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MODIFICATION OF PLASMA CHOLESTEROL PARAMETERS, LIPOPROTEINS, LECITHIN: CHOLESTEROL ACYL TRANSFERASE ACTIVITY AND TISSUE LIPIDS BY DIET AND EXERCISE IN PIGS AND CHICKENS

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ABSTRACT

MODIFICATION OF PLASMA CHOLESTEROL PARAMETERS, LIPOPROTEINS, LECITHIN: CHOLESTEROL ACYL TRANSFERASE ACTIVITY AND TISSUE LIPIDS BY DIET AND EXERCISE IN PIGS AND CHICKENS

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While elevated plasma cholesterol levels are one of the major risk factors associated with the development of atherosclerosis, the effects of diet and exercise on plasma cholesterol levels are still controversial. Experiments were undertaken, in pigs and chickens, to investigate how different dietary components (type of protein, type of fat, and type of fiber) or an aerobic exercise program affect cholesterol parameters.

Two experiments, utilizing young male pigs, tested the effects of varying the polyunsaturated (Puf) to saturated (Sat) fat ratio (P:S) and substituting plant (Pl) for animal (An) protein. The diets provided 16% of the energy as protein and 42% as fat with a P:S ratio of 3.0 in high-Puf diets and 0.3 in the high-Sat diets. In the first experiment, changing the P:S ratio from 0.3 to 3.0 reduced plasma cholesterol levels by 44 mg/dl. The high density lipoprotein (HDL) to low-density lipoprotein (LDL) ratio was 1.84 in the high-Puf diet and 1.42 in the high-Sat diet.

A second experiment tested the effects substituting plant (50% from soybean and 25% each from corn and wheat) for animal (90% casein and 10% lactalbumin) protein on these same parameters.

Increasing the P:S ratio reduced plasma cholesterol levels by 30% and also reduced the molar LCAT activity. Plant protein (compared to animal protein) reduced plasma total cholesterol levels by 23%. Other changes induced by substituting plant protein for animal protein were: increased fractional and molar LCAT rate; increased HDL:LDL ratio; and decreased plasma amino acid levels of lysine, threonine, valine, isoleucine, and leucine. Another important finding in this experiment was the ability of the dietary protein source to affect plasma cholesterol levels independent of the dietary P:S ratio.

In another set of experiments, both pigs and chickens were used as experimental models to study the effect two different sources of dietary fiber have on body composition, plasma cholesterol parameters and LCAT activity. Wheat bran (WB) or rolled oats (RO) were fed as 6.5% neutral detergent fiber (NDF) in diets for 17 weeks to weanling castrated pigs and for 5 weeks to mature "broiler type" roosters. Diet composition was (% energy): 18% protein, 42% fat (P:S = 0.3), and 40% carbohydrate with 0.05% added cholesterol.

Feeding RO reduced body fat in both pigs and chickens compared to feeding WB or a control diet (CN, < 1% NDF). While not affecting total cholesterol levels, in pigs, feeding WB increased HDL cholesterol levels, the percentage of cholesterol in the HDL fraction, and the HDL:LDL ratio. Feeding RO did not reduce plasma total cholesterol levels in pigs but did reduce levels in chickens (220 mg/dl) compared to CN (266 mg/dl). In both pigs and chickens RO increased

HDL cholesterol levels and the molar LCAT activity compared to the other two treatments.

In another experiment, young male castrated pigs were exercised aerobically; alternately 3 mph for 20 minutes per day or 3.3 mph for 9 minutes per day, seven days a week. During the first five weeks of the experiment, which consisted of 3 weeks training and 2 weeks of maximum exercise, the pigs were fed a corn-soy grower ration. The relatively short exercise program had no effect on any plasma cholesterol parameter. After 5 weeks the pigs were fed the high-fat (P:S = 0.3), high cholesterol diet fed as the control diet in the fiber experiments. Ten weeks of exercise significantly reduced plasma total and unesterified cholesterol levels. HDL cholesterol levels were not changed by exercise but the percentage of total cholesterol in the HDL fraction was significantly greater in the exercised compared to non-exercised pigs. Exercise also altered the plasma lipoprotein profile; increasing the proportion of HDL and decreasing the percentage of LDL.

In total, these experiments have shown that dietary changes or an aerobic exercise program can significantly affect body composition, plasma cholesterol parameters, including total and HDL cholesterol concentrations, and plasma LCAT activity.

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PART III

EFFECT OF FEEDING ROLLED OATS OR WHEAT BRAN ON PLASMA LIPIDS, LIPOPROTEINS, LCAT AND TISSUE LIPIDS IN PIGS AND CHICKENS

INTRODUCTION

Cardiovascular disease is one the the major health problems in the United States and the etiology of the disease has been intensively investigated in recent years (1). Most investigators will agree that plasma cholesterol levels are perhaps the most important single parameter involved in the development of atherosclerosis. Zilversmit (2) has recently reviewed this area, concluding that evidence supporting cholesterol involvement in atherogenesis are: 1) accumulation of free and esterified cholesterol in human atherosclerotic plaques; 2) increased incidence of coronary heart disease (CHD) earlier in life in persons with genetic hypercholesterolemia; 3) epidemiological studies have consistently shown high correlations between CHD and plasma cholesterol levels; and 4) animal studies have shown that cholesterol accumulation into arteries follows induced hypercholesterolemia.

What is still debatable is whether dietary changes can alter plasma cholesterol levels sufficiently to affect atherogenesis. The Senate Select Committee on Nutrition and Human Needs (3) after reviewing data linking diet to plasma cholesterol levels suggested these changes in the American diet as regarding atherosclerosis: 1) reduce caloric consumption; 2) reduce cholesterol consumption to 300 mg/day; 3) reduce overall fat consumption from 40 to 30 percent

of dietary calories; and 4) reduce saturated fat consumption to 10 percent of dietary calories and balance with 10 percent of the calories from monounsaturated and 10 percent of the calories from polyunsaturated fats. While prominent scientists (Hegsted (4); Glueck and Connors (5)) support these changes, other, equally as prominent scientists (Ahrens (6); Reisser (7)), suggest that such changes are unwarranted.

Clearly whether the diet has the capacity to affect the development of atherosclerosis needs further investigation. The primary objective of these experiments was to investigate whether altering dietary components in the presence of high-fat, high-cholesterol diets could beneficially change cholesterol parameters in pigs or chickens. The diet changes which were investigated were: 1) substituting plant protein for animal protein; 2) altering the polyunsaturated fat:saturated fat (P:S) ratio from 0.3 to 3.0; and 3) feeding 6.5% neutral detergent fiber (NDF) from either wheat bran or rolled oats in the diet. Also investigated was the effect a moderate exercise program had on these same parameters in pigs.

PART I

REVIEW OF LITERATURE

CARDIOVASCULAR DISEASE

OCCURRENCE

Cardiovascular disease (CVD) is the leading cause of death in the United States. While death rates (death/100,000 persons) have been falling slightly since 1968 (1), cardiovascular disease was still responsible for 1,037,000 deaths or 52.9% of total deaths in 1974 (8)(latest year for complete figures). By comparison the next two leading causes of death combined claimed fewer lives; malignancies (351,000 deaths) and accidents (115,000 deaths)(8). Another indication of the severity of CVD is the death rate in 45-64 year old males, the major wage earners in the United States. The incidence of coronary heart disease (CHD) deaths in males 25-44 years old is 48 per 100,000 persons. But in males 45-64 years the incidence jumps to 621 deaths per 100,000 persons. Although CHD is a degenerative disease, this increase in the incidence of deaths in the major wage earners in the United States has tremendous economic impact. Kolata has estimated the economic loss approaches 40 billion dollars annually (9).

The United States is not the only country with a high incidence of CHD. Keys (10) in an analysis of a World Health Organization (WHO) report on death in 34 countries, listed six countries with a high incidence of CHD: Australia, Canada, Finland, New Zealand, Scotland, and United States. Eight counties reported a low

incidence of CHD deaths: Bulgaria, Greece, Italy, Japan, Poland, Portugal, Romania, and Taiwan. Countires were eliminated where deaths from ill-defined or unknown causes were high. Keys suggests that the data on the above countries is accurate and reflects actual CHD deaths. Data from Keys' seven country survey supports the WHO report (11). For instance, while both Japan and Italy have total mortality rates similar to the United States and Finland, they have CHD death rates of 30% or less than that of the United States or Finland. These and other reports have led to many epidemiological studies to elucidate differences responsible for the differences in CHD mortality between countries. While results from epidemiological studies cannot prove cause and effect, they can provide evidence that the incidence of CHD correlates with the occurrence of various parameters, which have been called risk factors.

RISK FACTORS

The earliest epidemiological studies were primarily retrospective in nature. Keys (10) has recently reviewed epidemiological evidence linking CHD to diet. In 1916, C.D. DeLangens, a Dutch physician, reported that natives of Java had a lower incidence of atherosclerosis than the Dutch. He related this decreased morbidity to lower blood cholesterol levels. Similarly, retrospective studies were undertaken to investigate the reasons a decreased incidence in CHD deaths occurred after World War II in both Finland and Norway. While there were many

changes in lifestyle, Malmros (12) concluded that the factor that correlated most highly with the decline in CHD was the decrease in saturated fat consumption.

In 1948, the first major prospective study was undertaken to study CHD in Framingham, Massachusetts (13). This study initially surveyed over 5200 persons free from CHD and is still ongoing. Results from this study indicated three major risk factors in the development of atherosclerosis (14): elevated serum cholesterol levels, hypertension, and cigarette smoking. Other minor risk factors identified have been obesity, inactivity and diabetes mellitus.

Kannel et al. (14) has reported on the relative incidence of the above risk factors in the United States population (Table 1). Reports from Gordon et al. (15) have indicated that the morbidity increases geometrically when two or more risk factors are present in the same person. Men aged 30-59, with all three risk factors present have eight times the probability of developing CHD than similar aged men with no risk factors present (16). On the average, 18% of the men and women in the United States are at risk for the three major risk factors.

While all three risk factors are important, this review of literature concentrates on hypercholesterolemia. Epidemiological studies have reported that, in addition to the amount of plasma cholesterol, the lipoprotein fraction that carries cholesterol is also important in the development of atherosclerosis. An increased low density cholesterol lipoprotein (LDL) cholesterol level correlates with an increased incidence of CHD (17, 18) and an

Table 1. Percent prevalence of selected "risk factors" in the United States.[1]

Age (yr)	Inactivity[2]	Obesity[3]	Hypertension[4]	Cigarette Smoking[5]	Hyper-cholesterolemia[6]
			Men		
35-44	12.1	12.5	13.5	48.6	20.2
45-54	16.9	14.7	18.3	43.1	25.7
55-64	21.0	12.5	22.3	37.4	23.5
65-74	27.1	12.7	27.1	22.8	21.6
			Women		
35-44	13.3	20.1	8.5	38.8	12.9
45-54	19.3	24.2	18.2	36.1	28.0
55-64	30.8	30.9	31.2	24.2	49.7
65-74	39.0	27.2	47.6	10.2	51.0

[1]After Kannell et al. (14).

[2]Inactivity is average oxygen consumption less than 0.30 liter/min (1954-58).

[3]Obesity is weight 20 percent or more above median (1960-62).

[4]Hypertension is a blood pressure at least 160/90 (1960-62).

[5]Cigarette smoking refers to current habits (1970).

[6]Hypercholesterolemia is serum cholesterol concentrations 260 mg/100 ml or greater (1960-62).

increased high density lipoprotein (HDL) cholesterol level, correlates with a decreased incidence of CHD (17, 18). Thus any changes which can increase the ratio of HDL/LDL cholesterol could be beneficial in the prevention of CHD. This review first discusses lipoprotein and cholesterol metabolism. Then discussed are the effects diet (cholesterol, protein and fiber) and exercise exert on plasma cholesterol and lipoprotein metabolism.

LIPOPROTEIN METABOLISM

The composition of the four major classes of lipoproteins, chylomicra, very low density lipoprotein (VLDL), LDL and HDL are presented in Table 2. The lipoproteins are generally classified according to their flotation in salt gradients (19). Because of their importance in cholesterol metabolism and the development of atherosclerosis, intensive investigations into their structure, composition and metabolism have been undertaken in recent years.

Nascent chylomicra, the lowest density lipoprotein class, are synthesized in the intestine to transport exogenous triglycerides from the small intestine. The composition of chylomicrons is 90% triglycerides, 5-7% phospholipids and 1-2% of cholesterol and protein (20). Apoprotein B (apoB), the major apoprotein of chylomicra, is also synthesized in the intestine (21) and is necessary for binding and transport of lipids (22). In individuals with abetalipoproteinemia there is an absence of apoB and triglycerides, while synthesized by intestinal cells, are not transported from the intestinal cells to the blood (22). Once nascent chylomicra

Table 2. Composition and properties of human plasma lipoproteins.[1]

Properties	Chylomicrons	VLDL	LDL	HDL
Density, g/ml	0.95	0.95-1.006	1.006-1.063	1.063-1.210
Major apoproteins	apoB apoC-I apoC-II apoC-III	apoB apoC-I apoC-II apoC-III apoE	apoB	apoA-I apoA-II
Minor apoproteins	apoA-I apoA-II	apoA-I apoA-II apoD		apoC-I apoC-II apoC-III apoD apoE
Major lipids	triglycerides	triglycerides	cholesterol esters phospholipids	phospholipids
Minor lipids	phospholipids	phospholipids cholesterol esters	unesterified cholesterol triglycerides	cholesterol esters unesterified cholesterol

[1]Adapted from Morrisett et al. (26).

reach the blood, Havel et al. (23) have shown that apoprotein C (apoC) is transferred from HDL to complete the chylomicron particle. Smith et al. (24) have demonstrated that apoC-II binds to and activates lipoprotein lipase (LPL) associated with endothelial membranes. LPL then hydrolyzes the triglycerides in chylomicra to produce a chylomicron remnant. ApoC-II then returns to the HDL particle and the chylomicron particle is catabolized by the liver (19). Anderson and Dietschy (25) suggest that the cholesterol in the remnant is important in the regulation of hepatic cholesterolgenesis. Zilversmit (20) also suggests that the remnant is a major factor in the production of atherosclerotic plaques.

VLDL is synthesized primarily in the liver to transport endogenously synthesized triglycerides (27). The composition of VLDL is similar to chylomicra with 90% of its mass as triglycerides. A small amount however, designated iVLDL, is synthesized in the intestine (27). While both sources contain apoB, only hepatic VLDL contains apoC-II (29). iVLDL, in a manner similar to chylomicra, receives its apoC-II from HDL particles (28). After removal of triglycerides, intermediate density lipoproteins (IDL), precursors to LDL, are formed (28). IDL exchanges apoC with HDL and either in the liver or the periphery, is further delipidated to form LDL.

LDL carries cholesterol to extrahepatic tissues and cholesterol comprises approximately 60% of the lipid and over 40% of the total mass of LDL; mostly in the form of cholesterol esters (19). ApoB, which constitute more than 98% of the total apoproteins in LDL, has

been shown to interact specifically with cell surface receptors in many types of cultured cells, including human fibroblasts, lymphocytes, aortic smooth muscle cells and endothelial cells (29). The LDL particle is then degraded by internalization and catabolized by a mechanism elucidated by Brown and Goldstein (29-31). The significance of the LDL receptor system on cholesterol metabolism will be discussed in the next section. LDL is not extensively metabolized by the liver (32). In fact, Sniderman et al. (33) reported an increase in the catabolism of LDL after removal of livers in pigs.

