EFFECT OF DIETARY PROTEIN OF PLANT AND ANIMAL ORIGIN ON PLASMA, AORTIC AND LIVER LIPID LEVELS AND PLASMA LIPOPROTEIN FRACTIONS IN THE JAPANESE QUAIL

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IN THE JAPANESE QUAIL

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CHAPTER I

INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death and disability in the United States and other industrialized countries (Consensus Conference, 1985; Lipid Research Clinics Coronary Primary Prevention Trial Results, 1984; Guyton, 1981). From the mid-1940's until the mid-1960's, the drastic increase in CHD indicated that the disease had the potential of becoming the greatest epidemic of mankind (Woolf, 1982; Atherosclerosis Study Group, 1970). The National Health Examination Survey of 1960-62 estimated that 3.1 million Americans between the ages of 18 and 79 had definite CHD, while another 2.4 million were suspect. Among persons under the age of 65, nearly 1.8 million diagnoses were definite and 1.6 million were suspect (Atherosclerosis Study Group, 1970). Currently, more than 5.4 million Americans have symptomatic heart diseases and there are many undiagnosed cases (Consensus Conference, 1985).

Between 1970 and 1974, the annual CHD mortality rate exceeded one million, but fell to 962,000 by 1978 (News From the American Heart Association: AHA Committee Report, 1980). In the United States alone, more than one million heart attacks occur annually, resulting in a death rate of more than 550,000. Despite recent declines in mortality rates, CHD remains the major cause of death and disability in countries with a high standard of living such as the United States, Canada, Great Britain and Scandinavia. CHD accounts for more annual deaths than any other disease and costs the United States an estimated 60 billion dollars each year in direct and

indirect costs (Lipid Research Clinics Coronary Primary Prevention Trial Results, 1984; Consensus Conference, 1985; Brisson, 1981).

CHD is not a random occurrence, but rather, individuals at high risk are characterized by certain traits or habits. Among the most clearly defined risk factors associated with atherosclerotic coronory heart disease are elevated plasma cholesterol, hypertension and cigarette smoking (Consensus Conference, 1985). Other risk factors include diabetes, obesity, lack of exercise, behavior pattern, male sex, age, genetics and environmental factors (Ernst and Levy, 1984; News From the American Heart Association: AHA Committee Report, 1980).

The basic pathophysiologic condition underlying CHD is atherosclerosis. The primary characteristic of the atherogenic process is the accumulation of lipids, particularly cholesterol, in the walls of medium and large arteries (Atherosclerosis Study Group, 1970; Brisson, 1981; Woolf, 1982). The disease is one of slow and silent progression, with the most devastating effects on the coronary arteries. Symptoms may not appear until as much as two-thirds of the vessel lumen is occluded by plaque, and the capacity of the vessel to dilate becomes limited. Without warning, angina, heart attack or sudden death may occur. For approximately one-fourth of individuals with coronary atherosclerosis, sudden death is the first and final manifestation of the disease (Consensus Conference, 1985; Ruch and Patton, 1966).

The major etiological suppositions of atherosclerosis and CHD are the injury, transformation and lipid hypotheses. The injury hypothesis maintains that the atherogenic process is initiated when the arterial endothelium becomes damaged by high blood pressure, elevated plasma cholesterol or viral infection. This results in formation of scar tissue and the accumulation of fibrous plaque that protrude into the vessel lumen, causing

complete or partial blockage of blood flow (Time Magazine, 1984; Guyton, 1981; Olson, 1979). According to the transformation hypothesis, smooth muscle cells migrate to the damaged endothelium and undergo uncontrolled proliferation. This multiplication is caused by a carcinogen-induced transformation. Over a period of years, the mass of smooth muscle cells result in vessel occlusion, leading to heart attack or stroke (Olson, 1979; Time Magazine, 1978). The lipid hypothesis maintains that elevated plasma lipids, especially cholesterol, accumulate in the vessel wall, causing CHD (Olson, 1979; Kritchevsky, 1976).

Epidemiological and experimental studies have revealed a direct correlation between the incidence of CHD and blood cholesterol levels. Persons who migrate to a country whose inhabitants have a higher mean cholesterol level gradually acquire the dietary habits, blood cholesterol concentration and CHD rate characteristics of that population (Consensus Conference, 1985; Nichols et al., 1976; News From the American Heart Association: AHA Committee Report, 1980). A number of animal models, including nonhuman primates, develop atherosclerosis as a result of hypercholesterolemic diets. Long-term studies have shown that hypercholesterolemic primates, rabbits, turkeys and other species develop atherosclerotic lesions that progress from simple fatty streaks to complicated plaques resembling those in humans (Scott et al., 1967; Morrissey and Donaldson, 1977; Guyton, 1981).

Hypercholesterolemia is sometimes familial. A genetic basis for concentration of blood cholesterol has been reported in various animals. Heritability for blood cholesterol lies between 0.17 and 0.34 in chickens; between 0.68 and 0.99 in beef cattle; and is estimated at about 0.92 in squirrel monkeys (Arave, Miller and Lamb, 1973; News From the American Heart Association: AHA Committee Report, 1980). Children with inherited

hypercholesterolemia exhibit accelerated and severe CHD because they lack specific receptors that remove cholesterol (carried by low density lipoproteins or LDL) from plasma. As a result, these children have high cholesterol levels from birth and often die in early childhood. CHD, accompanied by less severe cholesterol elevation, may be due to congenital deficiencies of functioning LDL receptors, as well as to deficiencies indicated by dietary and lifestyle factors (Consensus Conference, 1985).

Although the range of cholesterol concentration in the general population is wide and influenced by genetic factors, the high average level is generally of dietary origin. Many studies involving humans and experimental animals have indicated that serum cholesterol concentration can be increased by saturated fat and cholesterol and reduced by lowering their intake (Kritchevsky, 1976; Lipid Research Clinics Coronary Primary Prevention Trial Results, 1984; AHA Committee Report, 1980; Consensus Conference, 1985).

The association between elevated plasma cholesterol and increased CHD risk is derived mainly from the plasma LDL fraction which transports most of the circulating cholesterol. While increased LDL-cholesterol consistently shows a positive correlation with the incidence of CHD, increased concentration of high density lipoprotein (HDL) cholesterol shows a negative correlation. Thus, LDL and HDL fractions are of clinical significance in determining CHD risk (News From the American Heart Association: AHA Committee Report, 1980).

Arterial diseases and increased CHD risk may involve triglycerides as well as cholesterol (Martin, Mayes and Rodwell, 1983; News From the American Heart Association: AHA Committee Report, 1980). The very low density lipoprotein fraction (VLDL) which transports most of the body's endogenous triglyceride may be related to CHD through its association with reduced

HDL, obesity and diabetes. The metabolism of VLDL and HDL appears to be linked, and an independent contribution of VLDL or triglycerides to CHD incidence has not been established to a degree of consistency for general populations (News From the American Heart Association: AHA Committee Report, 1980).

Since nutritional factors are recognized as major contributors to hypercholesterolemia and atherosclerosis, the first step in the treatment of high-risk individuals is diet therapy and caloric restriction. High-risk individuals are identified as having plasma cholesterol levels above the 95th percentile. The American Heart Association and the Consensus Conference Committee recommended that all Americans follow a "prudent" diet that reduces total dietary fat intake from the current level of 40% to 30% of total calories, reduces saturated fat intake to less than 10% of total calories, increases polyunsaturated fat intake to no more than 10% of total calories and reduces daily cholesterol intake to about 300 mg or less (Dairy Council Digest, 1984a; Consensus Conference, 1985). The American public consumes about three times more saturated fatty acids than polysaturated fatty acids, and about 266 mg (females) or 405 mg (males) cholesterol per day (Dairy Council Digest, 1984a).

In assessing the effects of dietary components on hypercholesterolemia, the majority of studies have focused on dietary fats (Kritchevsky, 1976; Carroll, 1978). More recently, dietary protein has been recognized for its ability to alter plasma lipids (Carroll, 1978; Kritchevsky, 1976; Sacks et al., 1983; Forsythe et al., 1980; Hevia and Visek, 1979; Terpstra and Sanchez-Muniz, 1981). The focus, however, has been mainly on qualitative rather than quantitative aspects. Evidence indicates that both are important factors (Carroll and Hamilton, 1975: Terpstra, 1981).

The objective of this research is to determine the effects of both quality and quantity of dietary protein on plasma, aorta and liver lipids and on the concentration of lower density lipoprotein fractions in plasma, using the Japanese quail as the experimental model. It is generally accepted that animal protein in the diet elevates plasma cholesterol while plant protein lowers cholesterol, with a direct relationship existing between level of protein in the diet and cholesterol concentration (Carroll, 1981; Kritchevsky, 1976; Park and Liepa, 1982; Mol et al., 1982; Howard et al., 1965). In contrast, Terpstra et al. (1983) reported that hypercholesterolemia is prevented in chickens when a high level of protein, whether of plant or animal origin, is incorporated into cholesterol-enriched diets. In chickens fed the low protein diets, the excess plasma cholesterol was carried mainly in the VLDL fraction. Plasma triglycerides and phospholipids were also found to be inversely related to the concentration of the dietary protein.

Although a link between dietary protein and lipid metabolism has been established, underlying mechanisms have not been elucidated. Speculations focus on mode of protein digestion, alteration in amino acid sequence, alteration in cholesterol synthesis, fecal steroid excretion, effect of undigested protein on bile acids, cholesterol turnover rate and alterations in activity of the lecithin: cholesterol acyltransferase (LCAT) enzyme (Terpstra et al., 1983; Hevia and Visek, 1979; Carroll, 1981).

CHAPTER II

REVIEW OF LITERATURE

Introduction

The etiology of atherosclerosis is multifactorial; however, elevated serum cholesterol represents one of the major risk factors associated with the development of coronary heart disease (Consensus Conference, 1985; News From the American Heart Association: AHA Committee Report, 1980; Committee Report, 1980; Research Clinics Coronary Primary Prevention Trial Results, 1984; Ernst and Levy, 1984). Because nutritional factors are recognized as important contributors to the development of hyperlipidemia, dietary changes have become major regimens in clinical practice, representing the first step in the control of hyperlipidemia (Consensus Conference, 1985).

The earliest report that implicated dietary components in the atherogenic process was made by Ignatowski in 1909. As a result of feeding eggs, meat and milk to rabbits, athersclerotic lesions, cirrhosis and anemia were observed. Ignatowski proposed that the disorders were pathological manifestations of the protein in the diet.

The fact that the diets were cholesterol-rich, the prior observation by Vogel that lesions contained excess cholesterol and the subsequent report by Anitschow and Cholatow that cholesterol per se was atherogenic, caused investigators to focus on cholesterol rather than protein as a cause of atherosclerosis (Brisson, 1981; Kritchevsky, 1976; Kritchevsky and Czarnecki, 1982). Dietary protein was definitely linked to the disease when

hypercholesterolemia and lesion development were induced in rabbits as a result of feeding cholesterol-free casein diets (Lambert et al., 1958; Malmros and Wigand, 1959).

Meeker and Kesten (1940, 1941) demonstrated that dietary protein of animal and vegetable origin exert different atherogenic effects. When rabbits were fed diets containing 38% protein (with or without added cholesterol) for six months, the casein diets were more atherogenic in the absence of cholesterol than the soy protein diets that incorporated cholesterol. Hamilton and Carroll (1976) supported this observation by demonstrating that a variety of animal proteins could induce atherosclerotic lesions in rabbits.

The mechanisms underlying the effects of dietary protein on lipid metabolism have not been elucidated. However, it has been shown that rabbits fed soy protein absorb less cholesterol and excrete more cholesterol than casein-fed rabbits (Huff and Carroll, 1980). Kritchevsky and Czarnecki (1982) have suggested that the lysine:arginine ratio, which is high in animal protein and low in plant protein, may account for the differential effects of soy protein and casein.

It has been demonstrated that dietary protein influences cholesterol synthesis. When the level of protein was increased in a diet fed to chickens, Yeh and Leveille (1972) observed increased cholesterol synthesis, decreased plasma cholesterol concentration and decreased fatty acid synthesis. Increased cholesterol synthesis might stimulate a higher rate of cholesterol degradation to bile acids, thereby reducing cholesterol concentration in plasma.

The type of protein in the diet may interact with other dietary components, such as fiber, to alter lipid metabolism. Semi-purified cholesterol-free diets containing casein and cellulose were 20% more

atherosclerotic in rabbits than diets containing soy protein and cellulose. When alfalfa replaced cellulose, the atherogenic effects of soy protein and casein were virtually the same (Hamilton and Carroll, 1976).

In addition to protein, fat and carbohydrate, other dietary constituents known to alter plasma lipids include plant sterols, lecithin, alcohol, certain vitamins and minerals. The total interactions of these constituents and their effects remain to be determined (Ernst and Levy, 1984).

Histopathology of Atherosclerosis

Structural Features of the Arterial Wall and Lesion Location

Atherosclerosis affects the arterial system of the body. According to DeBakey (1978), the main arterial beds affected are the: (1) coronary arteries, (2) major branches of the aortic arch, (3) celiac axis, superior mesenteric and renal arteries and (4) terminal abdominal aorta and its major branches. Lesions vary at different ages, in different races and sexes and even in the same individual (Gresham, 1981). Arteriographic examination of more than 15,000 patients have led to the recognition that clinical patterns and rates of atherosclerotic progression differ in the various areas of localization. In some patients, there is no evidence of further progression following corrective surgery. This suggests that atherosclerosis is not necessarily a continuously progressive disease. Rather, it may progress slowly to a certain point and is then retarded or arrested (DeBakey, 1978).

The arterial walls consists of the tunica intima, tunica media and tunica adventitia. The intima begins at the luminal edge. It consists of:

(1) a single layer of endothelial cells that line the vessel, (2) a subendothelial layer of collagenous and elastic fibers with few smooth muscle cells and (3) the internal elastic lamina which is a fenestrated band of elastic fibers forming a boundary between the intima and the media. The media is the thickest layer of the artery wall with elastic fibers predominating in large arteries and smooth muscle cells being more abundant in medium arteries. The adventitia or the outermost layer is a loose network of connective tissue fibers containing blood vessels, nerves and lymphatics (Krause and Cutts, 1981; Woolf, 1982).

The process leading to atherosclerosis involves proliferation of smooth muscle cells within the intima, production of collagen and elastic fibers by the smooth muscle cells and accumulation of lipid within the intima (Woolf, 1982). The disease is generally asymptomatic until lesions are large enough to cause ischemia (Blankenhorn, 1984).

Histological Aspects of Fatty Streak and Fibrous Plaque Lesions in the Arterial Wall

The earliest form of lesion most commonly observed is the fatty streak, which may appear throughout elastic and muscular arteries in early childhood (Blankenhorn, 1984). Although fatty streaks differ in terms of intracellular and extracellular lipid concentration, amount of connective tissue and prevalance of smooth muscle and foam cells, they are generally characterized as flat or slightly raised lesions of the arterial intima.

According to McGill (1974), variation in fatty streak lesions occurs at different stages of life. The "juvenile" streak generally occurs in childhood and adolescence. Usually, the lipid content is intracellular and there is little to no accumulation of new connective tissue. A second type of fatty streak, prevalent in young adults, contains a large amount of

extracellular lipid and increased connective tissue elements. This type lesion is often considered a progressing lesion, and therefore, a precursor of the more severe fibrous plaque. The third type is common among persons of middle-age or older. It is characterized by diffuse lipid infiltration, few cells and little extracellular lipid.

In some species, fatty streaks occur spontaneously. Baboons, monkeys, swine and newly hatched chicks may show varying degrees of fatty streaks, but have normal lipid levels. This indicates that hyperlipidemia is not a requirement for lipid deposition with the arterial intima (McGill, 1974)

Gelatinous lesions and microthrombi are also early forms of atherosclerotic lesions. Gelatinous lesions, which appear at the periphery of fatty streaks, are translucent, pinkish, blister-like elevations. They are considered the intermediate stage between the fatty streak and the plaque lesion (Gresham, 1981; Woolf, 1982; Blankenhorn, 1984). Microthrombi are minute aggregations of platelets and fibrin that form at the site of injury as a part of the normal hemastatic mechanism. Small microthrombi are completely absorbed, whereas larger ones can lead to an accumulation of subendothelial fat.

Although fatty streaks, gelatinous lesions and microthrombi are reversible, they are often considered the principal pathways to more advanced lesions (Blankenhorn, 1984). According to McGill (1974), the extent of coronary artery fatty streaks in young Caucasians parallels the extent of plaque formation in older persons of the same group. Young adults show a prevalence of lesions having characteristics of both fatty streaks and fibrous plaques.

Foam cells, containing fat droplets, are common in atherosclerosis (Geer and Haust, 1972). At the fatty streak stage, monocytes (macrophages) make up most of the foam cell population, whereas modified smooth muscle

cells predominate in foam cells of fibrous plaques (Adams and Bayliss, 1976; Woolf, 1982). According to Gerrity (1981), the efficiency of a monocyte clearing system determines the degree of atherosclerosis. Upon entering the arterial intima, the monocytes engulf lipid and become foam cells. Some of these lipid-rich cells migrate back to the blood stream, decreasing the monocyte:foam cell ratio. When a 1:1 ratio is achieved, the lesions do not progress further. Gerrity further proposed that advanced plaques do not show these features and their formation may partially result from failure of the monocyte clearing system.

The raised lesion (fibrous plaque) is the precursor of arterial occlusion. The typical fibrous plaque consists of a core of lipid surrounded by smooth muscle and connective tissue. The lipid core contains mainly cholesterol (McGill, 1974; Sabine, 1977; Woolf, 1982). Plaques are more common in the abdominal than in the thoracic aorta and are elevated above the surface of the intima (Woolf, 1982). As these raised lesions increase in size, cholesterol esters accumulate more rapidly and form crystals that interfere with healing. As growth occurs, the lesion may become embedded within the media, replacing normal smooth muscle cells. Raised lesions also grow into the vessel lumen, eventually obstructing blood flow (Blankenhorn, 1984).

Fibrous plaques begin to develop in the abdominal aorta and coronary arteries in the third decade and in the cerebral arteries around the fourth decade of life (McGill, 1974). When at least 60% of the surface of the coronary arteries is covered by lesions, various events such as thrombosis, plaque rupture and embolization can cause myocardial infarction or sudden death (Blankenhorn, 1984).

In severe atherosclerotic disease, fibrous plaques may become calcified. Eggen (1968) associated the extent and severity of calcification

with the severity of atherosclerosis. Calcification is often associated with the lipids of the arterial media. Since the media receives little to no blood supply from the vasa vasorum, it is vulnerable to ischemia. Also, thickening of the intima impedes the diffusion of nutrients from the plasma. Therefore, cell necrosis commonly occurs within the media and might enhance the calcification process.

