number of factors. During the processes of extracting neutral sterols, fecal extracts formed a precipitate when the silyating reagent was added. In order to obtain a clear solution that could be injected into the capillary column, we had to centrifuge the samples. Some of the neutral sterols could have been trapped in the precipitate.

In the capillary column that was used for the separation of the samples, a high temperature of 325°C called for in the original procedure could not be reached and this might have prevented the proper separation of the samples. Another disadvantage of using the capillary column was that we were not able to inject 3μL as in a mesh column

as described in the procedure. The separation of samples could have also affected because of this low volume.

The amount of total bile acids excreted by the ovx control group was less than the sham and the Iso groups. This effect on total bile acids could not be analyzed statistically as the number of animals in the ovx control group was very small (N = 4). The results of the total bile acids suggest that the hypocholesterolemic effect of the soy isoflavones could be due to the increased excretion of the total bile acids, as the amount of total bile acids excreted by the isoflavone groups was higher than the ovx control group and was not significantly different from the sham control group.

Conclusion

The results of this study show us that soy isoflavones exhibit a beneficial effect on ovariectomy-induced hypercholesterolemia. Soy isoflavones significantly prevented the ovariectomy-induced hypercholesterolemia. A dose-dependent reduction in total cholesterol was seen among the groups. Our observations indicated that Iso4 and Iso8 dose levels were more effective in lowering plasma cholesterol levels compared to the Iso2. These findings indicate a possibility of using soy isoflavones as a therapeutic agent in preventing hypercholesterolemia.

However, further studies are needed to determine the best dose of isoflavones that is suitable for human beings to lower cholesterol and the effect of long-term intake of isolated isoflavones in humans. Additional studies are also needed to elucidate the hypocholesterolemic mechanisms of soy isoflavones.

REFERENCES

Abbott RD, Curb DJ, Rodriguez LB, Masaki KH, Yano K, Schatz JI, Ross GW, Petrovitch H. Age-Related Changes in risk factor effects on the incidence of coronary heart disease. *Ann. of Epi.*. 2002;12 (3): 173-81.

Alexandersen P, Haarbo J, Breinholt V, Christiansen C. Dietary phytoestrogens and estrogen inhibit experimental atherosclerosis. *Climacteric* 2001; 4(2):151-59

Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin. Chem.*1974;113: 290-91..

American Heart Association. 2000 Heart and Stroke Statistical Update. Dallas, Tex: *American Heart Association*; 2002.

Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med* 1995;333: 276-82.

Anthony MS. Soy and cardiovascular disease: cholesterol lowering and beyond. *J. Nutr.* 2000; 130: 654s- 55s.

Anthony. MS, Clarkson TB, Hughes CL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of the peripubertal Rhesus monkey. *J. Nutr.* 1996;126: 43-50.

Arca M, Vega GL, Grundy SM. Hypercholesterolemia in postmenopausal women: metabolic defects and response to low use of lovastatin. *JAMA.* 1994; 271: 453-59.

Arjmandi BH, Ahn J, Nathani S, Reeves RD. Dietary soluble fiber and cholesterol affect serum cholesterol concentration, hepatic portal venous short-chain fatty acid concentrations and fecal sterol excretion in rats. *J.Nutr.* 1992 ;122(2):246-53

Arjmandi BH, Khan DA, Juma SS, Svanborg A. The ovarian hormone deficiency-induced hypercholesterolemia is reversed by soy protein and the synthetic isoflavone, ipriflavone. *Nutrition Research.* 1997; 17 (5): 885-94.

Badimon JJ, Badimon L, Fuster V. Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. *J. Clin. Invest.* 1990 ;85(4):1234-41.

Baird DD, Umbach DM, Lansdell L, Hughes CL, Setchell KDR., Weinberg CR, Haney AF, Wilcox AJ, Mclachlan JA. Dietary intervention study to assess

estrogenicity of dietary soy among postmenopausal women. *J.Clin. Endocrinol. Metab.*1995; 80:1685-90.

