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THE EFFECT OF INCREASING LEVELS OF DIETARY SOY PROTEIN OR OF RICE BRAN OIL ON BLOOD LIPID LEVELS, BODY WEIGHT, AND ABDOMINAL FAT MASS IN OVARIECTOMIZED RETIRED BREEDER RATS

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LIST OF ABBREVIATIONS

ACAT	acetyl-CoA : cholesterol transferase
AHA	American Heart Association
AIN	American Institute of Nutrition
AMI	acute myocardial infarction
ANOVA	analysis of variance
apo	apolipoprotein
BHT	butylated hydroxytoluene
BMI	body mass index
C7S	7% soy protein treatment group
C14S	14% soy protein treatment group
°C	degrees Celsius
C	control group
cc	cubic centimeters
CAD	coronary artery disease
CE	voluntary running treatment group
CETP	cholesterol ester transfer protein
CHD	coronary heart disease
CHF	congestive heart failure
CVA	cerebrovascular accident
CVD	cardiovascular disease
CW	swimming treatment group
d	day
DART	Diet and Reinfarction Trial

DEXA	dual x-ray absorpimetry
dL	deciliter
DM	diabetes mellitus
EKG	electrocardiogram
FFQ	food frequency questionnaire
FSH	follicle stimulating hormone
g	gram, grams
GPO	glycerol phosphate oxidase
HDL	high-density lipoprotein
HMG-CoA	3-hydroxy-3-methyl glutaryl CoA
HRT	hormone replacement therapy
HTN	hypertension
ICD-9	International Classification of Diseases, Ninth Revision
IDL	intermediate density lipoprotein
IHD	ischemic heart disease
kg	kilogram
L	liter, liters
LDL	low density lipoprotein
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
LSU	Louisiana State University and Agricultural and Mechanical College
mg	milligram, milligrams
MH	Maren Hegsted
MI	myocardial infarction

µl	microliter, microliters
µmol	micromole, micromoles
ml	milliliter, milliliters
mmHg	millimeters mercury
mmol	millimole, millimoles
mRNA	messenger ribonucleic acid
NCEP	National Cholesterol Education Program
nm	nanometer, nanometers
OVX	ovariectomy, ovariectomized
RT	room temperature
SC	standard control
ShC	sham operated control group
TC	total cholesterol
TG	triglycerides
TIA	transient ischemic attack
TRF	tocotrienol rich-fraction
VLDL	very low density lipoprotein
WC	waist circumference

LIST OF DEFINITIONS

- ***Acute myocardial infarction***: sudden, severe death of the heart muscle due to ischemia; heart attack
- ***Aneurysm***: dilatation or "ballooning" of the wall of the heart or coronary vessels
- ***Angina pectoris***: acute, choking chest pain due to lack of oxygen in the heart which can be precipitated by excitement or exertion
- ***Apolipoprotein***: protein constituent of a lipoprotein
- ***Atherosclerosis***: lipid deposits in the intima of the vessels; distributed in large and medium-sized arteries; associated with fibrosis and calcification; may lead to heart attack, stroke, and angina pectoris
- ***Cardiovascular disease***: any disease of the heart and its surrounding vasculature
- ***Coronary heart disease***: a specific type of cardiovascular disease that refers to a lack of, or decrease in, blood flow to the coronary arteries which manifests itself through other conditions
- ***Coronary occlusion without MI***: interruption of blood flow to a portion of the heart without death of heart tissue
- ***Intermediate coronary syndrome***: heart pain more frequent and of longer duration than in post-myocardial infarction syndrome
- ***Ischemia***: lack of or decrease in blood flow resulting in lack of oxygen
- ***Lipid***: substance that is insoluble in water; includes fatty acids, fatty acid esters, and substances capable of forming esters

- ***Lipoprotein***: substance consisting of a triglyceride and cholesterol ester core surrounded by phospholipids and protein
- ***Myocardial infarction***: heart attack
- ***Old myocardial infarction***: healed myocardial infarction or past myocardial infarction diagnosed on an electrocardiogram but presenting no additional symptoms
- ***Post-myocardial infarction syndrome***: fever, inflammation, or pain within 6 weeks following a myocardial infarction

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ABSTRACT

Coronary heart disease is the leading cause of morbidity and mortality in women over 55 years of age. Risk factors associated with coronary heart disease are high blood lipid levels, overweight and obesity, family history, age, gender, diet, cigarette smoking, and hypertension. In women, menopause causes changes in hormone levels, which may raise blood lipid levels, leading to an increased risk of coronary heart disease. Intake of soy may improve blood lipid profiles; this may be important in decreasing risk of coronary heart disease in women. The objective of this study was to determine the effects of short-term feeding of soy protein or rice bran oil on total cholesterol, high-density lipoprotein, triglyceride levels, abdominal fat, and body weight in seventy-six ovariectomized retired breeder rats, as a model for postmenopausal women. Rats were fed increasing levels of soy protein (7%, 14%, or 21%) and rice bran oil; total cholesterol, high-density lipoprotein, and triglyceride levels were measured to gather data needed for this study. Results suggested that there were no significant differences among mean total cholesterol levels. High-density lipoprotein levels for the rice bran treatment group and the 7% soy treatment group were significantly higher than the sham-operated group; there were no other significant differences. There were no significant differences among triglyceride levels, mean abdominal fat weights, or final body weights. These results suggest that there was no effect of soy or rice bran oil on total cholesterol or triglycerides. The 7% soy diet and the rice bran oil diet were effective in increasing HDL levels, suggesting a beneficial effect on blood lipid levels.

CHAPTER 1

INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in men and women in the United States. Death rates are declining in men, but they are increasing in women. This is because more women are reaching age 50-55, which is the average age at which menopause occurs. Postmenopausal women are at higher risk for CHD than other women; this is due, in part, to increased lipid levels and decreased estrogen production. Thus, researchers continue to search for the best preventitive measure to offset increased risk of CHD in postmenopausal women.

Soy protein has become widely noticed for its lipid-lowering effects (1-6). The mechanism by which soy protein lowers blood lipid levels is unknown. Soy has also been suggested to be beneficial in providing estrogen to postmenopausal women in the form of phytoestrogens; these estrogens are believed to lower lipid levels (1-6), thereby decreasing CHD risk (3-6). Rice bran oil has also been suggested to have a lipid-lowering effect (7-9), but the mechanism by which this occurs is not known. Rice bran oil and its effects on blood lipids have not been well studied.

Objectives

The initial objective of this study was to determine the effects of increasing levels of soy protein or rice bran oil on total cholesterol, high-density lipoprotein, and triglyceride levels in ovariectomized retired breeder rats. The second objective of this study was to determine the effect of increasing levels of soy protein and rice bran oil on body weight and abdominal fat mass in ovariectomized retired breeder rats.

Hypotheses

It was hypothesized that soy protein would decrease total cholesterol, high-density lipoprotein, and triglyceride levels in ovariectomized rats. In addition, it was hypothesized that rice bran oil would also lower these levels.

Limitations

Rat models are limited in their usefulness in the study of heart disease or cholesterol status. The use of rats in this study was justified, however, by the fact that rats are very appropriate for use in bone density studies. The number of animals used in this study was small. Some of the measurements were variable and produced inconsistent results. The final limitation of this study was the addition of large amounts of fat, cholesterol, and cholic acid to the rat diets, which may have allowed small effects of these constituents to go undetected.

Assumptions

Rats were assumed to be an adequate model for menopause and measurement of lipid levels. It was assumed that ovariectomy in rats would produce the same hormonal effects as menopause in women. Lastly, it was assumed that the sample size of our study was adequate.

Justification

The importance of this study lies in the realization that coronary heart disease (CHD) is the leading cause of mortality in postmenopausal women (10). The average CHD death rate of postmenopausal women is approximately 487 deaths per 100,000 persons; this is 20 times higher than that of breast cancer (11). Decreased estrogen levels have been shown to increase serum cholesterol levels in women (12, 13). Therefore, these two factors work together to increase CHD risk in postmenopausal women; the

increase in risk often due to an increase in serum cholesterol levels (14). Another factor considered to contribute to the increase the risk of CHD is decreased estrogen levels (12).

Prevention of increased CHD risk has traditionally been implemented by the use of hormone replacement therapy (HRT), in which women to take exogenous estrogen to increase their estrogen levels; thereby decreasing serum cholesterol levels (13). There are, however, risks associated with HRT. A 40% increase in breast cancer development has been reported in women using HRT (15). Further, a threefold to fourfold increase in the risk of endometrial hyperplasia, a premalignant condition that may predict endometrial carcinoma, has been described in women taking estrogen (15). This risk can diminish greatly with the addition of the hormone progestin to the HRT treatment (15). The risk of thromboembolic events (pulmonary embolism and deep venous thrombosis) increases threefold in women using HRT (15). Hormone replacement therapy may also increase risk of cholelithiasis and hypertriglyceridemia (15). Many women are turning to alternate therapies (with or without HRT use) to help reduce the risk of CHD

Diet is one of the therapies used to assist postmenopausal women in lowering their risk of CHD (13). Soy is emerging as a beneficial food. It has been shown to decrease total cholesterol levels in humans (1-6, 31, 41-45). Further, phytoestrogens found in soy products are believed to help replenish estrogen levels during menopause (3). Increased estrogen levels, in turn, decrease serum cholesterol levels and decrease CHD risk (3,17).

It is important that the effect of soy be studied thoroughly to determine if it is cardioprotective. The purpose of this research was to determine the effects of ovariectomy on body weight, abdominal fat weight, and food intake; and to determine the effects of increasing concentrations of soy protein or rice bran oil total cholesterol, high-

density lipoprotein, and triglyceride levels in ovariectomized retired Sprague-Dawley breeder rats as a model of postmenopausal women.

CHAPTER 2

REVIEW OF LITERATURE

Exogenous (Dietary) and Endogenous Lipids

Introduction

There are some important terms that apply to the discussion of exogenous and endogenous lipids. Lipids (fats) are a heterogeneous group of substances that are insoluble in water and include fatty acids, fatty acid esters, and substances capable of forming esters (18). Lipoproteins have a hydrophobic core of triglycerides and cholesterol esters surrounded by phospholipids and protein. Lipoproteins are graded in size and lipid content, and their density is inversely proportionate to their lipid content: the more lipid, the less dense, and vice versa (19). Apoproteins are the protein constituents of lipoproteins. As discussed in Endogenous Lipids, the major apoproteins are apo E, apo C, and apo B (18).

Exogenous Lipids

What is dietary fat?

In the typical Western diet, triglycerides, cholesterol esters, and phospholipids (primarily lecithin), comprise the dietary fat component of the meal (19). The term fat commonly refers to triglycerides, since they comprise 95% of total dietary fat. Triglycerides consist of one glycerol molecule and three fatty acid chains (20). Cholesterol esters and phospholipids comprise the other 5% of dietary fat (18). Cholesterol esters consist of free cholesterol and one molecule of fatty acid. Phospholipids also contain fatty acid chains within their molecules (20). All of these fats are transported from the intestine to the liver via an exogenous lipid transport system.

Digestion of Dietary Fat

Fat digestion begins in the stomach, where the fat components of a meal are partially broken down by the enzyme lingual lipase (19). The majority of fat digestion, however, occurs in the small intestine (20). The first step in fat digestion is the breakdown of fat globules into smaller sized particles so digestive enzymes can act on the globule surfaces (20). This process is called fat emulsification, and it occurs with the assistance of bile, a secretion of the liver. Bile does not contain digestive enzymes, but it does contain bile salts (20). The major function of bile and bile salts is to prepare the fat globules to be broken down by agitation in the small intestine (20). The polar areas of the bile salt molecule are highly water-soluble, while the remaining portions are highly fat-soluble (18). The fat-soluble portions of the bile salts dissolve in the fat globule, while the polar portions project outward and remain soluble in surrounding fluids (20). This effect greatly decreases the surface tension of the fat globule. When the surface tension of a fat globule is low, the globule can be broken up into many particles via agitation (20). This emulsification process increases the total surface area of the fats by 1000-fold, making the particles more readily available for absorption (18).

As the emulsification process occurs, the digestive enzymes cholesterol ester hydrolase, phospholipase A_2, and pancreatic lipase split cholesterol esters, phospholipids, and triglycerides into cholesterol, free fatty acids, and fatty acids and 2-monoglycerides respectively (20). Because fatty acids and 2-monoglycerides are formed so rapidly, they tend to block further globule digestion by crowding the fat globules (18). Crowding is rarely a problem in digestion, because bile salts remove fatty acids and 2-monoglycerides from the vicinity of the fat globules as fast as these products are formed. Removal of fatty acids and 2-monoglycerides occurs through the formation of micelles, which are

molecules composed of 20 to 40 molecules of bile salt (20). Since bile salts contain sterol nuclei, which are highly fat-soluble, and polar regions, which are highly water-soluble, the nuclei can aggregate and form a small fat globule in the center of the micelle (18). The fatty acids and 2-monoglycerides, along with cholesterol, become dissolved in the central fatty portion of the micelle, which immediately reduces the amount of fatty acids and 2-monoglycerides in the vicinity of the fat globules that will be digested (20).

Absorption of Dietary Fat

In addition to removing the fatty acids, 2-monoglycerides, and cholesterol from digestive fluids, the micelles also carry these products to the brush border of the intestine for absorption (20). At the brush border of the intestinal cells (enterocytes), the lipid center of the micelle diffuses into the enterocyte (19). The bile salts are then absorbed through the ileal segment of the small intestine and returned to the liver via the portal vein to be resecreted in the bile (19).

Once incorporated into the enterocyte, the fatty acids and 2-monoglycerides, along with cholesterol, are re-formed into triglycerides and cholesterol esters (20). These resynthesized lipids are collected in the endoplasmic reticulum of the enterocyte as large fat particles (19). While in the endoplasmic reticulum, the fat particles are coated with a protein layer, which stabilizes the particles upon their entrance in circulation (18). Fat particles are then pinched off from the cells and fuse with the Golgi apparatus, where they are coated with carbohydrate (19). The complete particles, called chylomicrons, are then released from the cell membrane via exocytosis, and dumped into the lymphatic circulation (20). Resynthesized triglycerides are then deposited into fat cells by the chylomicrons. Chylomicrons are cleared from lymphatic circulation by lipoprotein lipase; this enzyme is located on the surface of the inner layer (endothelium) of the

capillaries (18). Lipoprotein lipase catalyzes the breakdown of triglycerides in chylomicrons into free fatty acids and glycerol, which enter the adipose cells and are reesterified (18).