Implication of Intimal Thickness in the Atherogenic Process

Although not firmly established, there is evidence that diffuse intimal thickness predisposes to atherosclerosis (Woolf, 1982; McGill, 1974). In comparing the histopathological features of the human fatty streak with experimentally induced early lesions of the rhesus monkey, the major difference was in the type of cell that contained the lipid. In young humans, smooth muscle cells were the predominant lipid-containing cells, whereas macrophages were the lipid-rich cells in hypercholesterolemic monkeys. Duration and extent of plasma cholesterol elevation may have contributed to the difference.

In humans, intimal thickness becomes evident during the first year of life. However, changes leading to its onset may begin as early as the 34th week of gestation (Woolf, 1982). The initial event is the splitting of the internal elastic lamina, followed by the migration of smooth muscle cells, collagen, elastic fibrils and mesenchymal cells into the open spaces (Woolf, 1982; Gresham, 1981). By the end of the second decade, the intima of the coronary arteries equals the thickness of the media. Similar changes occur in the abdominal and thoracic aorta, but at a later age in the thoracic aorta (McGill, 1974).

The aortic intima is usually well developed by age 25. There is, however, a steady increase in thickness that seems to be age-related until around age 40, when a more variable pattern becomes evident (Woolf, 1982). In the coronary arteries, fibromuscular intimal thickness prior to age 20 is considered the normal process, representing an adaptation to organ growth and increased blood flow (McGill, 1974).

Generally, arteries that are sites of severe atherosclerosis are those that show a high degree of intimal proliferation. The aortic intima, which is not uniform in thickness, is thickest at those sites which are prone to lesion development. The iliac arteries show more extensive intimal thickening than the carotids or innominates when compared in the same individual. A similar pattern has been observed in the tibial and radial arteries (Woolf, 1982).

A probable cause of non-atherosclerotic intimal thickness is hemodynamic stress. Focal thickness, commonly observed at the branching points of arteries, and endothelial damage have contributed to the speculation that alterations in the physical processes of blood flow are important in intimal changes (McGill, 1974; Woolf, 1982). Nicosia, Krouse and Mobini (1981) linked hemodynamic stress with endothelial damage by studying newborn twins afflicted with placental transfusion syndrome. The hypervolemic twin showed pronounced intimal thickness in the aorta and iliac arteries, whereas the hypovolemic twin showed no abnormal thickness of the intima.

Endothelial Damage and Atherogenesis

As early as 1856, Virchow proposed that atherosclerotic lesions result from injury to the arterial wall (Ardlie, 1981). It is now well established that endothelial damage and subsequent atherosclerosis can be induced by physical, chemical, immunological and nutritional factors.

Physical methods of endothelial damage include cauterization, pinching the arterial wall, freezing, heating, exposure to X-rays and endothelial abrasion. The degree of injury is important in tissue response. Small injured areas in rabbits and rats show complete healing without thrombi formation, while large injured areas are often complicated by thrombi that interfere with healing. It has been observed in normocholesterolemic rabbits that delayed tissue repair results in lesion formation similar to early atherosclerosis in humans (Vesselinovitch and Fischer-Dzoga, 1981)

Schwartz, Stemerman and Benditt (1975) observed that endothelial injury was followed by platelet aggregation, accumulation of modified smooth muscle cells and delayed healing. It is possible that injury stimulates proliferation of smooth muscle cells that retard the healing process.

The arterial wall may be injured by hyperactive platelets that interact with subendothelial cells upon entering the vessel wall. These platelets are characterized by increased sensitivity to aggregating agents, increased tendency to aggregate spontaneously, increased coagulation activity and altered prostaglandin synthesis (Ardlie, 1981). Lesion formation may result from the stimulatory effect that platelet products and plasma constituents have upon the migration and proliferation of smooth muscle cells. Platelets may also participate in atherogenesis by forming thrombi on pre-existing lesions, thereby increasing lesion severity and causing further obstruction of blood flow. Both thrombosis and atherosclerosis are pathological counterparts of hemostasis resulting when platelet plugs and masses of fibrous tissue form at undesirable locations within the blood vessels (Woolf, 1982; Ardlie, 1981).

Endothelial damage alters endothelial permeability (Woolf, 1982; Gresham, 1981). Altered permeability is believed to disrupt the structural and metabolic barrier against the influx of plasma components, allowing

these constituents to gain access to the arterial intima. Among the infiltrating substances are the beta-lipoproteins that stimulate proliferation of smooth muscle cells. Smooth muscle cells, which produce collagen and elastin, are the central figures in atherogenesis (Ardlie, 1981).

Among the chemical stimulants known to induce atherosclerosis are certain hormones, enzymes, surfactant, turpentine, calciferol and products of cigarette smoke (Vesselinovitch and Fischer-Dzoga, 1981). Smoking has been identified as a major risk factor in cardiovascular disease, but the effects on the endothelium are not well established. When rabbits and rats were exposed to smoke, Woolf (1982) observed swelling of the endothelial cells, appearance of processes on the luminal membrane and formation of microthrombi. Frost (1973) observed a loss of endothelial cells when rabbits were exposed to cigarette smoke. In children borne of mothers who smoked, the endothelium appeared blister-like and edema was evident in the subendothelial cells (Woolf, 1982).

Smoking has been reported to decrease plasma high-density lipoprotein cholesterol (HDL-C) by approximately 4 mg% in sedentary men and 6 mg% in sedentary women; the degree of reduction being proportional to the degree of smoking (Blankenhorn, 1984). Accelerated interconversion of very low-density lipoprotein particles to low-density lipoproteins and increased concentrations of plasma-free fatty acids are among the known effects of cigarette smoking (Woolf, 1982).

A number of investigators have shown that endothelial injury does not play a significant role in the initial stages of atherosclerosis (Klimov and Nagornev, 1983; Stemerman, 1981; Davies et al., 1976). Electron microscopic examination has revealed that some areas of endothelium are more prone to infiltration by plasma components than other areas (Klimov and Nagornev, 1983). Repin et al. (1982) observed that areas of the arterial

wall having a density of 300 to 500 cells per square millimeter exhibit maximal predisposition to atherosclerosis compared to areas having a density of 2,000 to 2,500 cells per square millimeter. These areas did not coincide with areas of injured endothelium. In contrast to findings by Repin, other studies have demonstrated a greater accumulation of cholesterol in areas of regenerating endothelium than in areas showing endothelial loss (Minick, Sterman and Insull, 1977; 1979). Stemerman (1981) demonstrated that hypercholesterolemia in rabbits causes disturbance in functional activity of endothelial cells in the absence of endothelial injury.

Excess deposition and accumulation of macromolecules, such as LDL, in the arterial wall may be due to alteration in the intercellular junctions of endothelial cells (Pfeffer et al., 1980; Weinbaum et al., 1980). In some areas, endothelial cells contact each other by tight junctions, whereas in other areas, gap junctions are present. These latter junctions contain spaces three to four nanometers in diameter and are involved in the intercellular transport of ions and low molecular weight metabolites (Huttner, Boutet and More, 1973). In certain pathological conditions, particularly hyperlipidemia, activity of the gap junctions increase. This change may be due to structural rearrangement of the endothelium and to opening of the interendothelial junctions (Klimov and Nagornev, 1983).

In the arterial wall, gap junctions serve as a structural basis that enables the endothelium to function as a barrier. If these junctions are widened, changes in permeability occur, with increased capacity for lipoprotein uptake and cholesterol accumulation (Vlodavsky et al., 1978; Vlodavsky, Johnson and Gospodarowicz, 1979). Opening of the intercellular junctions can be accomplished by contraction of endothelial cells and enhanced by infiltrating monocytes (Klimov and Nagornev, 1983). It is

possible that a number of risk factors may act through the widening of interendothelial junctions.

Relation Between Lipid Transport, Lipoprotein Metabolism and Hyperlipidemia

Lipoprotein Classes

The major families of plasma lipids are cholesterol and cholesterol esters, triglycerides, phospholipids and fatty acids. Except for the fatty acids, which are transported in a complex with alubumin, the plasma lipids are transported by lipoproteins (Gotto, 1984).

The lipoproteins are aggregates of protein and lipid that solubilize in plasma. The clinically important classes are the: (1) high-density (HDL) or alpha lipoproteins, which transport mainly phospholipid and cholesterol; (2) low-density (LDL) or beta lipoproteins, carrying one-half to two-thirds of the total plasma cholesterol; (3) very low-density (VLDL) or pre-beta lipoproteins and (4) chylomicrons, which are the body's major triglyceride carriers. The intermediate-density lipoproteins (IDL) are products of VLDL metabolism that are further hydrolyzed to LDL (Fredrickson, Levy and Lees, 1967; Woolf, 1982; Gotto, 1984). The basic lipoprotein structure consists of a core of neutral lipid, cholesterol ester and triglyceride surrounded by an outer coat of protein, phospholipid and cholesterol (Gotto, 1981).

The protein components (apoproteins) of the lipoproteins are synthesized within the liver and the intestine. They aid in maintaining lipoprotein structure and in regulating metabolic processes. The maintenance of lipoprotein structure may be mediated through binding of an apoprotein with a phospholipid to form a stable complex that can solubilize and, therefore,

carry out the transport function. Certain apoproteins activate enzymes involved in regulating lipid metabolism. Lecithin cholesterol acyltransferase (LCAT), under the influence of apoprotein A-I or C-I, catalyzes the formation of cholesterol ester. Apoprotein C-II activates lipoprotein lipase, which hydrolyzes triglyceride. In cases of apoprotein C-II deficiency, plasma triglyceride level is severely elevated (Gotto and Jackson, 1981; Gotto, 1984; Davidsohn and Henry, 1974).

The principal apoproteins of plasma lipoproteins are A, B, C, D, and E. The A apoproteins (A-I, A-II and A-IV are found largely in HDL and, to a lesser extent, in chylomicrons. Apoproteins of the B subgroup (B-48 and B-100) are predominantly associated with LDL. The C's (C-I, C-II and C-III) are found in chylomicrons, VLDL and HDL, with C-III being present also in LDL. Trace amounts of apoproteins D and E are present in HDL; E is also found in FLDL and LDL. The major apoproteins of IDL are B, C, and E (Nestel and Fidge, 1981; Gotto and Jackson, 1981; Gotto, 1984).

The density of lipoproteins is proportional to protein content. HDL is made up of approximately 50% protein and the density ranges between 1.063 and 1.21. LDL, which contains at least 25% protein, has a density between 1.006 and 1.063. The protein content and density range of VLDL are approximately 15% and 0.94-1.006, respectively, while chylomicrons contain only about 2% protein with density being less than 0.94. The density of IDL lies between 1.006 and 1.019 (Burstein and Legmann, 1982; Brisson, 1981; Christie, 1982; Nestel and Fidge, 1981).

Distribution of Cholesterol, Phospholipid and Triglyceride in the Atherosclerotic Artery

A large number of epidemiological, clinical and experimental studies have provided evidence that the concentration of plasma lipids plays a

centrol role in the development and progression of atherosclerosis. The basis for the association stems from the following results: (1) lesions of the vessel wall contain lipid concentration that exceed lipid levels in normal areas of the intima; (2) individuals with clinical evidence of atherosclerosis have higher levels of plasma cholesterol, and often triglyceride, than those without clinical manifestation and (3) dietary induction of hyperlipidemia in experimental animals can result in development of lesions with a high content and some histological resemblance to human atherosclerosis.

The Consensus Conference (1985) pinpointed elevated blood cholesterol as a major cause of CHD. When cholesterol level in persons above age 20 averages 200-240 mg/dl, they are at moderate CHD risk, while those with cholesterol levels between 220 and 260 mg/dl are at high risk.

The total lipid content of the artery wall normally increases with age. During childhood and adolescence, the artery wall contains about 35% total cholesterol, with nearly equal portions existing in the free and esterified form. The main cholesterol ester is cholesterol-oleate (Woolf, 1982). Phospholipid and triglyceride concentrations average 42 and 25%, respectively (Sabine, 1977). After age 20, esterified cholesterol increases rapidly, resulting in a concentration that is 42% of the total lipid by age 40. Cholesterol-linoleate makes up at least 35% of the esterified cholesterol. Phospholipid and triglyceride levels decline (Woolf, 1982; Sabine, 1977).

In all types of atherosclerotic lesions, cholesterol increases drastically, reaching levels as high as 65-80% of the total lipid concentration. Most of the cholesterol in the fatty streak is esterified to oleic acid and to linoleic acid in the fibrous plaque.

The major phospholipids present in mammals are the lecithins, cephalins and sphingomyelins, which are largely formed in the liver. Their functional roles include involvement in lipoprotein synthesis, structure and function of nerve tissue and formation of cell membranes (Guyton, 1981). The total phospholipid content within the artery wall shows a significant increase with age until the fourth decade (Woolf, 1982). Phosphatidyl choline is synthesized in greatest amounts, with sphingomyelins being formed in smallest quantities. However, higher concentrations of spingomyelin appear in lesions (Newman and Zilversmit, 1966; Nakatani et al., 1967; Woolf, 1982). The accumulation may be due to enzyme inhibition (Eisenberg, Stein and Stein, 1969).

The role of phospholipid accumulation in atherosclerosis is unclear (Woolf, 1982). It has been hypothesized that an increase in phospholipid synthesis may guard against the dispersal of infiltrated cholesterol (Adams, 1967; Rossiter and Strickland, 1960).

Although elevated triglyceride concentration in plasma is associated with atherosclerosis (Martin, Mayes and Rodwell, 1983), triglyceride does not constitute a major proportion of lipid in either fatty streaks or fibrous plaques (Woolf, 1982). Some investigators, however, have reported a higher concentration of triglyceride in lesions of the coronary arteries than in the aorta (Dayton, Hashimoto and Pearce, 1965; Meyer et al., 1966).

Mechanisms Involved in the Metabolisnm of Exogenous and Endogenous Cholesterol

Cholesterol is a steroid lipid present in all cells of the body as a required metabolite for normal cell structure and function (Martin, Mayes and Rodwell, 1983; Grundy, 1983). It is a major component of cell membranes as well as the precursor of vitamin D, bile acids and the steroid

hormones. Only a small proportion of cholesterol is utilized for structural and functional purposes; the remainder is stored in tissue or is excreted (Guyton, 1981; Grundy, 1983; Blankenhorn, 1984).

Cholesterol is generally present in the diet. It is not an adult dietary essential, as the rate of endogenous synthesis is adequate for meeting the body's needs. The average rate of cholesterol synthesis is 1.0 grams per day, while 0.5-0.7 grams per day is of exogenous origin (Davidsohn and Henry, 1974; Blankenhorn, 1984).

Virtually all tissues are capable of cholesterol synthesis. In humans, rats, chickens and squirrel monkeys, de novo synthesis occurs largely in the liver, with a significant contribution by the intestine (Yeh and Leveille, 1971; Blankenhorn, 1984). Cholesterol is synthesized from acetyl coenzyme A within the microsomal and cytosol fractions of the cell. The most important step in the pathway is the irreversible conversion of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) to mevalonate, which is catalyzed by HMG-CoA reductase. The reductase enzyme is an important control site in cholesterol biosynthesis (Stryer, 1981; Richardson, 1978).

It is postulated that a feedback mechanism operates to control cholesterol synthesis and the level of cholesterol in the plasma (Martin, Mayes and Rodwell, 1983; Stryer, 1981). Absorbed cholesterol is transported by chylomicrons which are subsequently hydrolyzed by lipoprotein lipase. The chylomicron remnants are removed by the liver and the cholesterol is secreted in bile or converted to bile acids. During this process of hydrolysis, dietary cholesterol is recognized by hepatic cells and the rate of cholesterol synthesis by the liver is increased or decreased, due to alterations in the activity of HMG-Co-A reductase (Martin, Mayes and Rodwell, 1983; Blankenhorn, 1984). Because of feedback control, plasma cholesterol concentration is not changed by more than 15% as a result of altering

cholesterol intake. However, a cholesterol-rich diet could alter the plasma concentration by as much as 30% (Guyton, 1981).

In a study by Quintao, Grundy and Ahrens (1971), some patients failed to show reduction in cholesterol synthesis when large amounts of cholesterol were ingested. This indicates that the degree of feedback control varies from individual to individual. Feedback control of cholesterol synthesis is possibly one mechanism whereby some individuals can consume unrestricted amounts of cholesterol and maintain normal plasma cholesterol levels (Blankenhorn, 1984). The possiblity also exists that dietary cholesterol supresses the synthesis of new reductase in liver or stimulates the synthesis of enzymes that degrade existing reductase (Martin, Mayes and Rodwell, 1983).

Approximately 50 percent of intestinal cholesterol is absorbed (Blankenhorn, 1984). When cholesterol intake is 1 gram per day, about one-half is absorbed. At higher intakes, the proportion absorbed tends to decrease (Quintao, Grundy and Ahrens, 1971).

At least one-third of synthesized cholesterol is converted to cholic and chenodeoxycholic acids--the two primary bile acids. When conjugated with glycine or taurine, the salts are secreted in bile and function to enhance the digestion and absorption of fats (Blankenhorn, 1984). Both bile acids and dietary fat are required for cholesterol absorption. When patients with biliary obstruction were given one gram of cholesterol daily for a month, none of the cholesterol was absorbed. Similarly, no cholesterol was absorbed in patients on a fat-free diet. High fiber diets and plant sterols reduce cholesterol absorption. When cholesterol absorption and enterohepatic circulation were blocked by feeding large amounts of plant sterols, cholesterol synthesis significantly increased. In the

normal state, cholesterol synthesis may be inhibited by continual reabsorption of endogenous cholesterol (Quintao, Grundy and Ahrens, 1971).

In dogs and rats, absorbed cholesterol is rapidly converted to bile acids. This mechanism may serve as a protection against hypercholesterolemia. Humans, on the other hand, convert only a small proportion of hepatic cholesterol to bile acids. This low conversion rate may contribute to the relatively high cholesterol concentrations that frequently exist in man (Grundy, 1983).

More than 90% of bile acids are reabsorbed and returned to the liver for re-secretion into the bile. Bile acids that reach the colon are acted upon by intestinal bacteria and further metabolized to lithocholic acid. Fecal excretion of lithocholic acid is the principal pathway for removing cholesterol from the body (Guyton, 1981; Blankenhorn, 1984).

Evidence of an Inverse Relationship Between Plasma HDL-Cholesterol and the Incidence of Coronary Heart Disease

The hypothesis that an inverse relationship exists between the plasma HDL concentration and coronary heart disease has persisted since the 1950's (Gotto and Rifkind, 1982). Evidence of the relationship is statistical rather than causal, as no known experiment has shown that raising the HDL concentration prevents CHD or that lowering HDL increases the disease (Miller and Miller, 1978; Gotto and Rifkind, 1982).