Barnes S, Messina M. The role of soy products in reducing cancer risk. *J. Natl. Cancer Inst.* 1991; 83: 541-46.

Barnet-Connor E, Bush TL. Estrogen and coronary heart disease in women. *JAMA.*1991; 20:47-63

Beakey PA, Cerda JJ, Burgin CW. Grapefruit pectin inhibits hypercholestrolemia and atherosclerosis in miniature swine. *Clin. Cardiol* .1988;11: 595-00.

Boker LK, Van der Schouw YT, De Kleijn MJ, Jacques PF, Grobbee DE, Peeters PH. Intake of dietary phytoestrogens by Dutch women. *J. Nutr.* 2002;132(6):1319-28

Bonithon-kopp C, Scarabin PY, Darne B, Malmejac A, Guize L. Menopause related changes in lipoproteins and some other cardiovascular risk factors. *Int. J. Epidemiol.* 1990;12: 287-98.

Boring CC, Squires TS, Tong T, Montgomery S. Cancer statistics 1994 *CA-Cancer J. Clin.* 1995;44: 7-26.

Brown SA, Hutchinson R, Morriset J, Boerwinkle E, Davis CE, Gotto AMJr, Patsch W. Plasma lipid, lipoprotein cholesterol, and apolipoprotein distributions in selected US communities. The atherosclerosis Risk in Communities (ARIC) study. *Arterioscler. Thromb.* 1993; 8: 1139-58.

Bush TL. Evidence of primary and secondary prevention of coronary artery disease in women taking oestrogen replacement therapy. *Eur. Heart J.* 1996;17: S9-14.

Crouse JR, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. *Arch. Intern. Med.* 1999; 159:2070-76.

Dietschy JM, Siperstein MD. Cholesterol synthesis by the gastrointestinal tract: localization and mechanisms of control. *J. Clin.Inves.* 1965;44: 1311-27.

Dodge A, Glasebrook AL, Magee DE, Phillips LD, Sato M, Short LL, Bryant HU. Environmental estrogens: Effects on cholesterol lowering and bone in the ovariectomized rat. *J.Steroid Biochem. Molec. Biol.* 1996; 59(2): 155-91.

Doi H, Kugiyama K, Oka H, Sugiyama S, Ogata N, Koide SI, Nakamura SI, Yasue H. Remnant lipoproteins induce proatherothrombogenic molecules in endothelial cells through a redox-sensitive mechanism. *Circulation* 2000;102: 670-76.

Duane WC Measurement of bile acid synthesis by three different methods in hypertriglyceridemic and control subjects. *J. Lipid Res.* 1997 ;38(1):183-88

Duncan AM, Merz BE, Xu X, Nagel TC, Phipps WR, Kurzer MS. Modest hormonal effects of soy isoflavones in postmenopausal women. *J.Clin. Endocrinol. Metab.* 1999; 84: 3479-84.

Enmark E, Gustafsson JA. Newly discovered estrogen receptor. New therapeutic possibilities in postmenopausal symptoms, osteoporosis, cancer of the breast and prostate. *Lakartidningen.* 1998; 95(17):1945-9

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA.* 2001;285: 2486-24.

Expert Panel. Detection, evaluation and treatment of high blood cholesterols in adults: Summary of the second report of The National Cholesterol Education Program (NCEP) Expert panel on detection evaluation and treatment of high blood cholesterols in adults. *JAMA.*1993; 269:3015-23.

Fletcher SW, Colditz GA. Failure of estrogen plus progestin therapy for prevention. *JAMA.* 2002; 288(3): 366-68.

Foxall TL, Shwaery GT, Stuchi AF, Nicolosi RJ, Wong SS. Dose-related effects of doxazosin on plasma lipids and aoric fatty streak formation in the hypercholesterolemic hamster model. *Am. J. Pathol.* 1992; 40: 1357-63

Folch J, Lees M, Sloane-Stanley GH. A simple method for isolation and purification of total lipids from animal tissue. *J Biol Chem* 1957;226:497-09.