Chylomicrons depleted of their triglycerides remain in the circulation as cholesterol-rich molecules called chylomicron remnants (18). These remnants are carried to the liver, where they bind to receptors on the hepatic cells and are internalized by endocytosis (20).

Endogenous Lipids

Endogenous Lipid Transport

The endogenous lipid synthesis and transport system forms and transports triglycerides and cholesterol throughout the body (20). Endogenous lipid synthesis and transport includes four lipoproteins: very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (18).

The first lipoprotein synthesized in the endogenous system is VLDL. The apoproteins associated with VLDL are apo B-100, apo C, and apo E (21). Excess fatty acids and carbohydrates in the liver are formed into triglycerides, which are incorporated into VLDL via apo B-100 (18). Very low-density lipoprotein particles are responsible for transporting triglycerides from the liver to peripheral tissues for use (21). When VLDLs enter the capillaries, they come into contact with lipoprotein lipase (activated by apo C), which hydrolyzes triglycerides into their fatty acid components (21). These components are then used by the tissues for energy (18).

After most of the triglycerides are removed from VLDL, a residual particle, called a VLDL remnant (also known as IDL), remains in the circulation (21). Some of the IDL

particles are removed from circulation through the interaction of apo E with the LDL receptors on the surface of the liver (18). Also, the triglycerides in IDL can be hydrolyzed further by hepatic lipase to form LDL (22).

Low-density lipoprotein is responsible for transport of endogenous cholesterol to the tissues (18). The core of the LDL molecule consists primarily of cholesterol. The surface consists primarily of apo B (21). The apo B on the surface of the LDL molecule is recognized by the LDL receptors on the tissue cells. This recognition allows the LDL particle to enter the tissue cell via receptor-mediated endocytosis (20). Once in the cell, the LDL particle enters a lysosome, which is responsible for the release of free cholesterol into the cell (18). This free cholesterol is used in critical cell processes, such as the formation and maintenance of cell membranes (21).

Cholesterol, once integrated into cells, inhibits intracellular cholesterol synthesis in three ways. The first way is by inhibiting HMG CoA-reductase, which is the rate-limiting enzyme in cholesterol biosynthesis (18). By inhibiting this enzyme, the synthesis of cholesterol slows greatly. Cholesterol in the cell is also formed into cholesterol esters by the enzyme acetyl-CoA: cholesterol acyltransferase (ACAT), the activity of which is stimulated on the entrance of a satisfactory amount of cholesterol into the cell (20). Lastly, synthesis of new LDL receptors is inhibited until further cholesterol is required by the cell (21).

In the steady state, cholesterol both enters and leaves the cell (18). Free cholesterol is transported across the cell membrane and into the HDL particle. The HDL particle is synthesized in the liver and intestine, and consists mostly of phospholipids and apoprotein A-1, with very little cholesterol (22). After free cholesterol reaches HDL, it is esterified by lecithin-cholesterol acyl transferase (LCAT) (18). As a result, the liver

through the interaction of HDL may take up the cholesterol ester in the HDL particle via HDL receptors on the hepatocytes (22). Approximately 50% of cholesterol esters in HDL are delivered to the liver through HDL receptors (22). The remaining 50% are transferred from HDL to VLDL, IDL, or LDL. The LDL receptors internalize the esters and incorporate them into the hepatic circulation (22). This process is known as reverse cholesterol transport. Since HDL is responsible for removing excess cholesterol from the cells, it is considered cardioprotective at increased serum levels (22).

Cardiovascular Disease

What is Cardiovascular Disease?

Cardiovascular disease (CVD) is a generic term referring to any disease of the heart and its surrounding vasculature. Coronary heart disease (CHD) is included in the blanket term CVD. The term CHD is often used interchangeably with coronary artery disease and ischemic heart disease. Additional conditions included in the term CVD include: intermittent claudication (which is characterized by lack of sufficient blood flow and muscle cramping in the extremities), congestive heart failure, cerebrovascular accident or stroke, or transient ischemic attack (23). For this report, the focus is on CHD as a particular type of CVD; and CHD is used.

The International Classification of Diseases, Ninth Revision (ICD-9) defines CHD as a manifestation of any of the following conditions: acute myocardial infarction (AMI) - sudden, severe death of the heart muscle due to decreased coronary blood flow (ischemia); old myocardial infarction (old MI) - healed MI or past MI diagnosed on an electrocardiogram (EKG) but presenting no additional symptoms; angina pectoris - acute, choking thoracic pain due to lack of oxygen in the heart muscle and precipitated by excitement or exertion; atherosclerosis - plaque deposits in vessels; and aneurysm -

dilatation of the wall of the heart or coronary vessel. Other acute/subacute forms of CHD are also considered as CHD: post-MI syndrome - fever, pain, or inflammation within 6 weeks following an MI; intermediate coronary syndrome - heart pain more frequent and longer in duration than in post-MI syndrome which is triggered by lesser stimuli; coronary occlusion without MI - interruption of blood flow to a portion of the heart without death of heart tissue; and other chronic CHD not otherwise specified (24).

Who gets Coronary Heart Disease?

Coronary heart disease is the leading cause of death for both men and women of all races/ethnicities in the United States (11). An estimated 59.7 million Americans have at least one type of CHD (25). Coronary heart death rates were highest among white men (440 deaths per 100,000 individuals) and second highest among black men (421 deaths per 100,000 individuals) in 1998 (11). In 1998, black women had the highest death rates among women from CHD (302 deaths per 100,000 individuals), followed by white women (264 deaths per 100,000 individuals) in 1998 (11). In both genders, Asian/Pacific Islander men and women had the lowest CHD death rates in 1998 (247 and 148 deaths per 100,000 individuals, respectively) (11).

In both genders and races, CHD death rates increased as age increased. In persons aged 85 years or greater, the death rate (3,744 deaths per 100, 000 individuals) was three times higher than that of those aged 75-84 years (1,252 deaths per 100, 000 individuals), seven times higher than the rate of those aged 65-74 years (487 deaths per 100, 000 individuals), and 21 times higher than among persons aged 55-64 years (181 deaths per 100,000 individuals) (11). Men are at increased risk for CHD at the age of 45 years or older. Women are considered at increased risk at the age of 55 years or older (26). The increase in risk with age for women is much more variable than for men, due

primarily to the variability in the age of onset of menopause (12), which is discussed in Menopause.

By the year 2015, 45% of the women in the United States will be age 55 or older; thus, the numbers of women at risk for CHD will increase greatly (27). Further, there is increased risk for CHD at the age in which premature menopause occurs if estrogen replacement therapy is not used (26). The fatality rate for women from MI (due to atherosclerotic CHD) is 58.5 per 100,000 (11). Coronary heart disease claims more women's lives than cancer and car accidents combined (12).

Risk Factors Associated with Coronary Heart Disease

Types of Risk Factors

Risk factors associated with CHD can be divided into two groups: noncontrollable and controllable (25). Noncontrollable risk factors are: a family history of premature CHD (defined as MI or sudden death before 45 years of age in the father or other male first-degree relative, or before 55 years of age in the mother or other female first degree relative); age; race; and genetic influences. Risk factors that can be controlled, at least to a degree, are: elevated total cholesterol (TC) levels, elevated LDL levels, low HDL levels, obesity, hypertension, homocysteinemia, and diabetes mellitus (DM) (26). Hyperlipidemia is considered the primary controllable risk factor that contributes to increased CHD risk (27).

Risk factors that are completely within the control of an individual are: a diet high in saturated fat, cigarette smoking, and physical inactivity (27). Controllable risk factors are the focus of reducing risk of CHD, because these risk factors can be reduced or alleviated (28).

Hyperlipidemia as a Risk Factor for Coronary Heart Disease

In all individuals, the optimal total cholesterol level is <200 mg/dl (29). As the serum TC level increases, risk of CHD increases (11, 28, 29). Emphasis is placed, however, on LDL levels as a better indicator of CHD risk than is TC, as it is the most atherogenic lipoprotein (28).

What is cholesterol?

Cholesterol is a sterol, and it is an essential compound in human metabolism and cell function. The basic structure of cholesterol consists of a sterol nucleus, with an eight-carbon side chain (19). One of the eight carbons in the cholesterol side chain is attached to the sterol nucleus at carbon number 17 (19). The remaining carbons on the side chain of cholesterol can undergo reactions with other molecules to form other products, including sex hormones and cell membranes, essential to the human body (30).

Carriers of Cholesterol/Main Components of Carriers

Lipids associate with specific proteins to form plasma lipoproteins (30). Plasma lipoproteins are complexes that carry water insoluble lipids between various organs via the blood (19). Plasma lipoproteins differ in their ratios of lipid to protein. They also differ in their concentrations of various lipids; thus, the densities of lipoproteins differ. Density is the physical characteristic used to differentiate and classify the various lipoproteins (19). There are four major classes of human plasma lipoproteins, two of which carry the majority of endogenous cholesterol: LDL and HDL (30). These lipoproteins are composed of cholesterol, phospholipids, apolipoproteins, and triglyceride (19).

Phospholipids are lipids that contain phosphate and one or more fatty acid residue. The protein components of lipoproteins are apolipoproteins. They stabilize the

lipoproteins as they circulate in the blood. More importantly, apolipoproteins allow the lipoproteins to be recognized by receptors on cell surfaces (22). The predominant apolipoprotein in LDL is apo B-100, and the primary apolipoprotein in HDL is apo E (19).

Low-density lipoprotein is the major carrier of cholesterol, binding approximately 60% of serum total cholesterol. Low-density lipoprotein cholesterol is composed of 47% cholesterol, 23% phospholipids, 21% apolipoprotein B-100, and 9% triglyceride (19). High-density lipoprotein cholesterol is composed of 50% apolipoproteins, 28% phospholipids, 19% cholesterol, and 3% triglyceride (19).

Actions and Purposes of Cholesterol

Cholesterol has a critical importance in cell structure, *i.e.*, in the formation of the phospholipid bilayer of cell membranes. The physical integrity of all human cell membranes is, in part, contingent upon the presence and action of cholesterol. Cholesterol is transported to the tissue cells via LDL. The LDL receptors on the cells are specific to apo B-100, and upon recognition, the cell allows the receptor and the LDL particle to enter the cell. Cholesterol is then released into the cell and used in membrane construction (19).

Cholesterol enhances the mechanical stability of the membrane and regulates its fluidity. The orientation of the cholesterol in the phospholipid bilayer of the membrane is such that the cholesterol can associate with both the hydrophobic and hydrophilic regions of the bilayer. Thus, cholesterol can stabilize and provide flexibility to the hydrophilic region. As a result, cell membrane permeability is regulated. Cholesterol exercises a degree of control over what passes into or out of the cell (19). Cholesterol has a

markedly slow turnover rate in nonhepatic environments, which greatly enhances the ability of cholesterol in its formation of the structural elements of all cells (30).

The major function of cholesterol is to form cholic acid in the liver. Cholic acid is conjugated to amino acids, sodium, potassium, and calcium to form bile salts, which, in turn, aid in the digestion and absorption of lipids (30). Additionally, other functions include use of cholesterol by the adrenal glands to form adrenocortical hormones, by the testes to produce the male sex hormone testosterone, and by the ovaries to produce the female sex hormones progesterone and estrogen (30). Cholesterol is also present in the corneum of the skin. Acting with other lipids, cholesterol serves to make the surface of the skin highly resistant to water soluble substances (30). Cholesterol is chemically inert to acids; therefore, it allows the skin to resist the actions of many chemical agents and solvents that would otherwise penetrate the surface of the skin. Cholesterol also works with other lipids to prevent the evaporation of water from the skin (30).

Desirable Levels of Low-Density Lipoprotein

Low-density lipoprotein levels from 130-159 mg/dL are borderline high. Levels > 160 mg/dL are high (26); a high serum LDL concentration is the foremost cause of atherosclerosis and, hence, of CHD (31-33). The optimal LDL level is <130 mg/dl (29). In individuals with CHD, the optimal LDL level is < 100 mg/dL (29).

As LDL levels increase, the risk of CHD greatly increases (26, 29, 31). In the absence of all other risk factors, individuals with genetic backgrounds that cause elevated serum LDL concentrations show the highest risk for CHD (34). A decrease in CHD rates in individuals undergoing LDL-lowering therapies has also been shown (33). Evidence suggests that in the general population, elevated serum LDL concentrations occur largely

from a diet high in saturated fat and cholesterol; both of which suppress the uptake of LDL into the cells by LDL receptors (explained in detail below) (35).

Actions and Purposes of Low-Density Lipoprotein

Cholesterol introduced into cells lowers the concentration of messenger RNA in the cell, which thereby suppresses the synthesis of LDL receptors. This prevents further entry of LDL into the cell. If an excess of endogenous cholesterol exists, LDL receptors will not facilitate the absorption of the excess particles. Thus, excess LDL will remain in the bloodstream, which results in elevated serum LDL and total cholesterol levels. (19).

Desirable High Denisty Lipoprotein Levels

Low serum HDL levels are also considered a major risk factor for CHD (28). High-density lipoprotein is thought to be protective against atherosclerotic development, and therefore reduces CHD risk (35). Low HDL levels are an indicator of excess LDL remnants, which are primarily responsible for atherogenesis. Additionally, low HDL levels are a marker for other risk factors, including physical inactivity and insulin-resistance (28).

An HDL level of <35 mg/dL is low and is associated with increased risk of CHD (34). Conversely, a HDL level of >65 mg/dL is high, and is cardioprotective (33). Low HDL levels are associated with high CHD risk, even if LDL levels are normal or low (33, 34). In addition, high HDL levels are associated with decreased CHD risk, even when LDL levels are elevated (33). However, LDL is still believed to be the best indicator of CHD risk because it is the most atherogenic lipoprotein (32-34).