A number of findings are consistent with the protective effect of HDL against CHD. In studying five populations within the United States totaling 6,859 men and women above age 40, Castelli et al. (1977) found the rate of CHD increased from 8 to 18 per 100 subjects as HDL-cholesterol decreased from 44 to 25 mg/dl. The relationship remained when the effect of

other risk factors were considered. Similar results were obtained in the Framingham Study. CHD incidence per 1000 subjects above age 49 increased from 25 to 100 in men and from 14 to 164 in women, as HDL-cholesterol decreased from 70 to 30 mg/dl. Again, the association was independent of other measured variables, including triglyceride and LDL concentrations, smoking and body weight (Gordon et al., 1977). The Tromso Heart Study involved 6,595 men between ages 20 and 49 (Miller et al., 1977). Despite the age difference, the results paralleled those of the Framingham Study.

A number of studies have correlated HDL concentration with various CHD risk factors. The level of physical activity is directly correlated with HDL concentration. The effect is more apparent between groups who differ greatly in degree of activity, such as long distance runners and sedentary controls (Hartung et al., 1980). However, Cobb and Short (1967) found an increase in HDL when healthy young men exercised only one leg for six weeks. A four-month program of moderately strenuous exercise was shown to reduce plasma triglyceride by 15 to 18% and raise HDL concentration by 10% (Blankenhorn, 1984).

Male smokers consuming more than 20 cigarettes per day were found to have a mean HDL concentration 5.3 mg/dl lower than non-smoking controls. Female smokers who consumed an excess of 20 cigarettes per day showed HDL cholesterol levels 9.4 mg/dl lower than non-smokers who used female hormones and 8.6 mg/dl lower than non-smokers who did not use hormones (Criqui et al., 1980). As early as 1955, it was found that sex hormones affected HDL levels. When survivors of myocardial infarction received estrogen, there was a definite increase in the HDL cholesterol and phospholipid levels. Opposite effects were produced by testosterone (Barr, 1955). More recent studies support Barr's findings. Oral contraceptives can either raise or lower HDL cholesterol, depending on the balance between estrogens

and progestin. Estrogens raise HDL concentration in plasma, whereas certain progestins reduce HDL. When ethinyl estradiol was combined with a neutral progestin, HDL cholesterol increased by 25 mg/dl (Blankenhorn, 1984).

The Lipid Research Clinics Study reported a strong inverse relationship between HDL-cholesterol and triglyceride and between HDL and VLDL-cholesterol. A similar relationship was observed between HDL-cholesterol and serum thyroxin. Alcohol consumption correlated positively with plasma HDL concentration, while individuals who had never consumed alcohol had particularly low HDL levels (Belfrage et al., 1977).

One theory attempting to explain the protective effect of HDL against atherosclerosis and CHD is that HDL might function as the vehicle for transporting cholesterol from peripheral cells to the liver (Glomset, 1968). Extrahepatic tissues continuously degrade low-density lipoproteins and incorporate a certain proportion of the released cholesterol. With the exception of adrenals and gonads, extra-hepatic tissues cannot catabolize cholesterol that is taken up. This load of cholesterol must somehow leave the cells and return to the liver (Steinberg, 1981; Glomset, 1968).

According to Myant (1973), free cholesterol from extrahepatic tissues is transferred to HDL and esterified by LCAT. This less polar, esterified cholesterol moves to the central core of HDL, allowing the surface of HDL to incorporate additional free cholesterol from the cells. The esterified cholesterol in HDL is transferred to LDL and transported to the liver. Upon degradation within the hepatic cells, the freed cholesterol may be excreted in bile, converted to bile acids or reincorporated into lipoproteins.

A second hypothesis relative to the protective action of HDL is that HDL might prevent cellular uptake of LDL (Gotto and Rifkind, 1982). When

cells were loaded with cholesterol by incubation with high concentrations of LDL, a proportion of the cholesterol was transferred to the HDL within the medium. When HDL was present during the cholesterol loading, less cholesterol accumulated in the cultured cells (Stein, Stein and Gorey, 1978). In a similar study by Miller and Miller (1975), tumor cells were loaded with free cholesterol and incubated with HDL. Cholesterol was released into the medium. These experiments provide evidence that HDL may act to inhibit LDL uptake or to stimulate cholesterol released from cells in vitro.

The negative correlation between HDL and CHD may be attributed to accelerated triblyceride clearance by HDL (Gotto and Rifkind, 1982). HDL contains apoprotein C-II that activates lipoprotein lipase for catalyzing the breakdown of chylomicrons and VLDL into remnant particles. Incorporation of the excess lipid by HDL might cause conversion of the more dense HDL_3 particles to the less dense HDL_2 particles (Patsch et al., 1978). It has been demonstrated by Kuusi, Saarinen and Nikkila (1980) that HDL_2 is a substrate for lipoprotein lipase. HDL_2 and HDL_3 are major subfractions of HDL, with HDL_2 being increased in familial hyperalphalipoproteinemia, in premenopausal females and clofibrate therapy. It is speculated that HDL_2 might correlate more strongly with protection against CHD than total HDL (Shepherd et al., 1978; Gotto and Rifkind, 1982).

Lower Density Lipoproteins and Their Effects Upon Hyperlipidemia and Atherogenesis

As a result of many experiments that document the atherogenicity of LDL, the Consensus Conference (1985) panel specified the lowering of LDL-cholesterol to reduce the risk of heart attacks caused by coronary heart

disease. The most convincing evidence implicating LDL in CHD risk resul from studying familiar hypercholesterolemia. In homozygotes, LDL concen tration is four to six times normal and atherosclerosis develops at such accelerated rate that myocardial infarctions often occur early in child-hood. Heterozygotes have LDL levels that are two to three times normal, with myocardial infarctions occurring in the fourth or fifth decade. Th primary genetic error responsible for this anomaly is the absence of LDL receptors on cell membrances, resulting in a decreased rate of LDL remov from plasma (Brunzell and Miller, 1981; Nestel and Fidge, 1981; Steinber 1981).

In studying LDL uptake in human fibroblast cells, Goldstein and Bro (1977) demonstrated that uptake is mediated by high-affinity receptors o the cell membrane. There is evidence that a similar system operates in arterial smooth muscle cells and endothelial cells. When LDL concentrat is high, the particles bind very tightly to the high-affinity receptors within tiny pits. When the LDL concentration is low, more of the partic bind to low-affinity sites which are undifferentiated smooth areas of th cell membrane. The bound LDL enters the cell by endocytosis with subse-quent formation of vesicles that fuse with lysosomes. Hydrolysis of the cholesterol esters within LDL possibly enhances the onset of specific regulatory mechanisms. These mechanisms include: (1) stimulation of ac CoA:cholesterol acyltransferase or ACAT that re-esterifies the freed cho lesterol for possible storage; (2) inhibition of HMG-CoA reductase causi a reduction in cholesterol synthesis and (3) suppression of the rate at which new receptors are formed.

LDL particles that are bound to high affinity receptors enter the cell at a much faster rate than LDL bound to low-affinity receptor sites It is theorized that the low-affinity mechanism operates in familial

hypercholesterolemia. Regardless of LDL concentration, particle degrada tion occurs at a rate that is less than 10% of the normal rate. Studies show no increase in ACAT activity or suppression of HMG-CoA reductase an no inhibition of the rate at which new receptors are formed (Steinberg, 1981; Goldstein and Brown, 1977).

Steinberg et al. (1978) observed that the interaction of LDL and th LDL receptor depends on the protein rather than its lipid content. Earlier, Shireman, Kilgore and Fisher (1977) had determined that extraction the lipid content did not alter binding and uptake. However, the intera tion of apoprotein B and LDL lipid might alter the configuration of the apoprotein, thereby changing the affinity of LDL for the receptor.

The LDL receptor is not specific for apoprotein B, which is the major protein component of LDL. The binding of VLDL to the LDL receptor can be attributed to the presence of apoprotein B (Steinberg, 1981). Mahley and Innerarity (1978) demonstrated that in the absence of the B apoprotein, some lipoprotein fractions containing high levels of apoprotein E or only E can bind to the LDL receptor. These studies not only revealed that the same receptor recognizes both B and E, but that the affinity for apoprotein E is more than 20 times greater than for B. Although E is normally present in VLDL and HDL as a minor component, its concentration increases in both humans and experimental animals when dietary cholesterol is increased. Implications are clear that apoprotein E has a role in lipoprotein uptake by cells.

Depending on species and nature of diet, HDL contains varying levels of apoprotein E. When E is absent, HDL does not bind to the LDL receptor of the fibroblast cells. However, in the presence of apoprotein E, HDL molecules compete with LDL for binding sites (Mahley and Innerarity (1978). Miller et al. (1977a) and Carew et al. (1976) found that the plasma of

humans and animals contain sufficient apoprotein E to cause similar competitive interaction. Cholesterol-enriched diets increase apoprotein E in the HDL fraction as well as in lower density lipoproteins. HDL then binds to the LDL receptor with an affinity exceeding that of LDL (Steinberg, 1981).

HDL and LDL competition for LDL receptor sites has been demonstrated in skin fibroblasts, aortic smooth muscle cells and endothelial cells. This interaction significantly lowers LDL uptake as the HDL:LDL ratio increases. It is possible that such competitive interaction is the basis for the protective effect of HDL (Stein and Stein, 1976; Reckless, Weinstein and Steinberg, 1978).

Hepatic synthesis of VLDL is stimulated by increased synthesis of cholesterol, triglyceride or fatty acids. These lipoproteins are also produced in the intestine and transport largely triglyceride that is endogenously synthesized (Nestel and Fidge, 1981). Hydrolysis of VLDL involve progressive removal of surface components and lipids to form a particle of intermediate density (IDL). In some animals, such as the dog and rat, IDL particles are removed from the circulation by the liver. In man, most VLDL is further metabolized to LDL (Grundy, 1983).

Primary hypertriglyceridemia or Type IV hyperlipoproteinemia is usually associated with increased VLDL. This disorder increases the frequency of ischemic heart disease but it is unclear whether increased triglyceride acts as an independent risk factor in CHD susceptibility. Normal or slightly decreased LDL usually accompanies primary hypertriglyceridemia, whereas both VLDL and LDL are elevated in Type IIb hyperlipoproteinemia with increased CHD risk (Woolf, 1982).

Dietary triglyceride is transported by chylomicrons and hydrolyzed by lipoprotein lipase to chylomicron remnants which are removed by the liver (Grundy, 1983). The tendency to disregard chylomicrons as contributors to

atherogenesis is due in part to their size, which prevents penetration to the extravascular spaces. The possibility exists that chylomicrons can exert atherogenic effects without entering the vessel wall. If chylomicrons are degraded at the endothelial surface, the high concentration of degradation products might damage the vessel wall. The large chylomicrons can then enter the subendothelial spaces of the artery wall (Zilversmith, 1973; Steinberg, 1981).

Probably the best line of evidence for removing chylomicrons from involvement in atherogenesis has been provided by studying persons with Type I hyperlipoproteinemia. This disorder is caused by congenital lipase deficiency. Despite extreme elevation of chylomicrons in plasma, these persons have a low incidence of atherosclerotic disease (Woolf, 1982; Grundy, 1983).

Even if intact chylomicrons are not atherogenic, the remnant particles might be involved. A large proportion of cholesterol in hypercholesterolemic rabbits is associated with chylomicron remnants. When the cholesterol content of these particles is high, there is a delay in remnant uptake by the liver (Ross and Zilversmith, 1977).

Dietary Factors and Plasma Lipids

Carbohydrate and Fiber

The energy content of the diet is approximately 3,540 kilocalories per capita per day (Welsh and Marston, 1982). In the United States, more than 40% of total energy is derived from carbohydrate and as much as 80-90% in some populations. Although the availability of total carbohydrate has decreased since the turn of the century, the amount provided by sugars has

increased to approximately one-half of the total carbohydrate available (Dairy Council Digest, 1984).

Simple and complex carbohydrates exert different effects upon plasma lipid concentrations. The complex carbohydrates contain various levels of non-nutritive fiber and the fiber, rather than the nature of the sugar, may account for the differential effects (Kritchevsky, 1976).

In a study by Hallfrisch et al. (1985), a group of men, and a group of premenopausal and postmenopausal women consumed a diet for 13 weeks that was low in cholesterol, saturated fat and salt, but high in fiber and complex or simple carbohydrate. Dietary fat provided 35% of the total calories, carbohydrate provided 50% and protein, 15%. When the starch content was high, plasma concentrations of total, LDL and VLDL cholesterol decreased. No change occurred in plasma triglyceride level. When 20% of the complex carbohydrate in the diets of the men and premenopausal women was replaced by foods high in fructose and glucose, triglyceride and VLDL cholesterol increased in both groups. Total cholesterol also increased in the premenopausal women. The increase in triglyceride may have resulted from decreased activity of lipoprotein lipase which mediates triglyceride clearance.

Kritchevsky, Sallata and Tepper (1968) found fructose and sucrose to be more atherogenic than glucose when rabbits were fed diets containing 40% carbohydrate, 25% casein, 15% cellulose and 14% coconut oil. Lactose produced no atherogenic effects in the cholesterol-free diet. In baboons, Kritchevsky et al. (1974) observed equal cholesteremic effects of fructose, glucose, sucrose and starch. However, glucose and starch increased triglyceride by 43%, while fructose and sucrose increased triglyceride by more than 55%.

Lang and Barthel (1972) fed diets containing 66% dextrin or sucrose with 0.5% added cholesterol to three species of monkeys. In one species, dextrin was more hypercholesterolemic and atherogenic. In another, dextrin did not induce hypercholesterolemia, but intimal changes in the coronary arteries were evident. In the third species, no differential effects were observed.

It is generally accepted that high fiber diets result in decreasd plasma cholesterol levels (Kritchevsky, 1976). Fiber includes the products of plant cell walls that cannot be digested by enzymes within the human digestive tract (Eastwood, 1984). Among these products are lignin and undigestible carbohydrates such as cellulose, hemicelluloses and pectin (Oakenfull and Fenwick, 1978). Fiber is commonly found in cereals, legumes, fruits and vegetables.

Various physiological roles are associated with dietary fiber. In addition to a feeling of satiety, fiber increases gastrointestinal transit time by causing a delay of gastric emptying and enhances bile acid excretion (Eastwood, 1984; Anderson and Chen, 1979). Epidemiological and experimental results have implicated dietary fiber in the etiology of diabetes, cancer, atheroscloerosis and heart disease (Kritchevsky, Tepper and Story, 1974).

Different dietary fibers have resulted in varying changes in plasma lipid concentrations. Pectins and gums have been found to lower plasma cholesterol in man and experimental animals, whereas cellulose has produced no cholesterol effect in humans (Kirtchevsky, 1976). In laying hens, Menge et al. (1974) observed that an increase in cellulose caused a significant decrease in serum cholesterol, with an accompanying increase in egg yolk cholesterol. In varying the level of wheat straw and cellulose in the diet, Moore (1967) found wheat straw to be less atherogenic than cellulose.

Kritchevsky, Tepper and Story (1974) fed isocaloric diets to rats, with 50% of the total calories provided by carbohydrate (dextrose or sucrose), corn oil or casein. Alfalfa or cellulose was incorporated in the diet as the non-nutritive fiber source. In the presence of alfalfa, serum and liver cholesterol were significantly lower than when the diet contained cellulose. Cookson, Altschul and Fedoroff (1967) had previously observed that dietary alfalfa inhibited hypercholesterolemia in rabbits when cholesterol intake was 600 mg/day. In Kritchevsky's study, the differential effects of carbohydrate and protein were evident. The dextrose and protein diets that incorporated alfalfa resulted in lower cholesterol levels than the sucrose diet plus alfalfa.

Wheat bran is the most widely consumed dietary fiber (Salvioli, Salati and Lugli, 1980) and is not generally associated with hypocholesterolemia (Thompson, 1981). A study by Salvioli, Salati and Lugli produced contrasting results. When human subjects consumed 60 grams of bran per day, fecal sterol and bile acid excretion increased, whereas total cholesterol and LDL cholesterol decreased.

Although various fibers have been shown to bind bile acids, it has been reported that the binding is dependent on the presence of saponins (Oakenfull and Fenwick, 1978). It has also been reported that saponin-rich foods (for example, soybean protein) are hypocholesterolemic, whereas foods free of saponin (for example, wheat bran) have no effect on cholesterol concentration in man (Truswell and Kay, 1976; Sirtori et al., 1977). In studying saponin effects on plasma lipids and bile acids in rats, Oakenfull, Fenwick and Hood (1979) observed that saponins significantly reduced plasma and triglyceride levels, increased the rate of bile acid secretion and increased fecal excretion of bile acids and neutral steroids.

Dietary Lipid

Between 1909 and 1982, the level of fat in the food supply increased by 37%. Approximately 90% of total fat comes from: (1) fats and oils, (2) meat, poultry and fish and (3) dairy products. Fats and oils provided 44% of the available fat in 1982; meat, poultry and fish contributed 34% and dairy products, 12%. In terms of intake percentage, meat provided 31.5%, fats and oils, 30.5% and dairy products, 19.2% (Dairy Council Digest, 1984). While most of the available fat is of animal origin, vegetable sources account for the increase (Welsh and Marston, 1982).

The consumption of diets with a high saturated fatty acid content is associated with increased CHD risk, whereas diets with increased polyunsaturated fatty acids are linked with decreased risk to heart disease (Kritchevsky, 1976; Consensus Conference, 1985). A P:S ratio of 1 is probably the most effective means of achieving optimum serum lipid levels (Thompson, 1981). Factors such as length of the fatty acid chain and stereoisomerism are also implicated in CHD. Lauric, myristic and palmitic acids with 12, 14 and 16 carbon atoms, respectively, elevate plasma cholesterol, while stearic acid (18 carbon atoms) does not. Oleic acid has no significant effect upon cholesterol concentration. Its trans-isomer, kelaidic acid, raises the concentration of cholesterol (Vergroesen, 1972).

The Consensus Conference (1985) recommended the lowering of dietary lipid as a step toward reduced CHD risk. The type of lipid lowered and the degree of lowering are dependent on the type of oil incorporated in the diet. Vegetable oils tend to lower plasma cholesterol, whereas marine fish oils appear to be more effective in lowering plasma triglyceride. The differential effects may relate to changes in lipoprotein secretion (Goodnight et al., 1982). When rats were fed a diet supplemented with 8% fish

oil, plasma-free fatty acids were significantly decreased and triglyceride concentration decreased by 60%. Plasma cholesterol was also lowest in rats consuming the fish oil diets (Illman et al., 1986).