The most dense lipoprotein fraction, HDL, contains about 50% protein, 30% phospholipid, and 20% cholesterol (32). ApoA constitutes approximately 88% of the apoproteins associated with HDL (34). ApoC is the other major apoprotein class. Although apoC accounts for only 5-10% of the total apoproteins in HDL, this amount is about one-half of the total apoC content in the blood (34). As previously described HDL contributes its apoC to chylomicra and iVLDL. The major function of apoA-I is to activate plasma LCAT (35). Brunzell et al. (36) suggest that LCAT in association with HDL catalyzes the conversion of IDL to LDL. The VLDL particle consists of a hydrophobic core of triglycerides and cholesterol esters surrounded by a "membrane" of phospholipids, protein and unesterified cholesterol (36). Brunzell et al. (36) hypothesize that as triglycerides are removed from the core the VLDL particle becomes smaller. Thus an excess membrane exists. HDL in conjunction with LCAT accepts lecithin and unesterified cholesterol from the IDL molecule. The net result is conversion

of LDL to LDL and an increase in cholesterol esters in the HDL particle. Glomset (37) proposes that a major function of HDL is transportation of cholesterol, in the form of cholesterol esters, to the liver. Swartz et al. (38) have recently provided evidence that, in vivo, the liver utilizes HDL cholesterol for bile acid synthesis.

Unesterified cholesterol has the capacity to exchange freely between lipoproteins and between lipoproteins and cell surfaces (19). After esterification by LCAT the resulting cholesterol ester becomes relatively fixed in the polar core of HDL (37). Both Stein et al. (39) and Jackson (40) have reported that HDL facilitates the net removal of cholesterol from cells in culture presumably also by esterifying the unesterified cholesterol.

Clearly a tremendous interaction exists between the lipoprotein classes. HDL exerts a most important influence on the metabolism of other lipoproteins. It is involved in the transfer of triglycerides to cells by contributing apoC-II to chylomicra and iVLDL. HDL may also facilitate the conversion of IDL and LDL in two ways: 1) activating plasma LCAT and 2) accepting phospholipids and unesterified cholesterol from the IDL membrane. Finally HDL, again interacting with LCAT, may transport cholesterol ester from the cells to the liver for excretion.

CELLULAR CHOLESTEROL UPTAKE

As previously discussed (Lipoprotein Metabolism) cholesterol is carried in the blood primarily in the LDL fraction. Brown and Goldstein (29, 30, 31) in the past 5 years have elucidated a mechanism by which LDL-cholesterol can regulate extrahepatic cellular cholesterol metabolism. Under normal conditions the cholesterol content of the cell is balanced between cholesterol uptake or cholesterol synthesis of the cell and cellular losses of cholesterol. Cholesterol may be effectively lost when it is used in membrane synthesis or it may be lost in cellular secretions. Some cholesterol can be lost from the cell when transferred to HDL as previously described.

Brown and Goldstein have shown that LDL plays a major role in regulating cellular cholesterol balance through a cell surface receptor mediated system. When the cellular cholesterol requirement is high, the number of LDL receptors increase on the cell surface. LDL binds to the surface receptors, probably facilitated by apoB (31). The lipoprotein is internalized by endocytosis and undergoes degradation in lysosomes. The lipoproteins are degraded to unesterified cholesterol and fatty acid. An accumulation of unesterified cholesterol causes a depression in the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, an enzyme that catalyzes the conversion of HMG CoA to mevalonate; the first committed step in the synthesis of cholesterol (41). While unesterified cholesterol effectively decreases cellular cholesterol synthesis, it increases acyl cholesterol acyl transferase (ACAT)

activity; an enzyme which esterifies cholesterol within the cell. ACAT produces a cholesterol ester containing predominantly oleate as the fatty acid (31).

As cellular cholesterol storage increases the number of cell surface receptors for LDL are reduced through an unknown mechanism. Thus the cell by increasing the number of cell surface receptors has the ability to regulate LDL uptake and hence cholesterol uptake in response to cellular cholesterol needs. Dietschy and Wilson (42) hypothesize that plasma LDL levels can be regulated by extrahepatic tissue. If low levels of LDL are circulating in the blood, the cells have the capacity to synthesize their own cholesterol. But since extrahepatic cells usually synthesize only a small amount of cholesterol, if there are high levels of circulating LDL, the cells have only limited capacity to decrease these levels by decreasing cellular synthesis and increasing the uptake of LDL.

Goldstein and Brown (43) hypothesize that LDL contributes to atherosclerosis through a mechanism similar to the receptor mediated process. Essentially aortic endothelial damage allows an increased infiltration of LDL into the intima of the aorta. There the LDL saturates the intimacytes' LDL receptors and undergoes internalization and degradation in the previously described manner. But because smooth muscle cells normally are not in contact with high LDL levels, metabolic feedback to reduce the number of cell surface receptors is not as rigorous as in other cell types (44). Thus the cholesterol esters continue to accumulate, resulting in foam cell formation. The fact that the major cholesterol ester found in atherosclerotic plaques is cholesterol oleate is cited

as evidence for this pathway (31, 45). If LDL simply deposited its cholesterol esters in the cells, one would expect cholesterol linoleate to be the predominate cholesterol ester within the cell.

FACTORS AFFECTING PLASMA CHOLESTEROL

CHOLESTEROL

A controversy is growing as to whether feeding dietary cholesterol causes any changes in plasma cholesterol levels in humans. Those who support the hypothesis that dietary cholesterol does not affect plasma cholesterol levels, such as Mann (46) basically argue that: 1) epidemiological data from America does not show any correlation between dietary cholesterol and plasma cholesterol values and 2) clinical studies, particularly those in which cholesterol has been fed with egg yolks, have failed to show that feeding cholesterol to free living subjects causes any appreciable increase in plasma cholesterol levels. A recent study by Flynn et al. (52) in which 1 or 2 eggs were fed daily to free living men is representative. In a cross-over design men were fed either 1 egg, 2 eggs or an egg-free diet; each period was for 12 weeks. The egg-free diet contained approximately 260 mg of cholesterol so that the diets with eggs contained either 580 or 800 mg of cholesterol when supplemented with 1 or 2 eggs respectively. No statistical difference in plasma cholesterol levels were observed between treatments in either experiment. Thus the authors argue that the recommendations of the Senate Select Committee on Nutrition

and Human Needs (3) to limit daily cholesterol intake to less than 300 mg will not significantly affect plasma cholesterol levels.

Experiments where dietary cholesterol has been shown to affect plasma cholesterol levels have been well controlled, clinical studies where dietary cholesterol is either very restricted or totally removed for a period of time before the readdition of cholesterol to the diet (48). From these types of experiments, Keys (49) has predicted that the change in serum cholesterol levels follows the equation:

$$\Delta\text{serum cholesterol, mg/dl} = 1.5(\Delta\text{diet cholesterol, mg/1000 kcal})^{0.5}.$$

Generally this equation overestimates the change in plasma cholesterol that occurs when large amounts of dietary cholesterol are fed.

The effects of dietary cholesterol feeding in humans on other aspects of cholesterol metabolism, such as lipoprotein or apoprotein alterations, has not been extensively investigated. Mahley (50) recently reported that supplementation of diets of healthy young men with 4 to 6 eggs per day for 4 weeks resulted in the generation of an HDL_C fraction. This change occurred in subjects with unchanged plasma total cholesterol levels. Mistry et al. (51) also reported an increase in the HDL_C fraction when humans were fed 1500 mg of cholesterol per day. In this study there was an increase in β-VLDL, a cholesterol rich VLDL fraction also. However, Applebaum-Bowden et al. (52) reported that feeding a liquid formula diet containing 5000 mg of egg yolk cholesterol to subjects for 30 days caused no increase in the HDL_C fraction. These diets caused

a plasma cholesterol increase of 33 mg/dl (less than one-half of the increase predicted by Keys' equation) which was mainly associated with an increase in LDL cholesterol.

The significance of increased HDL_C blood levels relates to its apoprotein content. HDL_C contains significant quantities of arginine rich apoprotein (ARP) which can compete with apoB for cell surface receptors (50). Thus, HDL_C is internalized and contributes cholesterol to the cell in the same manner as LDL (50). Incubating aortic smooth muscle cells for 24 hours with HDL_C resulted in similar amounts of unesterified cholesterol and esterified cholesterol in the cells as when the cells were incubated with LDL. But when the cells were incubated with normal HDL, unesterified cholesterol levels were reduced by two-thirds and cholesterol esters in the cell were decreased by 90% compared to the cell incubated with either LDL or HDL_C (50).

Thus, if consumption of cholesterol in humans causes an increase in HDL_C levels, this may not be beneficial even though this results in an increase in HDL cholesterol levels. Moreover what is the significance of small changes in plasma cholesterol levels when massive and unphysiological amounts of cholesterol are fed to humans? Perhaps it would be more important to investigate the lipoprotein changes that might occur if plasma cholesterol levels were reduced from "normal" levels of 220 mg/dl to 150 mg/dl or less.

The effects of cholesterol feeding in animal experiments have been researched more extensively. As the changes that occur depend on the species, I will review alterations that occur with cholesterol

feeding in pigs and chickens as these are the two species that I have used in most of my experiments.

The lipoprotein pattern of the pig is similar to man with approximately 50-60% of the cholesterol in the LDL fraction. Mahley et al. (53) have extensively studied the effect of cholesterol feeding on cholesterol and lipoprotein changes in the pig. Upon cholesterol feeding the percentage of cholesterol in the LDL or lower density fractions rises from 50% to approximately 80-90%. Cholesterol feeding in pigs, as in man generates both a β-VLDL and HDL_C fraction, both containing significant amounts of ARP. An IDL fraction, normally not seen in swine, is also generated containing a large amount of cholesterol.

The mechanism behind these changes is still speculative. Ross and Zilversmit (54) hypothesize that β-VLDL and IDL are remnants from cholesterol induced intestinal lipoprotein synthesis. The increase in IDL and β-VLDL then arise from incomplete lipolysis of these intestinal chylomicra. Mahley (50) suggests that HDL_C is produced when normal HDL is overloaded with cholesterol.

Feeding cholesterol to chickens causes a greater elevation in plasma cholesterol than does feeding the same amount of cholesterol to pigs. A 20% protein, low fat diet with 0.25% cholesterol will elevate plasma cholesterol levels to approximately 225 mg/dl in chickens (55). In the pig feeding a similar diet would cause cholesterol levels to reach only 125 mg/dl. The difference in response may be due to ability of the chicken to absorb up to 80% of dietary cholesterol compared to 40% absorption in the pigs (56).

When a 1% cholesterol diet was fed to chickens with 20%, 15% and 10% dietary protein plasma cholesterol levels were 900 mg/dl, 1836 mg/dl, and 2332 mg/dl, respectively (55).

The effect of cholesterol feeding on lipoprotein metabolism in chickens has not been as extensively investigated as it has been in pigs. Unlike the pig or human, chicken serum generally separates into only two classes of lipoproteins corresponding to LDL and HDL, when separated by agarose or polyacrylamide gel electrophoresis (57, 58). Similarly, pigeons also have been shown to have only two lipoprotein fractions when separated by electrophoresis (59). However, ultracentrifugation of chicken serum yields three lipoprotein classes; a small amount of VLDL and normal levels of LDL and HDL (60). Dangerfield et al. (58) have reported that occasionally electrophoretic separation of lipoproteins yields two bands in the LDL area which may correspond to the VLDL and LDL fractions.

The major change that occurs with cholesterol feeding is the increase in the LDL lipoprotein fraction. The ratio of HDL/LDL cholesterol was 11.3 in chickens fed high-fat diets without cholesterol but dropped to 2.3 when the same diets with 0.2% cholesterol were fed (Forsythe and Bennink, unpublished observations). Kurski and Narayan (60) found an increase in the VLDL fraction upon cholesterol feeding in chickens. They hypothesized that this increase was due to a decreased degradation of VLDL.

PROTEIN

Kritchevsky (61) cites the experiments of Ignatowski in 1909 as providing the first evidence that the type of dietary protein fed could influence plasma cholesterol levels. Ignatowski, feeding meat and eggs to rabbits, suggested that the animal protein portion was responsible for the increased plasma cholesterol levels. But because the ingredients he fed also contained cholesterol, his hypothesis that the hypercholesterolemic response was due to protein was not accepted. While there have been a few other reports on the effect of protein source on plasma cholesterol levels, most of these results were considered to be due to other dietary components, such as essential fatty acid deficiency, and not due to the protein source (58, 59). Recent reports by Carroll et al. (64-66) and Kritchevsky et al. (68) have reawakened interest in the effects of protein source on plasma cholesterol levels. Hamilton and Carroll (65) reported that rabbits fed semi-purified low-fat, low-cholesterol diets developed significant hypercholesterolemia when animal protein was fed compared to feeding vegetable protein. Plasma cholesterol levels in rabbits fed animal proteins ranged from 105 mg/dl when raw egg whites were fed to 235 mg/dl when lipid extracted whole egg was the protein source. Feeding casein resulted in plasma cholesterol levels of 200 mg/dl. When vegetable protein was substituted for the animal protein in the same diet plasma cholesterol levels ranged from 25 mg/dl for soy protein to 80 mg/dl for wheat gluten.

Supplementation of the diet with choline and/or methionine (animal proteins have a high level of methionine relative to vegetable proteins) produced similar response as without supplementation, although rabbits receiving both the choline and methionine supplement showed slightly higher levels than those not receiving supplements.

While these experiments showed that animal protein could raise plasma cholesterol levels relative to vegetable protein, questions were raised concerning the dietary ingredient responsible for this difference. That is, is it the protein component of the diet or an ingredient associated with the protein, such as fiber in vegetable proteins that causes the difference in response? Huff et al. (66) tested the effect of feeding mixtures of soy protein and casein, feeding casein or soy protein hydrolysate and feeding amino acid mixtures of either casein or soy protein. A 75-25% mixture (casein/soy isolate) reduced plasma cholesterol levels to approximately one-half those occurring when 100% casein was fed. A 50-50% mixture further reduced cholesterol levels to concentrations observed when 100% soy protein was fed. Feeding the enzymatic hydrolysate of either protein source caused a small decrease in plasma levels compared to feeding the original source. Finally, feeding the amino acid mixture of casein did not change cholesterol levels compared to whole casein but the amino acid mixture of soy protein produced cholesterol levels approximately twice that of the intact soy isolate. Still, the levels that occurred when the soy amino acid mixture was fed were about one-half those occurring in the casein fed rabbits. Yadav and Leiner (67) showed that

feeding whole protein or amino acid mixtures of soy or casein to rats in hypercholesterolemic diets caused similar levels of plasma cholesterol. These studies indicate that, while other components of the protein source may slightly affect plasma cholesterol levels, the primary effect seems to be caused by the protein or the amino acid compostion of the protein source.

Kritchevsky et al. (68) have hypothesized that the ratio of arginine to lysine, high in vegetable protein but low in animal protein, may be responsible for the differences in plasma cholesterol levels. Kritchevsky et al. (69) reported that supplementing soy protein with lysine slightly increased the severity of atherosclerosis, but did not appreciably affect plasma cholesterol levels. Supplementing casein with arginine slightly increased plasma cholesterol levels in one experiment and slightly decreased levels in a second experiment. Thus, currently no experiments conclusively show that the amino acid imbalances between animal and vegetable protein cause the differences in plasma cholesterol levels.

The interaction between the dietary fat and protein source has been reported in only a few studies. Fumagelli et al. (70) fed high-fat (20% beef tallow), low-cholesterol diets to rabbits and reported that feeding casein resulted in higher levels of plasma cholesterol than when soy protein was fed. They also reported that feeding soybean meal caused a significant increase in fecal neutral sterols but little change in the excretion of fecal bile acids. Kim et al. (71) reported that feeding pigs casein as the protein source in high-fat, high-cholesterol diets resulted in

increased plasma cholesterol levels compared to feeding soy isolate. In their study they found that both protein sources caused similar levels of fecal neutral sterol and bile acid excretions. However the results of their study are subject to question. In 6 weeks, the pigs fed the casein diets gained only 6 kg of weight, compared to 15 kg of gain in the pigs fed the soy diets. They fed each pig approximately 1 g of cholesterol daily in a small amount of feed. Since there was no difference in cholesterol excretion and since the pigs ate the same amount of cholesterol per day, one could conclude that the differences in growth between treatments contributed to the different plasma cholesterol levels.