.

Genest JJr, McNamara. JR, Ordovas JM, Silbereman SR, Anderson M., Wilson. PW, Salem DN, Schaefer EJ. Lipoprotein, cholesterol, apolipoprotein A-I and B and lipoprotein (a) abnormalities in men with premature coronary artery disease. *J. Am. Col.l Cardiol.* 1992;19: 792-02.

Gooderham MH, Adlercreutz H, Ojala ST, Wahala K, Holub BJ. A soy protein isolate rich in genistein and daidzein and its effects on plasma isoflavone concentrations, platelet aggregation, blood lipids and fatty acid composition of plasma phospholipid in normal men. *J. Nutr.* 1996;126(8):2000-06

Gordan T, Castelli WP, Hjortland MC, Kannel WB Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am. J. Med.* 1977;62: 707-14

Grady D, Herrington D, Bittner V, et al, for the HERS Research Group. Heart and estrogen/progestin replacement study follow-up (HERS II): Part 1. Cardiovascular outcomes during 6.8 years of hormone therapy. *JAMA.* 2002;288: 49-57.

Greaves KA, Wilson MD, Rudel LL, Williams JK, Wagner JD, Consumption of soy protein reduces cholesterol absorption compared to casein protein alone or supplemented with an isoflavone extract or conjugated equine estrogen in ovariectomized cynomolgus monkeys. *J. Nutr.*2000;130: 820-26.

Greendale GA, Lee NP, Arriola ER, The Menopause (seminar) *The Lancet.* 1999; 353 (13): 571(1).

Haijar KA, Gavish D, Breslow JL, Nachman RL. Lipoprotein(a) modulation of endothelial cell surface fibrinolysis and its potential role in artherosclerosis. *Nature.* 1989;339: 303-05

Hamm TE, Kaplan JR, Clarkson TB, Bullock BC. Effects of gender and social behaviour on the development of coronary artery atherosclerosis in cynomolgus macaques. *Atherosclerosis.* 1983; 48(3): 221-33.

Hargrove GM, Junco G and Wong NCW. Hormonal regulation of apolipoprotein AI.*Journal of Molecular* Endocrinology 1999;22: 103-111

Harlap S. The benefits and risks of hormone replacement therapy: an epidemiological overview. *Am. J. Obstet. Gyneco.l* 1992;166: 1986-92.

Harpel PC, Gordon BR, Parker TS. Plasmin catalyses binding of lipoprotein(a) to immobilized fibrinogen and fibrin. *Proc Natl Acad Sci U S A.* 1989;86: 3847-51.

Hodgson JM, Croft KD, Puddey IB, Mori TA, Beilin LJ. Soybean isoflavonoids and their metabolites inhibit in vitro lipoprotein oxidation in serum. *J. Nutr. Biochem.* 1996;7:664-69.

Honore E, Williams J, Anthony M, Clarkson T. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. *Fertility and Sterility.* 1997;67: 148-54.

http://www.cdc.gov/mmwr/preview/mmwrhtml/00017276.htm. November,2001

http://www.cdc.gov/nccdphp/cvd-posters.pdf July, 2002

Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: a critical review. *J. Am. Coll. Nutr.* 2001; 20(1):5-19.

Hulley S. Randomized trial of estrogen plus progestine for secondary prevention of coronary heart disease in postmenopausal women. *JAMA..*1998; 280(19): 605A.

Hulley S, Furberg C, Barrett-Connor E, et al, for the HERS Research Group. Heart and estrogen/progestin replacement study follow-up (HERS II): Part 2. Non-cardiovascular outcomes during 6.8 years of hormone therapy. *JAMA.* 2002;288: 58-66

Ikenoue N, Wakatsuki A, Sagara Y. Small low-density lipoprotein particles in women with natural and surgically induced menopause. *Obstet. Gynecol.* 1999; 93: 566-70.