Dyerberg, Bang and Hjorne (1975) examined the fatty acid composition of plasma lipids of Eskimos who consume large quantities of fish and have a low incidence of CHD. Plasma levels of eicosophentaenoic acid (EPA), found only in marine animals, were markedly elevated. Decosahexaenoic acid (DHA) is also a major fatty acid of fish oils. Several studies have provided evidence that EPA and DHA reduce plasma total cholesterol and increase HDL-cholesterol (Conner, Lin and Harris, 1982).

Serum cholesterol levels and the incidence of heart disease remain low in certain populations despite the consumption of foods high in saturated fat and cholesterol. One such group is the Maasai tribesmen of East Africa who subsist largely on meat and a daily intake of 4-5 liters of whole fermented milk. In studying this population, Mann and Spoerry (1974) hypothesized that a factor in the fermented milk inhibits cholesterol synthesis and the hypercholesterolemic effect of milk fat and cholesterol. The factor in milk that has cholesterol-lowering ability has not been identified; however, orotic acid and HMG have received speculation (Richardson, 1978). Orotic acid is known to alter lipid metabolism. When 1% orotic acid was incorporated in the diets of rats, serum cholesterol decreased by 25-50% and triglyceride, by 44-64% (Bernstein, Richardson and Amundson, 1975).

Dietary Protein

Some studies have indicated that dietary animal protein is hypercholesterolemic in humans. Other studies have produced contrasting results or no effect. Strict vegetarians, who consume no animal protein, have lower

plasma cholesterol levels than lactovegetarians. Lactovegetarians have lower cholesterol levels than non-vegetarians who consume unrestricted amounts of animal protein (Sacks et al., 1983). When young women consumed diets containing 45-50 grams of vegetable or animal protein, the plasma cholesterol concentration decreased by 25% when the diet contained vegetable protein, compared to a 17% decrease when the diet contained animal protein. The greater cholesterol decrease with the vegetable protein diet may be due to increased fiber (Walker, Morse and Overley, 1960). Carroll et al. (1978) found that plasma cholesterol decreased by 19% when female subjects consumed diets containing a 2:1 mixture of animal and vegetable protein.

Flynn et al. (1979, 1981) studied the effects of eggs, meat, poultry and fish on serum lipids in human subjects. Eggs, providing 35% of total cholesterol intake, are the major single source of cholesterol in the diet. Meat, poultry and fish provide an additional 35% (Blankenhorn, 1984). In two separate experiments, Flynn et al. (1979) investigated changes in plasma lipid concentrations when 116 men consumed one or two eggs per day for three months. Both studies produced the same results. Whole egg cholesterol (250-300 milligrams per whole egg) did not significantly alter plasma cholesterol concentration.

In studying the effects of beef, poultry and fish on plasma lipids, Flynn et al. (1981) used 129 male and female subjects. One-half of the group consumed a daily diet of one egg and at least five ounces of raw beef for three months, while the other half simultaneously consumed one egg plus at least five ounces of raw poultry or fish. Neither diet significantly changed total cholesterol, HDL-cholesterol or triglyceride concentration in the male subjects. In the females, the only resulting change was an

in crease in plasma triglyceride concentration after three months on the poultry/fish diet.

Numerous animal studies have correlated source and level of dietary protein with changes in plasma lipid metabolism. Casein, the major protein of bovine milk, has been the most widely used animal protein and soy protein has been the most commonly used vegetable protein in establishing the relationship between protein source and hyperlipidemia. These studies, along with others that incorporate different animal and plant proteins, have provided evidence of the atherogenicity of animal protein, regardless of level, in a variety of experimental models. Contrasting results have been reported regarding the level of protein in the diet of some species.

Ignatowski hypothesized in 1909 that the protein contained in meat, eggs and milk exerted atherogenic properties in rabbits (Kritchevsky, 1976, 1980). Newburgh and Squire (1920) observed atherosclerosis in meat-fed rabbits after four weeks of feeding. Meeker and Kesten (1940, 1941) found casein to be atherogenic in rabbits, whereas soy protein produced no adverse affects. Terpstra and Sanchez-Muniz (1981) studied the time-course of hypercholesterolemia in rabbits fed casein or soy protein. After one day, the cholesterol and phospholipid levels increased by 50% on the soy protein diet and more than doubled on the casein diet. After one month, a significant decline was observed in the soy protein group but not in the casein group. While receiving the basal diet, most of the cholesterol was carried in the HDL fraction, which shifted to the LDL fraction during feeding of the experimental diets. Casein diets have also induced elevated serum lipid levels in pigs (Forsythe et al., 1980) and rats (Vahouny et al., 1985).

In many experimental studies, the effect of dietary protein on plasma cholesterol level is only significant when the diet incorporates

cholesterol (Mol et al., 1982; Terpstra, 1982b). When Mol et al. (1982) fed cholesterol-free protein and casein diets to chickens, no differential effects were observed. Stamler (1958) fed cholesterol-enriched diets to chickens and found soy protein to be less atherogenic than casein and gelatin. Kritchevsky (1959) reported similar findings regarding soy protein and casein. A hypercholesterolemic effect of dietary protein on plasma lipids in the absence of cholesterol has been observed only in rabbits (Terpstra, 1982b).

Kritchevsky (1980) fed cholesterol-free diets to rabbits that incorporated 25% corn protein, wheat gluten or lactalbumin. The diets also contained 40% sucrose, 15% fiber and 14% coconut oil. Lactalbumin was more cholesteremic and atherogenic than either corn protein or wheat gluten.

The atherogenicity of animal protein can be modified by the type of fiber in the diet. Kritchevsky et al. (1977) fed diets to rabbits containing 25% casein or soy protein plus 15% cellulose, wheat straw or alfalfa. Cholesterol levels were higher in the casein-fiber groups than in the soy protein-fiber groups; however, the increase was not significant in the casein-alfalfa group. Plasma cholesterol in the rabbits fed casein plus alfalfa was significantly lower than in the groups fed casein plus wheat straw or cellulose. In the soy protein groups, the alfalfa-containing diet resulted in significantly lower cholesterol levels than the diets that incorporated wheat straw or cellulose. Triglyceride concentration was highest in rabbits fed the casein-cellulose diet.

Kritchevsky (1979) used modifications of the same diet to examine the effects of protein and fat interaction on plasma lipid concentrations. The diets incorporated 25% beef protein, casein, textured vegetable protein (TVP) or a 1:1 mixture of beef protein and TVP. Beef tallow (15%) replaced coconut oil. The beef protein and casein diets were equally cholesteremic

and atherogenic and TVP was significantly less atherogenic. In comparison to the beef and casein groups, the beef-TVP diet resulted in significantly lower cholesterol levels. The atherogenicity of the beef-TVP diet was similar to the TVP diet.

In some species, the atherogenic properties of a particular protein in the diet can be enhanced by incorporating a higher proportion of the protein in the diet. Newburgh and Clarkson (1923) reported that rabbits consuming a diet containing 36% beef protein, without added cholesterol, developed lesions earlier than rabbits receiving a diet with 26% of the same protein. Nath, Harper and Elvehjem (1958) obtained similar results when rats were fed diets consisting of 40% and 70% casein, as well as Hevia et al.'s (1980) results, when rats consumed diets with 7.5%, 15% and 30% casein. Lofland et al. (1966), Clarkson et al. (1962) and Middleton et al. (1967) carried out similar investigations with pigeons and squirrel monkeys. Serum cholesterol in the pigeons varied with type of fat and level of protein, with high protein diets being more atherogenic. Findings in the squirrel monkeys revealed that in the absence of cholesterol, a 15% protein diet was more atherogenic than either a 30% of a 5% protein diet. With the addition of 0.25% cholesterol, the 30% diet exerted the greatest atherogenicity.

When male and female rats were fed cholesterol-enriched diets (1.2% cholesterol) containing 20 or 50% casein or soy protein, Terpstra (1982b) found the casein diets to be hypercholesterolemic in female rats, compared to the soy protein diets. This effect was enhanced by increasing the proportion of casein in the diet. No significant differences in cholesterol level were observed when the proportion of soy protein was increased. In the male rats, no differential effect of casein and soy protein was

observed. These results suggest that female rats are more susceptible to induced hypercholesterolemia than male rats.

In a number of studies, differential effects of dietary casein and soy proteins have been observed in male rats. Discrepancies could relate to the strain of rat.

A number of studies have shown an inverse relationship between serum cholesterol and the quantity of dietary protein in chickens. In investigating the effects of protein level on serum cholesterol in chickens, Yeh and Leveille (1972) observed that chickens receiving the high protein diet showed an increase rate of cholesterol synthesis but a decrease in the level of serum cholesterol. This hypocholesteremic effect of dietary protein was attributed to an increased rate of cholesterol degradation to bile acids in the liver. Although serum cholesterol was lowered, carcass cholesterol increased. The possibility exists that cholesterol shifted from the liver-plasma pool to the carcass.

In a study by Terpstra, Schutte and West (1983), female chickens were fed cholesterol-enriched semipurified diets containing 20% casein or soybean protein for 29 days. These diets, compared to cholesterol-free diets, results in hypercholesteremia. The hypercholesterolemia was prevented when the protein content was increased to 50%. Serum triglycerides and phospholipids were also reduced by feeding the high protein diets. Terpstra, Schutte and West's findings indicated that in cholesterol-fed chickens, replacement of casein with soybean protein does not significantly alter serum cholesterol. This is in contrast with findings in other species that casein, in comparison with soybean protein, exerts a hypercholesterolemic effect. The high protein diets were effective in reducing cholesterol in cholesterol-fed chickens, irrespective of whether casein or soybean protein was fed.

The excess cholesterol and phospholipid in the serum of the chickens fed the cholesterol-enriched diets with 20% protein were carried largely in the VLDL and IDL fractions, indicating a shift of cholesterol from LDL compared with the groups fed 50% protein diets. A similar pattern has been found in rats made hypercholesterolemic by feeding cholesterol-enriched casein diets (Terpstra, Van Tintelen and West, 1982b). In rabbits and guinea pigs, hypercholesterolemia is primarily associated with elevated LDL levels (Terpstra, Schutte and West, 1983; Terpstra, Van Tintelen and West, 1982a).

The concentration of liver cholesterol paralleled the serum concentrations. In chickens fed the lower protein diets, liver cholesterol was significantly higher than in chickens fed the high protein diets (Terpstra, Schutte and West, 1983).

Possible Mechanisms Involved in the Alteration of Plasma Lipids by Dietary Protein

The mechanisms involved in the alteration of cholesterol metabolism by dietary protein have not been fully elucidated. It has been shown in chickens that the decrease in plasma cholesterol concentration by high protein diets is accompanied by increases in cholesterol synthesis, fecal steroid excretion and cholesterol turnover rate (Yeh and Leveille, 1972). The principal pathway for removing steroids from the body is through excretion of neutral steroids and bile acids in the feces (Dietschy, 1968). However, the secretion and reabsorption of bile acids and neutral steroids by enterohepatic circulation play an important role in regulating lipid metabolism. Most bile acids are reabsorbed and transported from the intestine to the liver. The hepatocytes reconjugate the deconjugated bile acids and rehydroxylate some of the secondary bile acids so that these restored

forms are secreted in bile along with newly synthesized bile acids (Kutchai, 1983). There is evidence that a less efficient reabsorption of bile acids can lower serum cholesterol (Terpstra, Schutte and West, 1983).

It has been reported by Sklan (1980) that the binding of bile acids to casein prevents bile acid reabsorption. The possibility exists that, on high casein diets, the undigested protein might interfere with bile acid reabsorption through binding action, which reduces the availability of bile acids for reabsorption. Cholesterol concentration can therefore be reduced.

Van der Meer (1983) and Van der Meer et al. (1985) have proposed that the hypercholesterolemic action of casein may relate to both its phosphorylation and its influence on enterohepatic circulation. The phosphoserine residues of casein can bind casein to insoluble calcium phosphate (Holt, Hasnain and Hukins, 1982). In the small intestine, bile acids can also bind to calcium phosphate but their binding is competitively inhibited by casein. This leads to increased availability of bile acids for reabsorption and decreased fecal output of steroids. Plasma cholesterol concentration therefore increases.

It has been speculated that dietary protein may influence cholesterol metabolism through an effect on LCAT (Forsythe et al., 1980). This enzyme esterifies unesterified cholesterol in plasma and is associated with the transfer of tissue cholesterol to the liver for removal from the body (Glomset, 1968). When diets containing polysaturated fat were fed to pigs, both LCAT activity and plasma cholesterol levels decreased in comparison to saturated fat diets. The feeding of animal protein, in comparison to plant protein, also resulted in decreased LCAT activity, but increased concentrations of unesterified cholesterol in plasma. Since low LCAT activity paralleled a low level of unesterified cholesterol when polyunsaturated

fats were fed, increased LCAT activity was expected to accompany an increased level of unesterified cholesterol when protein diets were fed. This differential effect suggests that factors other than level of unesterified cholesterol may be involved (Forsythe et al., 1980).

It has been suggested that the lysine:arginine ratio may account for differential effects of plant and animal protein on plasma lipid levels (Kritchevsky, Tepper and Story, 1978). Rabbits were fed diets containing: (1) casein, (2) casein plus added arginine to bring the lysine:arginine ratio to 0.9, 3) soy protein or (4) soy protein plus added lysine to raise the lysine:arginine ratio to 2.0. The soy protein diet with added lysine raised serum cholesterol from 124 to 190 mg/dl and increased the atherogenicity by 64%. The addition of arginine to casein did not alter cholesterol concentration, but decreased atherosclerosis by 24%. Changes were also observed in the lipoprotein pattern. When rabbits were fed the casein or soy protein diets, LDL and HDL fractions were in greatest concentration. The casein-arginine diet resulted in a decrease of VLDL and IDL and a significant increase in LDL and HDL. In contrast, the soy protein-lysine diet resulted in a rise in LDL and IDL and a decrease in HDL. A decrease also occurred in LDL but was not significant. Wallentin (1977) proposed that dietary protein may alter the metabolism of apoprotein A-I, which is an activator of LCAT.

Park and Liepa (1982) observed that the feeding of animal protein to rats resulted in both increased LCAT activity and increased cholesterol level. The differential results obtained in pigs and rats may be attributed to variation in homeostatic mechanisms.

CHAPTER III

INFLUENCE OF PLANT AND ANIMAL PROTEIN ON TOTAL CHOLESTEROL CONCENTRATION IN PLASMA OF JAPANESE QUAIL

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Summary

An experiment was conducted to determine the effects of different animal proteins and a plant protein on total plasma cholesterol concentration of male Japanese quail. Each experimental diet contained 24% protein, which is the maintenance requirement for adult Japanese quail (National Academy of Sciences, 1977). The experimental diets also incorporated 0.5% added cholesterol.

One hundred and twenty adult male birds were weighed, divided into four equal groups and fed one of the following dietary treatments: (1) a basal diet containing 24% soy (plant) protein without added cholesterol, (2) a 24% soy protein diet plus 0.5% added cholesterol, (3) a 24% casein diet plus 0.5% added cholesterol or (4) a 24% whey protein diet containing 0.5% cholesterol.

At weekly intervals (except week one), four birds from each group were reweighed and sacrificed. Approximately 3.0 milliliters of blood were collected from each bird in tubes containing 50 microliters of 10% EDTA.

Plasma was separated and total cholesterol determined by colorimetric procedure.

At the end of seven weeks, plasma cholesterol was significantly elevated in birds receiving the cholesterol-enriched diets. This effect, however, was not consistent throughout the experiment. At the third, fourth, fifth and sixth weeks, the cholesterol concentration in birds fed the soy protein diet plus 0.5% cholesterol was not significantly different from birds receiving the control diet. The difference was only significant at the second and seventh sampling periods.

The animal protein diets (whey and casein) exerted different effects upon cholesterol concentration. After the second week of dietary treatment, plasma cholesterol concentration was higher in birds receiving the whey protein diet. The difference was significant at three of the six sampling periods.

In comparing the effects of dietary protein on cholesterol concentration relative to protein origin, whey protein was more hypercholesterolemic than soy protein at weeks three, five, six, and seven. No differential effect was observed between casein and soy protein.

These results indicate that: (1) when the diet contains 24% soy protein, with or without 0.5% added cholesterol, no significant difference occurs in cholesterol concentration in plasma; (2) different proteins of animal origin (casein and whey) exert different effects upon plasma cholesterol concentration, with whey protein being more hypercholesterolemic than casein; and (3) in comparison to soy protein, whey protein significantly elevates plasma cholesterol, whereas casein does not.

Introduction

Dietary protein of plant and animal origin exerts differential effects

upon total cholesterol concentration in plasma. In some species, animal protein elevates plasma cholesterol, while plant protein lowers cholesterol concentration. The most widely used vegetable and animal proteins in such experiments have been soy protein and casein, respectively. The hypercholesterolemic effect of casein in cholesterol-enriched diets has been demonstrated in rabbits (Meeker and Kesten, 1940, 1941), rats (Terpstra et al., 1982b), pigs (Forsythe et al., 1980) and monkeys (Terpstra et al., 1984). In rabbits, casein-induced hypercholesterolemia has been demonstrated without the addition of cholesterol to the diet (Carroll, 1978). Mol et al. (1982) observed no differential effect of casein and soy protein when chickens were fed cholesterol-free diets. However, when 1% cholesterol was added to the diet, plasma cholesterol concentration significantly increased. The increase was greater in chickens receiving the basal diet than in chickens receiving the casein diet.

The objective of this experiment was to compare the effects of different animal proteins (casein and whey protein) with the effect of soy (vegetable) protein on plasma cholesterol concentration in Japanese quail.

Materials and Methods

Maintenance of Animals

Japanese quail were hatched and maintained in the Animal Science Department at Oklahoma State University. Eggs that had been stored for 10-20 days at 55°F were incubated at 100°F and 99% relative humidity for 16-17 days. Birds were housed in five-tiered metal batteries in a well ventilated room. Temperature was maintained at 75°F and light was constant. Feed and water were provided *ad libitum*.

Experimental Procedure

One hundred and twenty adult male Japanese quail were divided into four equal groups (four separate pens) and fed a diet for seven weeks that contained: (1) 24% soy protein without added cholesterol (control diet), (2) 24% soy protein plus 0.5% added cholesterol, (3) 24% casein + 0.5% cholesterol or (4) 24% whey protein +0.5% cholesterol (Table I).

On the day "0", four birds from each group were sacrificed. Approximately 3.0 mililiters of blood were collected in tubes containing 50 microliters of 10% EDTA. Total cholesterol concentration in plasma was determined to establish baseline levels. On days 14, 21, 28, 35, 42 and 49, a similar procedure was followed. Plasma was separated, stored at 4°C and subsequently analyzed for total cholesterol. All birds were initially weighed and a final weight obtained on the day of sacrifice.