Two recent reports have questioned the ability of soy protein to decrease plasma cholesterol levels when fed with high fat diets in humans. Shorey and Davis (72) reported that the effect of soy protein is decreased when fed in conjunction with animal fats. Sirtori et al. (73) also reported that soy protein had a reduced effectiveness in reducing plasma cholesterol levels in hypercholesterolemic subjects when fed with saturated fats compared to when it is fed with polyunsaturated fats. An analysis of their data, however, shows that while it was true that plasma cholesterol levels were decreased to a greater extent when soy protein diets with high P:S ratios (2.7) were fed compared to feeding soy protein diets with a low P:S ratio; the difference was most likely due to the type of fat. It appeared that the decrease in plasma cholesterol levels brought about feeding soy protein was approximately 50 mg/dl regardless of the fat source.

DIETARY FIBER

Burkitt et al. (74) and Trowell (75) are credited with initiating the interest in the effects of dietary fiber on cardiovascular disease (76). They hypothesized that the decreased incidence of certain degenerative disease, including cardiovascular disease and hemorrhoids in developing countries was due to the consumption of dietary fiber far in excess of that ingested in developed countries. Trowell and Burkitt (77) report that the crude fiber intake in developing countries is on the order of 24 g per day. In developed countries this value is approximately 4 g per day. While other changes in the diet may also relate to the development of atherosclerosis these hypotheses renewed interest into investigations on the physiological significance of dietary fiber.

Fiber is usually defined as indigestible plant materials. Its major components consist of cellulose, hemicellulose, pectin, and lignin (78). The amounts of each component not only varies between species but also depends on the maturity of the plant at harvest; the lignin content increasing with age (79). Because of the chemical characteristics of these components there is at present no single method to quantitate the fiber content of a substance. The original and the current A.O.A.C. method for fiber is the crude fiber analysis, which sequentially extracts the material with ether, acid and alkali. Van Soest (78) has reported that this method results in loss of about 85% of the hemicellulose

and between 50-90% of the lignin content of the fiber. In particular this method gives low values, compared to other methods, for cereals which are high in hemicellulose and lignin.

Dissatisfaction with the crude fiber method had led to development of two other procedures, acid-detergent residue (ADF) and neutral-detergent residue (NDF)(79). ADF measures primarily lignin and cellulose, as the hemicellulose component is soluble at low pH and lost in the extraction. NDF measures cellulose, hemicellulose and lignin. Thus the difference between the two procedures gives an estimation of the hemicellulose content. At present no adequate method exists to quantitate the pectin and gum component of the plant wall (80).

It is not surprising, considering the diverse composition of fiber, that its effects on plasma cholesterol levels are controversial. Usually cellulose has been found to not change plasma cholesterol levels in rabbits (64, 66), rats (77, 78) and humans (83). In fact, in rabbits, cellulose has been shown to increase the plasma cholesterol (64, 66). Wheat bran, another widely used fiber source has also been shown not to decrease plasma cholesterol levels in most experiments (64, 66, 81, 84). Among the fibers that have been consistently shown to reduce plasma cholesterol levels are pectin (83, 85, 86), alfalfa (66, 87, 88) and rolled oats (89-91).

Dietary fibers are hypothesized to exert their hypocholesterolemic effect by binding bile acids in the gut. This increases excretion and causes an increased hepatic synthesis of bile acids (76). In in vitro studies various fibers

were tested on their ability to bind bile acids. Story and Kritchevsky (92) reported that alfalfa and lignin bound significantly more (100%) bile acids than did wheat bran. The least effective fiber was cellulose. Forsythe and Bennink (unpublished observations) found that even in the absence of total cholesterol changes, feeding oat bran to rats significantly increased the excretion of fecal bile acids compared to a low fiber control diet or when microcrystalline cellulose was fed. Feeding wheat bran resulted in intermediate excretion values. In this same study oat bran increased the excretion of fecal neutral sterols compared to the other three treatments. This increased excretion of bile acids by oat bran resulted in a 60% decrease in both liver total lipids and liver sterols. Similar responses have been observed by other investigators (93, 94).

EXERCISE

Leon and Blackburn (95) have recently reviewed epidemiological studies relating physical activity and CHD stating that exercise produces a number of beneficial effects: 1) a reduction in heart rate and blood pressure; 2) increased muscular endurance; 3) reduced blood coagulability; 4) reduced body weight and adiposity; 5) increased insulin sensitivity, and 6) blood lipid changes. The effects of exercise on blood lipids are not completely understood.

Epidemiological and clinical studies have usually found that the major lipid changes associated with increased exercise are: a decrease in plasma triglyceride levels; a decrease in plasma total

Fukuda et al. (103) reports that exercise in rats caused an increase in the excretion of fecal neutral and acidic sterols compared to pair-fed sedentary rats. Exercise has also been reported to increase the turnover of cholesterol (105). Bobek et al. (106) has shown in rats that exercise causes an increased turnover and decreased size of the slow turnover cholesterol pool; primarily body cholesterol.

The mechanism by which exercise causes an increased cholesterol turnover is also unclear. It seems unlikely that increased excretion of fecal sterols is responsible for the increased turnover. More likely the increased turnover causes the increased excretion. Wallentin (107) has reported that increased molar LCAT activity correlates with increased plasma cholesterol turnover. It is hypothesized that LCAT activity increases in response to increased VLDL triglyceride utilization (107). LCAT then facilitates the transfer of cholesterol from VLDL to HDL by the mechanism described in the Lipoprotein Metabolism section. Thus, it is through this mechanism that HDL levels are hypothesized to be increased with exercise (108).

Lopez (99) reported increased molar LCAT activity after 7 weeks of intensive exercise in humans. Simko and Kelley (108) reported an increase in fractional LCAT activity in exercised rats, but, since free cholesterol levels were not reported it was not clear if molar LCAT activity was changed. Gilliam and Burke (109) showed that exercise in girls (40 minutes per day, 5 times per week for 7 weeks) decreased plasma triglyceride levels and increased HDL plasma cholesterol levels. But 9 days after cessation of

cholesterol levels and an increase in HDL cholesterol levels (96-100). Wood et al. (96) reported on the effects of exercise in men and women in a cross sectional study. The exercise group had to run at least 15 miles per week and be maintaining body weight. A sedentary control group was matched to the exercise group on the basis of age and blood pressure. The most significant difference found was lower plasma triglyceride levels (60% of control levels) in the exercise group. Total cholesterol levels were also significantly lower in the exercise group compared to the control group; 200 mg/dl to 212 mg/dl respectively. Exercising also resulted in increased HDL cholesterol levels and an increased ratio of HDL/LDL cholesterol. Analysis after the study ended showed that the groups were not matched for body leanness. The runners were near or at ideal weight but the sedentary group averaged 22% above ideal weight. This could account for some of the differences in plasma lipids observed. This inability to control all variables in human studies, plus differences in the amount of exercise, has caused a great deal of confusion in interpretation of the effects of exercise on plasma cholesterol. While a few reports have concluded that exercise does not cause changes in plasma cholesterol levels (101, 102) most reports have indicated a beneficial effect (94, 95, 96, 99).

The mechanism by which exercise lowers blood cholesterol levels is not completely understood. Hepatic cholesterol synthesis in exercised rats, as assayed by HMG CoA reductase activity (103) or by acetate 1-^{14}C incorporation into cholesterol (104) appears to be increased, even while plasma total cholesterol levels are decreased.

exercise, triglyceride and HDL cholesterol levels had returned to pre-exercise values. This suggests that an exercise program needs to be maintained continuously to produce beneficial blood lipid changes over time.

PART II

EFFECTS OF DIETARY PROTEIN AND FAT SOURCES ON PLASMA CHOLESTEROL PARAMETERS, LCAT ACTIVITY, AMINO ACID LEVELS AND TISSUE LIPID CONTENT OF GROWING PIGS

INTRODUCTION

Recent reports have concluded that plant proteins are less hypercholesterolemic than animal proteins (110-112). When low-fat, low-cholesterol diets are fed to rabbits, casein as the protein source, results in higher plasma cholesterol levels than when soy protein is fed (65, 66, 68). Fewer studies have been conducted feeding high-fat or high-fat and high-cholesterol containing diets in conjunction with varying protein sources.

Carroll et al. (113) reported that humans fed high-fat (40% of energy with a P:S ratio of 0.4 to 0.8) diets showed small decreases in plasma cholesterol levels when plant protein, primarily soy protein, replaced a mixture of plant and animal protein in the diet. Kim et al. (71) fed pigs high-fat (40% of energy predominantly from butter), high-cholesterol (1055 mg/pig daily) diets containing either soy protein or casein for six weeks. Plasma cholesterol levels were approximately twice as high in pigs consuming casein as in pigs consuming soy protein. But, pigs fed casein did not grow as well as pigs fed soy protein and this could, as Kim et al. (71) point out, account for some of the differences in plasma cholesterol levels between treatments.

Sirtori et al. (73) and Shorey and Davis (72) have both suggested that the hypocholesterolemic effect of soy protein is less when saturated-fat diets are fed in place of polyunsaturated-fat diets. However, in neither of their studies were direct comparisons

made of the hypocholesterolemic action of plant versus animal proteins in both polyunsaturated and saturated-fat diets.

The objectives of this study were to investigate both the primary effects and interactions feeding animal or plant proteins in high-fat (P:S = 0.3 or 3.0), high-cholesterol diets have on plasma cholesterol parameters, plasma lecithin:cholesterol acyl-transferase activity and tissue (liver and aorta) deposition of lipids and cholesterol in pigs. Since a proposed mechanism to explain the differences between plant protein and animal protein on cholesterol levels is their amino acid composition (67, 114), plasma amino acid levels were also profiled.

MATERIALS AND METHODS

ANIMALS

Two experiments were undertaken to investigate dietary effects on plasma cholesterol parameters. In both experimetns male (crossbred York x Duroc or York x Hampshire) pigs were weaned at 3 weeks of age and fed a corn-soy starter ration containing 20% protein for 1 week. At 4 weeks of age pigs were assigned to the dietary treatments. Three or four pigs were housed together in each pen in an environmentally controlled building. They had free access to food and water. Pigs were weighed and blood samples were obtained by vena cava puncture bi-weekly 24 hours after diets were removed. The experimental diets were fed for 12 weeks in Experiment 1 and 14 weeks in Experiment 2.

DIETS

A control corn-soy diet (Table 1) was included in each experiment to provide a reference to compare responses of pigs fed the experimental diets. This diet provided 8, 76 and 16% of energy as fat, carbohydrate and protein, respectively. The experimental diets supplied 42% of energy as fat, 42% as carbohydrate and 16% as protein. They were formulated to contain either animal protein or plant protein and either a high ratio of polyunsaturated to saturated fat (P:S = 3.0) or a low ratio (P:S = 0.3). Crystalline cholesterol was added (0.2%) to each experimental diet. In the first experiment animal protein was derived from a mixture of meat-meal and casein and plant protein was derived from wheat, corn and soybean meal (Table 1). Within the first two weeks of the experiment it became apparent that food intake of pigs fed the animal protein diets was low. In an attempt to increase their food intake the level of meat meal was first reduced to 3% of the diet and finally eliminated. Still food intake was not comparable to that of pigs fed plant protein diets. Once pigs refuse a diet it is very difficult to get them to consume a similar diet. We, thus, terminated these groups but continued to feed the control and plant protein diets for 12 weeks.

To improve consumption of diets containing animal protein a combination of dried skim milk and casein was used in the second experiment (Table 2). Because dried skim contains lactose which may affect plasma cholesterol levels (111) an equal amount of lactose replaced corn starch in the plant protein diets. The plant protein

Table 1. Diet formulation (Experiment 1).

	Control	Pl Sat	Pl Puf	An Sat	An Puf
			-kg-		
Ground corn	790	268	268	—	—
Soybean meal[1]	175	230	230	—	—
Ground soft winter wheat	—	268	268	—	—
Meat meal[2]	—	—	—	115	115
Casein	—	—	—	115	115
Corn starch	—	—	—	555	555
Corn oil	—	27	186	41	193
Tallow	—	173	14	159	7
Basal mix[3]	15	15	15	15	15
Deflourinated phosphate	10	15	15	—	—
Calcium carbonate	8	4	4	—	—

[1]Dehulled soybean meal (48% protein).

[2]Meat meal (50% protein, 5% fat, and 3% collagen).

[3]Basal mix contains: salt, vitamin-trace mineral mix, vitamin E, selenium premix, and antibiotic premix. See Table 2 for composition.

Table 2. Diet formulation (Experiment 2).

Ingredients	Control	Pl Sat	Pl Puf	An Sat	An Puf
			-kg-		
Ground corn	790	250	250	—	—
Soybean meal[1]	175	—	—	—	—
Ground soft winter wheat	—	250	250	—	—
Soybean isolate[2]	—	136	136	—	—
Lactose	—	128	128	—	—
Dried skim milk	—	—	—	270	270
Casein	—	—	—	110	110
Corn starch	—	—	—	343	343
Salt	5	3	3	3	3
Calcium carbonate	8	4	4	10	10
Defluorinated phosphate	10	15	15	—	—
Vitamin trace mineral premix[3]	5	5	5	5	5
Vitamin E-Se premix[4]	5	5	5	5	5
Antibiotic premix[5]	2	2	2	2	2
Corn oil	—	27	186	41	193
Tallow	—	173	14	159	7
Corn fiber	—	—	—	50	50
Cholesterol	—	2	2	2	2
	1000	1000	1000	1000	1000
Estimated fat saturation ratio[6]					
Polyunsaturated	2.30	0.30	3.01	0.30	3.03
Saturated	1.00	1.00	1.00	1.00	1.00
Monosaturated	1.78	0.95	1.66	0.95	1.67

[1]Dehulled soybean meal (48% casein).

[2]Soya assay protein, General Biochemicals, Chagrin Falls, OH.

[3]Supplies the following nutrients per kg of diet: vitamin A, 3300 IU, vitamin D, 660 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 17.6 mg; d-panthothenic acid, 13.2 mg; choline, 110 mg; vitamin B_{12}, 19.8 µg; zinc, 74.8 mg; iron, 59.4 mg; manganese, 37.4 mg; copper, 9.9 mg and iodine, 2.1 mg.

[4]Supplies 11 IU of vitamin E and 0.1 mg of Se per kg of diet.

[5]Supplies 88 mg of chlortetracycline, 88 mg of sulfamethazine and 44 mg of penicillin per kg of diet.

[6]Ratio of each to saturated fat.

diets were analyzed for dietary fiber (115). An amount of purified corn fiber[1] equal to the level of dietary fiber in the plant protein diets, replaced cornstarch in the animal protein diets. Diets were analyzed by standard AOAC methods (116) to determine crude protein, ether extract, calcium, phosphorus, magnesium, copper and zinc (Table 3). The mineral content of the diets was determined to ensure similar levels since some minerals, particularly calcium, copper and zinc, have been reported to affect plasma cholesterol concentrations (117-119). Both the animal protein basal diet and the plant protein basal diet were mixed before addition of fat. Analyses were conducted on the saturated fat diet from each source. In Experiment 2, eight pigs were assigned to each treatment: control; plant protein-saturated fat (Pl Sat); plant protein-polyunsaturated fat (Pl Puf); animal protein-saturated fat (An Sat); and animal protein-polyunsaturated fat (An Puf).

PLASMA AND TISSUE ANALYSES

Plasma total and unesterified cholesterol were determined enzymatically.[2] Esterified cholesterol was calculated as the difference between total and unesterified cholesterol values. Plasma high density lipoprotein (HDL) cholesterol was also determined enzymatically after the low density (LDL) and very low density (VLDL) lipoproteins were precipitated with

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[1] Clinton corn bran, Clinton Corn Processing Co., Clinton, Iowa 52732.

[2] Cholesterol Reagent Set, Biodynamics/BMC, Indianapolis, IN 46250.