Jenner JL, Ordovas JM, Lamon-Fava.S, Schaefer MM., Wilson PVF, Castelli WP. Effects of age, sex and menopausal status on plasma lipoprotein (a) levels. The Framingham offspring study. *Circulation.* 1993; 87: 1135-41.

Jeske DJ, Dietschy JM. Regulation of rates of cholesterol synthesis in vivo in the liver and the carcass of the rat measured using [3H] water. *J. Lipid Res.* 1980;21: 364-76.

Jones GS, Hormonal changes in perimenopause. *The Menopause: Comprehensive Management.* New York: Masson Publishing. USA Inc 1980.

Kanazawa T, Osanai T, Zhang XS, et al. Protective effects of soy protein on the peroxidizability of lipoproteins in cerebrovascular diseases. *J.Nutr.*1995;125: 639S-46S

Kannel WB. Metabolic risk factors for coronary heart disease in women: prospective from the Framingham Study. *Am. Heart. J.* 1987;114: 413-19.

Kass, Annese B. Alternative therapies for menopause. *Clin. Obstet. Gynecol.* 2000; 93 (1): 162-83.

Kirk EA, Sutherland P, Wang SA, Chait A, LeBoeuf RC. Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. *J. Nut.r* 1998;28 :954-59.

Kuiper GGJM, Shughrue PJ, Merchenthaler I, Gustafsson J. The estrogen receptor beta sub type: a novel mediator of estrogen action in neuroendocrine systems front. *Neuroendocrinol.* 1998;19:253-86.

Lamarche B, Lemieux I, Despres JP. The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, pathophysiology and therapeutic aspects. *Diabetes Metab.* 1999;28: 229-34.

Lee IR, Dawson SA, Wetherall JD, Hahnel R. Sex hormone binding globulin secretion by hepatocarcinoma cells is increased by estrogens and androgens. *J. Clin. Endocrinol. Metab.* 1987;64: 825-31.

Lichtenstein A. Soy protein, isoflavones and cardiovascular disease risk. *J.Nutr.* 1998; 128: 1589-92

Lissin LW, Cooke JP. Phytoestrogens and cardiovascular health. *J. Am. Coll. Cardiol.* 2000;35(6):1403-10

Lobo RA. Benefits and risks of estrogen replacement therapy. *Am. J. Obstet. Gynecol.* 1995 ;173: 982-89

Lucas EA, Khalil DA, Daggy BP, Arjmandi BH. Ethanol-extracted soy protein isolate does not modulate serum cholesterol in Golden Syrian hamsters: a model of postmenopausal hypercholesterolemia. *J. Nutr.* 2001;131: 211-14.

Mackness MI, Abbott C, Arrol S, Durrington PN. The role of high-density lipoprotein and lipid-soluble antioxidant vitamins in inhibiting low-density lipoprotein oxidation. *Biochem. J.* 1993;294: 829-34.

Mckinlay SM, Brambilla PJ, Posner JG. The normal menopause transition. *Maturitas.* 1992;14: 103-15.

Messina M, Persky v, Setchell KDR, Barner s. Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutr. Cancer.* 1994;21: 113-31

Miyazaki A, Rahim AT, Ohta T, Morino Y, Horiuchi S. High density lipoprotein mediates selective reduction in cholesteryl esters from macrophage foam cells. *Biochim. Biophy.s Acta.* 1992;1126: 73-80.

Miller M, Cosgrove B, Havas S. Update on the role of triglycerides as a risk factor for coronary heart disease. *Curr. Atheroscler. Rep.* 2002;4(6): 414-48

Murkey AL, Wilcox G, Davis SR. Phytoestrogens. *J. Clin. Endocrinol. Metab.* 1998; 83: 297- 03.