In the United States, women over 20 years of age have higher mean HDL levels than men; these levels are consistent across all age groups for men and women (34). Black men and women have higher mean HDL levels than white men and women;

however, white men are at highest risk for CHD due to their higher LDL levels (32). In the general adult population of the United States, approximately 15% of men and 5% of women have HDL concentrations <35 mg/dL (33). HDL levels decrease as age increases (34). Therefore, measures to prevent CHD risk from increasing, secondary to the decreased levels, must be taken (34). In postmenopausal women, HRT is one measure that is effective in increasing HDL levels (29).

Actions and Purposes of High Density Lipoprotein

In order for cholesterol transport to occur, the HDL particle must be able to bind to receptors. These receptors are able to bind HDL and LDL. The LDL receptor is called the apo B/E receptor, which indicates that it is specific to both apo B-100 and apo E. High-density lipoprotein can bind to the receptors via the use of apo E on its surface (19). The HDL particle must also stimulate lecithin: cholesterol acyltransferase (LCAT), the enzyme that promotes the transfer of cholesterol from nonhepatic cells to hepatic cells. This enzyme takes up free cholesterol and esterifies it, which promotes the transfer of the esterified cholesterol out of the tissue cell and back to the liver for excretion in the bile (19).

How Humans Make Cholesterol

Biosynthesis of cholesterol in the human body occurs primarily in the liver, although all cells form cholesterol in very small amounts (19). After production and transport from the liver to tissues, most cholesterol is esterified at the 3-hydroxyl group by long chain fatty acids and fatty acyl CoA. The liver contains cholesterol acyltransferase, an enzyme which allows cholesterol esters to be formed from cholesterol and fatty acyl CoA, resulting in the cholesterol ester and a CoA byproduct (35).

Soy Protein and Coronary Heart Disease

Introduction

The beneficial effect of soy protein in comparison to animal protein in the treatment of hyperlipidemia has been well established in studies with either laboratory animals (36-39), or humans (1-6). There are many proposed mechanisms for this effect, and there are also many proposed active components thought to contribute to this effect (36-38). The active component of soy, which decreases plasma lipid levels, has not yet been determined (39). Possible constituents include: amino acids and peptides, saponins, phytic acid, trypsin inhibitors, fiber, and isoflavones (39). It is likely that the lipid lowering effect of soy protein on lipids is actually the result of many components acting synergistically (39).

Animal Studies

Soy protein concentrate and isolated soy protein in comparison to casein, and the effects of these proteins on blood cholesterol and thyroid hormones were studied in hamsters (6). Twenty-nine male hamsters were fed diets containing 25g/100g of isolated soy protein, soy protein concentrate, or casein for 35-days (6). Serum cholesterol levels were lower in the soy protein concentrate or isolated soy protein groups than in the casein group (6.62 mmol/L and 7.03 mmol/L vs. 8.28 mmol/L, respectively) (6). However, thyroid hormone levels were highest in the isolated soy protein group when compared to the soy protein concentrate or the casein groups (74.5 mmol/L vs. 55.1 mmol/L and 60.4 mmol/L, respectively) (6). Results support a hypocholesterolemic effect of soy protein, while disproving the theory that thyroid hormone concentrations are responsible for the hypocholesterolemic effect (6).

In a study performed using hamsters and rats, effects on cholesterol and thyroid hormones of extracted soy protein, nonextracted soy protein, and casein were compared (37). In the first part of the study, 24 male Sprague-Daley rats were fed experimental diets; the only component in the diets that differed from the controls was the protein source: extracted isolated soy protein versus no extracted soy protein (37). The two control diets used were casein diets: one diet contained casein, the other contained casein with an ethanol-acetone extract (37). Triglyceride, TC, and HDL cholesterol were measured enzymatically; thyroid hormone concentrations were measured using solid-phase kits (37). Serum TC levels were significantly lower in both the extracted soy protein and nonextracted soy protein fed groups, as compared to the casein fed group (1.81 mmol/L and 2.06 mmol/L vs. 2.45 mmol/L, respectively) (37). High-density lipoprotein levels were lowest in the extracted soy protein group when compared to the nonextracted soy and casein groups (1.46 mmol/L vs. 1.7 mmol/L and 2.1 mmol/L, respectively) (37). Thyroid hormone concentrations were highest in the extracted soy protein group. In the second part of the study, 50 hamsters were fed the same diets for an 8-week period (37). Serum total cholesterol levels were significantly lower in nonextracted soy protein group than in the casein group (3.44 mmol/L vs. 4.61 mmol/L, respectively) (37). Thyroid hormone concentrations were increased in both of the soy groups. The results of this study imply that soy protein is hypocholesterolemic in comparison to casein, although the mechanism of this action is unknown (37).

Another study compared the effects of casein and soy protein on plasma lipids in gerbils. Male gerbils were fed diets for 4 weeks; these diets contained either 18% casein or soy isolate as the protein source (38). The dietary fat sources were lard (16%) and safflower oil (1%); the cholesterol content of the diet was 0.1% (38). Blood was

collected from each gerbil and analyzed to determine lipid levels. Plasma TC levels were found to be lower in the soy fed gerbils when compared to the casein fed gerbils (159 mg/dL vs. 190 mg/dL, respectively) (38). Absolute HDL concentrations were unaffected by the protein source, but LDL concentrations were lower with the soy protein source (76 mg/dL vs. 45 mg/dL, respectively) (38). Thus, the ratio of LDL to HDL was lower in the soy fed gerbils compared to the casein fed gerbils (0.42 vs. 0.70, respectively) (38). This study suggests that gerbils are good models for the study of the effects of diet on cholesterol parameters.

Tovar-Palacio and co-workers (36) performed a study to determine the effects of soy protein on lipid metabolism in gerbils in 1998. In this study, gerbils were assigned to one of five different groups (n=8/group) and fed experimental diets for 4 weeks. Diets contained either casein or soy protein isolate with 2.1, 3.6, or 6.2 mg isoflavones per gram of protein (36). All gerbils fed soy protein had reduced serum total and LDL cholesterol concentrations compared to those fed casein protein (36). Total cholesterol levels did not vary significantly among isoflavone concentrations, but were significantly lower in the gerbils consuming the 6.2 mg isoflavone diet in comparison to the casein fed group (2.06 mmol/L vs. 3.44 mmol/L, respectively) (36). Further, LDL concentrations did not differ significantly among the soy fed groups, but were significantly lower in the high isoflavone fed group than the casein group (2.16 mmol/L vs. 0.74 mmol/L, respectively) (36). This study suggests that varying levels of soy isoflavones lower total cholesterol and LDL levels, however, there were no significant differences among groups. This study also suggests that isoflavones in soy protein may be responsible for its hypocholesterolemic effect (36).

The cholesterol lowering effect of soy protein has also been studied in rabbits. One study examined the effects of soy protein and casein on cholesterol levels in 66 male rabbits. Baseline cholesterol measurements were collected, and the rabbits were separated into 8 groups depending on their baseline cholesterol measurements (39). There were 8 diets used in the study: 1) low cholesterol soy protein diet (0.09g cholesterol/kg diet and 400g cholesterol/kg diet); 2) low cholesterol casein diet (0.1 g cholesterol/kg diet and 348 g casein/kg diet); 3) low cholesterol casein plus F (formaldehyde treated)-casein diet (0.1 g cholesterol/kg diet, 174 g casein/kg diet, and 192 g F-casein/kg diet); 4) low cholesterol F-casein diet (0.1g cholesterol/kg diet, and 384 g F-casein/kg diet); 5) high cholesterol soy protein diet (1.06 g cholesterol/kg diet and 400 g soy protein/kg diet); 6) high cholesterol casein diet (1.08 g cholesterol/kg diet and 348 g casein/kg diet); 7) high cholesterol casein plus F-casein diet (1.09 g cholesterol/kg diet, 174 g casein/kg diet, and 192 g F-casein/kg diet); and 8) high cholesterol F-casein diet (1.07 g cholesterol/kg diet and 384 g F-casein/kg diet) (39). Serum cholesterol levels were measured, along with fecal bile acid secretion (39). Results show that F-casein fed rats gained the least weight, suggesting that they did not like the diet, and that these weights differed significantly from those of all other groups. Cholesterol levels were significantly different among casein fed and soy fed groups; the group fed the high cholesterol casein diet had the highest serum cholesterol levels (14.0 mmol/L) (39). The group fed the low cholesterol soy diet had the lowest serum cholesterol levels (2.4 mmol/L) (39). Bile acid secretion (an indicator of cholesterol metabolism) was highest in the low cholesterol soy fed group (120.6μmol/d), while excretion was lowest in the low cholesterol casein plus F-casein fed group (58.1 μmol/d). This implies that bile acid secretion slows upon administration of a casein diet, which

causes excess cholesterol in the blood. This study suggested that soy protein has a profound effect on cholesterol levels by increasing bile acid secretion.

In addition to rodents and rabbits, the effects of soy protein have also been studied in monkeys. A study by Wilson and co-workers in 1998 (31) addressed the addition of soy protein to the NCEP Step 1 cholesterol-lowering diet. In this study, 20 female cynomolgus monkeys were fed three types of diets: 1) the "average American diet" (36% fat, 15% saturated fat, 15% monounsaturated fat, and 6% polyunsaturated fat); 2) Step 1 diet (30% fat, 9% saturated fat, 14% monounsaturated fat, and 7% polyunsaturated fat); and 3) a modified Step 1 diet, which contained soy protein as the protein source and guar gum as the added fiber source (31). The modified Step 1 diet had the most pronounced cholesterol lowering effect (31). Total cholesterol levels were lowest in the modified diet fed group than in the Step 1 diet fed group, followed by the average American diet fed group (3.82 mmol/L, 5 mmol/L, and 6.16 mmol/L, respectively) (31). Low-density lipoprotein levels were also lowest in the modified diet fed group when compared to the Step 1 diet fed group and the average American diet fed group (1.98 mmol/L, 3.29 mmol/L, and 4.35 mmol/L, respectively) (31). The main finding was that a modified Step 1 diet, with the addition of soy protein, produces a significant decrease in TC and LDL levels in these animals (31).

In all of the species discussed above, soy protein has been shown to lower cholesterol levels. This effect has also been seen in nearly all human studies.

Human Studies

The effects of casein and varying levels of soy isoflavones on plasma lipid concentrations were examined in 156 men and women (1). Participants were assigned randomly to 1 of 5 diet groups: 1) 25 g casein control; 2) 25 g soy protein containing 3

mg isoflavones; 3) 25 g soy protein containing 27 mg isoflavones; 4) 25 g soy protein containing 37 mg isoflavones; or 5) 25 g soy protein containing 62 mg isoflavones (1). The subjects consumed these diets for 9 weeks. Blood samples were collected at baseline and after 9-weeks. Compared to the casein diet, the soy protein diet containing 62 mg isoflavones lowered TC and LDL levels by 4% and 6%, respectively (1). In the group that consumed the soy diet containing 37 mg isoflavones, both TC and LDL were decreased by 8% (1). There were no changes in TG levels or HDL levels in any of the diets. The soy diets containing 27 mg and 3 mg isoflavones did not significantly alter TC or LDL. The results of this study suggest that naturally occurring isoflavones in soy protein reduce TC and LDL levels without affecting TG and HDL levels (1).

The effects of soy protein and fiber added to a cholesterol-lowering diet were examined in another crossover study of 31 hyperlipidemic men and normolipidemic postmenopausal women. They consumed a test diet or a control diet for 1 month each, with a 2-week washout between diets. (2). The diets were designed according to NCEP Step 2 dietary principles (<7% of total calories from saturated fat and <200 mg cholesterol per day), but cholesterol in the diets was further reduced to <80 mg per day (2). In the test diet, 93% of the protein was replaced with vegetable protein, including 33 g of soy protein. Fasting blood samples were obtained at the beginning and end of each phase (2). Compared with the control diet, the test diet decreased total cholesterol by 6.2%; LDL by 6.7%; and the ratio of LDL to HDL by 6.3% (2). The authors concluded that vegetable protein (mainly soy) and soluble fiber significantly improve the lipid-lowering effect of a low total fat and low saturated fat diet (2).

The above findings are not unusual, but in one study, isoflavone supplementation did not alter serum lipid concentrations. In that study, 46 men and 14 postmenopausal

women were given either a tablet containing 55 mg of isoflavonoids or a placebo with the evening meal for 8 weeks (47). Food diaries monitored usual food intake of the participants; compliance was established through tablet counts and urine tests (47). The results showed no significant differences in any plasma lipid levels when the isoflavone supplement and the placebo were compared (47). The authors concluded that their results did not support the hypothesis that isoflavones lower plasma lipid levels.

Another study looked at the effects of varying levels of soy for 3 and 6 weeks on 81 hypercholesterolemic men. The men were divided randomly into 5 groups; each group received 50 g of protein per day, which included isolated soy protein and casein, in the following proportions: 50:0, 40:10, 30:20, 20:30, and 0:50, respectively (3). Blood samples were collected and analyzed at baseline and at weeks 3 and 6 of the study. Replacement of 20 g casein with 20 g soy protein (20:30 group) decreased total cholesterol levels significantly by 1.8% compared to baseline (3). Decreases in total cholesterol levels were seen with all groups that were fed soy protein. Adjusted mean changes of -0.163, -0.095, and –0.182 mmol/L were found in groups that received 30, 40, and 50 grams of soy protein, respectively (3). This study suggests that consuming as little as 20 g of soy protein per day for 6 weeks can reduce TC concentrations in hypercholesterolemic individuals.

Effects of long-term soy intake on the plasma lipids of hypercholesterolemic, postmenopausal women were examined in another study. Sixty-six women were assigned to one of three groups: 1) 56 mg isoflavones/40 g soy protein; 2) 90 mg isoflavones/40 g soy protein; and 3) casein and non-fat dry milk (control) (4). Study participants consumed their assigned diets for 6 months. Blood samples were collected at baseline and at the conclusion of the study (4). No significant changes in TC were

observed in any of the treatment groups in the 6-month period. However, both of the isoflavone fed groups had decreases in LDL cholesterol levels (–0.28 mmol/L for the soy diet with 56 mg isoflavones, and –0.25 mmol/L in the soy diet with 90 mg isoflavones) compared to the control group (4). There was also an increase in HDL levels with both isoflavone-containing diets (5.2% in the soy diet with 56 mg isoflavones and 3.6% in the soy diet with 90 mg soy isoflavones) when compared to the control group (Baum et al., 1998). This study suggests that long term intake of soy protein may decrease the risk of CHD in postmenopausal women by favorably altering plasma lipid levels.