Table 3. Analysis of selected nutrients in diets (Experiment 2).[1]

Analyses	Control	Pl Protein	An Protein
Crude protein, %	16.0	17.7	17.6
Ether extract, %	3.6	24.0	23.7
Calcium, %	0.75	0.75	0.80
Phosphorus, %	0.52	0.64	0.44
Magnesium, ppm	1000	800	600
Copper, ppm	13	16	13
Zinc, ppm	99	74	76

[1]See Table 2 for composition of diets.

heparin-manganese solution (120). Plasma lipoproteins were separated by electrophoresis on polyacrylamide gel.[1] Tubes were scanned on a densitometer at 610 nm, and percentages of VLDL, LDL and HDL fractions were calculated (121). Plasma triglycerides were extracted in isopropanol:heptane (5.6:3.0, v/v) and then determined colorimetrically (122). Plasma lecithin:cholesterol acyltransferase (EC 2.3.1.43) (LCAT) activity was assayed by the method of Stokke and Norum (123) with modifications suggested by Wallentin and Vilkrot (124). Amino acid levels in plasma were determined after precipitating 3 ml of plasma with 10 volumes of 20% sulfosalicyclic acid. Norleucine was added as an internal standard. Analyses were performed in a lithium citrate buffer system with ion exchange chromatography using DC-4A exchange resin. Liver and thoracic aorta lipids were extracted in chloroform:methanol (2:1, v/v) and determined gravimetrically. An aliquot of the extracted lipid was used to determine tissue cholesterol (125).

STATISTICAL ANALYSES

In Experiment 1 results were statistically tested by analysis of variance with means compared by Tukey's procedure (126). In experiment 2 data were compared by analysis of variance using orthogonal contrasts (126). Comparisons were: control versus experimental; animal protein versus plant protein; and saturated fat versus polyunsaturated fat. Results are presented as means for each group plus the pooled standard error for parameters.

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[1] Redi-Disc, Ames Co., Elkhart, IN 46514.

RESULTS

EXPERIMENT 1

An initial rise in plasma cholesterol concentrations was found in all groups (Figure 1). The control and plant protein, polyunsaturated fat groups reached their highest cholesterol levels at 4 weeks while the plant protein, saturated fat group showed its highest values at 6 weeks. Plasma cholesterol levels were highest in pigs fed the plant protein, saturated fat diet, intermediate in pigs fed the plant protein, polyunsaturated fat diet and lowest in pigs fed the control diet.

Final body weights and plasma lipid levels are shown in Table 4. Body weights were not affected by the diet. Pigs fed the saturated fat diet had the highest plasma cholesterol levels at the end of the experiment. Plasma cholesterol levels of pigs fed the plant protein, polyunsaturated fat diet were not higher than levels in pigs fed the low-fat, low-cholesterol, control diet. Pigs fed either of these diets had significantly lower plasma cholesterol levels than pigs fed the plant protein, saturated fat diet.

Plasma HDL-cholesterol levels were not significantly altered in pigs fed the experimental diets nor were plasma triglyceride levels changed (Table 4). The percentage of LDL was decreased (and HDL increased) in pigs fed the plant protein, polyunsaturated fat diet compared with values in pigs fed the control diet. Consequently, the ratio of HDL to LDL was higher in the plant protein, polyunsaturated group than in the control group. The percentage of

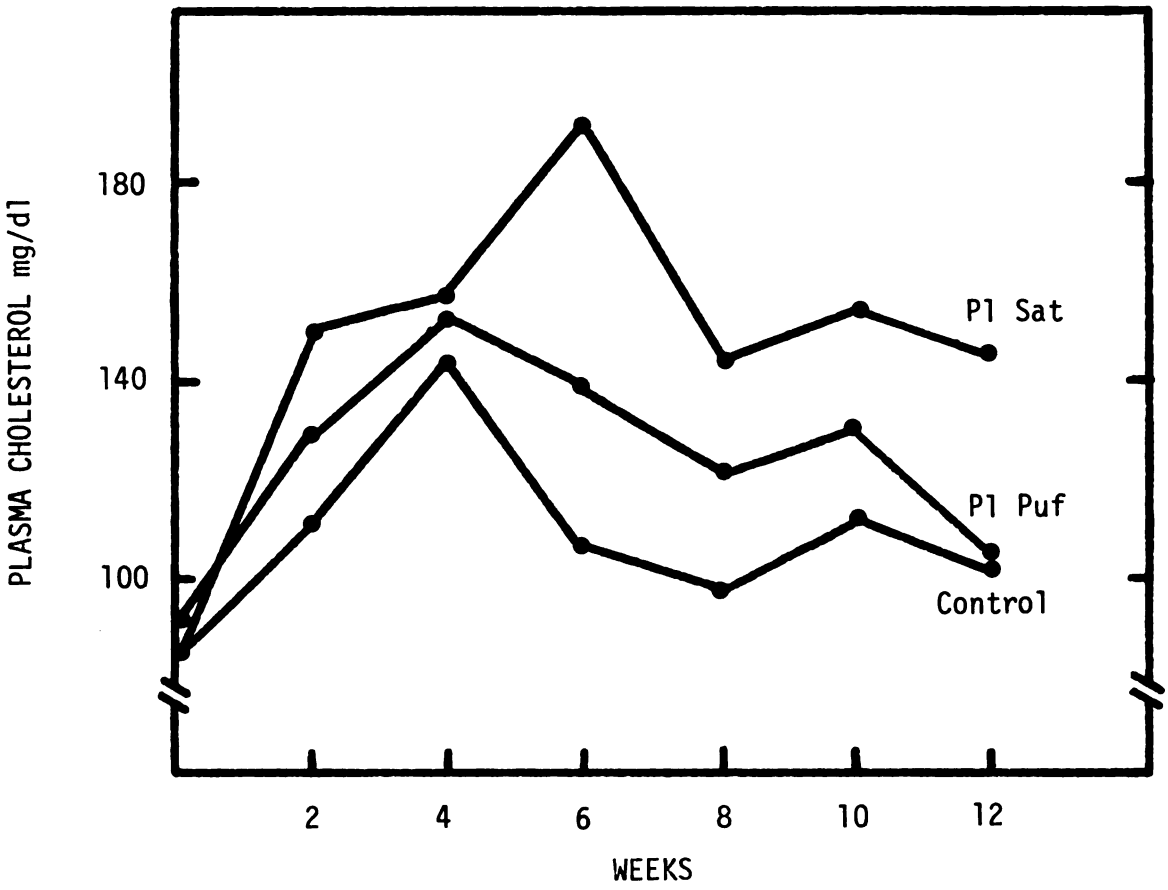


Figure 1. Effect of varying P:S ratio on bi-weekly plasma cholesterol levels in pigs (Experiment 1). Pooled SE ranged from 9 to 18 mg cholesterol/dl plasma for each collection period. Plasma cholesterol levels in pigs fed saturated fat were significantly greater ($p<0.05$) than those observed in pigs fed the control diet at weeks 2, 6, 8, 10, and 12. Consumption of saturated fat significantly increased plasma cholesterol levels compared with the polyunsaturated fat at weeks 6 and 12. There was no significant difference, at any collection point, in plasma cholesterol levels between the polyunsaturated fat group and the control group.

Table 4. Effects of dietary fat on body weights and plasma lipids of pigs fed plant protein diets (Experiment 1).[1]

Diet	Final body weight, kg	Plasma lipids (mg/dl)			Plasma lipoproteins (%)			
		Total cholesterol	HDL cholesterol	TG	VLDL	LDL	HDL	HDL/LDL
Control	98[2a]	100[a]	46[a]	32[a]	3.3[a]	44.2[a]	52.5[a]	1.20[a]
Pl Sat	88[a]	144[b]	533[a]	37[a]	3.1[a]	40.1[ab]	56.8[a]	1.42[ab]
Pl Puf	85[a]	102[a]	52[a]	39[a]	3.3[a]	34.1[b]	62.6[b]	1.84[b]
Pooled SE	± 5	± 10	± 3	± 6	± 0.3	± 1.8	± 1.8	± 0.1

[1]Means for six pigs weighing 9.4 ± 0.3 kg initially and fed the diets for 12 weeks. Blood samples were obtained at the end of the experiment.

[2]Values in columns with different superscript letters are significantly different ($p<0.05$).

LDL and HDL and the HDL/LDL ratio were intermediate in pigs fed the plant protein, saturated fat diet.

In summary, these results show the typical hypocholesterolemic response to an increased dietary P:S fat ratio which others have also observed (127, 128). A second experiment was designed to test whether protein source could affect changes in plasma cholesterol parameters and to examine possible interactions of protein source and fat source.

EXPERIMENT 2

The removal of meat and the addition of dried skim milk to the animal protein diets markedly improved their consumption relative to results obtained in Experiment 1. No effect of dietary treatments on final body weights (Table 5) was observed as all groups of animals gained approximately 0.7 kg daily. Food intake was also similar among treatments. Pigs fed the experimental diets consumed approximately 0.5 kg diet (1 g of cholesterol) per day initially and 2.3 kg diet (4.6 g of cholesterol) per day by the end of the experiment. Cholesterol content of the diet was 0.6 g per 1000 kcal. Since food intake and growth of the pigs were similar among treatments, changes in plasma cholesterol levels of pigs fed the experimental diets do not reflect differences in growth or cholesterol consumption.

Bi-weekly plasma total cholesterol values are plotted in Figure 2. Levels of circulating cholesterol tended to increase during the first 4 weeks in pigs fed the experimental diets.

Table 5. Final body weight, plasma cholesterol parameters, and LCAT activity in pigs fed different protein or fat for 14 weeks (Experiment 2).[1]

Diet	Final Body Weight, kg	Cholesterol, mg/dl				UC/CE	HDL Cholesterol %	Plasma LCAT Activity	
		Total	Unesterified	Esterified	HDL			Esterification %/hour	Molar rate µM/L/hour
Control	91	89	28	61	29	0.48	32	7.7	54
Pl Sat	84	158	52	105	43	0.57	28	4.4	59
Pl Puf	85	111	38	77	43	0.44	40	6.0	51
An Sat	82	205	64	141	67	0.47	32	3.0	45
An Puf	78	169	48	121	50	0.41	29	2.3	29
Pooled SE	± 2	± 9	± 3	± 5	± 2	± 0.03	± 1	± 0.2	± 2
Sign.[4] (p<0.05)	NS[5]	Exp. Prot. Fat	Exp. Prot. Fat	Exp. Prot. Fat	Exp. Prot.	NS	NS	Exp. Prot.	Prot. Fat

[1]Eight pigs per treatment weighing initially 12.0 ± 0.3 kg. Blood was drawn at 14 weeks.

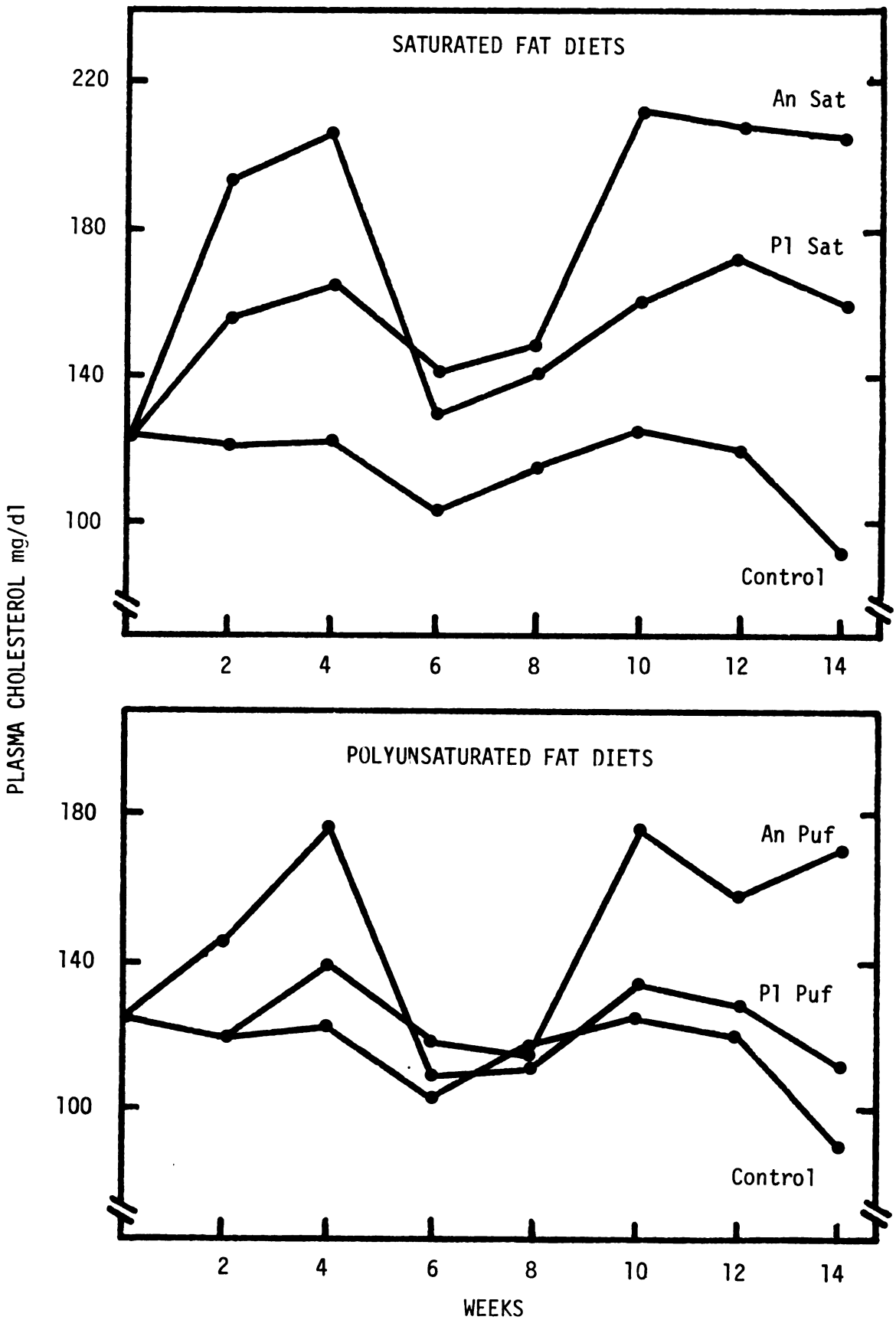
[2]UC/CE = Ratio of unesterified to esterified cholesterol.

[3]Percentage of total cholesterol in HDL fraction.

[4]Comparisons are control diet versus experimental; plant protein versus animal protein; and saturated fat versus polyunsaturated fat.

[5]Not significant.

Figure 2. Effect of animal or plant protein and varying P:S ratio on bi-weekly plasma total cholesterol levels in pigs (Experiment 2). Pooled SE ranged from 5 to 10 mg cholesterol/dl plasma for each collection point. Increasing the P:S ratio significantly decreased ($p<0.05$) plasma cholesterol values at weeks 2 to 14. Plant protein significantly increased plasma cholesterol levels compared with animal protein at weeks 2 and 4 but significantly decreased cholesterol levels at weeks 10, 12 and 14. The experimental diets increased plasma cholesterol levels compared with the control diet at weeks 2, 4, 10, 12, and 14.


SATURATED FAT DIETS
220
180
140
100
An Sat
Pl Sat
Control
2
4
6
8
10
12
14
PLASMA CHOLESTEROL mg/dl
POLYUNSATURATED FAT DIETS
180
140
100
An Puf
Pl Puf
Control
2
4
6
8
10
12
14
WEEKS

A similar response was noted in the first experiment. At both 2 and 4 weeks of the experiment plasma cholesterol levels were unexpectedly higher in pigs fed the plant protein experimental diets than in pigs fed animal protein. There was a transient decrease in cholesterol levels in both protein groups at 6 and 8 weeks. The reason for the initial elevation in plasma cholesterol levels of pigs fed the plant protein diets and for the subsequent drop in plasma cholesterol levels of all groups at 6 and 8 weeks is not clear. After 10 weeks pigs fed the animal protein diets had higher plasma cholesterol levels than pigs fed plant protein. Values in the animal protein groups remained approximately 50 mg/dl higher than values in the corresponding plant protein group until the end of the experiment.