Murphy HC, Burns SP, White JJ, Bell JD, Iles RA. Investigation of human low-density lipoprotein by 1H nuclear magnetic resonance spectroscopy: mobility of phosphatidylcholine and sphingomyelin headgroups characterizes the surface layer. *Biochemistry.* 2000;39: 9763-70.

Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's Biochemistry. 23rd edition.1993:250-55.

Nestel PJ, Yamashita T, Sasahara T, et al. Soy isoflavones improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. *Arterioscler. Thromb.Vas.c Bio.l* 1997;17: 3392-98.

Ng MK, Jessup W, Celermajer DS.Sex-related differences in the regulation of macrophage cholesterol metabolism. *Curr. Opin. Lipidol.* 2001;12(5): 505-10

Otto J, Ordovas JM, Smith D, van Dongen D, Nicolosi RJ, Schaefer EJ. Lovastatin inhibits diet induced atherosclerosis in F1B golden Syrian hamsters. *Atherosclerosis* 1995;114(1):19-28

Pasquali P, Gambineri A, Anconetani B, Vicennati V, Colitta, Caramelli E, casimirri F, Morselli-Labate AM. The natural history of the metabolic syndrome in young women with the polycystic ovary syndrome and the effect of long-term oestrogen-progestrone treatment. *Clin. Endo.* 1999; 50:517-27.

Pearson TC. The risk of thrombosis in essential thrombocythemia and polycythemia vera. *Semin. Oncol.* 2002;29 :16-21

Phillips JC, Wriggers W, Li Z, Jonas A, Schulten K. Predicting the structure of apolipoprotein A-I in reconstituted high-density lipoprotein disks. *Biophysical Journal.* 1997;73: 2337-46.

Potter SM, Baum J, Teng H. Soy protein and isoflavones. Their effects on blood lipids and bone density in postmenopausal women. *Am. J. Clin. Nutr.*1998; 68: 1375S-79S.

Quintao EC, Nakandakare E, Oliveira HC, Rocha JC, Garcia RC, de Melo NR. Oral estradiol-17 beta raises the level of plasma high-density lipoprotein in menopausal women by slowing down its clearance rate. *Acta. Endocrinol.*1991;125(6):657-61.

Raines EW, Ross R. Biology of atherosclerotic plaque formation: possible role of growth factors in lesion development and the potential impact of soy. *J. Nutr.* 1995; 125:624S-30S.

Ranby M. Studies on the kinetics of plasminogen activation by tissue plasminogen activator. *Biochim. Biophys. Acta.* 1982; 704: 461-69.

Reblin T, Meyer N, Labeur C, Henne-Bruns D, Beisiegel U. Extraction of lipoprotein(a), apoB, and apoE from fresh human arterial wall and atherosclerotic plaques. *Atherosclerosis.* 1995;113: 179-88.

Renaud S, Lanzmann-Petithory D. Coronary heart disease: dietary links and pathogenesis. *Public Health Nutr.* 2001;4: 459-74

Rosenberg L. Hormone Replacement Therapy: the need for reconsideration. *Am. J.Public Health.*1993;137: 54-63.

Ross R. Atherosclerosis is an inflammatory disease. *Am. Hear.t J* .1999;138: S419-20.

Rye KA, Clay MA, Barter PJ. Remodelling of high density lipoproteins by plasma factors. *Atherosclerosis.* 1999;145: 227-38.

Sakata N, Phillips TE, Dixon JL. Distribution, transport, and degradation of apolipoprotein B-100 in HepG2 cells. *J. Lipid. Res.* 2001;42: 1947-58

Sanada. M, Nakagawa H, Kodama I, Sakasita T, Ohama K. The effect of hormone replacement therapy on metabolism of lipoprotein remnants in postmenopausal women. *Maturitas.*2000; 34: 75-82.

Sandkemp M. Funke H, Kohler E, Asmann G. Lipoprotein(a) is an independent risk factor for myocardial infarction at young age.(1990) *Clin. Chem. 36: 20-23*

Sarrel PM. Ovarian hormones and the circulation. *Maturitas.* 1990; 12: 287-98.