Measurement of Total Cholesterol in Plasma

Fluorometric determination of total cholesterol is based on the Lieberman-Burchard reaction. To 20 microliters of plasma in a 15-milliliter centrifuge tube, 0.2 milliliter of acetic acid-choloform (3:2 mixture) was added and mixed. Five milliliters of chloroform-acetic anhydride (10:3 mixture) were added to the tube, followed by addition of 0.2 milliliter of concentrated sulfuric acid. The contents of the tube were immediately mixed upon adding the sulfuric acid and centrifuged for 10 minutes at 2700 rpm. The supernatant was decanted into flurometer tubes and read in the Model III Turner Fluorometer 40 minutes after the addition of sulfuric acid to the first tube.

A "blank" was run with each set of samples in which plasma was replaced by an equal volume of water. A standard was also included in each

TABLE I

DIET COMPOSITION

Component	Control	Control + 0.5% Cholesterol	Control + 0.5% Cholesterol + Whey Protein	Control + 0.5% Cholesterol + Casein
Cornstarch	58.72	58.22	54.93	58.58
Isolated Soy[a]	28.27	28.27	--	--
Whey Protein[b]	--	--	32.00	--
Casein[c]	--	--	--	28.33
Corn Oil[d]	7.00	7.00	7.00	7.00
Glista Salts[e]	5.37	5.37	5.37	5.37
Cholesterol[f]	--	0.50	0.50	0.50
Vitamin Premix[g]	0.20	0.20	0.20	0.20
DL-Methionine	0.44	0.44	--	0.02

[a]U.S. Biochemical Corporation, Cleveland, Ohio

[b]Express Foods Company, Inc., Louisville, Kentucky

[c]Erie Casein Company, Inc., Erie, Illinois

[d]Mazola corn oil

[e]55.94 g/kg $CaCO_3$; 522.102 g/kg $CaPO_4$, tribasic; 167.82 g/kg K_2HPO_4; 164 g/kg NaCl; 65.263 g/kg $MgSO_4 . 7H_2O$; 12.12 g/kg $MnSO_4 . H_2O$; 9.323 g/kg ferric citrate; 1.865 g/kg $ZnCO_3$; 0.373 g/kg CuSOs. $5H_2O$; 0.168 g/kg boric acid; 0.168 g/kg $Na_2MbO_4 . 2H_2O$; 0.746 g/kg KI; 0.019 g/kg $CoSo_4.7H_2O$; 0.004 g/kg Na_2SeO_3

[f]CH-USP cholesterol, Sigma Co.

[g](g/kg): 1.05 Vit. A/D3; 7.45 g Vit A; 6.05 g Hetrazeen; 5.0 g B_{12}; 60 g Thiamine Monitrate USP; 18.0 g Riboflavin; 56.5 g Niacin; 12.0 g d-Ca Panto USP; 4.15 g Pyridoxine HCl USP; 2.2 g Folic Acid USP; 33.0 g Biotin; 141.0 g Ascorbic Acid Coated; 55.0 g Inositol; 1.1 g PABA; 100.0 g Ethoxyquin; 497.5 g Dextrose

set of samples. Total cholesterol concentration (mg/dl) was calculated as follows:

$$\frac{\text{Fluorometric Value of Sample}}{\text{Fluorometric Value of Standard}} \times \text{Concentration of Standard}$$

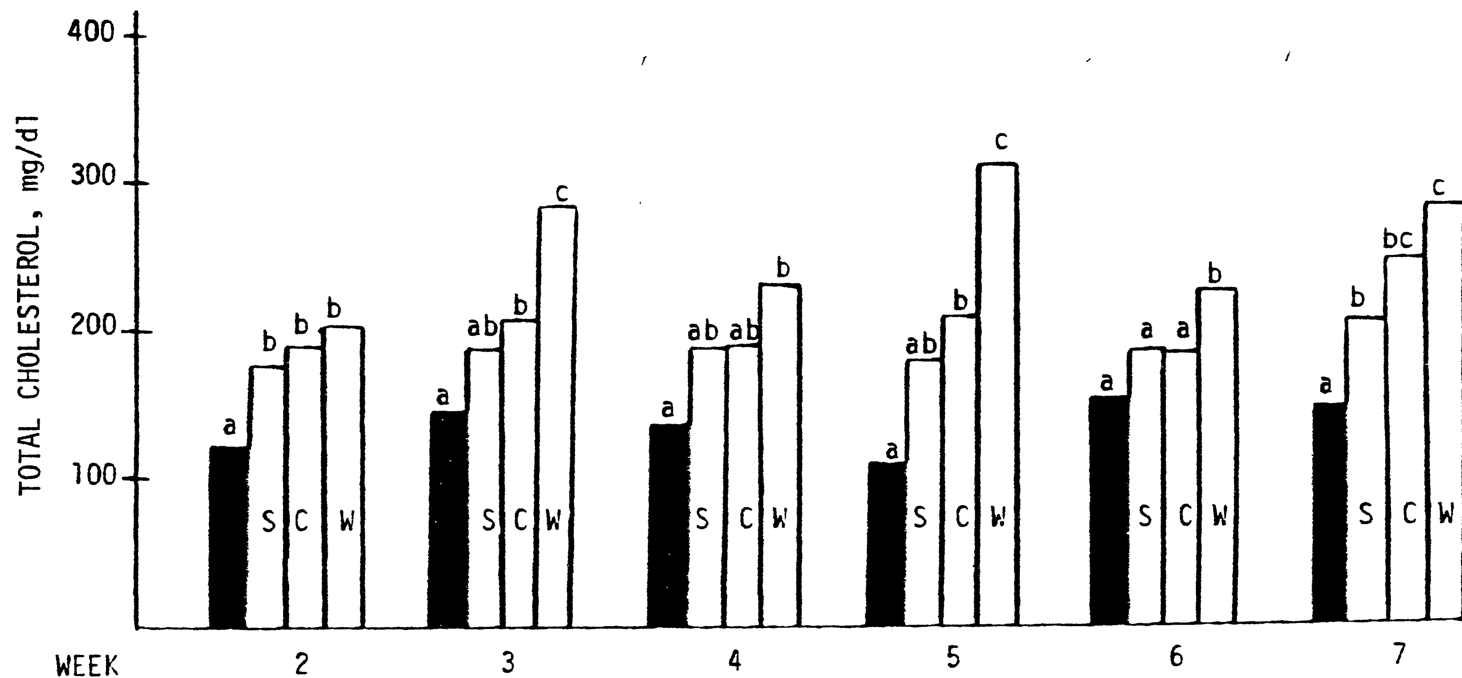
All data were analyzed by the difference between means, and statistical significance was based on Fisher's Least Significant Difference (LSD) multiple comparison Test (Ott, 1984).

Results and Discussion

The birds in this experiment were eight weeks old, with an overall mean weight of 117 grams. The mean weight change in the casein-fed birds (6.13 grams) was statistically greater than in birds fed the soy or whey protein diets (2.79 and 2.63 grams, respectively), but not statistically different from weight change in birds fed the control diet (3.33 grams).

A number of studies have shown that the addition of cholesterol to the diet elevates plasma cholesterol concentration regardless of type of protein in the diet (Morrisey and Donaldson, 1977; Mol et al., 1982; Cho, Egwim and Fahey, Jr., 1985). In contrast, it has been demonstrated that dietary protein of animal origin can induce hypercholesterolemia in rabbits in the absence of added cholesterol (Kritchevsky, 1980).

In this experiment, the addition of 0.5% cholesterol to diets containing 24% soy protein, casein or whey protein resulted in elevated cholesterol concentration in plasma (Figure 1). The elevation, however, was not consistently significant. The difference in the control diet and the cholesterol-enriched soy protein diet was significant (P=0.05) only at the second and seventh weeks. The control and casein diets were significantly different at weeks two, three, five, and seven, while the whey protein diet, in comparison to the control diet, resulted in significantly higher



Note: Shaded bars represent control diet; S = soy protein; C = casein and W = whey protein diet. If bars do not share a common letter (abc), the mean cholesterol levels are statistically different (P = .05). ADV F test indicates statistical differences due to treatment and week (P = .21). SEM = 18.23 to 21.05.

Figure 1. Mean Total Cholesterol Concentration for Each Dietary Treatment by Week

levels of plasma cholesterol throughout the experiment. Since no statistical differences resulted in cholesterol concentration due to interaction of treatment and week, cholesterol concentration from each dietary treatment was averaged over the entire experimental period (Figure 2).

In studying the effect of dietary protein on plasma cholesterol concentration, Kritchevsky (1980) found lactalbumen to be more cholesteremic in rabbits than either corn protein or wheat gluten. Terpstra and Sanchez-Muniz (1981) observed a greater degree of hypercholesterolemia than casein or soy protein. After the second week of dietary treatment, the whey protein diets increased cholesterol level. In comparison to the soy protein diet, the increase was significant at the third, fifth, sixth and seventh weeks. In comparison to casein, the effect of the whey protein diet resulted in significantly elevated plasma cholesterol at weeks three, five and six. No differential effects were observed between the soy protein and casein diets.

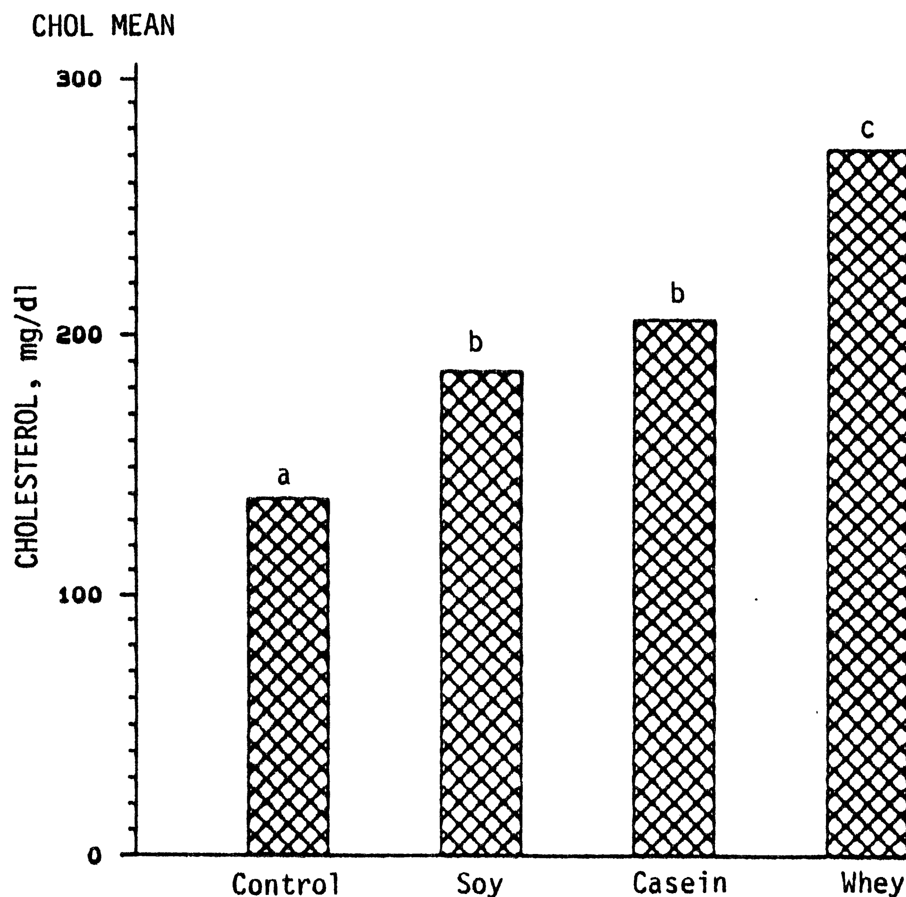
The conclusions drawn from this experiment were:

1. The addition of 0.5% cholesterol to the diet increases plasma cholesterol concentration.

2. Animal protein is not necessarily more hypercholesterolemic than plant protein. In the Japanese quail, the incorporation of soy protein or casein in the diet at the required protein level produces no differential effects upon cholesterol concentration in plasma.

3. Whey protein is more hypercholesterolemic than either soy protein or casein.

In attempting to elucidate the differential effects of dietary protein upon cholesterol metabolism, the lysine:arginine ratio has been found to play a role. Kritchevsky, Tepper and Story (1978) observed that when the lysine:arginine ratio in soy protein was increased to 2.0, serum



Note: Different letters at the top of bars represent statistical differences between mean cholesterol levels. SEM = 7.4 to 7.6.

Figure 2. Mean Cholesterol Levels in Plasma Resulting From Dietary Treatments Over Seven-Week Experimental Period

cholesterol concentration significantly increased in rabbits. In contrast, when the lysine:arginine ratio in casein was decreased to 0.9, serum cholesterol concentration did not change, but atherogenicity decreased by 24%. In this experiment, the lysine:arginine ratio of the soy protein, casein and whey protein diets was 0.85, 1.95 and 3.35, respectively. The degree of hypercholesterolemia observed in the birds paralleled the lysine: arginine ratio.

CHAPTER IV

INFLUENCE OF LOW AND HIGH LEVELS OF SOY AND WHEY PROTEIN IN CHOLESTEROL-FREE AND CHOLESTEROL-ENRICHED DIETS ON PLASMA LIPIDS OF JAPANESE QUAIL

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Summary

Japanese quail were fed a basal diet containing 16 or 40% soy protein without added cholesterol, 16 or 40% whey protein without added cholesterol, 16 or 40% soy protein + 0.5% cholesterol, or 16 or 40% whey protein + 0.5% cholesterol. At two-, four- and six-week intervals, one group of birds receiving each diet was sacrificed. Plasma was analyzed for total cholesterol, HDL-cholesterol and triglyceride concentrations by standard colorimetric method. After two weeks of dietary treatment, plasma cholesterol of birds receiving the low protein diets with added cholesterol was significantly higher relative to birds receiving the other treatments. The elevation was greater when the diet contained whey protein. Plasma cholesterol in birds fed the cholesterol-enriched high protein diets was higher than in birds fed the cholesterol-free high protein diets. No difference was observed between the effects of the high protein diets + cholesterol and the low protein diets without cholesterol. HDL-cholesterol was higher

in birds receiving the low protein diets. At two weeks, plasma triglyceride level was higher when the diet contained soy protein and when the level was 16%. These differences were not observed at six weeks.

These results suggest that: (1) both plant and animal protein suppress hypercholesterolemia in Japanese quail when incorporated at a high (40%) level in diets containing 0.5% added cholesterol, (2) a high protein diet is inversely related to the concentration of HDL-cholesterol and (3) over a longer feeding period (two weeks versus six weeks), the source and level of protein in the diet result in no significant alteration of triglyceride concentration.

Introduction

Elevated plasma cholesterol is one of the major risk factors associated with atherosclerosis and coronary heart disease (Lipid Research Clinics Coronary Primary Prevention Trial Results, 1984). Since nutritional factors are known to contribute to the development of hyperlipidemia, dietary changes represent the first step in controlling the level of blood lipids (Consensus Conference, 1985).

Dietary protein has an effect upon lipid metabolism. Many studies have provided evidence that in some species animal protein elevates plasma cholesterol, while plant protein has a lowering effect (Meeker and Kesten, 1940, 1941; Hamilton and Carroll, 1976; Terpstra and Sanchez-Muniz, 1981). It is reported that when the level of animal protein in the diet is increased, its effect on plasma cholesterol is no different from the effect of plant protein (Terpstra, Schutte and West, 1983). There is evidence that dietary protein significantly alters cholesterol metabolism only when the diet incorporates added cholesterol (Mol et al., 1982).

This experiment was carried out to determine the influence of protein quality and quantity on plasma lipid concentrations in the Japanese quail using a plant protein (soy) and an animal protein (whey) at 16 and 40% levels in cholesterol-free or cholesterol-enriched diets.

Materials and Methods

One hundred and ninety-two adult male Japanese quail were divided into eight groups (three subgroups each), with each of the 24 subgroups housed in a separate pen. Each group was fed a diet containing 16 or 40% soy protein, with or without 0.5% added cholesterol or a diet containing 16 or 40% whey protein, with or without 0.5% added cholesterol (Table I). At two-, and four- and six-week intervals, one group of birds receiving each diet was sacrificed. Blood was collected from each bird and plasma was alyzed for total cholesterol, HDL-cholesterol and triglyceride.

Total Cholesterol Determination

Total cholesterol concentration was determined fluorometrically by standard colorimetric procedure. To 20 microliters of plasma, 0.2 ml of acetic acid-choloroform (3:2) was added and mixed. Five ml of choloroform-acetic anhydride (10:3) were added, followed by the addition of 0.2 ml of concentrated sulfuric acid. The contents were immediately mixed following the addition of the sulfuric acid and centrifuged at 2700 rpm for 10 minutes. The supernatants were decanted into appropriate fluorometer tubes and read in the Model III Turner Flurorometer 40 minutes after sulfuric acid was added to the first tube. A blank was run with each set of samplesin which plasma was replaced by an equal volume of distilled water. A standard (100 mg/dl) was run with samples. Total cholesterol was

TABLE I

DIET COMPOSITION

	W/OUT CHOLESTEROL				WITH CHOLESTEROL			
	16% SOY	40% SOY	16% WHEY	40% WHEY	16% SOY	40% SOY	16% WHEY	40% WHEY
SOY PROTEIN [a]	19.0	47.4	-	-	19.0	47.5	-	-
WHEY PROTEIN [b]	-	-	21.2	53.3	-	-	21.3	53.3
DL-METHIONINE	0.4	-	-	-	0.4	-	-	-
CELLULOSE [c]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
CORNSTARCH	65.0	37.0	63.1	31.1	64.5	36.4	62.6	30.6
CORN OIL [d]	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
VITAMIN TM PMX [e]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
GLISTA SALTS [f]	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
CHOLESTEROL [g]	-	-	-	-	0.5	0.5	0.5	0.5

[a] United States Biochemical Corporation, Cleveland, Ohio

[b] Express Foods Company, Inc., Louisville, Kentucky

[c] Solka-floc (powdered cellulose); crude fiber content, 87%; dietary fiber, 99.5%.

[d] Mazola corn oil

[e] (g/kg): 1.05 g Vit A/D_3; 7.45 g Vit A; 6.05 g Hetrazeen; 5.0 g B_{12}; 60 g Thiamine Mononitrate USP; 18.0 g Riboflavin; 56.5 g Niacin; 12.0 g d-Ca Panto USP; 4.15 g Pyridoxine HCl USP; 2.2 g Folic acid USP; 33.0 g Biotin; 141.0 g Ascorbic Acid; 55.0 g Inositol; 1.1 g PABA; 100.0 g Ethoxyquin; 497.5 g Dextrose

[f] 55.94 g/kg $CaCO_3$; 522.102 g/kg $CaPO_4$, tribasic; 167.82 g/kg K_2HPO_4; 164 g/kg NaCl; 65.263 g/kg $MgSO_4 \cdot 7H_2O$; 12.12 g/kg $MnSO_4 \cdot H_2O$; 9.323 g/kg ferric citrate; 1.865 g/kg Zn carbonate; 0.373 g/kg $CuSO_4 \cdot 5H_2O$; 0.168 g/kg boric acid; 0.168 g/kg $Na_2MoO_4 \cdot 2H_2O$; 0.746 g/kg KI; 0.019 g/kg $CoSO_4 \cdot 7H_2O$; 0.004 g/kg Na_2SeO_3

[g] CH-USP cholesterol, Sigma Co.

calculated as follows: (fluorometric value of sample/fluorometric value of standard) x concentration of standard (McDougal and Farmer, 1957).