The effects of soy protein on the plasma lipids of normocholesterolemic, premenopausal women were examined in another study. In this study 13 premenopausal women were assigned to one of 3 diets: 1) 10 mg isoflavones (control); 2) 65 mg isoflavones; and 3) 129 mg isoflavones (5). Each group consumed their assigned diet for 3 menstrual cycles. Total cholesterol, HDL, and LDL were measured at 4 phases of each menstrual cycle: 1) early follicular phase; 2) midfollicular phase; 3) periovulatory phase; and 4) midluteal phase (5). When compared with the control diet, the high isoflavone diet lowered LDL cholesterol significantly by 7.6% to 10%, depending on the phase of the menstrual cycle (5). The high isoflavone diet also lowered the ratio of TC to HDL by 10.2%, and the ratio of LDL to HDL by 13.8% (5). However, no significant changes were seen in HDL or TC levels among diets. This study suggests that isoflavones significantly improved the lipid profile of normocholesterolemic, premenopausal women. These effects could contribute to a lower CHD risk in people who consume soy for long-term periods (5).

Rice Bran Oil and Coronary Heart Disease

Introduction

Rice bran oil is believed to be a hypocholesterolemic substance, due to its high concentration of unsaponifiable matter (7). This fraction of rice bran oil includes tocotrienols, γ-oryzanol, β-sitosterol, and unsaturated fatty acids, all of which may contribute to cholesterol reduction (7-9).

Animal Studies

Quershi and co-workers (9) studied the effects of a tocotrienol-rich fraction (TRF) and novel tocotrienols on total cholesterol, LDL, and apo B in 15 4-month old hypercholesterolemic swine. The swine were divided into 5 groups; one group was fed a control diet, and the other four groups were test groups. These groups were fed a corn-soybean control diet supplemented with 50 mg of TRF, γ-tocotrienol, d-P (21)-T3 (novel tocotrienol), or d-P (25)-T3 (novel tocotrienol) (9). After 6 weeks, serum cholesterol was reduced by 32-38%, LDL was reduced by 35-43%, and apo B was reduced by 20-28% in the treatment groups when compared to the control group (9); these effects persisted 10 weeks after the end of the study. The authors concluded that high tocotrienol levels in the blood of the treatment groups were responsible for this effect (9).

Human Studies

One study by Gerhardt and Gallo in 1998 (7) compared the effects of rice bran and oat bran on elevated total cholesterol levels in humans. Fifty-two moderately hypercholesterolemic subjects were used in this study (7). All subjects added 84 g/d of rice bran, oat bran, or rice starch placebo to their diets for 6-weeks. Total cholesterol, triglycerides, HDL, and LDL measurements were taken at baseline and at the end of the study (7). Total cholesterol levels decreased significantly in the rice bran and oat bran

groups (8.3% and 13%, respectively) (7). The change in total cholesterol levels was attributed to the decrease in LDL for the rice bran and oat bran groups (13.7% and 17.1%, respectively). There was no consistent effect on triglycerides or HDL (7). In general, rice bran and oat bran decreased the lipid levels of the subjects by 78% (7). The authors concluded that overall, rice bran and oat bran should be added to the diets of hypercholesterolemic individuals.

Overweight and Obesity

Overweight and obesity are increasingly prevalent disorders affecting the population of the United States. An estimated 97 million Americans (59.4% of American males, and 50.7% of American females) are considered overweight or obese (40). Prevalence of overweight or obesity in blacks and Hispanics is higher than that of white individuals, constituting 66% of black women, 65.9% of Hispanic women, and 63.9% of Hispanic males (40). Women in the United States with low income or education are more likely to be obese than those of higher socioeconomic status; this finding is not consistent in males (40). Yearly total health costs attributed to obesity amount to approximately $99.2 billion; $51.6 billion of this amount is due to direct costs of diseases associated with obesity (40).

Weight Gain

A cause of increased CHD risk in women is total body weight; this is more important in postmenopausal women (41). Body weight reaches its maximum point very close to menopause (41); for any given body weight, there is an increase in body fat and abdominal fat (see Body Fat Distribution below) with advancing age (41).

In a five-year study of 541 women, weight gain at the time of menopause was examined (42). Lipid levels, blood pressure, weight, and body mass index (BMI) were

measured for each participant over the five-year period (42). Results suggested that LDL levels were higher and HDL levels were lower in postmenopausal women than in premenopausal women (+12.8 mg/dL and –4 mg/dL, respectively) (42). Postmenopausal women gained an average of 2.25 kg over the study period; this amount was the same as in premenopausal women (42). A correlation was seen between weight gain and increased lipid levels; in the women that showed the largest weight gain, cholesterol and triglyceride levels showed the greatest level of increase (+17.2 mg/dL and +. 23 mmol/L, respectively) (42). Postmenopausal women taking HRT were found to have the greatest increase in BMI compared with premenopausal women (+1.72) (42). It was concluded that in women, weight gain, increased BMI, and increased blood lipids increase CHD risk (42).

Body Fat Distribution

An android pattern of fat distribution refers to the ventral or upper body fat, which is found primarily in the abdomen, arms, and torso. This type of fat is found primarily in men, and is associated with an increased risk of CHD (44). Gynoid fat distribution refers to lower body fat, particularly in the hips and thighs. This type of fat is found primarily in women, and is associated with a lower CHD risk than the android pattern of fat distribution (43). Android fat has been related directly to an increase in the development of CHD (44); further, android fat has been associated indirectly, through lipid profiles, with increased CHD risk (44). Android fat percentages have been shown to increase in women after menopause (44). Conversely, gynoid fat has been shown not to increase CHD risk (44). Android obesity is associated with greater health risks than fat in peripheral regions of the body (40). Abdominal fat contains three compartments:

visceral, retroperitoneal, and subcutaneous. Visceral abdominal fat is most strongly correlated with the risk factors associated with overweight and obesity (40).

Overweight and Obesity as a Risk Factor for Coronary Heart Disease

Genetics is thought to be responsible for 25-40% of individual differences in body mass and body fat distribution (40). The risk of obesity for a first-degree relative of an overweight individual is 2 times that of the general population. The risk increases to 3-4 times for first-degree relatives of a moderately obese individual, and to 5 times for relatives of a severely obese individual (40). However, diet is the principal cause of overweight and obesity in the United States (40). The most important element of diet is excess energy intake; this is commonly paired with reduced physical activity (42).

Body mass index (BMI) is the current measure of overweight and obesity. A BMI greater than or equal to 30 is considered clinically obese (42); and a BMI between 25 and 29.9 is considered overweight (40). Body mass index does not address body fat distribution into account, which is its major limitation (40). Waist circumference (WC) is, however, considered to be a more accurate indicator than BMI of abdominal obesity, in particular. Measurements indicating high risk of CHD are a WC >40 inches in men and > 35 inches in women (40). The best direct measurement of fat distribution is dual x-ray absorpitometry (DEXA), because it is rapid and measures fat mass in absolute terms. Thus, many researchers are using to DEXA as a measure of fat distribution and obesity, although BMI is still commonly used to define obesity (42).

Obesity and body fat distribution are linked directly to many of the comorbidities associated with CHD (42). Co-morbid conditions associated with obesity are: HTN, dyslipidemia, and impaired glucose tolerance/type 2 DM (55). Above a BMI of 30, comorbidities become more prevalent and more severe as BMI increases, but they are

also related directly to body fat distribution (40). Variance in body fat distribution was evaluated in a study of 103 men and 131 women, all of similar ages (42). Dual x-ray absorpitometry was used to measure body composition of each volunteer. Men had a 50% greater lean tissue mass and a 13% lower fat mass than the women. The proportion of android body fat was significantly greater in men (48.6%) than in premenopausal women (38.3%) (42). The reverse was found for gynoid fat distribution. These results suggested a gender difference in fat distribution and fat mass in men and women. The data also illustrate that changes in fat distribution parallel the gender differences in CHD (42).

Effects of menopause on fat distribution were also examined in the study by Ley and co-workers (42). In the study, 70 of the 131 women in the study were postmenopausal (42). They were found to have a significantly higher incidence of android fat distribution (42.1%) than premenopausal women (38.3%) (42). Total fat mass was also greater in postmenopausal women. Thus, compared to premenopausal women, fat distribution in postmenopausal women is similar to that seen in men (42). Android fat distribution is associated with increased LDL, total cholesterol, blood pressure, and presence of DM; it is evident why this type of distribution may be associated with increased CHD risk.

The relation of body fat distribution to complications of obesity in women was addressed in another study (44). The study involved 25 obese women; these women were separated into two groups, one containing 16 individuals with predominantly upper body obesity and the other containing 9 individuals with predominantly lower body obesity (44). Adipocyte receptor activity, cholesterol levels, and fat cell size were measured. Those subjects with upper body obesity had significantly higher cholesterol levels than

the other group (187 mg/dL and 183 mg/dL, respectively) (56). Fat cell size was found to be larger in the group with upper body obesity. These findings were associated with increased glucose levels in this group, which can be a diagnostic criterion in determining DM risk. (44). These findings support the increased lipid levels in the group with upper body obesity (44), thereby helping to explain the increased CHD risk for individuals with this type of obesity (40).

Hypertriglyceridemia as a Risk Factor for Coronary Heart Disease

Triglyceride-rich lipoproteins in plasma originate either in the liver or the intestine; these lipoproteins also contain cholesterol, cholesteryl ester, and phospholipid (45). The contents of these lipoproteins, in excess, are believed to be a risk factor for CHD risk; triglycerides are considered to play a role in atherosclerosis (45). Confusion does not lie in whether hypertriglyceridemia is a CHD risk factor, but in whether it is a risk factor that is independent from other risk factors (47). One study examined the interrelationships of fasting plasma triglyceride levels, other lipids, and risk of MI. The study consisted of 340 subjects, who were separated into quartiles based on fasting triglyceride levels; quartile 1 represented the lowest levels, and quartile 4 represented the highest (47). All quartiles were comprised of at least 70% male subjects. This was due to a higher number of men being recruited for the study. Triglyceride, total cholesterol, and LDL levels all increased as quartile number increased. In quartile 1, mean triglyceride levels were 70.1 mg/dL, cholesterol levels were 206.3 mg/dL, and LDL levels were 127.9 mg/dL (47). In quartile 4, mean triglyceride levels were 278.9 mg/dL, cholesterol levels were 245 mg/dL, and LDL levels were 140 mg/dL (47). High –density lipoprotein levels decreased with increasing quartile number; in quartile 1, HDL levels were 51.6 mg/dL, and in quartile 4, HDL levels were 37.1 mg/dL (47). These results

suggested that fasting triglyceride levels are an important marker for CHD risk, particularly when HDL levels are considered. This means that as triglyceride levels increase, HDL will decrease; both of these factors will, in turn, contribute to a higher CHD risk. Therefore, fasting triglycerides are considered an important marker of CHD risk, but are not considered an independent risk factor in this study.

Another study addressed triglyceride level, LDL particle diameter, and MI risk (46). This was a prospective study that included 14, 916 men, and the outcome measurement used was MI diagnosed during a 7 year follow up period (46). The men were divided into a test group and a control group. The test group included men who were at risk for CHD. Blood samples were collected at baseline and again in the follow up period for each group (46). Of the total number of test subjects, 266 men were diagnosed with MI in the follow up period; they were matched to 308 individuals from the control group. The triglyceride levels of those diagnosed with MI were remeasured upon notification of diagnosis. These men had significantly smaller LDL particle diameters than did the controls (25.6 nm vs. 25.9 nm, respectively) (46). The 266 men with MI also significantly higher mean triglyceride levels (168 mg/dL vs. 132 mg/dL) (46). After adjustments for other risk factors, including HDL and TC levels, triglyceride levels remained a statistically significant risk indicator, with a relative risk of 1.4 per 100 mg/dL increase. Conversely, LDL diameter did not remain significant after controlling for other risk factors (46); LDL carried a relative risk of 1.09 with each 0.8 nm decrease in LDL particle size. Subjects with the highest triglyceride levels were found to have a 2.5 times higher risk of CHD than those with lower levels (46). These results suggest that triglyceride levels appear to be an independent predictor of future CHD events, particularly MI. Therefore, elevated triglyceride levels may help identify individuals at

high risk for MI (46). This study supports the theory that hypertriglyceridemia is an independent risk factor for CHD. These results differ from the previous study, which suggests that triglyceride levels are a risk marker, but are not an independent risk factor, for CHD risk.

Diet and Coronary Heart Disease

Total Fat, Saturated Fat, and Unsaturated Fat

Saturated fatty acids have been identified as the major dietary factor that raises serum cholesterol concentrations (48). Approximately one-third of the saturated fatty acids in the American diet come from dairy products, one-third from red-meat fat, and one-third from other sources (48). There are three saturated fatty acids that are thought to be hypercholesterolemic: lauric, myristic, and palmitic (49). Conversely, unsaturated fats have been shown to protect against increased serum cholesterol concentrations (43).

Dietary intervention trials have demonstrated that restriction of dietary total and saturated fat, or the replacement of the latter with polyunsaturated fatty acids, is of great benefit with respect to CHD risk (43). One large study that supported this was the Finnish Mental Hospital study, a crossover study that involved more than 10,000 inpatients at two mental health institutions (50). The study employed an experimental diet in which milk was replaced by an emulsion of soybean oil in skim milk, and butter was replaced by margarine (50). These treatments resulted in 12% and 18% reductions in plasma total cholesterol levels (50). In men, CHD mortality decreased by 53%; in women, CHD mortality decreased by 34% (50).

Another large study, the Oslo Diet-Heart study, involved 412 survivors of MI who were randomized to a diet low in saturated fat (8.4% of total calories) and cholesterol (264 mg/dL), but increased levels of polyunsaturated fats (15.5% of total calories) (51).