Schaefer EJ. Lipoproteins, nutrition, and heart disease. *Am. J. Clin. Nutr.* 2002; 75(2): 191-12.

Schonherr E. Genistein selectively inhibits platelet-derived growth factor-stimulated versican biosynthesis in monkey arterial smooth muscle cells. *Arch. of Biochem. and Biophy.* 1997;339: 351-61.

Searcy RL, Bergquist LM. A new color reaction for the quantitation of serum cholesterol. *Clin. Chim. Acta.* 1960;5: 192-9.

Seman LJ, McNamera JR Schaefer EJ. Lipoprotein(a), homocysteine and remnant like particles : emerging risk factors. *Curr.Opin.Cardiol.* 1999;14: 186-191.

Sempos CT, Cleeman JI, Carroll MD, Johnson CL, Bachorik PS, Gordon DJ, Burt VL. Prevalence of high blood cholesterol among US adults: an update on the guidelines from the second report of The National Cholesterol Education Program (NCEP). 1993;269: 3009-14.

Senoz S, Direm B, Gulekli B, Gokmen,O.. Estrogen deprivation, rather than age, is responsible for the poor lipid profile and carbohydrate metabolism in women. *Maturitas.* 1996; 25: 107-14.

Setchell KDR. Absorption and metabolism of soy isoflavones from food to dietary supplements and adults to infants. *J. Nutr.* 2000;130: 654s- 655s.

Setchell. KDR, Nechemias-Zimmer L, Cai J, Heubi JE. Exposure of infants to phytoestrogens from soy infant formulas. *The Lancet.* 1997;350: 23-37.

Shefer S, Nguyen LB, Salen G, Ness GC, Chowdhary IR, Lerner S, Batta AK, Tint GS. Differing effects of cholesterol and taurocholate on steady state hepatic HMG-CoA reductase and cholesterol 7 alpha-hydroxylase activities and mRNA levels in the rat. *J. Lipid. Res.* 1992 ;33:1193-00.

Sohn E, Daggy BP, Arjmandi BH. Ovariectomized hamster: a potential model of postmenopausal hypercholesterolemia. *J. Nutr. Biochem.* 1999;10: 660-63.

Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med* . 1989;320: 915-24.

Subbaiah TR, Kessel B, Agarwal M, Rajan.R, Abplanalp W, Rymaszewski.Z. Antioxidant potential of specific estrogens on lipid peroxidation in postmenopausal women. *J. Clin. Endocrinol. Metab.* 1993; 77(17): 324-30.

Sullivan JM, Fowlkes LP, Estrogens, menopause and coronary heart disease. *Cardiology Clinics.*1996; 14: 105-16.

Sznajderman M, Oliver MF, Spontaneous premature menopause, ischaemic heart disease and serum lipids. *The Lancet.* 1963;1: 962-65.

Teede HJ. Hormone replacement therapy and the prevention of cardiovascular disease. *Hum. Reprod. Update.* 2002;8(3): 201-15

Terpstra AH, Holmes JC, Nicolosi RJ. The hypocholesterolemic effect of dietary soybean protein vs. casein in hamsters fed cholesterol-free or cholesterol-enriched semipurified diets. *J. Nutr.*1991;121:944-47

Tikkanen MJ, Adlercreutz H. Dietary soy-derived isoflavone phytoestrogens. Could they have a role in coronary heart disease prevention? *Biochem. Pharmacol.* 2000;60(1) :1-5

Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. The effect of soybean phytoestrogen intake on low-density lipoprotein oxidative resistance. *Proc.Natl.Acad.Sci.* 1998;95: 3106-10.

Tsang S.M.T, Barnes. M. E, Gersh. B.J, Hayes .S. N. Risks of coronary heart disease in women; Current understanding and evolving concepts. *Mayo Clinical Proceedings.* 1996;75(12): 0025-26.