Measurement of HDL-Cholesterol

Plasma LDL and VLDL fractions were selectively precipitated by magnesium phosphotungstate and removed by centrifugation. Fifty microliters of the precipitating reagent were added to 400 microliters of plasma and allowed to stand for 15 minutes. The mixture was centrifuged at high speed for 10 minutes, or long enough to obtain a clear supernatant. Twenty microliters of the supernatant were analyzed for HDL-cholesterol using the above procedure for total cholesterol (Sigma Technical Bulletin No. 350-HDL, 1982).

Triglyceride Determination

To a 10 ml capacity screw-cap test tube, 0.8 g triglyceride purifier, 5 ml isopropanol and 200 microliters of plasma were added. The tubes were agitated for at least five minutes, allowed to settle and centrifuged at high speed for five minutes to obtain a clear supernatant. Two ml of the supernatant were transferred from each tube to correspondingly labeled tubes. To each tube, 0.5 ml potassium hydroxide was added and the contents mixed. Tubes were incubated in a 60 degree C water bath for five minutes and then cooled. Sodium periodate solution (0.5 ml) was added to each tube (125 mg sodium periodate in 50 ml 2N acetic acid). Contents were mixed. Ten minutes after the sodium periodate was added, 3 ml of color reagent were added (20 ml 2N ammonium acetate, 40 ml anhydrous isopropanol and 0.15 ml acetylacetone). Upon mixing, the tubes were loosely capped and incubated in the 60 degree C water bath for 30 minutes. The tubes were cooled and contents transferred to appropriate spectrophotometer cuvets and read

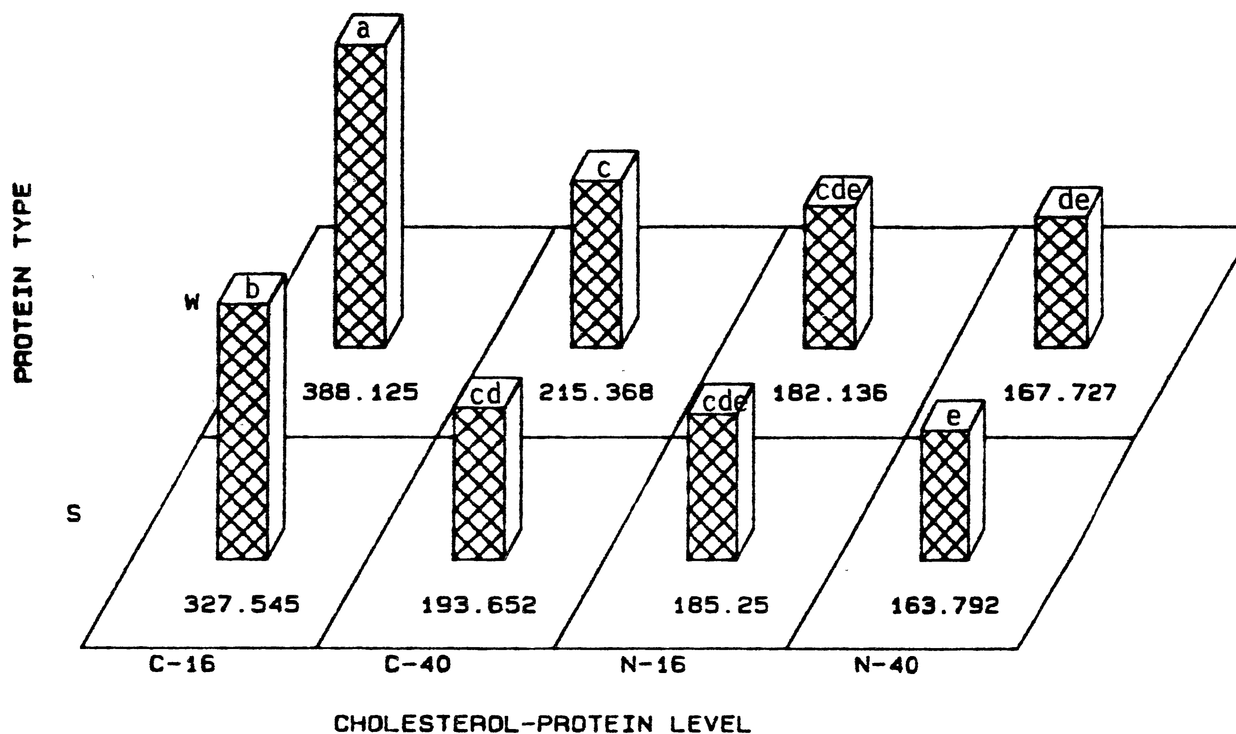
at 410 nm within 20 minutes. (A blank containing 5 ml isopropanol and 200 microliters of water and a standard containing 4.8 ml isopropanol, 200 microliters water and 200 microliters of triolein standard were run with each sample set.) The concentration of triglyceride was calculated as for total cholesterol (Sigma Technical Bulletin No. 405 (4-77), 1980).

In this 2 x 2 x 2 factorial experiment, results were tested by an analysis of variance F test. The difference in means was determined by comparing the level of significance (P=.05) with the observed significance level (OSL) using SAS.

Results and Discussion

The total cholesterol concentration in plasma differed relative to protein source (soy versus whey), protein level (16% versus 40%) and the presence or absence of 0.5% added cholesterol in the diet. Since no difference was observed in plasma cholesterol due to time of sampling (two-week intervals), the values were averaged over the entire experimental period (Figure 1). The low plant and animal protein diets with added cholesterol resulted in significantly higher cholesterol levels than the other dietary treatments, with the elevation being statistically greater when the diet contained whey (animal) protein. The cholesterol-enriched high protein diets resulted in higher cholesterol levels than the corresponding cholesterol-free diets. No difference was observed in cholesterol response with the high protein + cholesterol diets and the low protein diets without added cholesterol.

The inverse relationship observed beween the level of protein in the diet and plasma cholesterol concentration parallels results obtained by other researchers. When Yeh and Leveille (1972) fed high protein diets to chickens, the rate of cholesterol synthesis increased, but serum



Note: Bars not sharing a common letter (abc) represent statistically different mean cholesterol values (P = .05). Range of SEM: 10.9 to 12.8.

Figure 1. Mean Total Cholesterol Concentration in Plasma of Quail Fed Diets Containing Low (16%) or High (40%) Levels of Soy (S) or Whey (W) Protein, With Cholesterol (C) or Without Added Cholesterol (N)

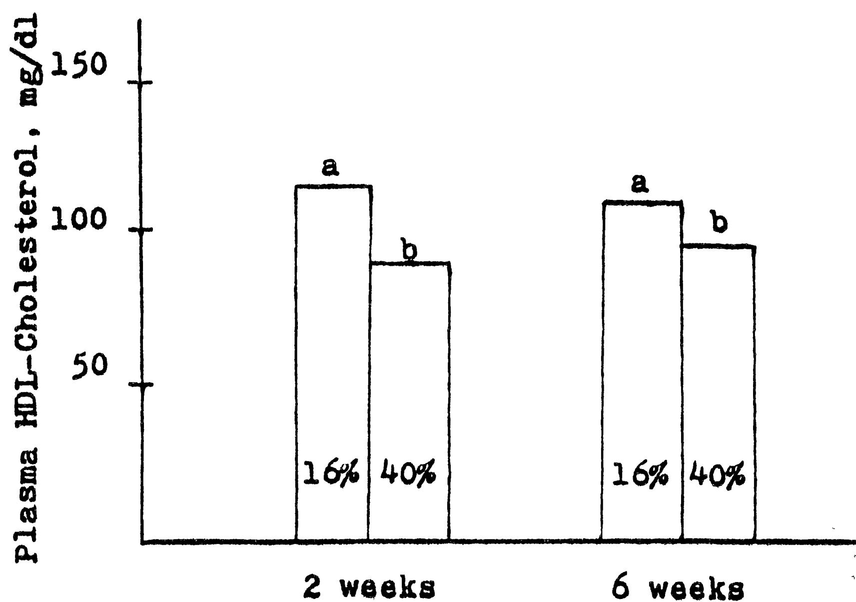
cholesterol decreased. Similar results were observed in chickens by Terpstra, Schutte and West (1983).

Bile acids are known to affect cholesterol metabolism. Some cholesterol is removed from the body through fecal excretion of bile acids. Most of the bile acids, however, are reabsorbed and reconjugated or rehydroxylated by hepatocytes (Kutchai, 1983). It has been speculated that a less efficient process of bile acid reabsorption may be a mechanism by which protein diets reduce plasma cholesterol. Sklan (1980) reported that casein binds bile acids, reducing their availability for reabsorption and thereby lowering cholesterol.

Kritchevsky, Tepper and Story (1978) reported that the lysine:arginine ratio in plant and animal protein may account for alterations in plasma cholesterol. When the lysine:arginine ratio in soy protein was raised, cholesterol increased in rabbits. When the ration was lowered in casein, no change was observed in plasma cholesterol level.

In this experiment, cholesterol in the HDL fraction differed in regard to the level of protein in the diet. When the diet contained 16% protein, HDL-cholesterol was significantly higher than when the diet contained 40%. These results were the same at two and six weeks (Figure 2). When diets were fed to chickens for six weeks that contained plant or animal protein, with or without added cholesterol, no change occurred in HDL-cholesterol (Mol et al., 1982). However, the feeding of cholesterol-enriched diets to rats that contained low or high levels of soy protein or casein resulted in lower HDL-cholesterol than the corresponding cholesterol-free diets (Terpstra, Van Tintelen and West, 1982b).

Plasma triglyceride concentration was significantly higher in quail fed the soy protein diets compared to the whey protein diets after two weeks of feeding. Results were similar when the diets contained 16% rather



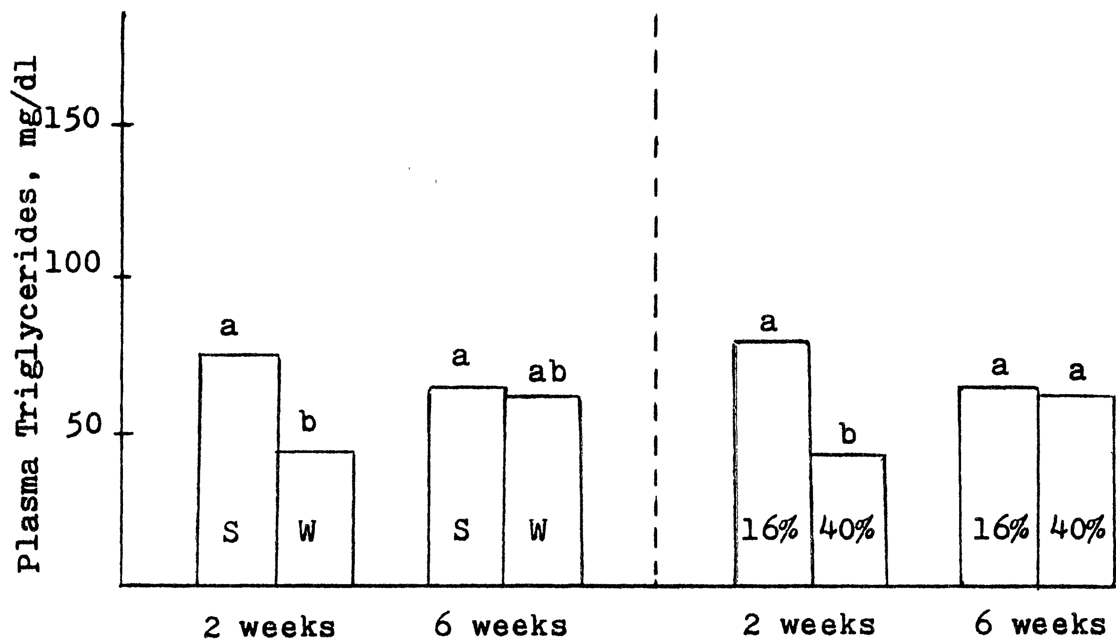
Note: Different letters represent statistical differences between the means (P=.05

Figure 2. Mean HDL-Cholesterol Concentration in Plasma Due to Interaction of Protein Level in the Diet and Week of Sampling

than 40% protein. These differences were not observed after six weeks of feeding (Figure 3).

The differential effects in triglyceride concentration at two and six weeks may relate to feed intake. After two weeks of dietary treatment, birds fed the high protein diets had a greater mean weight loss (7.5 g) than did birds fed the low protein diets (1.95 g). This suggests a lower intake of the high protein diet during the first two weeks and therefore, a lower triglyceride concentration. After six weeks, the change in weight due to the low and high protein diets was nearly equal. Similar triglyceride levels were consistent with the similar changes in weight, suggesting that feed intake was about the same.

It is concluded from this experiment that: (1) both plant (soy) and animal (whey) protein suppress plasma cholesterol level in Japanese quail when incorporated in a cholesterol-enriched diet at a high (40%) level; (2) a low protein diet (16%) results in higher HDL-cholesterol than a high protein diet and (3) the source and level of protein in the diet produce no significant change in plasma triglyceride concentration over a longer feeding period (two weeks versus six weeks).



Note: Different letters indicate statistical differences between the means (P=.05)

Figure 3. Mean Triglyceride Concentration in Plasma Due to Interaction of Protein Source (Soy or Whey) and Week of Sampling and Interaction of Protein Level (16 or 40%) and Week of Sampling

CHAPTER V

INFLUENCE OF SOY PROTEIN, WHEY PROTEIN AND CASEIN ON PLASMA, LIVER AND AORTA LIPIDS IN MALE JAPANESE QUAIL

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Summary

Japanese quail were fed cholesterol-enriched diets containing low (16%) or high (40%) levels of soy protein, whey protein or casein for six weeks. Total cholesterol concentration in plasma did not differ significantly from the baseline level when birds were fed the high soy or high casein diets. The cholesterol level significantly exceeded the baseline level with the low soy or casein diets and the low and high whey protein diets. The high soy and high casein diets also resulted in cholesterol levels that were statistically lower than the corresponding diets containing the low protein level. The low whey protein diet elevated cholesterol in the HDL fraction, while no difference occurred due to the high protein diets. Triglyceride concentration was also higher when birds were fed the low whey protein diet. No significant differential effects of soy protein and casein on HDL-cholesterol and triglyceride concentrations were observed. Liver total cholesterol was significantly higher when the diet contained either level of whey protein or casein. Total cholesterol in the

aorta was highest when the high casein diet was fed and lowest with the high soy protein diet, relative to other treatments. Each of the low/high protein diet combinations resulted in a differential response of total cholesterol in the aorta. Plasma concentrations of free cholesterol were higher when the diet contained low soy protein, low and high whey protein or low casein. These results indicate that: (1) significant differences occur in the level of plasma total cholesterol relative to protein source, protein level and interaction of protein source and level in the diet; (2) low and high levels of protein in the diet significantly alter plasma concentrations of HDL-cholesterol, free cholesterol and triglyceride and (3) the source of dietary protein results in significant differences in the concentrations of total cholesterol in the liver and aorta.

Introduction

Experimental studies have provided evidence that source and level of protein in the diet influence cholesterol metabolisma and the process of atherogenesis. Meeker and Kesten (1940, 1940) observed greater atherogenic effects of casein in rabbits compared to soy protein. Newburgh and Clarkson (1923) observed more rapid atherosclerosis in rabbits when the diet contained 36% beef protein in comparison to 26% of the same protein. In more recent studies, Terpstra and Sanchez-Muniz (1981) observed that plasma cholesterol in rabbits increased 50% after one day of feeding on a cholesterol-enriched soy protein diet and more than 100% on a cholesterol-enriched casein diet. The subsequent cholesterol reduction in the soy-fed group did not occur in the casein-fed group. When female rats were fed diets containing 20 and 50% levels of casein or soy protein, hypercholesterolemia increased as the proportion of casein increased. No difference was observed when the proportion of soy protein was similarly increased

(Terpstra, VanTintelen and West, 1982b). Hevia et al. (1980) obtained similar results in rats when the diets contained 7.5, 15 or 30% casein levels. Park and Liepa (1982) reported a greater elevation of plasma total cholesterol and HDL-cholesterol in rats when the diet contained casein rather than cottonseed protein. Similar increases were observed in plasma triglyceride level when rats were fed casein (Vahouny et al., 1985).

An inverse relationship between plasma cholesterol and the level of protein in the diet was observed in chickens. When the diet contained a high level of casein or soy protein, hypercholesterolemia was prevented. The suppression of plasma cholesterol level did not occur with the low protein diets (Terpstra, Schutte and West, 1983).

This experiment was designed to determine the influence of cholesterol-enriched diets containing 16 or 40% levels of soy protein, whey protein or casein on plasma, liver and aorta lipid concentrations in the Japanese quail.

Materials and Methods

Forty-eight adult male Japanese quail were weighed and divided into six equal groups. Each group was fed a different diet for six weeks that contained 16 or 40% soy protein + 0.5% cholesterol, 16 or 40% whey protein + 0.5% cholesterol or 16 or 40% casein + 0.5% cholesterol (Table I). At the end of the experimental period, birds were re-weighed and sacrificed. A group of eight birds fed the basal diet of Purina Game Bird Layene was also sacrificed. Approximately three ml of blood per bird were collected in vials containing 50 microliters of 10% EDTA. Livers and aortas were also removed. All samples were stored at 4 degrees C for subsequent analysis.