These values were judged as low when compared to the dietary recommendations of that time. A 5-year follow up showed a mean decrease in the TC levels of the intervention group of 14%, and an overall decrease in CHD mortality of 26% (51). The Los Angeles Veterans Administration study also employed a diet high in polyunsaturated fatty acids (52). This study involved 846 males, assigned to two experimental groups and one control group. Both groups were fed a diet consisting of 40% total energy as fat (52). The treatment groups were given a diet consisting of 3 times more polyunsaturated fat (equaling 16% of calories) than the control diet. The above diet also contained 40% less cholesterol (365 mg) than the control diet, which contained 912 mg cholesterol (52). Saturated fat intake was 11% of total energy in the treatment group and 18% in the control group. A 31% decrease in CHD morbidity and mortality was reported in the treatment group (52). All three of these early studies paved the way for further exploration of the relationship between dietary fat and CHD risk.

More recent studies have been larger. The Minnesota Coronary survey assessed diets of comparable total fat contents in 9,057 patients in mental health clinics and nursing homes (53). Both the treatment diet and the control diet derived 39% of total energy from fat, but the treatment diet was higher in polyunsaturated fat, lower in saturated fat (15% vs. 5% and 9% vs. 18%, respectively), and lower in cholesterol (166 mg/d vs. 446 mg/d) (53). No significant differences were noted in the study's endpoints of MI and sudden death, but mean TC levels still decreased by 14% in the treatment group (53).

The Diet and Reinfarction Trial (DART) was conducted at about the same time as the Minnesota Coronary Survey. In the DART study, 2,033 men who had recovered from an MI were allocated to receive or not receive advice on each of three dietary

factors: 1) a reduction in fat intake; 2) an increase in fish (and therefore, polyunsaturated fat) intake; or 3) an increase in cereal fiber intake (54). The study results indicated a mean reduction of 2.8% in mean TC levels for the group that received advice on how to lower fat intake, but no difference in the groups advised on fish or fiber (54). However, there were significant differences in total mortality rates in the fish fed group, with a 29% decrease in the group advised to increase fish intake (54).

These studies have evaluated the effects of reduced intake of total and saturated fat, or increased intake of polyunsaturated fat on CHD risk. The results of these trials clearly suggest that restriction of saturated fat significantly reduces the risk of CHD, and a relative increase in polyunsaturated fats (particularly n-3 fatty acids) decreases the risk of CHD. In general, consumption of a diet low in total and saturated fat should be encouraged for the reduction of CHD risk (55).

Trans Fatty Acids

The carbon-carbon double bonds of fatty acids can exist in either the *cis* or *trans* configuration. When the two hydrogen atoms are on opposite sides of the double bond, the configuration is termed *trans*; when the two hydrogen atoms are on the same side of the double bond, the configuration is termed *cis* (57). The bond angle is larger for a trans than a cis double bond. Therefore, the *trans* double bond results in acyl chains that can pack together more tightly (57).

Trans double bonds occur as such in nature. They are the result of anaerobic bacterial fermentation in ruminant animals and are thereby introduced into the food chain (57). Humans consume trans fatty acids in the form of meat and dairy products (57). The trans fatty acid content of both of these foods combined comprises approximately 5% of total fat content (58). Trans fatty acids are mostly formed in the hydrogenation of

oil, a process used to transform oil from a liquid to a semisolid state (59). Hydrogenated fat is used in the manufacture of margarines and vegetable shortening and is therefore in foods prepared with these products (59). Hydrogenation also results in the saturation of some double bonds in the acyl chain of the fatty acid (59). *Trans* fatty acids constitute approximately 5% to 6% of dietary fat in the average American diet (58). *Trans* fatty acids have been shown to increase the ratio of LDL cholesterol to HDL cholesterol, which is believed to increase CHD risk (60).

Several studies have been conducted that support the claim that *trans* fatty acids increase CHD risk by increasing the LDL: HDL cholesterol ratio. One such study involved 47 men and women with diagnosed CHD, and a reference group (n=56) with no evidence of CHD (58). Plasma samples from all subjects were analyzed using fatty acid extraction and cholesterol assays (58). Total plasma *trans* fatty acids were 24% higher in the group with diagnosed CHD (58). In addition, TC and LDL were higher in subjects with CHD than in the reference subjects (212 mg/dL vs. 185 mg/dL, and 146 mg/dL vs. 115 mg/dL, respectively) (58). A significant difference in HDL levels was also seen, with the group with CHD having lower HDL levels than the reference group (34 mg/dL vs. 52 mg/dL) (58). The results of this study are consistent with the hypothesis that dietary *trans* fatty acids increase total cholesterol and LDL levels, while lowering HDL levels, and may therefore increase CHD risk.

Another study addressed the effects of *trans* fatty acids on risk of MI. A total of 521 subjects were used in the study; 239 had been diagnosed with acute MI, and 282 were used as a control group (59). All subjects were given a semiquantitative food frequency questionnaire (FFQ) to determine *trans* fatty acid intake (59). Responses to the FFQ were adjusted for age, sex, and energy intake. The mean *trans* fatty acid intake

was 4.36 g/d (4.3% of total fat intake) in men, and 3.61 g/d (4.8% of total fat intake) in women (68). The relative risk for CHD related to *trans* fatty acid intake was highly significant when the highest and lowest quintiles were compared (95% confidence interval). This significance remained after adjustment for other coronary risk factors. This study supports the hypothesis that *trans* fatty acids increase MI risk by increasing CHD risk.

Judd and co-workers (60) studied the effects of *cis* and *trans* fatty acids on plasma lipids of 29 men and 29 women in 1994. This was a crossover study that included 4 diets: 1) high oleic acid (monounsaturated) (16.7% of total energy); 2) moderate *trans* fatty acids (3.8% of total energy), 3) high *trans* fatty acids (6.6% of total energy); and 4) saturated fat (16.2% of total energy) (60). Compared with the oleic diet, LDL cholesterol increased 6% with moderate *trans* fatty acids, 7.8% with high *trans* fatty acids, and 9% with saturated fats (60). HDL cholesterol was unchanged after the moderate *trans* fatty acid diet, but was 2.8% lower after the high *trans* fatty acid diet. This study suggests that dietary *trans* fatty acids raise LDL cholesterol, but to a lesser degree than saturated fats; also, higher concentrations of *trans* fatty acids may result in decreased HDL levels. This study also supports the hypothesis that *trans* fatty acids increase CHD risk by altering LDL and HDL levels.

A small-scale study examined varying levels of *trans* fatty acids and their effects on serum cholesterol levels. This study included 18 men and 18 women, who consumed one of 5 diets that were randomly provided for 35-day periods; a sixth diet was administered first to allow collection of lipid measurements for comparison to the other diets (55). The diets were identical with the exception of the fat source. Each source contributed 30% of total energy from fat. The sources of fat were: soybean oil,

semiliquid margarine, soft margarine, shortening, stick margarine, and butter (55). The theory behind use of these fat sources was that the more solid sources would provide higher levels of trans fatty acids. The mean LDL level of the group that consumed the butter-containing diet was 177 mg/dL, and the mean HDL level for this group was 45 mg/dL. The diet that contained butter as a fat source was given first to cause the greatest increase in cholesterol levels with saturated fat; the effects of the other 5 fats would then be determined using the lipid values from those consuming the butter-containing diet (55). Low density lipoprotein cholesterol was reduced by 12%, 11%, 9%, 7% and 5%, after subjects consumed the soybean oil, semiliquid margarine, soft margarine, shortening, and stick margarine sources, respectively; the HDL levels were reduced by 3%, 4%, 4%, 4%, and 6%, respectively (55). Ratios of HDL to TC were highest after the diet containing stick margarine (6.03) and lowest after the diets containing soybean oil and semiliquid margarine (5.43 and 5.47, respectively) (55). This implies that lower *trans* fatty acids and lower saturated fats have a beneficial effect on plasma lipids.

The intake of *trans* fats and CHD risk in women was examined in a large-scale study. This study evaluated questionnaires from 85, 095 women without diagnosed CHD (56). The mean reported *trans* fatty acid intake, determined from answers to questionnaires, was 5.8% of dietary fat (4 g); 60% of this value was from vegetable fats and 40% was from animal sources, primarily beef and dairy fat (56). Of the total number of subjects, 431 cases of CHD were reported in follow-up. *Trans* fatty acid intake was related directly to a higher risk of CHD; the relative risk for the highest versus the lowest quartile was 1.50 (56). These results were adjusted for age, smoking, and other standard risk factors; however, relative risk was not substantially changed. The association was even stronger for those women who consumed four or more teaspoons of margarine per

day (relative risk of 1.66), due to the increased *trans* fat content (56). These findings support the hypothesis that consumption of *trans* fats may contribute to CHD risk.

Age as a Risk Factor for Coronary Heart Disease

This increase is due to the progressive accumulation of coronary atherosclerosis over time, the effects of which are more pronounced in men at an earlier age. This progression of atherosclerosis is believed to be due to the increase of cholesterol levels with age (28). The age of increase in CHD risk is believed to be approximately 55 years for women because menopause occurs at approximately this age; women are then at increased risk for CHD (61). Estrogen production by women is believed to offset the increase in CHD risk by approximately 10 years, which explains the age of increased risk in women as being approximately 10 years greater than that of men (61). Most individuals with newly diagnosed CHD are over the age of 50 years; this diagnosis is especially pronounced in women (28).

Hypertension as a Risk Factor for Coronary Heart Disease

Elevated blood pressure is a potent risk factor for CHD (28). Moderate elevations of blood pressure in women are a particularly strong risk factor for not only CHD, but also organ damage and stroke (62). Therapy for hypertension should begin at >140/90 millimeters mercury (mmHg) (desirable blood pressure < 120/80 mmHg) (29). Diet therapy is preferred for adults. If not successful in a 6-month period, drug therapy is usually considered (62); if values for blood pressure are very high, drug therapy may be implemented immediately. This, however, depends on the patient's other risk factors (62).

Cigarette Smoking as a Risk Factor for Coronary Heart Disease

Cigarette smoking is a powerful risk factor for CHD that predisposes the smoker to CHD in several ways (62). Smoking acts synergistically with other risk factors, such as HTN and hyperlipidemia, thereby increasing risk of CHD (62). The major risk associated with smoking is its promotion of atherosclerotic plaque formation (28). As many as 30% of CHD deaths in the United States each year are attributed to cigarette smoking. This risk is strongly dose related: the more cigarettes an individual smokes, the higher the CHD risk (62). Smoking cessation rapidly and markedly reduces risk of MI (28). Those individuals with already diagnosed CHD will experience a 50% reduction in risk of MI, sudden death, and total mortality if they quit smoking. This occurs in as little as one year (62).

Diabetes Mellitus as a Risk Factor for Coronary Heart Disease

Individuals with diabetes mellitus (DM) have an increased risk for CHD (28). More than 60% of total patient hospital days due to DM-related conditions were attributed to cardiovascular complications (28). Coronary heart disease is the leading cause of death in over 60% of diabetics (28). Factors associated with DM that affect risk of CHD are hyperglycemia, albuminuria, hypertension, low HDL levels, and hypertriglyceridemia. (28).

Hyperglycemia (fasting blood glucose level >126 mg/dL) is characteristic of individuals with DM (28). Hyperglycemia is considered an independent risk factor for CHD in DM patients because it is known to promote macrovascular disease (atherosclerosis). The mechanism by which hyperglycemia promotes macrovascular disease is not understood thoroughly (28).

In diabetics, risk associated with HTN increases greatly when compared to those without DM (28). Hypertension can ultimately lead to stroke, kidney failure, and congestive heart failure; these disorders cause more complications than DM alone (28).

Low HDL levels are manifested as a risk factor for CHD in patients with DM; however, the more critical lipid level is the plasma triglyceride level (TG) (28). Desirable TG levels are set at <200 mg/dL (28). In diabetics, blood glucose levels are often abnormally high, which causes TG levels to remain increased (28). Although TG are not atherogenic alone, they are proatherogenic – they assist LDL in forming atherogenic plaque (28). More importantly is the fact that hypertriglyceridemia works with other lipids to form atherogens; the reduction of TG levels in individuals with DM will thereby decrease risk of atherogenesis, and therefore, CHD (28).

Female Reproductive Hormones/Ovarian Cycle/Menopause

Female Reproductive Hormones and Functions

Three types of female reproductive hormones are hypothalamic releasing hormone (lutenizing hormone releasing hormone – LHRH); anterior pituitary hormones (follicle stimulating hormone – FSH, and luteinizing hormone – LH); and ovarian hormones (estrogen and progesterone). These hormones are not released in constant amounts during the month; their secretion rates depend on the times of the menstrual cycle (65). The duration of the sexual cycle averages 28 days; however, it may be as short as 20 days or as long as 45 days (65). The menstrual cycle has two components. First, an ovum is released from the ovaries, producing several hormonal changes; these changes prepare the body to allow fetal growth (65). Second, the endometrium of the uterus is prepared for the implantation of the ovum at the required time in the cycle (65).

The anterior pituitary (gonadotropic) hormones are secreted in response to the secretion of LHRH from the hypothalamus (35). Changes in the menstrual cycle are dependent on the secretion of the anterior pituitary hormones; thus, they are dependent on the release of LHRH (35). Ovaries not stimulated by these hormones remain inactive; for example, in children that have not yet begun menstruating (65). Two anterior pituitary hormones essential to ovarian function are FSH and LH. During each month of the menstrual cycle, there is a cyclical increase of FSH and LH, which causes follicular growth (65). Ovulation occurs as a result of the effects of FSH and LH on the ovarian follicles.

After ovulation, LH stimulates the empty follicle with the transformation of the follicle into a new structure – the corpus luteum (35). The corpeus luteum secretes estradiol and progesterone (65). Progesterone secretion peaks during the formation of the corpeus luteum; this effect combined with estradiol produces a negative feedback inhibition of FSH and LH secretion (35, 65). This inhibition retards new follicle development so ovulation does not reoccur during the cycle (35). Near the end of the cycle, new follicles develop in preparation for the next cycle; the corpeus luteum breaks down due to the lowered LH levels (65). As a result, estrogen and progesterone levels fall, which causes menstruation and allows a new cycle to begin.