Uesugi T, Toda T, Tsuji K, Ishida H. Comparative study on reduction of bone loss and lipid metabolism abnormality in ovariectomized rats by soy isoflavones, daidzin, genistin, and glycitin. *Biol. Pharm. Bull.* 2001; 24(4): 368-72.

Van Tol A, Van Gent T, Scheek LM. Lipoprotein structure and metabolism during progression and regression of atherosclerosis in pigs fed with fish oil-derived fatty acids. *Adv. Exp. Biol.* 1991;285: 417-21.

Verdeal K, Brown RB, Richardson T, Ryan DS. Affinity of phytoestrogens for estradiol-binding proteins and effect of coumestrol on growth of 7, 12-dimethylbenz(a) anthracene induced rat mammary tumors. *J. Natl. Cancer.Inst.* 1980; 64: 285-89.

Wade GN, Zucker I. Modulation of food intake and locomotion activity in female rats by diencephalic hormone implants. *J.Comp. Physiol. Psychol.* 1970;88: 183-93.

Wakatsuki A, Ikenoue N, Sagara Y. Effects of estrogen on susceptibility to oxidation of low-density and high density lipoprotein in postmenopausal women. *Maturitas.* 1998;28: 229-34.

Wakatsuki A, Sagara Y. Lipoprotein metabolism in postmenopausal and oopherectomized women. *Obstet Gynecol.* 1995;85: 523-28

Wakatsuki A, Okatani Y, Ikenoue N, Fukaya T. Different effects of oral conjugated equine estrogen and transdermal estrogen replacement therapy on size and oxidative susceptibility of low-density lipoprotein particles in postmenopausal women. *Circulation* 2002;106(14): 1771-76

Wallen WJ, Belanger MP, Wittnich C. Sex hormones and the selective estrogen receptor modulator tamoxifen modulate weekly body weights and food intakes in adolescent and adult rats. *J. Nutr.* 2001; 131(9): 2351-57.

Wang TTY, Sathyamoorthy N, Phang JK. Molecular effects of genistein on estrogen receptors mediated pathways. *Carcinogenesis.* 1996;17: 271-75.

Wasling C. Role of the cardioprotective diet in preventing coronary heart disease. *Br. J. Nurs.* 1999;8(18):1239-48.

Wilcox JN, Blumenthal BF. Thrombotic mechanisms in atherosclerosis:potential impact of soy proteins. *J. Nutr.* 1995;125: 631S-38S.

Williams JK, Clarkson TB. Dietary soy isoflavones inhibit in-vivo constrictor responses of coronary arteries to collagen-induced platelet activation. *Artery Disease.* 1998;9: 759-64.

World Health Organization. Research on the menopause in the 1990s. *WHO Tech. Rep. Ser.* 1996; No 866.

Writing group for the PEPI trial. Effects of estrogen/progestin regimens on the heart disease risk factors in postmenopausal women. *JAMA.* 1995;273: 2307-15.

Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA.* 2002;288: 321-33.

Wroblewski L, Cooke JP, Phytoestrogens and cardiovascular health. *Amer Coll of Cardio.* 2000;35: 1403-10.

Yamaguchi K, Honda H, Wakisaka C, Tohei A, Kogo H. Effects of phytoestrogens on acetylcholine- and isoprenaline-induced vasodilation in rat aorta. *Jpn. J. Pharmacol.* 2001; 87(1): 67-73.

Zhang X, Wang L, Zhang H, Guo D, Qiao Z, Qiao J. Estrogen inhibits lipopolysaccharide-induced tumor necrosis factor-alpha release from murine macrophages. *Methods Find. Exp. Clin. Pharmacol.* 2001;23(4)169-73

Zumoff B. Does postmenopausal estrogen administration increase the risk of breast cancer? Contributions of animal, biochemical and clinical investigative studies to a resolution of the controversy. *Proc.Soc.Exp.Biol.Med.*1998; 217: 30-37.

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