TABLE I

DIET COMPOSITION

	16% Soy	40% Soy	16% Whey	40% Whey	16% Casein	40% Casein
Soy Protein[a]	19.0	47.5	--	--	--	--
Whey Protein[b]	--	--	21.2	53.3	--	--
Casein[c]	--	--	--	--	18.5	46.2
DL-Methionin	0.4	--	--	--	0.2	--
Cellulose[d]	3.0	3.0	3.0	3.0	3.0	3.0
Cornstarch	64.5	36.4	62.6	30.6	65.2	37.7
Corn Oil[e]	7.0	7.0	7.0	7.0	7.0	7.0
Vitamin PMX[f]	0.2	0.2	0.2	0.2	0.2	0.2
Glista Salts[g]	5.4	5.4	5.4	5.4	5.4	5.4
Cholesterol[h]	0.5	0.5	0.5	0.5	0.5	0.5

[a]United States Biochemical Corporation, Cleveland, Ohio

[b]Express Foods Company, Inc., Louisville, Kentucky

[c]Erie Casein Conmpany, Inc., Erie, Illinois

[d]Solka-floc (powdered cellulose)

[e]Mazola corn oil

[f](g/kg): 1.05 g Vit A/D3; 7.45 g Vit A; 6.05 g Hetrazeen; 5.0 g B_{12}; 60 g Thiamine Mononitrate USP; 18.0 g Riboflavin; 56.5 g Niacin; 12.0 g d-Ca Panto USP; 4.15 g Pyridoxine HCl USP; 2.2 g Folic acid USP; 33.0 g Biotin; 141.0 g Ascorbic Acid; 55.0 g Inositol; 1.1 g PABA; 100.0 g Ethoxyquin; 497.5 g Dextrose

[g](g/kg): 55.94 g $CaCO_3$; 522.102 g $CaPO_4$, tribasic; 167.82 g K_2HPO_4; 164 g NaCl; 65.263 g $MgSO_4.7H_2O$; 12.12 g $MNSO_4.H_2O$;9.323 g ferric citrate; 1.865 g Zn carbonate; 0.373 g $CuSO_4.5H_2O$; 0.168 g Na_2MbO_4 $.2H_2O$; 0.746 g KI; 0.019 g $CoSO_4$; 0.004 g Na_2SeO_3

[h]CH-USP cholesterol, Sigma Co

Total Cholesterol Determination

Total cholesterol concentration was determined fluorometrically by standard colorimetric procedure. To 20 milcoliters of plasma, 0.2 ml of acetic acid-chloroform (3:2) was added and mixed. Five ml of chloroform-acetic anhydride (10:3) were added, followed by the addition of 0.2 ml of concentrated sulfuric acid. The contents were immediately mixed following the addition of the sulfuric acid and centrifuged at high speed for 10 minutes. The supernatants were decanted into appropriate fluorometer tubes and read in the Model III Turner Fluorometer 40 minutes after sulfuric acid was added to the first tube. A blank was run with each set of samples in which plasma was replaced by an equal volume of distilled water. A standard was also run with each sample set. Total cholesterol was calculated as follows: (fluorometric value of sample/fluorometric value of standard) x concentration of standard (McDougal and Farmer, 1957).

Measurement of HDL-Cholesterol

Plasma LDL and VLDL fractions were selectively precipitated by magnesium phosphotungstate and removed by centrifugation. Fifty microliters of the precipitating reagent were added to 400 microliters of plasma and allowed to stand for 15 minutes. The mixture was centrifuged at high speed for 10 minutes or long enough to obtain a clear supernatant. Twenty microliters of the supernatant were analyzed for HDL-cholesterol using the above procedure for total cholesterol (Sigma Technical Bulletin No. 350-HDL, 1982).

Triglyceride Determination

To a 10-ml capacity screw-cap test tube, 0.8 g triglyceride purifier,

5 ml isopropanol and 200 microliters of plasma were added. The tubes were agitated for at least five minutes, allowed to settle and centrifuged at high speed for five minutes to obtain a clear supernatant. Two ml of the supernatant were transferred from each tube to correspondingly labeled tubes. To each tube, 0.5 ml potassium hydroxide was added and the contents mixed. Tubes were incubated in a 60 degree C water bath for five minutes and then cooled. Sodium periodate solution (0.5 ml) was added to each tube (125 mg sodium periodate in 50 ml 2N acetic acid). Contents were mixed. Ten minutes after the sodium periodate was added, 3 ml of color reagent were added (20 ml 2N ammonium acetate, 40 ml anhydrous isopropanol and 0.15 ml acetylacetone). Upon mixing, the tubes were loosely capped and incubated in the 60 degree C water bath for 30 minutes. The tubes were cooled and contents transferred to appropriate spectrophotometer cuvets and read at 410 nm within 20 minutes. (A blank containing 5 ml isopropanol and 200 microliters of water and a standard containing 4.8 ml isopropanol, 200 microliters water and 200 microliters triolein standard were run with each sample set). The concentration of triglyceride was calculated as for total cholesterol (Sigma Technical Bulletin No. 405 (4-77), 1980).

Lipid Extraction

To analyze animal tissues for lipid fractions, it was first necessary to isolate and purify the total lipid concentration. The procedure followed was developed by Folch, Lees and Stanley (1957).

The tissue was weighed, blotted, cut into very fine pieces and placed in a 50-ml beaker. Nineteen volumes of 2:1 chloroform-methanol per gram of wet weight were added to the beaker. The beaker was covered with aluminum foil and refrigerated overnight (or until the tissue no longer floated on top of the solution). Following the extraction, the solvent was filtered

and the filtrate collected in 50-ml centrifuge tubes. Ten additional milliliters of chloroform-methanol were added to the extracted tissue and allowed to stand for one-half hour and then filtered. This filtrate was added to the centrifuge tubes. The extracting beaker was rinsed with another 10 volumes of the extracting solution and also added to the filtrate. A volume of 0.04% $CaCl_2$ equal to 22% of the final volume of chloroform-methanol required for the amount of tissue extracted was added to the centrifuge tube. The tube was stoppered, vigorously shaken and centrifuged for 10 minutes at high speed (until the contents separated into two distinct phases). The upper phase was removed.

A volume of "equilibrated solvents upper phase" equal to the volume of 0.04% $CaCl_2$ was added to each tube. This solution was a 3:48:47 mixture of chloroform, methanol and 0.04% $CaCl_2$. Centrifugation and upper phase removal were repeated.

The chloroform layer and any remaining upper phase were made into one phase by the addition of 3 ml of ethanol. Contents were transferred to a round bottom flask and the solvents evaporated using a rotating vacuum evaporator. The lipid was transferred to a 25-ml volumetric flask and brought to volume with hexane. An aliquot of the extract was analyzed for total cholesterol, HDL-cholesterol and triglyceride.

Free Cholesterol

Two hundred microliters of plasma were pipetted into a 13 x 100 ml test tube and three ml of alcohol-acetone mixture (1:1) added. Contents were mixed and tubes centrifuged. Two ml of the supernatant were transferred to a 12 x 15 ml centrifuge tube and two ml of 1% digitonin solution (1.0 g of digitonin in 100 ml of 50% ethyl alcohol) was added. Contents were mixed and placed in a 50 degree C water bath for five minutes (or

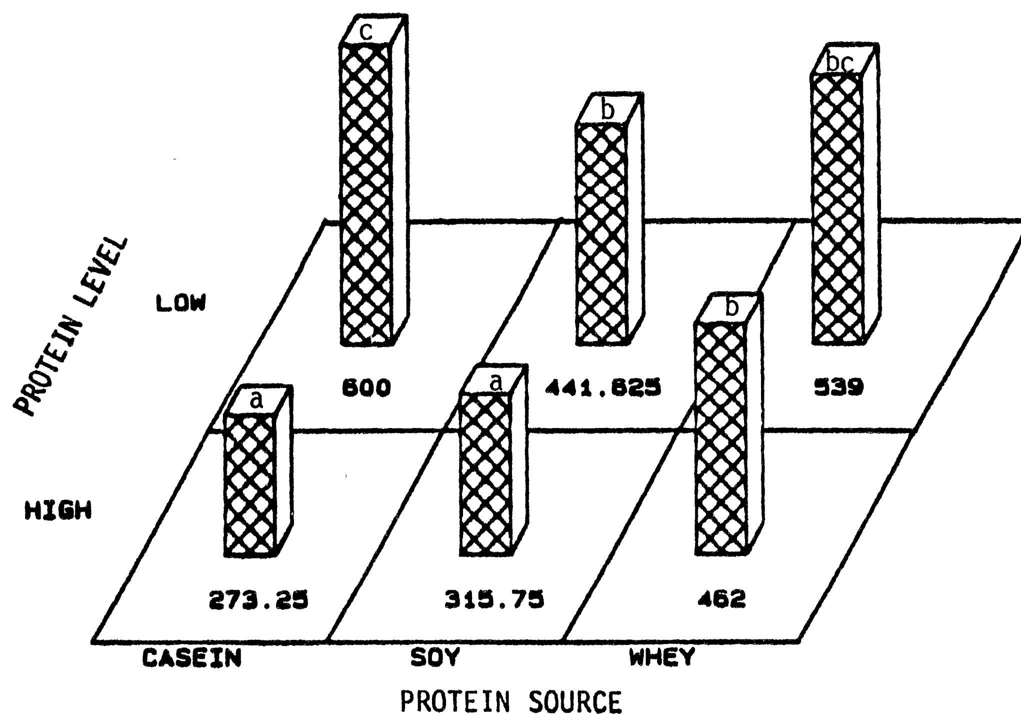
until flocculation occurred). Tubes were cooled at room temperature and centrifuged at high speed for 10 minutes. The supernatant was decanted. Three ml of acetone were added to the tubes, which were again centrifuged. The supernatant was again decanted. Four ml of glacial acetic acid were added to tubes and contents were mixed. Upon adding 3 ml of color reagent, the contents were mixed by inversion of the tubes. (The color reagent was prepared by diluting eight ml of stock iron reagent to 100 ml with concentrated sulfuric acid; the stock iron solution consisted of 2.5 g ferric chloride in 100 ml of concentrated phosphoric acid.) Tubes were allowed to cool for one-half hour. Contents were transferred to appropriate cuvets and read in the Spectrophotometer 20 at 560 nm. A cholesterol standard was run with each set of samples (10 mg/dl). Free cholesterol was calculated as for total cholesterol. Esterified cholesterol was determined by difference between total cholesterol and free cholesterol concentrations (Caraway, 1960).

In this 3 x 2 factorial experiment, results were tested by an analysis of variance F test. The difference in means was determined by comparing the level of significance (P=0.5) with the observed significance level (OSL) using SAS.

Results and Discussion

Change in body weight differed significantly with respect to protein level. When the diet contained 16% protein, the mean weight change was -0.04 grams, compared to -4.04 grams when the diet contained 40% protein.

The effects of the low and high protein diets on total cholesterol concentration in plasma are shown in Figure 1. Plasma cholesterol significantly increased when the diet contained low soy protein or low casein, compared to the corresponding high protein diets. No statistical



Note: Bars not sharing a common letter (abc) represent statistically different mean cholesterol levels (P = .05); pooled SEM = 42.04. AOV F test indicates statistical differences due to protein type (P = .0001); protein level (P = .001); and interaction between protein type and level (P = .01).

Figure 1. Mean Cholesterol Concentrations in Plasma of Quail Feeding on Cholesterol-Enriched Diets Containing Low or High Levels of Soy Protein, Whey Protein or Casein

difference was observed in cholesterol response due to contrasting levels of whey protein. In a previous experiment, plasma cholesterol was significantly reduced when quail were fed a high whey protein diet. The difference may relate to the greater weight loss in the prior experiment and therefore, a greater intake of whey protein per gram of body weight that was effective in suppressing plasma cholesterol.

Differences in HDL-cholesterol were not significant with respect to protein source, but were significant relative to the level of protein in the diet (Table II). When the diet contained 16% protein, HDL-cholesterol was higher than with the 40% protein diets. In some experiments, differences in HDL-cholesterol have been observed due to protein source rather than protein level. Sautier et al. (1983) reported that, in Wistar rats, casein diets increased cholesterol in the HDL fraction compared to soy and whey protein, whereas diets containing casein decreased HDL-cholesterol in Zucker rats (Terpstra, Van Tintelen and West, 1982b). The response of plasma triglycerides (Table III) to the dietary treatments was similar to the HDL-cholesterol response.

In this experiment, total cholesterol in liver differed relative to protein source (Table IV). The concentration was highest in birds fed the whey protein and low casein diets. Results of this study do not confirm Sautier et al. (1983), who observed a lower concentration of liver cholesterol when rats were fed whey protein compared to soy protein or casein. The cholesterol lowering effect may relate to decreased cholesterol synthesis in the liver by certain types of protein in the diet.

The high soy and whey protein diets resulted in significantly lower total cholesterol (Table V) in the aorta than the corresponding low protein diets. Opposite effects resulted from the low and high casein diets.

TABLE II

HDL-CHOLESTEROL CONCENTRATIONS (mg/dl) IN PLASMA OF BIRDS FEEDING ON DIETS CONTAINING LOW (16%) OR HIGH (40%) LEVELS OF SOY PROTEIN, WHEY PROTEIN OR CASEIN FOR SIX WEEKS

Protein Level	Protein Source		
	Soy	Whey	Casein
16%	157[ab]	187[b]	164[ab]
40%	127[a]	118[a]	142[a]

Note: Mean values; pooled SEM = 16.08; eight birds per treatment. Mean values not sharing a common superscript are statistically different (P = .05). AOV F test indicated significant differences between the means due to protein level (P = .003); differences due to protein source not significant (P = .1); interaction between protein source and level not signifcant (P = .31).

TABLE III

TRIGLYCERIDE CONCENTRATIONS (mg/dl) IN PLASMA OF BIRDS FED DIETS CONTAINING LOW (16%) OR HIGH (40%) LEVELS OF SOY PROTEIN, WHEY PROTEIN OR CASEIN FOR SIX WEEKS

Protein Level	Protein Source		
	Soy	Whey	Casein
16%	68[a]	134[b]	92[ab]
40%	62[a]	33[a]	53[a]

Note: Pooled SEM = 21; eight birds per treatment. Mean values not sharing a common superscript are statistically different (P = .05). AOV F test indicates statistical differences due to protein level (P = .007). Differences due to protein source not significant (P = .65); differences due to interaction between protein source and level not significant (P = .08).

TABLE IV

TOTAL CHOLESTEROL (mg/dl) IN LIVER OF QUAIL FED DIETS CONTAINING LOW (16%) OR HIGH (40%) LEVELS OF SOY PROTEIN, WHEY PROTEIN OR CASEIN FOR SIX WEEKS

Protein Level	Protein Source		
	Soy	Whey	Casein
16%	320^{ab}	502^{bc}	627^{c}
40%	245^{a}	514^{bc}	343^{ab}

Note: Pooled SEM = 82; four birds per treatment. Mean values not sharing a common superscript are statistically different (P = .05). AOV F test indicates significant differences due to protein source (P = .0094); differences due to protein level not significant (P = .09); differences due to interaction between protein source and level not significant (P = .2).

TABLE V

TOTAL CHOLESTEROL (mg/dl) IN AORTA OF QUAIL FED DIETS CONTAINING LOW (16%) OR HIGH (40%) LEVELS OF SOY PROTEIN, WHEY PROTEIN OR CASEIN FOR SIX WEEKS

Protein Level	Protein Source		
	Soy	Whey	Casein
16%	208^{c}	215^{c}	193^{bc}
40%	115^{a}	172^{b}	315^{d}

Note: Pooled SEM = 7.9; two pooled samples from eight birds. Mean values not sharing a common superscript are statistically different (P = .05). AOV F test indicates significant differences due to protein source (P = .0001) and interaction between protein source and level (P = .0001); differences due to protein level not significant (P = .49).

Plasma concentrations of free cholesterol (Table VI) were measured and esterified cholesterol was determined by difference between total and free cholesterol concentrations (Table VII). When the basal diet was fed, 26% and 75% of total cholesterol existed in the free and esterified forms, respectively. In comparing the effects of low and high levels of each protein on the concentration of esterified cholesterol, only the high casein diet produced significantly lower levels than the corresponding low protein diet.

The enzyme, lecithin:cholesterol acyltransferase (LCAT), esterifies free cholesterol in plasma and is also associated with the transfer of tissue cholesterol to the liver for removal from the body (Glomset, 1968). Dietary protein influences cholesterol metabolism through an effect on LCAT. When animal protein was fed to pigs, the concentration of free cholesterol increased, but the activity of LCAT decreased. The unexpected decrease in LCAT activity suggested that factors other than the level of free cholesterol may be involved in the effect of LCAT on cholesterol metabolism (Forsythe et al., 1980).

In this experiment, animal protein had a similar effect on free cholesterol as in pigs. The feeding of the low whey protein and the low casein diets significantly increased the concentration of free cholesterol above the baseline level. The low soy and high whey protein diets caused an increase, but without significance. The low soy, low and high whey and low casein diets increased esterified cholesterol above the baseline level. These results suggest that the activity of LCAT in the Japanese quail is influenced by the origin and the level of protein in the diet.

In conclusion, this experiment shows that: (1) animal protein, as well as plant protein, can reduce plasma cholesterol in Japanese quail when incorporated in cholesterol-enriched diets at a high (40%) level; (2) a low

TABLE VI

CONCENTRATION OF FREE CHOLESTEROL (mg/dl) IN PLASMA OF QUAIL FED DIETS CONTAINING LOW (16%) OR HIGH (40%) LEVELS OF SOY PROTEIN OR CASEIN FOR SIX WEEKS

Protein Level	Protein Source		
	Soy	Whey	Casein
16%	125^{abc}	206^{c}	152^{bc}
40%	62^{a}	124^{abc}	84^{ab}

Note: Pooled SEM = 28.6; five birds per treatment. Mean values not sharing a common superscript are statistically different (P = .05). AOV F test indicates significant differences due to protein level (P = .005); differences due to protein source and interaction between protein source and level not significant (P = .06) and (P = .94).

TABLE VII

CONCENTRATION OF ESTERIFIED CHOLESTEROL (mg/dl) IN PLASMA OF QUAIL FED DIETS CONTAINING LOW (16%) OR HIGH (40%) LEVELS OF SOY PROTEIN, WHEY PROTEIN OR CASEIN FOR SIX WEEKS

Protein Level	Protein Source		
	Soy	Whey	Casein
16%	305^{ac}	336^{bc}	409^{c}
40%	255^{ab}	355^{bc}	173^{a}

Note: Pooled SEM = 46.2; five birds per treatment; esterified cholesterol estimated by difference between total and free cholesterol. Different superscripts represent significant differences between means (P = .05). AOV F test indicates significant differences due to protein level (P = .02) and interaction between levels and source (P = .02).

level of animal protein (whey) significantly increases HDL-cholesterol as well as triglyceride in plasma; (3) total cholesterol in the liver is in-increased when the diet contains animal protein, regardless of level; total cholesterol in the aorta is influenced by the interaction of protein source and level and (5) the level of protein in the diet alters free cholesterol concentration in plasma while the interaction of protein source and level alters esterified cholesterol level in plasma.