Menopause

Menopause refers to the cessation of ovarian activity that occurs at approximately 50 years of age (35). During postmenopausal years, the ovaries are depleted of follicles and halt in the excretion of estradiol (35, 65, 66). The decrease in estradiol is not due to altered pituitary function, but is rather due to changes in the ovaries themselves (67). The secretion of FSH and LH is elevated due to the lack of negative feedback inhibition from

estradiol (35). Estrogen continues to be produced in small quantities, due to the aromatization of the androgen androstenedione (35). This hormone is converted into a very weak estrogen called estrone. After a few years of production of small amounts of estrone, atrophy of the urethra, vaginal walls, and vaginal glands occurs (65). The few remaining follicles become atretic, and as a result, estrogen production by the ovaries falls almost to zero (68).

The withdrawal of estrogen secretion is responsible for the characteristic adverse changes that occur physically in women during menopause (35). Vasomotor disturbances cause the "hot flashes" which cause a fall in core body temperature, followed by perfuse perspiration and intense feelings of extreme heat (35, 65). The urogenital tissues become atretic, causing vaginal dryness (65). Also, night sweats, breast tenderness, and difficulty sleeping have been reported by postmenopausal women (67).

There is also increased risk of cardiovascular disease as a result of estrogen loss; this loss is due to menopause (35, 65, 67). In fact, postmenopausal women experience 92% of all CHD mortality in women; this may be associated with estrogen loss (67). The exact causative factors behind the postmenopausal acceleration of CHD are poorly understood (67). Plasma levels of LDL increase by 10% to 15% after menopause; HDL is increased after menopause by 10% to 15% if estrogen replacement therapy is administered (13). If no therapy is administered, the HDL will continue to decrease with age; this negatively affects CHD risk (36). Adverse changes in lipoprotein profiles, which are associated with declining endogenous estrogen levels, account for only 25% - 50% of the increased postmenopausal risk for CHD (67). Recent research has shown that dietary therapy may not appreciably alter lipoprotein levels in postmenopausal women (53); but when paired with HRT or exercise, effects may be more profound (61). In one

study, 177 women (ages 45-64) and 190 men (ages 30-64) were assigned randomly to low-fat diet, aerobic exercise, a low-fat diet and aerobic exercise, or no treatment (68). Compliance was determined through food diaries; activity diaries were also included for the two groups including exercise. Results showed that the only significant differences in cholesterol levels were in the diet and exercise group, which exhibited decreases in LDL and total cholesterol levels of –14.5% and –17.5%, respectively (68). This supports the theory that diet alone does not appreciably alter the plasma lipids of postmenopausal women. Loss of estrogen also appears to reverse vasodilatation in the coronary arteries of postmenopausal women; this vasodilation is due to endogenous non-steroidal hormone action in the vessels (13). Mechanisms of the protective action of estrogen are not understood; however, estrogen replacement therapy is considered the best option in reducing the various effects of menopause. Postmenopausal estrogen replacement is associated with a 35% to 50% reduction in CHD risk (12).

Rats as Models for Menstruation and Menopause

Rats experience puberty at approximately 50-60 days (2 months) of age (69). The vagina opens (as a result of puberty) between 34 and 109 days, with an average of 72 days; ovulatory estrous cycles begin at about 77 days, but may occur between 45 and 147 days of age (69).

Vaginal changes in the rat are closely related to the estrous cycle. The estrous cycle of female rats is comparable to the menstrual cycle in human females (69). However, there are many differences between the two cycles. The estrous cycle of rats is much shorter in duration than the human cycle, lasting approximately 102 hours (69). There are many stages in the estrous cycle of rats: proestrus (where ovarian follicle becomes enlarged), estrus (where large follicles produced and egg matures), metestrus I

(where ovulation occurs), metestrus II (where eggs are in oviduct), and diestrus (where corpus lutea are formed) (69).

Menopause begins in rats at approximately 450-540 days of age (69). However, efficient breeding life ceases long before the onset of menopause. Because of the lack of available data and the poor understanding of the role of estrogen in rat cardiovascular physiology, rats are a poor model for the cardiovascular changes associated with menopause (70). However, the rat is a particularly good model for estrogen protection from ovariectomy dependent bone loss (70). That is why rats were chosen for this study.

Rats as Models for Disease

Introduction

The rat model is a generally appropriate model for the study of human health and disease (71). In many instances, the rat model is the most appropriate experimental model of human disease (71). The rat is an easily available, low-cost, well-studied animal model (70). Presently, rats comprise 28% of laboratory animals and provide important models for cardiovascular, pulmonary, renal, endocrinology, reproduction, and parasitology (71).

Features of the Rat Digestive System

The rat most closely approximates the form and function of the digestive system of humans when compared to other rodents used in laboratory research (72). The gastrointestinal transit time for rats is 12-24 hours (72). Lighting schedules may affect the rate of intake and defecation of the rat (72). The rat stomach is divided into nonglandular and glandular portions (72). The rat is unique because it has no gallbladder and a weak Oddi's sphincter, which shows that the rat liver stores very little bile; this may affect fat digestion, and may become a limiting factor in the comparison of rat

digestion to human digestion (72). Also, the rat cecum differs from that of other rodents because it has no internal septa; it is constricted in the middle to form two parts (72). These differences in the rat anatomy may allow for decreased general nutrition requirements, *i.e.* a lower fat diet, in comparison to those of humans (72).

Rats as Models for Coronary Heart Disease and Hyperlipidemia

Rats are very resistant to the development of atherosclerotic lesions when placed on high-lipid diets (73). Lesions in the coronary arteries of rats are difficult to produce, due to the lack of intimal cell proliferation, and complicated lesions with hemorrhage, thrombosis, and resulting MI are rare (73). Therefore, rats are seldom used for studies of CHD.

Rats are not ideal models for the study of cholesterol levels and hyperlipidemia (88). The predominant cholesterol found in rats is HDL-C, which allows rats to resist diet-induced LDL and TC elevations (74). The typical lipoprotein profile of the rat differs greatly from the human profile; rats have high HDL and low LDL (70). Diet manipulations including feeding bile acids and high cholesterol diets are often used to induce hyperlipidemia (74). These manipulations are, at a minimum, necessary considering that rats have a very high rate of cholesterol synthesis, and can decrease hepatic sterol synthesis and biliary cholesterol secretion (resulting in very little change in TC levels) (74). With typical rat diets, TC and TG levels of rats range from 44 to 78 mg/dL and 39 to 87 mg/dL, respectively. Rats also lack cholesterol ester transfer protein (CETP), which has a major role in the transfer of cholesterol esters to LDL and VLDL in exchange for TG from HDL. Cholesterol-rich feedings, result in increased HDL levels due to the lack of CETP (74).

In manipulated rat diets, it is recommended that the cholesterol content be sufficient to challenge the capacity of cholesterol synthesis and metabolism to accommodate the cholesterol load; this is intended to cause the desired increases in plasma lipid levels (74). The amount of cholesterol to be used in to challenge the cholesterol synthesis and metabolism pathways is not to exceed 5 to 10 times the amount of cholesterol synthesized endogenously by the rat (74). For any diet manipulation, rats are not recommended as models. Those animal species with the major portion of their cholesterol in the LDL fraction are preferred (74). Despite these limitations, rats were chosen for this study because they are good models for bone analyses, which was the focus of our major study.

CHAPTER 3

MATERIALS AND METHODS

Animals and Treatment Assignments

The Institutional Animal Care and Use Committee approved this study on January 13, 2000 (Appendix A). Seventy-six retired female breeder Sprague-Dawley (Harlan Co., Minneapolis, MN) rats, approximately 9 months old, were used in this study. Rats were housed individually in stainless steel hanging wire cages, measuring 15½ centimeters (cm) wide x 26 cm long x 12½ cm high. Animals were housed in an environmentally controlled room in the Louisiana State University and Agricultural and Mechanical College (LSU) Life Sciences Live Animal Vivarium at 22° Celsius (C) and 60% humidity. Rats were on a twelve-hour on/off light cycle (lights on at 0700 hours/lights off at 1900 hours). Upon arrival at the facility and for six consecutive days thereafter, American Institute of Nutrition (AIN)-93 basal diet and water were provided *ad libitum*.

Upon arrival, rats were weighed and weights were recorded to the nearest gram (g). For two consecutive days post arrival, rats were placed in cages attached to running wheels for one hour each day to determine exercise proclivities. Following these exercise periods, total revolutions run were recorded for each rat.

To randomize assignment to treatment groups, rats were ranked from highest body weight to lowest body weight on day 4. The rankings were recorded. Blank pieces of paper, representing the eight treatment groups in the study, were numbered 1 through 8. These pieces of paper were placed face down on a table. A numbered piece of paper was drawn from the group and the number was assigned to the first rat on the list. After all eight slips were drawn and the first eight rats had been assigned, the process was

repeated. This method was continued until all rats had been assigned to the eight treatment groups; seven groups contained 9 rats each and the swimming group (CW) contained 10 rats. After assigning rats to treatment groups, the group with the most runners was labeled the voluntary running group (CE). Rats in this group that did not run sufficiently in the preliminary trials were exchanged with rats from other groups that were of similar weights and that did run. All rats in the running and swimming groups were given AIN-93 diet (21% casein).

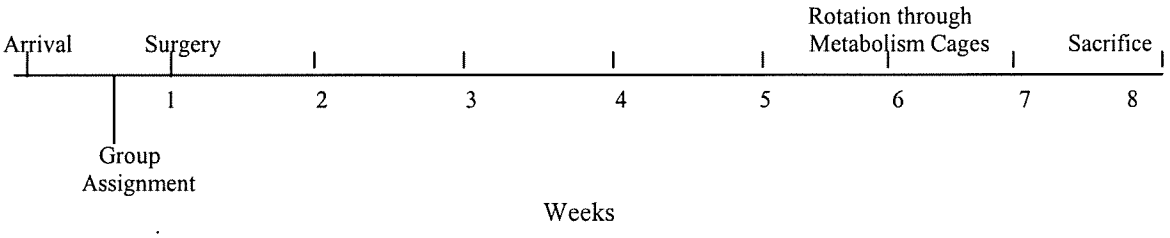

Figure 1. Timeline by week for study-range for weighing and feeding, dates and week ranges for arrival of rats, treatment group assignments, rat surgery, recovery, metabolism cage rotation, and sacrifice. Weighing and feeding animals continued throughout the study.

Table 1. Rat groups, surgery, diet, exercise, and treatment group assignments. Group names are as follows: C=control group, C7S=7% soy diet group, C14S=14% soy diet group, 21S=21% soy diet group, CRB=rice bran oil group, CE=running group, CW=swimming group, ShC=sham operated group, and OVX=ovariectomy surgery. (All groups, unless otherwise noted, received basal 21% casein diet.)

Group Name	Surgery	Diet	Exercise Type
C	OVX	21% casein	None
C7S	OVX	14% casein, 7% soy	None
C14S	OVX	7% casein, 14% soy	None
21S	OVX	21% soy	None
CRB	OVX	Rice bran oil	None
CE	OVX	21% casein	Voluntary Running
CW	OVX	21% casein	Swimming
ShC	Sham	21% casein	None

The research presented in this thesis is part of a larger study; this thesis will focus only on the dietary and lipid level component of the study.

Diet

Table 2 lists ingredients for the AIN-93 diet, as well as the adjustments made to modify the AIN-93 diet for specific diet treatment groups. The control diet (modified AIN-93) contained 21% casein. Treatment diets were made by substituting an increasing amount of soy protein isolate (a generous gift from Protein Technologies, Inc.) for all or part of the casein: 7% soy + 14% casein, 14% soy + 7% casein, or 21% soy + 0% casein. One hundred fifty grams of rice bran oil (a generous gift from Riceland Foods, Stuggart, AR) was substituted for 150 g soybean oil in the control diet to produce the rice bran treatment diet (Table 2a).

Diets were mixed in separate batches. The first batch was prepared prior to arrival of the rats. To prepare diets, 0.717 grams butylated hydroxytoluene (BHT) (ICN Biomedicals, Aurora, OH) were added (as a preservative) to a 3854-gram (1 gallon) container of soybean oil to achieve a final concentration of 200-mg/kg oil. Immediately prior to mixing the diet, soybean oil and Crisco shortening (Proctor & Gamble, Cincinnati, OH) were warmed separately. Macronutrients were added to a large mixing bowl and mixed using a Hobart mixer (Model A-200-FD) for ten minutes at low speed. The sides of the bowl were scraped. Macronutrients were mixed for another five minutes. Micronutrients, cholesterol, and cholic acid were sieved sequentially into a large bowl, and then mixed by hand until uniformly combined. The micronutrient mixture was added to the bowl containing the micronutrients. This was mixed for ten minutes. The sides of the bowl were scraped. Mixing continued for another five minutes. Crisco and soybean oil (BHT added) were mixed together quickly and

immediately added to the dry ingredients. These ingredients were mixed for five minutes. The sides of the bowl were scraped. Mixing was continued for an additional ten minutes.

Diets were stored frozen in tightly sealed Ziploc bags (S.C. Johnson, Racine) from which excess air was removed. Bags were labeled with diet type, date, batch number, and preparers' initials. Aliquots of each diet type and batch were placed in small bags and stored for further analysis. Treatment diets were adjusted by altering the amounts of casein, soy protein isolate, and rice bran oil; these adjustments are reflected in the table below.

Table 2. AIN-93 control diet ingredients, modifications, and treatment adjustments. Adjustments were made to the modified AIN-93 diet. The following is a formula to prepare 6 kilograms (kg) of diet.

INGREDIENT	AIN-93M Diet	MODIFIED AIN-93M Diet
Macronutrients	**g/kg diet**	**g/kg diet**
Sucrose	100 g	67 g
Casein		
100% Casein/ 0% Soy Protein Isolate (ShC, C)	140 g	210 g
93% Casein/ 7% Soy Protein Isolate (C7S)	N/A	140 g/ 70 g
86% Casein/ 14% Soy Protein Isolate (C14S)	N/A	70 g/ 140 g
0% Casein/ 100% Soy Protein Isolate (21S)	N/A	0 g/ 210 g
Cornstarch	465.69 g	313.33 g
Dextrinized cornstarch	155 g	104 g
Fiber[a]	50	50
Micronutrients		
L-cysteine	1.8 g	1.8 g
Mineral mix (AIN-93M-MX)[bc]	35 g	35 g
Vitamin mix (AIN-93-VX)	10 g	10 g
Choline bitartrate[b]	2.5 g	2.5 g
Cholesterol	0 g	5 g
Cholic acid	0 g	2 g
Fats		
Crisco®	0 g	50 g
Soybean oil (BHT added as a preservative)	40 g	150 g
Rice bran oil (CRB)	N/A	150 g

[a] cellulose
[b] crush with mortar and pestle
[c] sieve

Food Intake and Weight Measurements

Food intake was recorded on Monday, Wednesday, and Friday throughout the study. To determine intake, food cups were weighed empty, filled with appropriate diet, provided to rats, and re-weighed after 2 days. The difference between the filled cup weights and the cup weights after feeding was calculated. Spillage, which was the amount of food spilled onto colored paper placed beneath each cage, was subtracted from the above difference. The resulting measure was recorded as food intake.