Increasing the dietary P:S ratio from 0.3 to 3.0 lowered plasma cholesterol values in both the animal and plant protein groups by approximately 40 mg/dl (Figure 2). This effect was evident by the second week of the study. As in Experiment 1, pigs that consumed the plant protein, polyunsaturated fat diet had plasma cholesterol levels similar to the control, low-fat, low-cholesterol group; despite consuming approximately 4.5 g of cholesterol per day.

Blood lipid values from the final collection period (14 weeks) are presented in Table 5. Pigs fed the experimental diets had higher plasma cholesterol (total, unesterified, esterified and HDL-cholesterol) levels than did pigs fed the low-fat, low-cholesterol, control diet. Plasma total, unesterified and esterified cholesterol

levels were significantly higher in pigs fed the animal protein diets than in pigs fed the plant protein diets; likewise, values were higher in pigs fed the saturated fat diets than in pigs fed the polyunsaturated fat diets. Thus, the highest total cholesterol levels were in the animal protein, saturated fat group (205 mg/dl) and the lowest values in the plant protein, polyunsaturated fat group (111 mg/dl). The ratio of plasma unesterified to esterified cholesterol was not significantly affected by dietary protein or fat. Plasma HDL cholesterol (Table 5) was elevated significantly in pigs fed animal protein; this was a reflection of total cholesterol levels as neither the type of protein nor fat fed caused a difference in the percentage of total cholesterol in the HDL fraction. As in Experiment 1, plasma triglyceride levels were unaffected by dietary treatments.

Lecithin:cholesterol acyl transferase esterifies unesterified cholesterol in plasma which presumably facilitates transport of cholesterol from tissue pools to the liver (129). The fractional esterification rate, which reflects the percentage of cholesterol esterified per hour, was negatively correlated ($r = -0.75$) with free cholesterol levels (Table 5). This observation is in agreement with previously reported results (130) and may explain part of the decrease in fractional esterification rate that occurred when animal protein was fed; these animals had higher unesterified cholesterol levels and thus, on a percantage basis, esterified less cholesterol than animals with lower cholesterol levels. However, the molar LCAT rate, which is an absolute rate that takes into account the level of unesterified cholesterol, was also significantly

reduced in pigs fed the animal protein diets and in the pigs fed polyunsaturated fat diets. Thus, the decrease in the fractional LCAT rate in pigs fed animal protein is greater than that accounted for by an increase in unesterified cholesterol levels. The effect of polyunsaturated fat on molar LCAT activity in our experiment agrees with other reports (131, 132) but to our knowledge this is the first report that dietary protein can affect LCAT activity.

The distribution of plasma lipoproteins (Table 6) was unaffected by dietary treatment. The plasma HDL/LDL ratio was significantly lower in pigs fed the animal protein diets than in pigs fed plant protein. As in the first experiment, increasing the P:S ratio tended to elevate the HDL/LDL ratio.

Kritchevsky (112) has postulated that the amount of arginine and lysine in diets may influence plasma cholesterol levels. The plant protein diets fed contained 1.10% arginine and 0.85% lysine (calculated values) while the animal protein diets contained 0.63% arginine and 1.36% lysine. The amino acid content of the control diet was similar to that of the plant protein diets. The ratio of arginine to lysine in the plant protein diets was almost three times that in the animal protein diets (1.29 to 0.46, respectively). Although plasma lysine levels were significantly higher in pigs fed the animal protein, no differences were found in plasma arginine levels or in the ratio of arginine to lysine between treatments. Plasma levels of branched-chain amino acids, leucine, isoleucine and valine, and of threonine were higher in pigs fed experimental diets than in pigs fed the control diet (Table 7). Feeding pigs animal protein also resulted in higher

Table 6. Effect of dietary protein and fat sources on plasma lipoprotein distributions (Experiment 2).

Diet	Percentage			
	VLDL	LDL	HDL	HDL/LDL
Control	17.3	39.4	44.3	1.13
Pl Sat	11.6	40.1	49.3	1.26
Pl Puf	12.1	38.1	49.8	1.37
An Sat	12.2	42.5	45.3	1.11
An Puf	11.7	42.4	46.9	1.15
Pooled SE	0.3	1.0	0.9	0.06
Sign.[2] ($p<0.05$)	NS[3]	NS	NS	Prot.

[1]Eight pigs per treatment. Blood drawn at 14 weeks of the experiment.

[2]See Table 5 for explanation of statistical comparisons.

[3]Not significant.

Table 7. Plasma amino acid levels in pigs fed different protein and fat sources (Experiment 2).[1]

Diet	Plasma Amino Acids (nmoles/ml)						
	Lysine	Arginine	Threonine	Serine	Valine	Isoleucine	Leucine
Control	130	90	118	105	238	74	140
Pl Sat	109	77	140	96	261	95	152
Pl Puf	116	85	134	137	314	108	178
An Sat	132	92	167	126	385	132	203
An Puf	131	96	168	149	480	115	210
Pooled SE	± 3	± 3	± 7	± 6	± 24	± 5	± 9
Sign.[2] ($p<0.05$)	Prot.	NS[3]	Exp. Prot. Fat	Fat	Exp. Prot.	Exp. Prot.	Exp. Prot.

[1]Eight pigs per treatment.

[2]See Table 5 for explanation of statistical comparisons.

[3]Not significant.

levels in the plasma of threonine and the branched chain amino acids, leucine, isoleucine and valine. Pigs fed the polyunsaturated fats had higher levels of serine and valine than pigs fed the saturated fat diets.

Feeding the high-fat, high-cholesterol experimental diets increased liver cholesterol and total lipid and cholesterol in aortas of pigs (Table 8). Neither of the experimental treatments, fat or protein, caused any change in liver or aorta lipid content. Both the liver and aorta had slightly higher ($p<0.1$) cholesterol contents in animals fed the polyunsaturated fat diets than pigs fed the saturated fat diets. This would tend to support the hypothesis that one mechanism by which polyunsaturated fats lower plasma cholesterol levels is by redistribution to the tissue pool (133). Neither protein treatment affected the amount of cholesterol in either tissue. Longer term feeding experiments than those employed in the present study may be necessary to produce changes in tissue lipid.

DISCUSSION

Increasing the dietary P:S ratio from 0.3 to 3.0 decreased plasma cholesterol concentrations of the pigs in two different experiments. In the first experiment the decrease was 42 mg/dl and in the second experiment levels decreased by 40 mg/dl. Polyunsaturated fats decrease plasma cholesterol levels in humans as well (127, 128, 134) and in pigs, Greer et al. (135) reported that plasma cholesterol levels decreased by 25 mg/dl when

Table 8. Effect of dietary protein and fat sources on liver and aorta lipid composition (Experiment 2).[1]

	Liver, mg/g wet weight		Aorta, mg/g dry weight	
	Total Lipids	Cholesterol	Total Lipids	Cholesterol
Control	41	3.07	19	4.06
Pl Sat	47	3.72	22	4.24
Pl Puf	43	4.28	23	4.85
An Sat	46	4.42	21	4.58
An Puf	37	4.44	23	4.77
Pooled SE	2	0.1	0.4	0.1
Sign.[2] ($p<0.05$)	NS[3]	Exp.	Exp.	Exp.

[1]Eight pigs per treatment.

[2]See Table 5 for explanation of statistical comparisons.

[3]Not significant.

soybean oil replaced tallow as the fat source in high-fat, cholesterol-containing diets similar to the diets in our experiments.

Feeding the pigs plant protein decreased plasma cholesterol levels by 50 mg/dl compared with feeding animal protein. Kim et al. (71) reported that in pigs fed high-fat diets containing cholesterol, replacement of casein with soy isolate reduced plasma cholesterol levels by 107 mg/dl. One reason for the greater treatment response in the experiment of Kim et al. (71) than observed in our experiments may have been the sources of plant and animal proteins. Whereas we fed a mixture of plant proteins (soy, 50%; wheat, 25%; and corn, 25%) and milk proteins (casein, 90% and lactalbumin, 10%), Kim et al. (71) fed either 100% casein or 100% soy protein. At least in rabbits, wheat protein was found to be less hypocholesterolemic than soy protein (111). A second possibility may relate to growth responses of the pigs. In their experiment, pigs fed casein gained considerably less weight than those fed soy isolate (71) whereas treatment did not affect weight gain of our pigs. The lower weight gain of pigs fed casein and consequently increased intake of cholesterol per kg body weight may have contributed to the larger response of plasma cholesterol levels to protein source seen in their study (107 mg/dl) compared with that seen in our study (50 mg/dl).

Carroll et al. (113) reported that plasma cholesterol levels of humans decreased by 16 mg/dl when soy protein replaced a mixture of animal proteins (meat and casein) in high-fat diets. Sirtori et al. (73) also reported that consumption of a mixture of plant proteins (63% soy protein) in diets containing 25% of energy as fat reduced

plasma cholesterol levels an average of 50 mg/dl in hypercholesterolemic patients compared to feeding a mixture of plant and animal proteins.

When a mixture of soy protein and casein (half and half) was fed to pigs plasma cholesterol levels were intermediate, but not equal distant, between levels observed when soy and casein were fed separately (71); levels were closer to those observed when soy was fed alone than when casein was fed as the sole source of protein. Although consumption of plant proteins, as a group, generally produce lower plasma cholesterol levels than does consumption of animal proteins, it is evident that not all plant proteins are equally effective in this regard (111). Additionally it appears that responses of plasma cholesterol levels to mixtures of plant and animal proteins may not be directly proportional to levels of the two protein sources in the diet.

We observed no interaction between dietary fat source and protein source on plasma cholesterol levels in pigs. Others have suggested that the hypocholesterolemic effect of soy is reduced when fed with saturated fat (72, 73). Feeding diets with low P:S fat ratios did not reduce the effectiveness of plant protein in lowering plasma cholesterol levels in pigs. Plasma cholesterol levels decreased by 50 mg/dl in pigs fed the polyunsaturated fat diets when plant protein replaced animal protein. Plant protein was equally effective when pigs were fed saturated fat diets; plasma cholesterol levels were also 50 mg/dl lower in pigs fed plant protein than in those fed animal protein. Carroll and Hamilton (111) fed casein or soy isolate protein diets with either 15% butter or

15% corn oil to rabbits. In rabbits receiving 15% butter, consumption of soy protein resulted in plasma cholesterol levels approximately 75 mg/dl lower than when casein was consumed (estimated from Figure 3 of reference 111). Similarly in rabbits fed corn oil, switching from casein to soy isolate caused an approximate 60 mg/dl drop in plasma cholesterol levels. Thus their results show, like our results, that there is little interaction between dietary fat source and protein source in the regulation of plasma cholesterol levels.

Shorey and Davis (72), in an experiment with mildly hypercholesterolemic young men, concluded that saturated fat negated the hypocholesterolemic effect of soy protein. They, however, did not compare protein sources in diets high in polyunsaturated fat. Thus, it is possible that soy protein may not have exhibited a hypocholesterolemic effect in their subjects under these latter conditions, either. Sirtori et al. (73) also suggested a decreased effectiveness in the ability of soy protein to lower plasma cholesterol levels in hypercholesterolemic humans when soy protein was fed with saturated fats. Their results clearly show the expected hypocholesterolemic action of polyunsaturated fat, but they did not make direct comparisons between animal and plant proteins. Until direct comparisons of protein and fat sources are made in the same experiment, it is not possible to state unequivocally that saturated fat negates the hypocholesterolemic effect of soy protein in humans. Since both fat and protein can affect plasma cholesterol levels, conditions could exist where their effects on plasma cholesterol offset each other, however, this was not observed in our study with pigs.

The mechanism by which dietary protein sources alter plasma cholesterol values is unclear. Plasma LCAT activity has been suggested to be involved in the turnover and clearance of plasma cholesterol (130). Effects of dietary fat and protein source on LCAT activity were observed in our study. When polyunsaturated fats were fed to pigs, both molar LCAT activity and plasma cholesterol levels were decreased compared to when saturated fats were fed. Feeding animal protein, compared to plant protein, also decreased plasma molar LCAT activity but increased plasma cholesterol levels.

Decreases in molar LCAT activity by polyunsaturated fats have been observed in humans (131, 132) and rats (136). Larking and Sutherland (136) reported decreased levels of unesterified cholesterol when polyunsaturated fats were fed to rats. They suggested that decreased unesterified cholesterol levels were responsible for decreased molar LCAT activity. This is possible since the method used to assay molar LCAT activity depends on endogenous substrate availability, and does not differentiate between changes in enzyme activity and substrate availability (129, 130). Changes in molar LCAT activity in humans positively correlates with changes in unesterified cholesterol, triglyceride, and phospholipid blood levels (35). Feeding polyunsaturated fat diets decreased plasma unesterified cholesterol levels in pigs but did not alter plasma triglyceride levels. Thus in this experiment, the effect of feeding polyunsaturated fats on molar LCAT activity and unesterified cholesterol levels are consistent

with decreases observed in other experiments in which polyunsaturated fats have been fed (129, 130, 134).

The reason plasma molar LCAT activity decreased when animal protein was fed is also unclear. Animal protein caused an increase in unesterified cholesterol levels compared to plant protein. Based on results obtained when polyunsaturated fats were fed (low LCAT activity and low plasma unesterified cholesterol levels), one would expect that pigs fed animal protein to exhibit higher molar LCAT rates than pigs fed plant protein. Since the opposite effect occurred (low LCAT activity and high unesterified cholesterol levels) it is apparent that LCAT activity is influenced by additional factors besides the concentration of unesterified cholesterol. Feeding animal protein reduced the ratio of HDL to LDL compared to values in pigs fed plant protein. While the absolute level of HDL cholesterol in the plasma was higher in the animal protein fed pigs the percentage of total cholesterol was increased in the animal protein fed pigs. Wallentin (107, 130) has reported little correlation between HDL lipid changes and molar LCAT activity so that these changes in the pig may not be responsible for the decreased LCAT activity. Possibly feeding animal protein altered the metabolism of apoprotein A, an activator of LCAT (129), but this was not investigated in this experiment. Finally, it must be emphasized that while the differences in LCAT activity between treatments is statistically significant, the physiological significance, particularly since there was no difference in the percentage of esterified cholesterol, is unknown.

One other mechanism proposed to explain the varying effect of dietary protein source on plasma cholesterol levels is the amino aicd composition of the proteins (66, 67). Kritchevsky has suggested that the dietary ratio of arginine to lysine, high in plant proteins but low in animal proteins is important in the development of atherosclerosis (112). He hypothesizes that high lysine levels inhibit hepatic arginase activity, perhaps resulting in greater production of arginine-rich lipoproteins (112). Kritchevsky et al. (69) reported an increased incidence of atherosclerosis in rabbits when lysine was added to soy protein diets. But the additional lysine did not significantly increase plasma cholesterol levels in their study compared to groups fed soy protein diets without lysine. Our animal protein diets contained more lysine than the plant protein diets. While this increase was reflected in plasma lysine levels in pigs fed animal protein, there was no dietary effect on plasma arginine levels or on the ratio of arginine to lysine in plasma. The lower methionine content of soy protein, relative to animal proteins, has also been hypothesized to be a factor in the hypocholesterolemic action of soy protein (65, 137). Kim et al. (111) reported no effect of supplementation of soy diets with methionine in pigs on plasma cholesterol levels compared to pigs fed soy protein alone. No difference in plasma methionine levels were observed in our pigs with any of the treatments. It must be noted that the amino acids were determined from samples obtained from the peripheral circulation. These samples may not reflect the flux

of amino acids from the intestine to the liver. This initial liver influx of amino acids into the liver could influence plasma cholesterol metabolism.