CHAPTER VI

INFLUENCE OF DIFFERENT LEVELS OF SOY AND WHEY PROTEIN IN CHOLESTEROL-FREE AND CHOLESTEROL-ENRICHED DIETS ON PLASMA CONCENTRATIONS OF LOW DENSITY LIPOPROTEIN FRACTIONS

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Summary

Adult male Japanese quail were fed diets containing 16% or 40% soy protein without added cholesterol; 16 or 40% soy protein + 0.5% added cholesterol; 16 or 40% whey protein without added cholesterol; or 16 or 40% whey protein + 0.5% added cholesterol. At the end of six weeks, the birds were sacrificed and blood samples collected. The concentration of chylomicron, VLDL and LDL particles was determined turbidimetrically by treating aliquots of plasma with appropriate concentrations of calcium or magnesium ions and heparin sodium. The concentration of LDL particles was significantly lower when the diet contained 40% whey protein + cholesterol. The concentration of LDL particles with the high soy + cholesterol diet was not statistically different from the levels observed with the low soy or low whey diets without cholesterol. No significant alteration occurred in the concentration of VLDL particles due to dietary treatment. Chylomicron

particles were significantly reduced when the diet contained high soy protein + cholesterol compared to high whey + cholesterol. Since the low density lipoproteins (chylomicrons, VLDL and LDL) are potentially atherogenic, these results suggest that dietary protein of animal origin is no more atherogenic in the Japanese quail than is plant protein when incorporated in the diet at a 40% level.

Introduction

Lipoproteins are the plasma-soluble complexes of lipid and protein that transport blood lipids. Chylomicrons and VLDL are the main carriers of exogenous and endogenous triglyceride, respectively. LDL transport one-half to two-thirds of total cholesterol in plasma, while HDL transport phospholipids and a lower level of cholesterol (Gotto, 1984). Elevated concentrations of lower density lipoproteins, particularly LDL, are associated with increased risk of atherosclerosis and coronary heart disease (CHD). Fractional levels of lipoproteins in plasma can be altered by dietary factors such as fat, cholesterol and protein (Kritchevsky, Tepper and Story, 1978; Terpstra, Van Tintelen and West, 1982b).

This experiment was carried out to determine the influence of high and low levels of dietary protein of plant and animal origin on the concentration of low density lipoprotein particles in the plasma of Japanese quail.

Materials and Methods

Forty adult male Japanese quail were divided into eight groups and fed a diet that contained: (1) 16% soy protein without cholesterol; (2) 16% soy protein + 0.5% added cholesterol; (3) 40% soy protein without cholesterol; (4) 40% soy protein + 0.5% cholesterol; (5) 16% whey protein without

cholesterol; (6) 16% whey protein + 0.5% cholesterol; (7) 40% whey protein without cholesterol or (8) 40% whey protein + 0.5% cholesterol (Table I). After six weeks, birds were sacrificed and blood samples collected in vials treated with 10% EDTA. Plasma was separated and the concentration of lower density lipoprotein particles in each plasma sample was determined.

Lipoprotein Precipitation

The method followed was developed by Scholnick, Burstein and Eder (1971). The plasma concentration of chylomicrons, VLDL and LDL particles was estimated turbidimetrically by selective precipitation with heparin sodium in the presence of calcium and magnesium ions. The salt solution of low ionic strength + 1% heparin sodium resulted in the combined precipitation of chylomicron + VLDL + LDL particles. When the salt solution was of intermediate ionic strength in the presence of 5% heparin sodium, chylomicron + VLDL particles were precipitated, and with a high ionic strength salt solution and 5% heparin sodium, only chylomicrons were precipitated. For each plasma sample, three precipitations were carried out.

Tube one contained 2 ml of 0.025M calcium chloride, 0.2 ml plasma and 0.04 ml of 1% heparin sodium in normal saline. Prior to the addition of heparin, the optical density (OD) of the sample was determined in the Spectrophotometer 20 at 640 nm. After the heparin was added, the sample was mixed and four minutes later, the OD was determined again. The difference in turbidity represented the concentration of chylomicron + VLDL + LDL particles.

To the second tube for each plasma sample, two ml of 0.1M magnesium chloride in 0.7% saline and 0.2 ml plasma were added and the OD determined. Following the addition of 0.04 ml of 5% heparin sodium, OD was again

TABLE I

DIET COMPOSITION

	W/OUT CHOLESTEROL				WITH CHOLESTEROL			
	16% SOY	40% SOY	16% WHEY	40% WHEY	16% SOY	40% SOY	16% WHEY	40% WHEY
SOY PROTEIN [a]	19.0	47.4	-	-	19.0	47.5	-	-
WHEY PROTEIN [b]	-	-	21.2	53.3	-	-	21.3	53.3
DL-METHIONINE	0.4	-	-	-	0.4	-	-	-
CELLULOSE [c]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
CORNSTARCH	65.0	37.0	63.1	31.1	64.5	36.4	62.6	30.6
CORN OIL [d]	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
VITAMIN TM PMX [e]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
GLISTA SALTS [f]	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
CHOLESTEROL [g]	-	-	-	-	0.5	0.5	0.5	0.5

a United States Biochemical Corporation, Cleveland, Ohio

b Express Foods Company, Inc., Louisville, Kentucky

c Solka-floc (powdered cellulose); crude fiber content, 87%; dietary fiber, 99.5%.

d Mazola corn oil

e (g/kg): 1.05 g Vit A/D_3; [illegible].45 g Vit A; 6.05 g Hetrazeen: 5.0 g B_{12}; 60 g Thiamine Mononitrate USP; 18.0 g Riboflavin; 56.5 g Niacin; 12.0 g d-Ca Panto USP; 4.15 g Pyridoxine HCl USP; 2.2 g Folic acid USP; 33.0 g Biotin; 141.0 g Ascorbic Acid; 55.0 g Inositol; 1.1 g PABA; 100.0 g Ethoxyquin; 497.5 g Dextrose

f 55.94 g/kg $CaCO_3$; 522.102 g/kg $CaPO_4$, tribasic; 167.82 g/kg K_2HPO_4; 164 g/kg NaCl; 65.263 g/kg $MgSO_4 \cdot 7H_2O$; 12.12 g/kg $MnSO_4 \cdot H_2O$; 9.323 g/kg ferric citrate; 1.865 g/kg Zn carbonate; 0.373 g/kg $CuSO_4 \cdot 5H_2O$; 0.168 g/kg boric acid; 0.168 g/kg $Na_2MbO_4 \cdot 2H_2O$; 0.746 g/kg KI; o.019 g/kg $CoSO_4 \cdot 7H_2O$; 0.004 g/kg Na_2SeO_3

g CH-USP cholesterol, Sigma Co.

determined. The difference in OD represents the sum of chylomicrons + VLDL.

The OD of the third tube, containing 2 ml of 0.1M magnesium chloride in 1% saline and 0.2 ml plasma, was determined. After adding 0.04 ml of 5% heparin, the second determination was made. The difference in OD is indicative of chylomicron concentration.

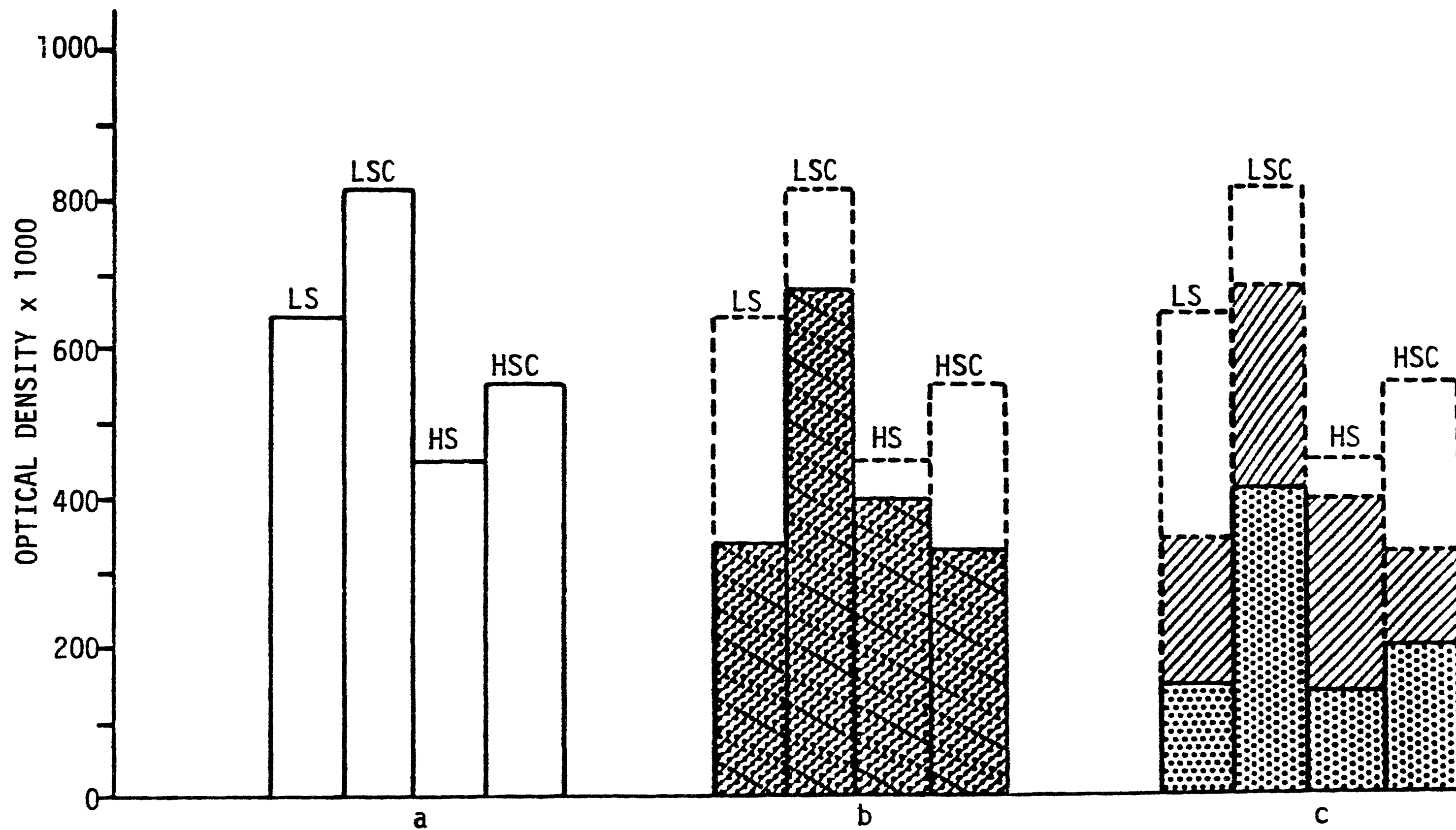
The OD for each tube was multiplied by 1000. The difference in OD between tubes 1 and 2 gives an estimate of the concentration of LDL particles and the difference between tubes 2 and 3 estimates the concentration of VLDL particles.

In this 2 x 2 x 2 factorial experiment, results were tested by an analysis of variance F test. The difference in means was determined by comparing the level of significance (P=.05) with the observed significance level (OSL) using SAS.

Results and Discussion

Figure 1-a shows the total concentration of precipitated lower density lipoproteins (chylomicrons + VLDL + LDL) when the quail were fed the different soy protein diets. The high soy protein + cholesterol diet resulted in a combined concentration of chylomicrons, VLDL and LDL particles that was significantly lower than the low soy + cholesterol diet. No significant difference in particle concentration is observed between soy and whey as the protein source when the dietary protein is low (16%).

The combined concentration of chylomicron and VLDL particles is shown by the shaded area of Figure 1-b, and the estimated concentration of LDL particles by the broken lines in the same figure. The estimated LDL concentration resulting from the high soy + cholesterol diet was significantly different only in comparison to the cholesterol-free high soy protein diet.



Note: a=chylomicron + VLDL + LDL particles; b=chylomicron + VLDL particles (shaded bars) and estimated concentration of LDL particles (broken lines) by difference between a and b; c=chylomicron particles (dotted bars), estimated VLDL (diagonal bars) and estimated LDL particles

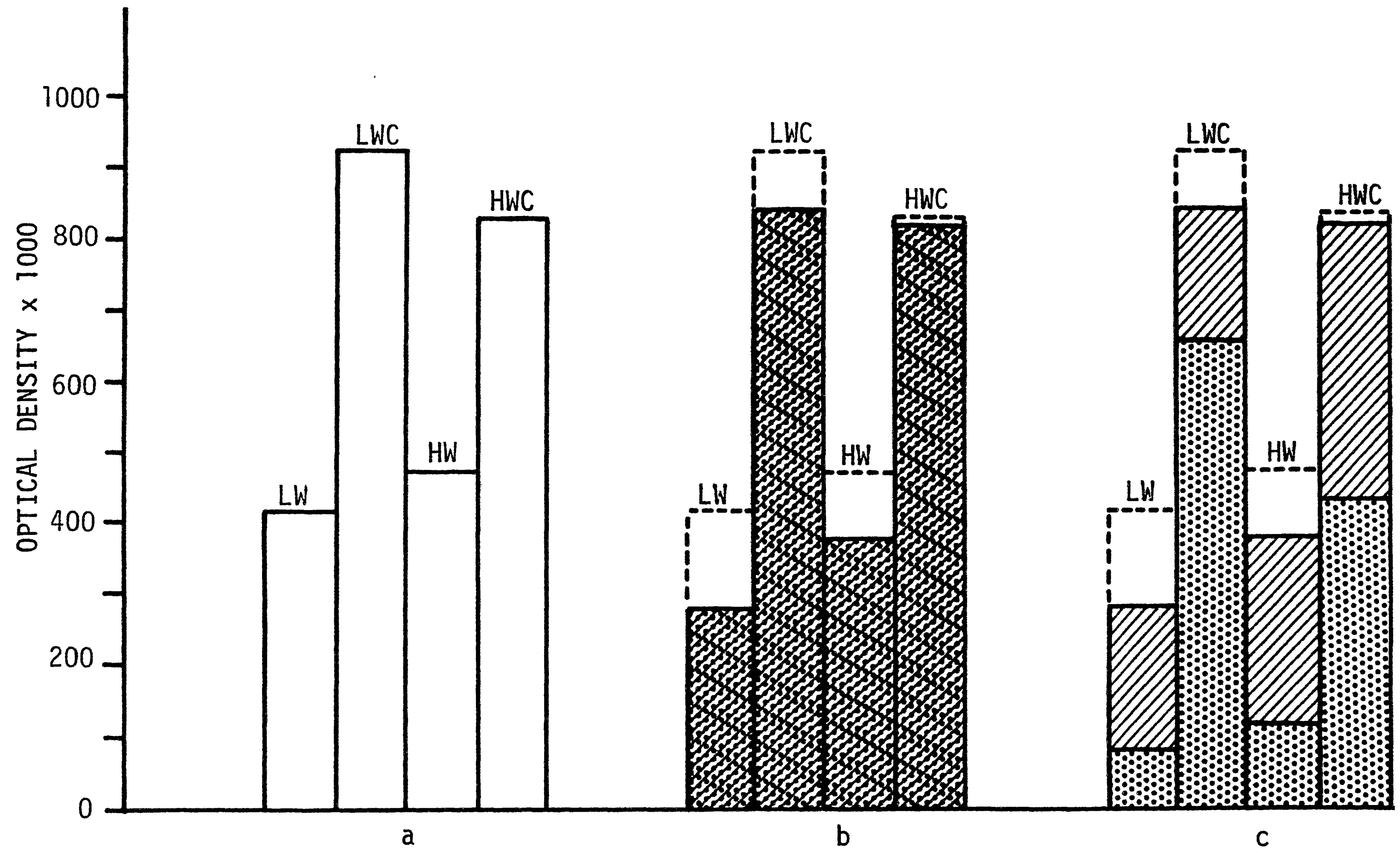
Figure 1. Selective Precipitation of Lower Density Lipoproteins When Quail Were Fed Diets Containing Different Levels of Soy Protein, With or Without Added Cholesterol

When chylomicrons alone were precipitated (Figure 1-c, dotted bars), the cholesterol-enriched high soy protein diet resulted in a concentration of particles that did not differ significantly from the concentration with the high soy or low soy diets without cholesterol, but was significantly lower than when the birds received the low soy diet with cholesterol. No difference occurred in the level of VLDL particles due to dietary treatment.

The cholesterol-enriched diets containing both low and high levels of whey protein resulted in an elevated total concentration of low density lipoproteins in comparison to the cholesterol-free diets (Figure 2-a). This elevation was due to the combined concentration of chylomicron + VLDL particles, as shown in Figure 2-b. The estimated concentration of LDL particles (broken lines, Figure 2-b) did not differ significantly, due to alterations in the whey protein diets as with the soy protein diets.

Chylomicron particles were significantly lower in concentration when the high whey + cholesterol diet was fed relative to the low whey + cholesterol diet, and significantly higher compared to the cholesterol-free whey protein diets (Figure 2-c).

Because of the large size of chylomicron particles relative to other lipoproteins, they are often disregarded as contributors to atherosclerosis, since they cannot enter the normal vessel wall. This incapacity to enter the vessel wall can be altered if chylomicrons are degraded at the endothelial surface and the high concentration of degradation products cause damage to the vessel wall. The large chylomicron particles can then enter the vessel wall and possibly participate in the atherogenic process (Zilversmit, 1973; Steinberg, 1981). In terms of the resulting high chylomicron levels, diets that contain 16% or 40% whey protein + 0.5%



Note: a=chylomicron + VLDL + LDL particles; b=chylomicron + VLDL particles (shaded bars and estimated concentration of LDL particles (broken lines) by difference between a and b; c=chylomicron particles (dotted bars), estimated VLDL (diagonal bars and estimated LDL particles

Figure 2. Selective Precipitation of Lower Density Lipoproteins When Quail Were Fed Diets Containing Different Levels of Whey Protein, With or Without Added Cholesterol

added cholesterol or 16% soy protein + 0.5% cholesterol are potentially atherogenic.

The concentration of VLDL particles did not differ significantly when the soy or whey protein diets, with or without added cholesterol, were fed to the birds. High levels of VLDL and LDL cholesterol are positively correlated with atherosclerosis (Castelli et al., 1977) and cholesterol feeding has been shown to cause a pronounced increase in VLDL in quail (Day, Barker and Stafford, 1974). The observation in this experiment that animal or plant protein in cholesterol-free or cholesterol-enriched diets caused no difference in the concentration of VLDL particles suggests that, relative to VLDL, animal protein is no more atherogenic than is plant protein in the Japanese quail.

The concentration of LDL particles was significantly higher when birds were fed the high soy + cholesterol diet versus the high whey + cholesterol diet or the low soy diet without cholesterol versus the low whey diet without cholesterol. The results suggest that soy protein is potentially more atherogenic than whey protein relative to the concentration of LDL particles when the diet incorporates a 40% level + 0.5% added cholesterol or a 16% level without added cholesterol.

The results of this experiment indicate that high or low levels of soy or whey protein and the presence or absence of 0.5% cholesterol in the diet alter the concentrations of LDL and chylomicron particles in plasma of Japanese quail without altering the concentration of VLDL particles.

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