Weights were recorded on Monday, Wednesday, and Friday throughout the study up to the date of sacrifice. To weigh rats, a bench scale (Toledo Model 4030) was zeroed, and then tared using a plastic ice-cream bucket; rats were placed in the bucket and weights were recorded to the nearest gram.

Surgery

Veterinarians and a veterinary student from the LSU School of Veterinary Medicine performed ovariectomies (OVX) on the rats. Surgeries were conducted on days 7 and 8 after the arrival of the rats. Rats in running (CE) and swimming groups (CW) received surgery first to allow maximum recovery before beginning exercise. Remaining rats were then ovariectomized in the following order: rats with the number 1 from each group were ovariectomized on the same day; rats with the number 2 from each group were ovariectomized on the same day; the same order was followed until all rats numbered 1-9 were ovariectomized.

An induction chamber supplied with oxygen and Isoflurane (Abbott Labs, Chicago, IL) was used to induce anesthesia. Following the onset of anesthesia, rats were removed from the box and fitted with a nose cone, which continuously supplied Isoflurane. This was used to prevent the rats from regaining consciousness during

surgery. Buprenex (Buprenorphine hydrochloride – Reckitt and Colman, Hull, England) was administered subcutaneously after anesthesia at 0.05-mg/kg final body weight to provide post-operative analgesia. Rats were clipped dorsally from under the shoulder blades to the tail. Following clipping, nose cones were removed from rats to allow transport of rats to the operating room.

Nose cones were re-applied when rats reached the operating room. Bilateral incisions, approximately ½ inch in length, were made dorsally, superior to each hindquarter. Ovaries were excised. Muscle tissue was then sutured using 5-0 nylon (Ethilon™ -- Ethicon, Inc., Somerville, NJ). The nose cone was removed; and rats were transported to the post-operative area where 15 cubic centimeters (cc) of Lactated Ringer's solution (Abbott Labs, Chicago, IL) was injected subcutaneously between the shoulder blades of each animal to prevent dehydration. Skin incisions were closed with Super Glue (Loctite Corp., Cincinnati, OH). Immediately following the closure of incisions, rats were placed on a bed of towels in a plastic box. Rats remained in the box until they reached a semi-conscious state, after which they were returned to their respective cages. This procedure was continued until all rats except the sham group (ShC) had surgery.

The same basic procedure was followed for the ShC group; however, the ovaries were not excised. They were pulled through the incisions, visualized, and returned to the body cavity. Wound closure and post-operative recovery followed the same procedure as above.

Sacrifice

Sacrifice was carried out during week 8 of the study. Rats numbered 1 in all treatment groups were sacrificed first; then all rats numbered 2 were sacrificed. This

order was continued until all rats through number 9 were sacrificed. Rats were anesthetized using Isoflurane. In a process similar to that described above for surgery, rats were placed individually in an induction chamber attached to the anesthesia machine via plastic tubing. Isoflurane was administered; rats were removed from the box and fitted with a nose cone to continue anesthesia. Cardiac puncture was performed on each rat using a 10 cc syringe fitted with a 22-gauge needle. Approximately 9 to 10 cc of blood were drawn from each rat and transferred immediately into red-topped Vacutainer tubes (B & D Labs, Franklin Lakes, NJ). Care was taken to transfer the blood slowly to avoid hemolysis. Blood was allowed to clot at room temperature (RT), placed on ice to maximize clot retraction, and transported to the laboratory.

Blood samples were centrifuged at 2490 revolutions per minute (rpm) for 10 minutes in the laboratory using a Clay Adams centrifuge (Parsippany, NJ). Serum was removed from each sample, aliquotted, and frozen for later chemical analyses: serum cholesterol, high-density lipoprotein, and triglyceride levels.

Cervical dislocation was performed to ensure the humane sacrifice of each rat. A ventral incision was made from the mid-point of the hindlegs to the midpoint of the front legs. The breastbone was split and the heart was removed and weighed; and weight was recorded to the nearest milligram. Next, the liver and uterus were removed and weighed to the nearest milligram. Livers were frozen and stored. Uterus weights and the presence or absence of ovaries were used to confirm OVX. All visible abdominal fat was removed from the body cavity (care was taken not to disrupt the intermesenteric fat); fat was then weighed and recorded to the nearest milligram.

Serum Total Cholesterol and HDL-C Analysis

To measure serum total cholesterol (TC), a colorimetric assay, using commercially available kits (Sigma Diagnostics, St. Louis, MO), was used. Briefly, duplicate tubes were labeled with each rat number. Serum was examined visually for presence of red color; if serum was excessively red, this suggested hemolysis. For serum that was hemolyzed to a large degree (as determined by one of the investigators – MH), a third test tube was labeled as a serum control (SC). The hemolyzed serum was aliquotted into the third tube and diluted with 1 ml of water. The absorption value of the SC tube was subtracted from the values of the other tubes to determine actual values, controlling for hemolysis. One milliliter (ml) of pre-prepared INFINITY™ cholesterol reagent (Sigma Diagnostics, St. Louis, MO) was added to each duplicate tube; and 10 µl of rat serum were added (this was also done to the SC tube if present). Tubes were covered with Parafilm (American Natural Canners, Nenah, WI) and mixed. Samples were incubated for 15 minutes at room temperature (RT). Absorbances were recorded at 500 nm on a Hitachi (Model U-2000) spectrophotometer. Absorbances were measured in triplicate, and the mean of the three values was used to calculate final values. Milligrams/deciliter of total cholesterol were calculated using the following formula:

$$\text{Total cholesterol (mg/dL)} = [(A_{sample} - A_{SC} / A_{standard}) \times \text{Standard}$$

To assure accuracy of the readings from the spectrophotometer, an ACCUTROL control (Sigma Diagnostics, St. Louis, MO) was used with each sample group analyzed. The spectrophotometer was calibrated using 10 microliters (µl) each of 100 mg/deciliter (dL), 200 mg/dL, and 400 mg/dL cholesterol calibrators (Sigma Diagnostics, St. Louis, MO) with each sample group analysis.

The method for HDL level measurement was identical to that for total cholesterol, except the serum was spun at 2490 rpm for 15 minutes to allow precipitate to form. The remaining supernant was used for analysis. The following formula was used:

$$\text{Total HDL-C (mg/dL)} = [(A_{sample} - A_{SC}) / A_{standard}] \times 50 \times 1.1$$

Triglyceride Levels

The same general method used for quantitation of total and HDL levels was used to calculate TG levels; however, the reagent for the TG assay was prepared by adding 20 ml of deionized water to each bottle of powdered glycerol phosphate oxidase (GPO)-Trinder reagent (Sigma Diagnostics, St.Louis, MO). GPO-Trinder was reconstituted immediately prior to use. Duplicate test tubes were labeled with each rat number. For samples that were hemolyzed, a third test tube was labeled as a serum control (SC) to which 1 ml water was added. The absorption value of the SC tube was subtracted from the values of the other tubes to determine TG levels. One milliliter GPO Trinder reagent was added to each duplicate tube; and 10 μl of rat serum were added (this was also done to the SC tube if present).

The spectrophotometer was calibrated using 10 microliters (μl) each of 125 mg/deciliter (dL), 250 mg/dL, and 500 mg/dL calibrators (Sigma Diagnostics, St. Louis, MO) with each sample group analysis. Tubes were covered with Parafilm and mixed gently by inversion. Samples were incubated for 15 minutes at RT. Absorbances were recorded at 540 nm. Milligrams/deciliter of triglycerides were calculated using the total cholesterol formula stated above. Absorbances were measured in triplicate for each sample; the mean of the three values was used to calculate final values. To assure the accuracy of the readings from the spectrophotometer, an ACCUTROL control was used

with each sample group analyzed. The following formula was used in the calculation of triglyceride levels:

Serum triglycerides (mg/dL) = [(A_{sample} - A_{SC}/ $A_{standard}$) x Concentration of Standard

Statistical Analysis of Data

Adjusted liver and abdominal fat weights were calculated prior to SPSS analysis. Actual liver and fat weights were divided by the final body weight of each rat; the resulting values were used as adjusted liver weights and adjusted abdominal fat weights.

Statistical analysis was performed using the SPSS analysis program (SPSS Incorporated, Chicago, IL). Data were entered into the program spreadsheet and descriptive statistics were calculated; these included means and standard deviations. One-way analysis of variance (ANOVA) was performed on weight, food intake, total cholesterol levels, HDL-C levels, and TG levels among groups to determine significant differences. Means and standard deviations of each test were calculated. Tukey's post-hoc tests were also performed on these data sets to determine differences among groups. Data are presented as means ± standard deviations; both were rounded to the nearest whole number.

CHAPTER 4

RESULTS

1. Treatment Effects

Final Body Weight/Total Weight Gain

There were no significant changes in final body weight of any of the groups during this study. The greatest mean numerical final body weight was recorded for the C14S group (359 g ± 1.87), and the lowest numerical final body weight was recorded for the ShC group (331 g ± 7.08) (Table 3).

There were no significant changes in total weight gain of the groups during this study. The greatest numerical weight gain was recorded in the C14S group (16.1 g ± 24.04), while the lowest weight gain was recorded in the ShC group (8.11 g ± 10.92) (Table 3). Figure 2 shows changes in mean body weights over time.

Table 3. Rat groups [sham-operated control (ShC), OVX control (C), casein and rice bran oil diet (CRB), 7% soy diet (C7S), 14% soy diet (C14S), and 21% soy diet (21S)], final body weights per group (g), and total weight gain per group (g ± standard deviation).

Rat Group	Final Body Weight	Mean Weight Gain
ShC	331.0 g	8.1 ± 10.92
C	350.9 g	13.2 ± 16.77
CRB	351.2 g	12.4 ± 13.82
C7S	356.6 g	14.2 ± 19.42
C14S	358.9 g	16.1 ± 24.04
21S	351.0 g	13.5 ± 17.08

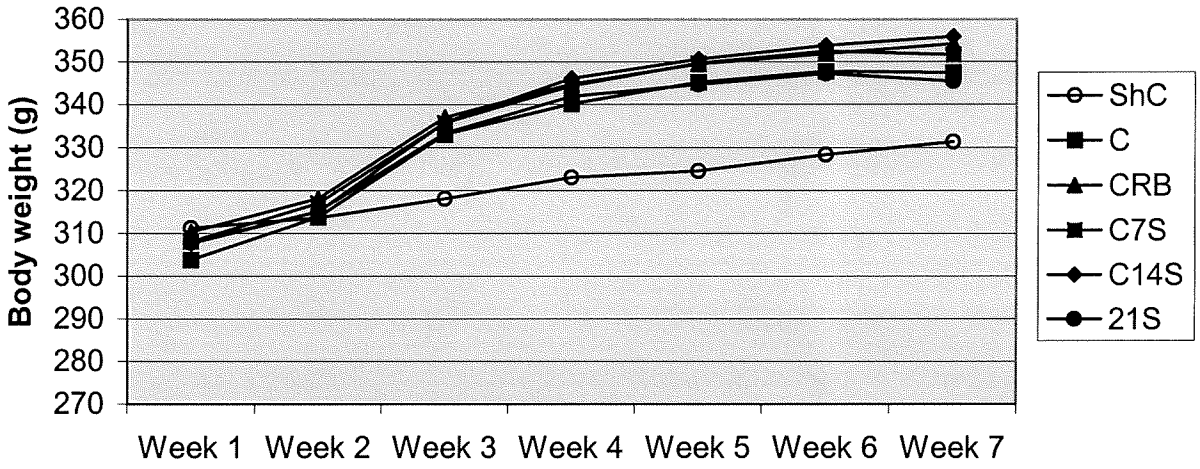

Figure 2. Mean weekly body weights in rat groups(g)

Intakes

There were no significant differences in mean total intakes among groups. The greatest numerical total average intake was recorded in the C14S group (721 g ± 71.61), and the lowest numerical total average intake was recorded in the ShC (666 g ± 39). Table 4 contains mean total intake (g) ± standard deviations. Figure 3 shows intake by week over the course of the study.

Table 4. Rat groups (ShC, C, CRB, C7S, C14S, 21S) and mean total intake of assigned groups.

Rat Group	Average Intake
ShC	666 ± 38.99
C	669 ± 43.90
CRB	695 ± 77.46
C7S	703 ± 66.12
C14S	721 ± 71.61
21S	717 ± 47.01

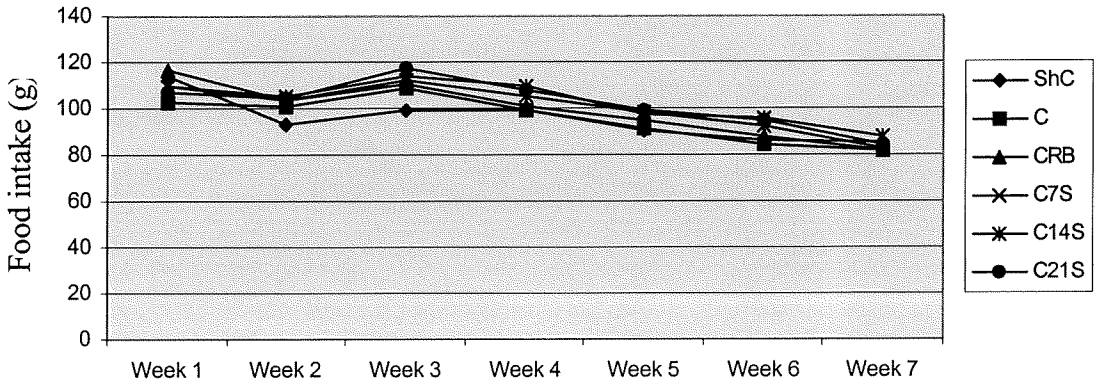

Figure 3. Mean weekly food intakes for rat groups (g).