In conclusion, our studies clearly show that feeding plant protein, compared to animal protein, reduces plasma cholesterol levels when fed in conjunction with high-fat, high-cholesterol diets. Furthermore, we observed no interaction between the type of dietary fat fed and the type of dietary protein fed. The hypocholesterolemic effect of plant protein was significant even though the protein source was mixed (soy, wheat and corn) so that soy isolate provided only 50% of the diet protein. This study while confirming previous reports that polyunsaturated fat lowers plasma molar LCAT activity and offers the first evidence that molar LCAT activity is reduced in the plasma of pigs fed animal protein.

PART III

EFFECT OF FEEDING ROLLED OATS OR WHEAT BRAN ON PLASMA LIPIDS, LIPOPROTEINS, LCAT AND TISSUE LIPIDS IN PIGS AND CHICKENS

INTRODUCTION

Burkitt (74) and Trowell (75) have hypothesized that the lower incidence of coronary heart disease (CHD) in developing countries, compared to Western nations, is due to the higher intake of dietary fiber in the developing countries. They also suggest that the high fiber intake in developing countries contributes to the lower plasma lipid levels compared to the developed countries. Experimental studies in both animals and humans have shown that individual fiber types uniquely affect plasma cholesterol levels. Wheat bran has been shown to be ineffective in lowering plasma cholesterol levels in many studies (65, 68, 138-140), while other fibers, such as pectin or rolled oats, have been shown to lower plasma total cholesterol levels (86, 89).

In the present experiments wheat bran or rolled oats were chosen, because of their seemingly different effects on plasma cholesterol levels, to be fed at levels of 6.5% neutral detergent fiber (NDF) to either pigs or chickens. Pigs were chosen as an experimental model because they metabolize cholesterol in a manner similar to man, have similar lipoprotein characteristics as man (53) and have been shown to develop atherosclerotic lesions similar to man (141). Since pigs generally exhibit low levels of plasma cholesterol the diets were also fed to chickens. Plasma cholesterol concentrations increase to much higher levels in chickens than pigs when fed the same diet (55).

While the major objective of these experiments was to investigate the effect wheat bran or rolled oats have on plasma cholesterol levels, other parameters, such as body composition, plasma HDL cholesterol and plasma lecithin:cholesterol acyl transferase (LCAT) activity were also investigated.

MATERIALS AND METHODS

ANIMALS

Two experiments were undertaken to investigate the effects feeding wheat bran or rolled oats have on plasma cholesterol and lipoprotein parameters and tissue lipid deposition. In Experiment 1 crossbred (Yorkshire x Duroc or Yorkshire x Hampshire pigs) castrated male pigs were assigned at seven weeks of age to the dietary treatments. Ten pigs in each treatment were housed together in an environmentally controlled building with slatted floors. They had free access to diet and water. Pigs were weighed and blood samples obtained by vena cava puncture at weeks 4, 7, 10, and 13, after a 24 hour fast. At slaughter (17 weeks after the start of dietary feeding) aorta, liver, heart, and hams were obtained and stored for subsequent analysis.

In the second experiment broiler strain roosters were assigned to the same dietary treatments as in Experiment 1. Prior to dietary treatments, the chickens were fed a low-fat, low-cholesterol semi-purified diet for 8 weeks. Average weight of the chickens at the beginning of the experiment was 2.9 ± 0.1 kg. As in the first experiment the chickens had free access to food and water.

Blood samples were taken via cardiac puncture at 3 and 5 weeks of the experiment after an overnight fast.

DIETS

Composition of the diets fed in both experiments are shown in Table 1. Both fiber diets were formulated to contain 6.5% neutral detergent fiber (NDF) while the control diet contained less than 1% NDF. Neutral detergent fiber was determined as described by Goering and Van Soest (115). The diets were plant protein based, and high fat, with a P:S ratio of 0.3. Crystalline cholesterol was added (0.05%) to each diet. Dietary fatty acids were extracted in chloroform:methanol (2:1, v/v). The non-saponifiable lipids were extracted as described in the section on hepatic sterol synthesis. Fatty acids were extracted with petroleum ether after acidifying with 3N HCl. After solvent evaporation the fatty acids were converted to their methyl esters by adding excess etheryl diazo-methane. The fatty acids were determined by gas-liquid chromatography (GLC) equipped with a flame ionization detector and an electronic integrator. GLC conditions were: injector temperature, 135°C; detector temperature, 175°C; column temperature was initially held at 130°C for 5 minutes after injection and programmed from 130-160°C at 2°/min. Temperature was held at 160°C until the last methyl ester was eluted; N_2 carrier gas flow 30 ml/min; H_2 flow, 15 ml/min; and air flow, 240 ml/min. Fatty acids were separated on a 1 meter long, 2 mm ID glass Supelco column packed with

Table 1. Composition of diets, %.

Ingredients	Diet		
	Control	Wheat Bran	Rolled Oats
Wheat bran[1]	—	16.0	—
Rolled oats[2]	—	—	50.0
Soybean meal	37.0	32.0	22.0
Corn starch	39.6	30.1	7.1
Mineral and vitamin supplement[3]	1.3	1.3	1.3
Tallow	18.0	17.0	16.0
Corn oil	1.0	1.0	1.0
Cholesterol	0.05	0.05	0.05

[1]Standard wheat bran from American Association of Cereal Chemists (AACC), St. Paul, MN.

[2]Rolled oats from Quaker Oats, Chicago, IL.

[3]Supplying the following per kilogram of diet: vitamin A, 3300 IU; vitamin D, 660 IU; 11 IU of vitamin E; menadione sodium bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 17.6 mg d-pantothenic acid, 13.2 mg; choline, 110 mg; vitamin B_{12}, 19.8 μg; zinc, 75 mg; iron, 60 mg; manganese, 37 mg; copper, 10 mg; iodine, 2.8 mg, and selenium, 0.1 mg.

10% SP 2340 coated on chromosorb WAW (100/120 mesh). Fatty acids were identified based on retention times of standard fatty methyl esters. Values are expressed as percentage of fatty acid, by weight, of total fatty acids in the diet (Table 2).

Dietary cholesterol and plant sterol concentrations were determined by gas-liquid chromatography. Cholesterol composition of the diets was (mg/100 g diet): control, 40; wheat bran, 39; and rolled oats, 65. Plants sterol content was (mg/100 g diet): 2.3; wheat bran, 4.8; and rolled oats, 5.1.

PLASMA AND TISSUE ANALYSES

Plasma total and unesterified cholesterol were determined enzymatically.[1] Esterified cholesterol was calculated as the difference between total and unesterified cholesterol values. Plasma high density lipoprotein (HDL) cholesterol was also determined enzymatically after the low density (LDL) and very low density (VLDL) lipoproteins were precipitated with heparin-manganese solution (120). Plasma lecithin:cholesterol acyl transferase (LCAT, EC 2.3.1.43) activity was assayed by the method of Stokke and Norum (123) with modifications suggested by Wallentin and Vilkrot (124). Plasma lipoproteins were separated by electrophoresis on polyacrylamide gel,[2] quantitated by densitometry at 610 nm, and percentages of VLDL, LDL

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[1]Cholesterol reagent set, BMC, Indianapolis, IN 46250.

[2]Redi-Disc, Ames Company, Elkhart, IN 46514.

Table 2. Fatty acid composition of diets, %.[1]

Fatty acid	Diet		
	Control	Wheat Bran	Rolled Oats
C_{14}	3.13	2.15	3.06
C_{15}	0.87	0.34	0.91
$C_{16:0}$	19.56	18.19	18.66
$C_{16:1}$	4.54	6.65	5.20
C_{17}	1.54	0.26	1.78
$C_{18:0}$	14.07	12.13	13.06
$C_{18:1}$	40.76	36.24	35.43
$C_{18:2}$	9.19	17.96	16.53
$C_{18:3}$	1.12	1.46	1.76
C_{20} (or greater)	0.28	0.18	1.40

[1]Percent of total fatty acids, based on weight.

and HDL fractions were calculated (121). Plasma triglycerides were extracted in isopropanol:heptane (5.6:3.0, v/v) and determined gravimetrically. Hams were separated from the carcasses and trimmed according to commercial practices; the meat separated from bone and connective tissue, ground to pass a 5 mm screen, and vacuum dried at 60°C. The extracted lipid (chloroform:methanol, 2:1) was used to determine total tissue fat (gravimetrically) and tissue cholesterol (colorimetrically)(125).

HEPATIC STEROL SYNTHESIS

In vivo liver sterol synthesis was determined in fed chickens in the 5th week of the experiment. One ml of saline containing 7.7 millicuries of tritiated water was injected into the heart of each chicken. Fifteen minutes after injection the chickens were killed by cervical dislocation and livers were removed quickly and frozen in a dry ice-acetone bath. Duplicate 3 gram samples were homogenized in 3 ml of distilled water. From each sample two 0.5 ml aliquots were added to 3 ml of 30% KOH and brought up to 10 ml with 95% ethanol and heated in a water bath at 70°C for 3 hours. After cooling, 10 ml of distilled water was added and the nonsaponifiable lipids were extracted 3 times with petroleum ether. The pooled extracts were backwashed with distilled water to remove any tritiated water. Any traces of remaining water were removed with anhydrous sodium sulfate. After the petroleum ether was evaporated, 10 ml of scintillation cocktail (4 g 2,5-diphenyl-oxazole and 200 mg 1-4 bis (2-(4-methyl-5-phenylxazdyl))-benzene in 667 ml toluene

and 333 ml of triton-X 100) was added and the samples were counted in a liquid scintillation counter. Fifteen minutes after injection of tritiated water a blood sample was taken to determine the specific activity of body water. This was used to calculate the moles of ^3H_2O incorporated into nonsaponifiable lipids per gram wet tissue per minute. Total body water was estimated by tritiated water dilution from the specific activity of plasma at 50 min.

STATISTICAL ANALYSES

The values are reported as mean ± standard error of mean (SEM). In both experiments data were analyzed by analysis of variance with means compared by Tukey's procedure (126).

RESULTS

Neither the final body weights nor the carcass weights were affected by feeding dietary fiber (Table 3). Average daily feed consumption, computed for the entire experimental period, was 1.88, 2.02 and 1.88 kg food consumed/day for the control, wheat bran and rolled oats diets, respectively. Ham composition was used to estimate carcass composition since ham composition is highly correlated with overall body composition (141). The percentage of fat in hams of pigs fed rolled oats was significantly reduced in comparison to either the control or wheat bran fed pigs. And the percentage fat in the heart and thoracic aorta tended to be lower in pigs receiving the rolled oats diet than in pigs fed

Table 3. Dietary fiber effects on body, heart and aorta composition in pigs.

	Diets		
	Control	Wheat Bran	Rolled Oats
Final weight, kg	121 ± 2[1a2]	118 ± 3[a]	122 ± 3[a]
Carcass weight, kg	91 ± 3[a]	90 ± 4[a]	89 ± 3[a]
% Fat, ham[3]	48.2 ± 2[a]	46.1 ± 2[a]	41.7 ± 2[b]
Heart[3]			
% fat	36.3 ± 2[a]	37.7 ± 2[a]	35.6 ± 3[a]
total cholesterol, mg/g dry matter	15.2 ± 0.3[a]	15.5 ± 1.1[a]	15.8 ± 1.3[a]
Aorta[3]			
% fat	28.1 ± 2[a]	33.2 ± 6[a]	26.3 ± 1[a]
total cholesterol, mg/g dry matter	3.4 ± 0.1[a]	3.6 ± 0.1[a]	3.3 ± 0.1[a]

[1]Mean ± SEM. Ten pigs per treatment. Blood drawn at 13th week of experiment.

[2]Values with different superscripts are different ($p<0.05$).

[3]Expressed as percent of dry matter.

either of the other diets. There was no effect of dietary fiber on the cholesterol content of either the heart or thoracic aorta.

Feeding dietary fiber did not cause any changes in plasma total or unesterified cholesterol levels or in the ratio of unesterified cholesterol to esterified cholesterol (Table 4). While the values reported in Table 4 are for the 13 week of the experiment, similar results were observed at the 7th and 10th weeks. Plasma triglyceride levels were unaffected by dietary fiber. Feeding wheat bran or rolled oats caused an increase in HDL cholesterol levels compared to the control diet. Since there was no difference in total cholesterol, the percentage of cholesterol in the HDL fraction was also significantly greater in pigs fed wheat bran or rolled oats.

Plasma fractional LCAT rate, which estimates the fraction of unesterified cholesterol converted to cholesterol esters, was not significantly affected by dietary treatment. Fractional LCAT activity is highly correlated with unesterified cholesterol levels (130). Since there was no difference in unesterified cholesterol levels between dietary treatments it was not surprising that the fractional rate was also similar among treatments. Molar LCAT rate, an absolute rate which takes into account the levels of unesterified cholesterol, was affected by diet. Feeding rolled oats increased molar LCAT activity compared to feeding either the control or wheat bran diets.

Feeding wheat bran caused a significant increase in the HDL fraction and a significant decrease in the LDL fraction (Figure 1).

Table 4. Effect of dietary fiber on plasma cholesterol and triglyceride levels and LCAT activity in pigs.

	Control	Wheat Bran	Rolled Oats
Plasma cholesterol, mg/dl			
total	134 ± 5[2a3]	132 ± 6[a]	133 ± 3[a]
unesterified	26 ± 2[a]	28 ± 2[a]	30 ± 2[a]
HDL	42 ± 3[a]	58 ± 3[b]	55 ± 4[b]
UC/CE[4]	0.19 ± 0.01[a]	0.21 ± 0.01[a]	0.22 ± 0.01[a]
HDL cholesterol, %[5]	31.3 ± 1[a]	43.9 ± 3[b]	41.2 ± 2[b]
Triglycerides, mg/dl	54 ± 3[a]	46 ± 5[a]	42 ± 4[a]
Plasma LCAT activity			
fractional, %CE/hour	4.7 ± 0.4[a]	4.4 ± 0.4[a]	5.3 ± 0.6[a]
molar μM/L/hour	31.4 ± 2.7[a]	29.4 ± 1.6[a]	41.8 ± 6.8[b]

[1]Ten pigs per treatment. Blood drawn at 13th week of the experiment.

[2]Mean ± SEM.

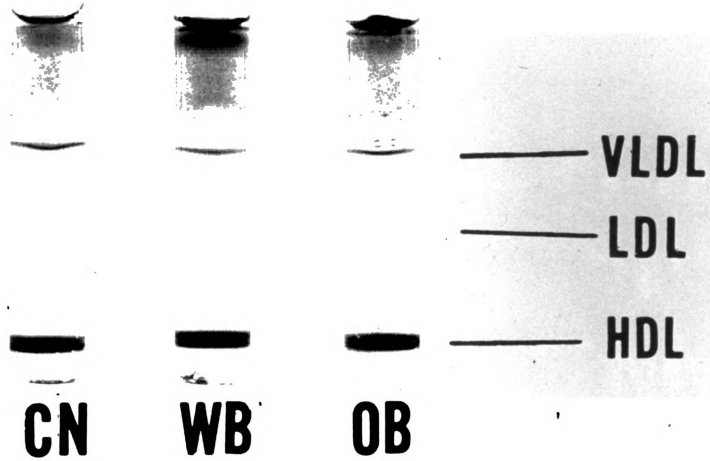
[3]Values with different superscripts are different ($p<0.05$).

[4]UC/CE = unesterified cholesterol/esterified cholesterol.

[5]Percentage of total cholesterol in the HDL fraction.

[6]Percentage of tritiated cholesterol converted to cholesterol ester/hour.

Figure 1. Effects of dietary fiber on plasma lipoprotein separations. Representative samples separated by polyacrylamide gel electrophoresis. CN = control; WB = wheat bran; and OB = rolled oats diets. HDL:LDL ratios are: CN, 1.27 ± 0.1; WB, 1.52 ± 0.1; and OB, 1.27 ± 0.1.


VLDL
LDL
HDL
CN
WB
OB

This same pattern was observed at the 7th and 10th weeks of the experiment as well. The ratio of HDL/LDL was also significantly greater in plasma of pigs fed wheat bran than in pigs fed either the control or rolled oats diets (1.52 and 1.27, Table 5).