Liver Weights/Adjusted Liver Weights

Mean liver weights were significantly lower in the CRB (11.7 g ± 1.51) and 21S (11.5 ± 0.88) groups when compared to the C (13.6 g ± 0.65) group (p =0.019). These differences did not remain when liver weight was adjusted for body weight. Mean liver weights and mean adjusted liver weights (g) ± standard deviations are shown in Table 5.

Table 5. Rat groups, mean liver weights (g) and mean adjusted liver weights (g).[1]

Rat Group	Mean Liver Weight	Mean Adjusted Liver Wt.
ShC	12.2 ± 1.49	0.037 ± 0.0039
C	13.6 ± 0.65 [ab]	0.039 ± 0.0032
CRB	11.7 ± 1.51 [a]	0.033 ± 0.0026
C7S	12.6 ± 1.25	0.036 ± 0.0033
C14S	12.3 ± 1.58	0.034 ± 0.0029
21S	11.5 ± 0.88 [b]	0.033 ± 0.0031

[1] Values expressed as means ± SEM.
[ab] Means with the same superscript are significantly different ($p<0.05$)

Abdominal Fat Weight/Adjusted Abdominal Fat Weight

There were no significant differences in mean abdominal fat weight or mean adjusted abdominal fat weight among the groups. Numerically, the greatest abdominal fat weight was recorded in the 21S groups (17.7 g ± 5.09) and the lowest abdominal fat

weight was recorded in the C group (13.4 g ± 2.57). The same pattern of numerical results were recorded with adjusted abdominal fat weight (0.050 g ± 0.0129 and 0.038 g ± 0.0069, respectively). Table 6 contains mean abdominal fat weights and mean adjusted abdominal fat weights (g) ± standard deviations.

Table 6. Rat groups, mean abdominal fat weights (g) ± standard deviations, and mean adjusted abdominal fat weights (g) ± standard deviations.

Rat Group	Mean Abdominal Fat Weight	Adjusted Abdominal Fat Wt.
ShC	14.3 ± 3.93	0.043 ± 0.021
C	13.4 ± 2.57	0.039 ± 0.007
CRB	16.7 ± 6.33	0.041 ± 0.009
C7S	15.6 ± 2.09	0.044 ± 0.006
C14S	16.6 ± 4.96	0.046 ± 0.012
21S	17.7 ± 5.09	0.050 ± 0.013

Serum Total Cholesterol Levels/HDL-C Levels/Triglyceride Levels

There were no significant differences in serum total cholesterol levels among any groups. Numerically, the lowest total cholesterol levels were recorded in the C14Sgroup (160.9 mg/dL ± 34.60), and the highest levels were recorded in the CRB group (173.4 mg/dL ± 20.16).

There was a significant increase in the HDL-C levels in the CRB (55.8 mg/dL ± 19.64) and C7S (68.9 mg/dL ± 49.71) groups when compared to the ShC group (30.4 mg/dL ± 16.70) ($p = 0.016$).

There were no significant differences in mean triglyceride levels among groups. Numerically, the CRB group had the highest TG level (94.2 ± 32.69), while the 21S group had the lowest level (73.9 ± 12.79). Mean total cholesterol, HDL-cholesterol, and TG levels (mg/dL) ± standard deviations are shown in Table 7.

Table 7. Rat groups, mean total cholesterol levels (mg/dL), mean HDL-C levels (mg/dL), and mean TG levels (mg/dL).[1]

Rat Group	Total Cholesterol	HDL-Cholesterol	Triglycerides
ShC	192 ± 35	30 ± 17 [ab]	74 ± 19
C	176 ± 38	35 ± 9	75 ± 17
CRB	199 ± 64	56 ± 20 [a]	94 ± 33
C7S	192 ± 57	54 ± 24 [b]	76 ± 8
C14S	161 ± 29	42 ± 12	82 ± 20
21S	174 ± 20	50 ± 11	74 ± 13

[1] Values expressed as means ± SEM.

[ab] Means with the same superscript are significantly different ($p<0.05$)

CHAPTER 5

DISCUSSION

This research assessed the effect of increasing levels of soy protein or of a single concentration of rice bran oil on body weight, food intake, abdominal fat weight, liver weight, total cholesterol (TC), high-density lipoprotein (HDL) levels, and triglyceride (TG) levels of ovariectomized (OVX) retired breeder rats. In our study, there was no significant difference in overall food intake among the groups. There were no significant differences in final body weight or total weight gain among groups. No significant differences were found in absolute or body weight adjusted mean abdominal fat weights. Mean liver weights were significantly lower in the 21S and casein rice bran oil (CRB) groups than in the control (C) group; however, no difference was seen after adjusting liver weight for total body weight. No significant differences were found in mean TC or TG levels among groups. The group fed 7% soy (C7S) and the CRB group had significantly higher HDL levels when compared to those of the other groups.

The results of our study did not support the hypothesis that ovariectomy in rats would increase significantly total food intake. For all groups, intake was numerically highest during week one of this study and was lowest during week seven. This pattern has been seen before in our laboratory. In 2000, food intakes of OVX rats were examined for a 5-week period (83). Results suggested a dramatic decrease in food intake after week one of the study, and a relatively steady intake until the end of the study. The sharp decrease seen in the intake after week one may be due to the rats adjusting to the diet and to their surroundings. Rats were deprived of water and food during shipping, and this may explain the increased intakes in the first week of the study.

When intake was compared on a week-to-week basis, the only significant difference was shown between the 21S group the ShC group at week three. If viewed as a block, rather than in

separate groups, the OVX rats had mean intakes approximately 9-18 grams more than the ShC group at week 3. This was also seen by Stanciu (2000): in that study, sham operated rats ate less numerically than did OVX rats (83). This may be a direct result of the ovariectomy in the rats. Bellino (70) has shown that food intake is higher in OVX rats than non-OVX rats; this is attributed in part to the lack of hormone production seen with OVX. Numerically, the ShC had the highest intake during week one and had the greatest decline in intake for week two. For weeks 3-7, the ShC group had the lowest mean intake among groups. Overall findings are consistent with previous research done in our laboratory.

We hypothesized further that OVX would significantly increase final body weight and total weight gain in rats when compared to the sham-operated group. In the present study, however, no significant differences in final body weight or total weight gain were seen among groups. This finding does not support our original hypothesis; but, it does agree with a previous study in our lab in which OVX rats gained weight, regardless of dietary status, when compared to sham-operated rats (83). The differences in weight gain among groups were not significant, but the OVX block had higher numerical weights overall (83). In the present study, if all OVX rats are considered as a block, the final body weight of the OVX rats is approximately 20 grams higher than the ShC group. This is probably a result of OVX, because rats have been shown to have an increase in total body weight and weight gain after OVX (70).

The lack of significant results can be attributed, in part, to the large variability seen in the total body weight and weight gain, which in turn, is probably due to the small sample size. If the sample size were increased, our results may have been less variable and may have reached statistical significance. Further, the animals in our study were still growing, so differences in growth rates may have contributed to the variability. A wide range of body weights at baseline

may have added to the variability. Technical errors may have added to the variability. Lastly, the duration of the treatment period may not have been long enough.

The second part of our first hypothesis was that OVX would increase abdominal fat mass in rats when compared to the ShC group. There were no significant differences found among groups. Numerically, mean abdominal fat weights were highest in the 21S group, and lowest in the C group. The same pattern remained after adjustment for total body weight. If the OVX rats assigned to soy treatment diets are considered as a single block, mean abdominal fat weights are 3 to 4 grams higher than the ShC or C groups, suggesting that soy protein may increase abdominal fat mass. It is not clear whether OVX was the cause of the increase in abdominal fat weight in the soy groups, or if soy compounded the increase in abdominal fat in these groups. A dose-response effect was noted among the soy fed groups, with the C7S group having the lowest abdominal fat weights of the three and the 21S group having the highest abdominal fat weights of the three. The reasons behind the findings on abdominal fat weights are unclear; the most likely reason for our results is that there were no significant differences in body weights. Other studies, including those from our lab, have produced conflicting results (83-85).

It was also hypothesized that mean liver weights would be decreased in animals fed a soy containing diet or a rice bran containing diet. Mean liver weights for rats in the 21S group and the CRB group were significantly lower than the mean liver weight for the C group. When liver weights were standardized using the body weight of the animal, no differences in mean liver weights were observed. Thus, the increase in liver weights was related to the body sizes of the rats. This differs from the findings of Madani and co-workers (1998); they showed that liver weights were higher in rats fed a casein diet with added cholesterol when compared to a soy protein diet (75). That group of investigators worked with 5-week old male Wistar rats; this may

account for the differences observed between the studies. In our study, livers were excised, weighed, and frozen with the original intent of measuring liver cholesterol and triglyceride levels. Thus was not done for our study.

It was hypothesized further that increasing levels of soy protein in the diet would decrease TC levels in a dose-dependent fashion. When considering the impact that soy protein could have on cholesterol levels, an alternate hypothesis could have been that there would be a plateau-effect, with a maximum cholesterol lowering effect seen at either the lowest or intermediate level of soy intake. It was also hypothesized that a single concentration of rice bran oil in the diet would decrease TC levels. None of the hypotheses were supported by our data, since no significant differences in TC levels were found among the groups. Numerically, the C7S animals had the highest mean TC levels and were virtually identical to the ShC group. Animals in the C14S group had the lowest mean TC levels; and 21S animals had a TC value between the two levels. Overall, rice bran oil fed rats had the highest mean TC levels.

It was also hypothesized that increasing levels of soy protein in the diet would increase HDL levels, again in dose dependent fashion. It was hypothesized further than rice bran oil would increase HDL levels. There was a significant elevation in mean HDL levels in rats fed a diet containing 7% soy protein, when compared with sham-operated controls. Mean HDL levels in rats fed 7% soy were also elevated when compared with the C group; however, this difference was not significant. Rats fed rice bran oil also had significantly elevated mean HDL levels when compared with the ShC group. These findings suggest that soy protein or rice bran oil is effective in increasing HDL levels in OVX rats.

Lastly, it was hypothesized that increasing levels of soy protein would lower TG levels in a dose-dependent fashion. Due to the high amount of variability in TG values for the groups,

this hypothesis could not be supported. No significant differences were found among groups.

Rats, with the exception of Obese Zucker rats (81), are seldom used for studies of CHD since they are not ideal models for the study of cholesterol levels and hyperlipidemia (74). The predominant cholesterol found in rats is HDL-C, which allows rats to resist diet-induced LDL and TC elevations (74). Rats are also very resistant to the development of atherosclerotic lesions when placed on high-lipid diets (73). Lesions in the coronary arteries of rats are difficult to produce, due to the lack of intimal cell proliferation, and complicated lesions with hemorrhage, thrombosis, and resulting MI are rare (73). As has been stated previously, the rat was used as a model in this study, as this was an ancillary project in a study designed to look at the effects of soy protein on bone.

There are studies, however, that have been published looking at the effect of dietary soy proteins or soy protein isolates on lipid levels in rats (37, 75-82, 86). The study of Choi and co-workers (1989) demonstrated that in adult rats, soy protein was effective in lowering serum cholesterol levels and triglyceride levels in adult rats fed a cholesterol containing chow.

To maximize the likelihood that there would be a soy effect on cholesterol levels in our study, all modified AIN-93M diets were made hypercholesterolemic, by the addition of 0.05% cholesterol, 0.02% cholic acid and 5% Crisco. For studies like ours, it has been recommended that the cholesterol content be sufficient to challenge the capacity of cholesterol synthesis and metabolism to accommodate the cholesterol load (74). The amount of cholesterol used to challenge cholesterol synthesis and metabolism pathways should not to exceed 5 to 10 times the amount of cholesterol synthesized endogenously by the rat (74). Mean TC levels of all groups of rats, including the control groups, in this study were approximately three times the normal values (44 to 78 mg/dL) for rats (74). Clearly, the goal of reaching the desired hypercholesterolemic

levels was reached.

Few studies examining the effect of soy protein in lipid levels have been performed in OVX rats (83, 84). The study by Kikuchi-Hayakawa and co-workers (1998) demonstrated that OVX in Wistar rats increased TC levels by 40% compared with sham-operated animals (84). When rats were fed fermented soy milk for 6 weeks, there was a 20% increase in TC levels when compared with control animals. These data suggested that soy could induce a decrease in mean TC levels. In a study of 11-week old Sprague Dawley rats (83), glycitin and daidzin prevented an increase in serum TC levels. The latter studies suggest that soy may be a potential treatment for hyperlipidimia associated with menopause.

Taken together, these studies suggest that, although not the ideal animal model for lipid metabolism, rats were at least adequate models. Results from our study suggest, however that this may not be the case. There are differences, however, between our study and other published studies using the rat model for lipid studies. It could be hypothesized that the age, strain, or gender of the rat used, or the concentration of soy, or the actual soy component used in our study was not optimal. Moreover, the cholesterol levels seen in our animals were considerably higher than those in the other reports; it may have been that dietary cholesterol and fat levels were too high and they simply "overwhelmed" lipid metabolism in the study animals. Finally, the reason that lack of significant differences, for TC an TG, and for most groups, for HDL levels, may be the large variability around the mean in the assay used to quantitate these lipid levels.

Conclusions

These data suggested that there were no differences in mean total cholesterol or triglyceride levels among the soy fed or rice bran oil fed animals, when compared with each other or to either the sham-operated control or the ovariectomized rat control. Casein with 7%

soy protein added or rice bran oil did increase high-density lipoprotein levels when compared with the sham-operated control group.

Future Directions

In future studies, a more suitable model for blood lipids, such as hamsters, should be used. Sample sizes should be larger to account for any intra-group variation, and to account for variation among groups. Further, diets should be less hypercholesterolemic so the potential effect of soy or rice bran oil will not be "overwhelmed" by the high fat content. A longer treatment period should be used to allow for a longer time for animals to adjust to our lab and the novel diet; this may allow a more pronounced effect to occur as a result of the treatment diets. Lastly, the same individual should conduct all lipid analyses to control for any variability due to differences in techniques.

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