A second experiment was undertaken to test the effect of feeding dietary fiber on plasma cholesterol levels in chickens. Usually plasma cholesterol levels are mich higher in chickens than in pigs if they are both fed a high fat diet which contains cholesterol. Other plasma cholesterol and lipoprotein parameters were investigated to determine if they were altered in chickens in the same manner as they were in pigs. As occurred in the pig study, the experimental diets had no affect on the final body weights of the chickens (Table 6). Chickens fed the rolled oats diet had a higher percentage of body water than chickens fed the control diet. Since body water and fat are inversely related, Experiment 2 confirms the results in Experiment 1; namely feeding rolled oats tends to decrease body fat compared to feeding the control, fiber-free diet. Feeding wheat bran had no effect on body composition.

In this experiment feeding dietary fiber to chickens did alter plasma total cholesterol levels (Table 6). Chickens fed the rolled oats diet had an 18% decrease in plasma total cholesterol levels compared to chickens fed the control diet (220 mg/dl vs. 266 mg/dl, respectively). Feeding wheat bran resulted in intermediate cholesterol levels (246 mg/dl) in comparison to chickens fed control and rolled oats diets. There was no significant effect of feeding

Table 5. Plasma lipoproteins in pigs as affected by dietary fiber.

	Diets		
	Control	Wheat Bran	Rolled Oats
Lipoproteins, %[1]			
VLDL	16 ± 1[a]	14 ± 0.3[a]	16 ± 1[a]
LDL	38 ± 2[a]	35 ± 1[b]	38 ± 2[a]
HDL	46 ± 2[a]	52 ± 1[b]	47 ± 2[a]
HDL/LDL	1.27 ± 0.1[a]	1.52 ± 0.1[b]	1.27 ± 0.1[a]

[1]Relative percentage of total lipoprotein.

[2]Mean ± SEM. Ten pigs per treatment. Blood drawn at 13th week of experiment.

[3]Values with different superscripts are different ($p<0.05$).

Table 6. Effect of dietary fiber on final body weight, body water, plasma cholesterol parameters and plasma LCAT activity in chickens.

	Diet		
	Control	Wheat Bran	Rolled Oats
Final body weight, kg	4.0 ± 0.2[a3]	4.1 ± 0.3[a]	4.0 ± 0.2[a]
Body water, %	49.9 ± 2.8[a]	53.5 ± 2.3[ab]	62.1 ± 4.9[b]
Plasma cholesterol, mg/dl			
total	266 ± 8[a]	246 ± 15[ab]	220 ± 15[b]
unesterified	83 ± 3[a]	75 ± 4[a]	69 ± 5[a]
HDL	104 ± 1[a]	103 ± 2[a]	98 ± 5[a]
HDL cholesterol, %[5]	39 ± 1[a]	43 ± 3[ab]	46 ± 4[b]
UC/CE[4]	0.46 ± 0.02[a]	0.44 ± 0.02[a]	0.47 ± 0.02[a]
Liver sterol synthesis			
μmoles ^3H_2O/g/min[6]	7.9 ± 1[a]	8.1 ± 2[a]	10.4 ± 1[a]
Plasma LCAT activity			
fractional rate, % CE/hour[7]	4.7 ± 0.6[a]	4.7 ± 0.6[a]	7.6 ± 0.9[b]
molar rate, μM/L/hour	106 ± 6[a]	95 ± 8[a]	143 ± 19[b]

[1]Eight chickens per treatment with an initial weight of 2.9 ± 0.1 kg. Blood was drawn after 5 weeks of dietary treatment.

[2]Mean ± SEM.

[3]Values with different superscripts are different ($p<0.05$).

[4]UC/CE = unesterified cholesterol/esterified cholesterol.

[5]Percentage of total cholesterol in the HDL fraction.

[6]Micromoles of ^3H_2O incorporated into unsaponifiable lipids/g of liver/min.

[7]Percent of tritiated cholesterol converted to cholesterol ester/hour.

Table 7. Effect of dietary fiber on plasma lipoprotein percentages in chickens.

	Diets		
	Control	Wheat Bran	Rolled Oats
Lipoproteins, %[2]			
LDL, 1	37 ± 4[3,4a]	30 ± 5[ab]	21 ± 4[b]
LDL, 2	10 (1)[5]	14 (3)	21 (4)
HDL	62 ± 4[a]	65 ± 4[a]	69 ± 3[a]
HDL/LDL	2.0 ± 0.4[a]	3.2 ± 0.9[a]	4.1 ± 0.6[a]

[1]Eight chickens per group. Blood was drawn at 5 weeks of experiment.

[2]Relative percent of each lipoprotein.

[3]Mean ± SEM.

[4]Values with different superscripts are different ($p<0.05$).

[5]Number in parentheses indicate number of chickens with 2 bands in the LDL fraction. The value is the average for the number showing a fraction.

dietary fiber on either unesterified cholesterol levels or the ratio of unesterified to esterified cholesterol. The percentage of cholesterol carried by the HDL fraction tended to be higher in both groups of chickens fed fiber. However, only the chickens receiving rolled oats had HDL cholesterol values which were significantly different from the control values. In the first experiment with pigs, both fibers caused an increased percentage of cholesterol in the HDL fraction. Liver sterol synthesis was unaffected by dietary fiber treatment. Plasma LCAT activity, both the fractional rate and molar rate were higher in chickens fed the rolled oats diet than in chickens fed either of the other diet treatments. In Experiment 1, feeding the rolled oats diet to pigs caused an increase in the molar, but not the fractional, LCAT rate.

The effect of dietary fiber on electrophoretic lipoprotein separations in chickens is shown in Table 7. The relative percentage of LDL was reduced in the Rolled oats diet compared to the control diet. Furthermore feeding fiber increased the number of bands observed in the LDL region compared to the control diet. Dangerfield et al. (58) have previously shown that occassionally electrophoretic separations do produce two bands in the LDL region although the significance of these bands is unknown.

DISCUSSION

The effect of dietary fiber on body composition has not been extensively investigated. In both experiments feeding rolled oats reduced body fat compared to feeding either the control or wheat bran diets. In pigs fed rolled oats the fat percentage in hams was 41.7% as compared to 46.1% or 48.2% fat in the hams of pig fed wheat bran or control diets, respectively. The fat content of the aorta and heart also tended to be lower in pigs fed rolled oats. In chickens, feeding rolled oats resulted in an increase in body water (62%) compared to either the control (50%) or wheat bran diet (53.3%). Since body water and fat are inversely related, chickens fed rolled oats would have the lowest percentage of body fat. Tsai et al. (143) reported that carragheenan and gum arabic reduced the relative dry body weight, which indicates an increase in body water and a relative decrease in body fat, compared to either a control (no fiber) or wheat bran diet. Sundaravalli et al. (144) reported that cellulose fed to rats did not affect body composition. Forsythe and Bennink (unpublished observations) have observed that liver total lipids were reduced when oat bran was fed to rats compared to a control diet (low fiber diet). The mechanism by which rolled oats lowers body fat is not clear. Southgate and Durin (145) reported that fecal lipids were increased when fiber was fed but whether this is a factor was not investigated in these experiments.

Wheat bran did not affect plasma total cholesterol levels in either pigs or chickens. Many other reports have shown that wheat bran does not decrease plasma cholesterol levels in either humans (138-140) or animals (65, 68, 143). In the pig, feeding wheat bran did increase plasma HDL cholesterol levels by 16 mg/dl over the control levels. The percentage of total cholesterol in the HDL fraction was increased from 31% in the pigs fed the control diet to 44% in the pigs fed the wheat bran diet. McDougall et al. (84) reported that feeding 50 g of wheat bran daily for 6 months, while not affecting total cholesterol levels, did increase HDL cholesterol levels compared to pre-treatment levels. Miettinen (146) also reported, again without any change in total cholesterol levels, that feeding wheat bran to humans resulted in a 36% increase in HDL cholesterol levels. In the second experiment, in chickens, wheat bran did not significantly increase the HDL cholesterol levels.

In Experiment 1, in pigs, wheat bran also increased the percentage of HDL by 15% and decreased the percentage of LDL by 10% compared to the other two diets; and the ratio of HDL/LDL was 20% greater in the wheat bran fed pigs than in either the control or rolled oats fed pigs. Hill et al. (147) has shown in pigs that the relative percentages of lipoproteins obtained by electrophoretic separations are similar to the percentages of lipoproteins obtained with ultracentrifugation and Sclieren optics. In Experiment 2, in chickens, the HDL/LDL ratio tended to be increased in the wheat bran diets compared to the control diet (3.2 to 2.0, respectively) but this was not a significant increase ($p<0.05$). Brodribb et al.

(148) reported feeding 24 g/day of wheat bran to free living humans for 6 months caused a 28% decrease in LDL fraction and a 68% increase in the HDL fraction, even though there were no changes in total cholesterol levels.

The mechanism by which wheat bran causes these changes is not at all clear. McDougall et al. (84) suggested that wheat bran causes an increased excretion of bile acids and neutral sterols resulting in liver cholesterol depletion; however they have no evidence for this contention. They contended that HDL cholesterol was utilized for bile acid synthesis and this caused a relative increase in HDL cholesterol levels. There are data in the literature which supports as well as refutes the hypothesis that wheat bran increases the excretion of fecal sterol. Neither Eastwood et al. (138) nor Walters et al. (149) found an increase in excretion of either fecal bile acids or neutral sterol when humans were fed 39 g or 30 g of wheat bran per day, respectively. Both experiments, however, did report increases in fecal weight and in fecal fat excretion. Pomare and Heaton (150) reported that wheat bran caused an increased excretion of bile acids when fed to rats. In our laboratory (Forsythe and Bennink, unpublished observations) feeding wheat bran to male rats did not increase the excretion of fecal neutral sterol but did increase the excretion of fecal bile acids compared to rats fed a low-fiber, control diet.

It appears, in the pig at least, that wheat bran has a beneficial effect on plasma cholesterol by increasing the HDL cholesterol levels and decreasing the LDL cholesterol levels even

though total cholesterol levels were unchanged. Furthermore the absolute levels of these lipoproteins were altered through as yet unknown mechanisms.

Rolled oats affected plasma cholesterol parameters to a greater extent than did wheat bran. Like wheat bran, it did not lower plasma cholesterol levels in pigs but it did decrease plasma cholesterol levels by 18% in chickens compared to the control diet. In one of the first papers on the effects of rolled oats on plasma cholesterol levels Degroot et al. (89) reported that rolled oats decreased plasma cholesterol levels to 20% of control values in rats fed hypercholesterolemic diets. In a study with hypercholesterolemic men, reported in this same paper, the addition of 140 g of rolled oats per day, as bread in the diet, decreased plasma cholesterol levels from 251 mg/dl to 223 mg/dl in 21 days. McNaughton (151) reported that feeding rolled oats (18%) in low-fat diets without cholesterol to chickens, decreased plasma cholesterol levels from 130 mg/dl (control) to 88 mg/dl. Degroot et al. (89) suggest that some of the hypocholesterolemic effect of the rolled oats is due to its relatively high vegetable fat content resulting in an increased P:S ratio. The fatty acid composition of each diet fed in this experiment shown in Table 2 indicates no major differences in fatty acid composition.

Rolled oats may bind more fecal sterols than does wheat bran. Balmer and Zilversmit (93) found that ground oats bound more sodium taurocholate than did ground wheat. Forsythe and Bennink (unpublished observation) have observed that feeding oat bran to rats increased fecal bile acid excretion 4 fold and neutral sterol

In conclusion, both of these experiments show beneficial affects due to feeding dietary fiber. Wheat bran, while not decreasing plasma total cholesterol, did increase plasma HDL cholesterol and HDL levels in pigs. Feeding rolled oats resulted in a decrease in body fat in both pigs and chickens. Rolled oats, also, decreased plasma total cholesterol in chickens and increased the percentage of cholesterol in the HDL fraction in both pigs and chickens. Finally, it increased the molar LCAT activity in both pigs and chickens. These changes should be beneficial in decreasing the severity of atherosclerosis.

excretion 3 fold as compared to control animals (low fiber diet). This resulted in a decrease in both total liver lipids and in liver sterols in rats fed oat bran compared to those fed the control diet.

Plasma molar LCAT activity was increased in plasma of both pigs and chickens fed the rolled oats diet. LCAT, activated by apoprotein A-I from HDL, transfer linoleate from lecithin to unesterified cholesterol (37). This cholesterol ester is transported to the liver by HDL where Schwarz et al. (38) have shown that HDL cholesterol is utilized for bile acid synthesis. While HDL levels are important in the reaction they have not been highly correlated with LCAT activity. Wallentin (107) has hypothesized that LCAT activity increases in response to an increase in the turnover of cholesterol esters. Lopez et al. (152) reported that intensive exercise, a treatment which has been shown to increase the turnover of body cholesterol (153), caused an increase in molar LCAT activity. This is consistent with the hypothesis that fiber (rolled oats) increases the turnover of cholesterol through an increased excretion of fecal sterols (154).

The method used to assay LCAT activity is a self-substrate method. As such it is impossible to relate changes in activity to an actual change in enzyme activity or a change in substrate availability. Also unclear is the actual physiological significance of altered molar LCAT rates, especially when there was no change in the ratio of unesterified to esterified cholesterol as there were in these experiments.

PART IV

EFFECT OF AN AEROBIC EXERCISE PROGRAM ON BODY COMPOSITION, PLASMA CHOLESTEROL PARAMETERS, LCAT ACTIVITY, LIPOPROTEINS AND TISSUE LIPIDS IN YOUNG PIGS

INTRODUCTION

The ability of exercise to lower plasma total cholesterol levels in humans is still controversial. While Lopez et al. (152) reported a small decrease in plasma cholesterol levels after a seven week intensive exercise program, many other studies have failed to show that exercise can lower plasma total cholesterol levels (100-102, 109). In animal stidies, neither Link et al. (155), exercising pigs, nor Kenealy et al. (156), exercising goats, were able to show that a moderate exercise program could reduce plasma total cholesterol levels. Fukuda et al. (103) has reported that a moderate exercise program in rats was able to significantly reduce plasma total cholesterol levels compared to pre-exercise levels.

While plasma total cholesterol levels have not been conclusively shown to decrease after an exercise program, other parameters of cholesterol metabolism, have been shown to beneficially change after exercise. Many studies have reported increases in plasma HDL cholesterol levels after exercise, even when total cholesterol concentrations have remained unchanged (101, 102, 109, 152). Lopez et al. (152) has reported that exercise increased plasma molar leçithin:cholesterol acyl transferase (LCAT) activity in humans and Simko reported that exercise in rats increased fractional LCAT activity. Plasma lipoprotein profiles have also been reported to be changed by exercise in humans, with an increase in HDL fraction and a decrease in LDL fractions after an exercise program.

Interestingly, except for the report by Link et al. (155) with miniature pigs, there are no other reports on how exercise affects plasma cholesterol levels in pigs. The pig is an excellent model in which to study cholesterol metabolism and exercise. It is physiologically and cardiovascularally similar to humans (141). The pig also responds to cholesterol feeding, has similar lipoprotein patterns and develops atherosclerotic lesions as do humans (53). And because its weight is similar to humans the energy response to exercise should also be similar to humans. Thus, experiments were undertaken to study how a moderate exercise program would affect plasma cholesterol levels, HDL cholesterol levels and plasma LCAT activity in pigs fed high-fat, high cholesterol diets similar to those consumed by Americans.

MATERIALS AND METHODS

ANIMALS AND DIETS

Twelve purebred (Yorkshire or Duroc) and four crossbred (Yorkshire x Hampshire) castrated male pigs were assigned to either an exercise or non-exercise group. The pigs were randomly assigned to treatments except where two pigs were from the same litter in which case one pig was assigned to each treatment. All pigs in each treatment were housed together in pens with slatted floors with free access to feed and water. The pigs weighed 23 kg at the start of the experiment.

Both treatments were fed the same diets (Table 1). For the first five weeks of the study, 16% protein corn-soybean meal grower diet was fed. After five weeks the pigs were fed a high fat-0.05% cholesterol diet for the remainder of the experiment. Food consumption was recorded while the pigs were fed the high-fat diet. Weights were recorded and blood samples were drawn from the vena cava at 5, 7 and 13 weeks of the experiment, 24 hours after the removal of feed.

EXERCISE PROTOCOL

The exercise program was primarily aerobic and an amount to which an average American could easily adapt. By the end of a three week training period, during which the time and distance the pigs were exercised was gradually increased, the pigs were accustomed to running on a treadmill. The exercise regimen was running 3.3 mph for 9 minutes one day and 3.0 mph for 20 minutes on alternate days at 0% grade. Except for two periods of two days each near the end of the experiment when the belt on the treadmill broke, the pigs were exercised every day throughout the experimental period. Initially the pigs received an electrical shock from a 9 volt prod if they did not run but after a few shocks the pigs adapted to the exercise and usually ran without shocks throughout the experiment.

Prior to beginning the exercise program all pigs under went a stress test. The test consisted of three consecutive three minute runs at three mph at 0%, 2.5% and 5% grade. At the end of the test

Table 1. Composition of diets, %.

Ingredients	Diet	
	Corn-soy	High fat
Corn	78.1	—
Corn starch	—	39.5
Soybean meal	17.5	37.5
Dicalcium phosphate	1.1	1.5
Calcium carbonate	1.3	1.5
Salt	0.5	0.3
Vitamin premix[1]	0.5	0.5
Vit E-Se premix[2]	0.5	0.5
Antibiotic premix	0.5[3]	0.2[4]
Corn oil	—	1.0
Tallow	—	18.0
Cholesterol[5]	—	0.05
	100%	100%

[1]Supplied the following nutrients per kg of diet: vitamin A, 3300 IU; vitamin D, 660 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 17.6 mg; d-pantothenic acid, 13.2 mg; choline, 110 mg; vitamin B_{12}, 19.8 μg; zinc, 75 mg; iron, 60 mg; manganese, 37 mg; copper, 10 mg; and iodine, 2.8 mg.

[2]Supplied 11 IU of vitamin E and 0.1 mg of Se per kg of diet.

[3]Supplied 110 mg of chlortetracycline per kg of diet.

[4]Supplied 88 mg of chlortetracycline, 88 mg of sulfamethazine and 44 mg of penicillin per kg of diet.

[5]As crystalline cholesterol.

maximal heart rate, obtained by palpitation, was 300-320 beats/minute. Within the experiment the maximum exercise (20 minutes at 3 mph) produced heart rates of 220 beats/minute; or 70% of maximum heart rate.

PLASMA AND TISSUE ANALYSES

Plasma total and unesterified cholesterol were determined enzymatically.[1] Esterified cholesterol was calculated as the difference between total and unesterified cholesterol values. Plasma high density lipoprotein (HDL) cholesterol was also determined enzymatically after the low density (LDL) and very low density (VLDL) lipoproteins were precipitated with heparin-manganese solution (120). Plasma LCAT (EC 2.3.1.43) activity was assayed by the method of Stokke and Norum (123) with modifications suggested by Wallentin and Vikrot (124). Plasma lipoproteins were separated by electrophoresis on polyacrylamide gel.[2] The tubes were then scanned on a densitometer at 610 nm, and the percentage of the alpha, pre-beta and beta lipoproteins were calculated. Triglycerides were extracted in isopropanol: heptane (5.6:3.9, v/v) and then determined colorimetrically (122).

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[1]Cholesterol Reagent Set, BMC, Indianapolis, IN 46250.

[2]Redi-Disc, Ames Company, Elkhart, IN 46514.

Total heart lipids were extracted with chloroform:methanol (2:1, v/v) and determined gravimetrically. An aliquot of the extracted lipid was assayed for total cholesterol (125). Carcass fat and water was determined by densitometry (157). Specific gravity measurement were made on chilled carcasses (2°C) 24 hours after slaughter. Thoracic aortas were stained with Sudan IV to assess lipid infiltration (158).

STATISTICAL ANALYSES

All values are reported as mean ± standard error of mean (SEM). Treatment means were compared by t-test (126).

RESULTS

Final body weights were not affected by the exercise treatments (Table 2). Since the pigs were allowed to eat ad libitum, the exercised pigs increased their food consumption to compensate for the energy expenditure of running. Because the pigs in each treatment were penned together no statistics can be computed for feed efficiency; however, the exercised pigs consumed more food per unit weight gain than did the non-exercised pigs (Table 2). Exercise did not affect the percentage of lipid in the carcass or heart or cholesterol content of the heart. Heart weight was heavier in the exercised pigs but when expressed as weight per relative body weight there was no difference. Lipid infiltration in the thoracic aorta, as assessed by sudan staining, was minimal and similar for both treatments.

Table 2. Final body weights, feed consumption, and body and heart lipids in non-exercised and exercised pigs.

	Non-exercised	Exercised	p value
Final weight, kg	95 ± 2[1]	100 ± 3	NS[2]
Feed, lb/gain, lb	2.65	2.86	—
Carcass lipid,[3] %	23.6 ± 0.9	24.3 ± 0.7	NS
Carcass water,[3] %	55.9 ± 0.6	55.4 ± 0.5	NS
Heart weight, g	310 ± 10	350 ± 9	$p<0.05$
Heart weight g/kg body weight	0.33 ± 0.01	0.35 ± 0.01	NS
Heart lipid, %	5.0 ± 0.5	5.9 ± 0.01	NS
Heart cholesterol, mg/100 g	1.5 ± 0.2	1.6 ± 0.2	NS
Aortic sudanophilia[4]	<1	<1	

[1]Mean ± SEM. Eight pigs per treatment.

[2]Not significant.

[3]Carcass lipid and water estimated from specific gravity.

[4]Lipid infiltration was graded on a relative scale; 0 to 4. 0 = no infiltration and 4 = maximal infiltration.

In the first five weeks of the experiment, which corresponds to 3 weeks of training and 2 weeks of full exercise, all pigs were fed a low-fat, corn soy protein diet. Because this diet was low-fat and did not contain cholesterol, blood lipid levels were low. Exercise did not produce any changes in plasma cholesterol, triglyceride or other parameters (Table 3). Two weeks later blood was again drawn. During this two weeks, and for the remainder of the experiment, the pigs were fed a high-fat (tallow) diet with added cholesterol. At this time, after 4 weeks of running, plasma total and HDL cholesterol levels were higher in the exercised pigs. The exercised pigs, however were consuming approximately 8% more food and consequently more cholesterol than the non-exercise group. Cholesterol consumption was approximately 0.68 g cholesterol/day for the exercised group and 0.60 g cholesterol/day for the non-exercised group at this time. The higher plasma cholesterol values seen in the exercised pigs probably reflects this increased consumption of cholesterol. The ratio of unesterified to esterified cholesterol was slightly reduced ($p<0.1$) in the exercised pigs but exercise had no effect on triglycerides, percentage of total cholesterol in the HDL fraction or plasma LCAT activity.

Switching from a low-fat diet with no added cholesterol to a high-fat diet with cholesterol resulted in increases in most plasma lipid levels. Plasma total and HDL cholesterol and plasma triglycerides were higher in the high-fat diets. The percentage of total cholesterol carried in the HDL fraction was also increased.

Table 3. Diet and exercise effects on plasma cholesterol levels, triglyceride levels and LCAT activity in pigs.

	Non-exercised	Exercised	P value
	Corn-soy Diet (Week 5[1])		
Cholesterol, mg/dl			
total	95 ± 5[2a3]	99 ± 4[a]	NS[4]
unesterified	27 ± 3[a]	23 ± 2[a]	NS
HDL	23 ± 1[a]	26 ± 3[a]	NS
UC/CE[5]	0.29 ± 0.03[a]	0.23 ± 0.02[a]	NS
HDL cholesterol, %[6]	25 ± 1[a]	26 ± 3[a]	NS
Triglycerides, mg/dl	26 ± 5[a]	33 ± 5[a]	NS
Plasma LCAT activity			
fractional rate, % CE/hour	6.5 ± 0.5[a]	6.9 ± 0.5[a]	NS
molar rate, μM/L/hour	46.4 ± 7[a]	40.8 ± 6[a]	NS
	High-fat Diet (Week 7[8])		
Cholesterol, mg/dl			
total	126 ± 6[b]	146 ± 6[b]	$p<0.05$
unesterified	32 ± 2[a]	31 ± 1[b]	NS
HDL	41 ± 2[b]	50 ± 3[b]	$p<0.05$
UC/CE[5]	0.35 ± 0.02[a]	0.29 ± 0.02[a]	NS
HDL cholesterol, %[6]	33 ± 2[b]	34 ± 2[b]	NS
Triglycerides, mg/dl	45 ± 4[b]	45 ± 5[b]	NS
Plasma LCAT activity			
fractional rate, % CE/hour	3.6 ± 0.2[b]	3.5 ± 0.2[b]	NS
molar rate, μM/L/hour	38.3 ± 3[a]	30.3 ± 2[a]	NS

Table 3 (cont'd.)

[1]Three week training period and 2 week exercise period.

[2]Mean ± SEM. Eight pigs per treatment.

[3]Values with different superscripts in each diet period are different ($p<0.05$).

[4]Not significant.

[5]Unesterified cholesterol/esterified cholesterol.

[6]Percentage of total cholesterol in the HDL fraction.

[7]Percentage of tritiated cholesterol converted to esterified cholesterol/hour.

[8]Four weeks of exercise concluded. The high-fat diet was consumed for two weeks.

The high-fat diet caused a decrease in the fractional LCAT activity, but no change was observed in the molar LCAT rate.

By the 13th week of the experiment (10th week of exercise) the exercise protocol produced a significant decrease in plasma total cholesterol levels (Table 4). The exercise group had plasma total cholesterol levels which were 30 mg/dl less than the non-exercised group. Again it should be noted that the exercised pigs were consuming more food and more cholesterol than the non-exercised pigs. The exercised pigs consumed approximately 2.4 kg of feed and 1.3 g of cholesterol per day while the non-exercised consumed approximately 2.2 kg of feed and 1.2 g of cholesterol per day. The unesterified cholesterol levels followed total cholesterol levels and were also reduced. No difference in the absolute amount of plasma HDL cholesterol was found but, the percentage of total cholesterol carried in the HDL fraction was significantly greater in the exercised pigs than the non-exercised pigs.

Relative plasma lipoproteins, are reported in Table 4. Exercise caused an increase in percentage of HDL and a decrease in percentage of LDL compared to the non-exercised pigs. Thus the ratio of HDL to LDL was significantly increased (1.40 to 1.08) in the exercised pigs.

Plasma fractional LCAT activity in the exercised pigs after 10 weeks of exercise, as was the case after 2 and 4 weeks of exercise, was not different from values of non-exercised pigs. The non-exercised pigs, however, did have higher molar LCAT activity compared to the exercised pigs after 10 weeks of exercise.

Table 4. Effect of exercise on plasma cholesterol levels, lipoprotein profile and LCAT activity in pigs.

	Non-exercised	Exercise	p value
Cholesterol, mg/dl			
total	194 ± 11[1]	163 ± 7	p<0.05
unesterified	39 ± 1	31 ± 2	p<0.05
HDL	46 ± 4	49 ± 4	NS[2]
UC/CE[3]	0.26 ± 0.01	0.23 ± 0.01	NS
HDL cholesterol, %[4]	22.2 ± 2	29.5 ± 2	p<0.05
Relative lipoproteins, %[5]			
VLDL	12 ± 1	11 ± 1	NS
LDL	43 ± 2	38 ± 2	p<0.05
HDL	45 ± 2	52 ± 3	p<0.05
HDL/LDL	1.1 ± 0.08	1.4 ± 0.13	p<0.05
Plasma LCAT rate			
fractional %CE/hour[6]	3.37 ± 0.26	3.11 ± 0.27	NS
molar μM/L/hour	33.9 ± 3.1	25.1 ± 1.8	p<0.05

[1]Mean ± SEM. Eight pigs per treatment. Blood drawn after 10 weeks of exercise.

[2]Not significant.

[3]Unesterified cholesterol/cholesterol ester.

[4]Percentage of total cholesterol in the HDL fraction.

[5]Relative percentage of each lipoprotein.

[6]Percentage of tritiated cholesterol converted to cholesterol ester/hour.

DISCUSSION

While regular exercise in humans can contribute to weight reduction and increase lean body mass (95, 159), the pigs undergoing exercise in this study had similar final body weights and percentage of body fat and water as the non-exercised pigs. Both Fitts et al. (161) and Weiss et al. (160) have reported that moderate exercise did not alter body weight or body composition in pigs. Link et al. (155) also reproted no effect of exercise on body composition in pigs run on a treadmill for 10 minutes at 10 mph 5 days per week compared to non-exercised pigs. Similar to results obtained in this study, Link et al. (155) reported that the exercised pigs consumed more food per day than the non-exercised pigs. Holloszy (161) using rats and Barnard et al. (163) using guinea pigs, have reported that exercise in these animals decreased food consumption; resulting in lower body weights than in control animals. Both these investigators used mature animals and fairly intensive exercise programs (approximately 30 m/minute for up to one hour daily). Fitts et al. (161) has suggested that the energy requirement for growth in young animals, as for pigs in the present experiment, stimulates the appetite and overrides the appetite depression that occurs with exercise. Leiberman et al. (163) has shown that exercised young guinea pigs gained weight at the same rate as control (sedentary) guinea pigs, but that exercised mature guinea pigs did not gain as much weight as non-exercised guinea pigs. It seems probably that the animal's age as well as the

exercise duration and intensity contribute to differences in body weight and composition.

Plasma total cholesterol levels were 18% lower in the exercised pigs compared with the non-exercised pigs. Link et al. (155) reported that a moderate exercise program (1.75 miles run per day) for 22 months did not affect plasma cholesterol levels in miniature pigs. In exercising goats, Kenealy et al. (156) also reported no changes in plasma cholesterol levels compared to sedentary goats. Fukada et al. (103) reported that moderate exercise in rats (11 m/minute for one hour daily) did decrease plasma cholesterol levels. Simko and Kelley (108) also using rats, showed that exercise decreased red blood cell cholesterol but they did not report wheather total cholesterol levels were affected. In cross-sectional studies, plasma cholesterol levels are usually lower in trained men compared to matched controls (96, 98). Wood et al. (96) reported that male runners (at least 15 miles per week) had lower plasma cholesterol levels than sedentary matched controls. However, while Wood et al. (96) matched the groups for age and blood pressure, the sedentary group were almost 20% above their ideal weight, compared to the runners who were close to their ideal weights. This difference could contribute to the difference in plasma cholesterol levels between treatment groups. Clinical studies, in which humans have started an exercise program, have been inconclusive as to the response of plasma cholesterol levels to exercise. Some reports have indicated that exercise decreases plasma total cholesterol levels (152, 165) but the majority have failed to show a relationship between exercise

and plasma total cholesterol levels. The differences in response may relate to the exercise duration and intensity. In our study, as in the study in rats by Fukuda et al. (104) which also showed a decrease in cholesterol levels with exercise, the animals were exercised daily.

While the effects of exercise on plasma total cholesterol levels is questionable, particularily in humans, it does appear that exercise increases HDL cholesterol levels (94, 109, 152). Gilliam et al. (109) reported that a moderate exercise program in girls (40 minutes per day of running and skipping rope for six weeks) increased HDL cholesterol levels and increased the percentage of cholesterol in the HDL fraction from 21.8% in the non-exercised girls to 28.8% in the exercised girls. These changes occurred even though plasma total cholesterol levels were not affected by exercise. Lopez et al. (152) also reported an increase in HDL cholesterol in young males after an intensive seven week exercise program. These results are similar to the results obtained in the present experiment. Although the absolute level of HDL cholesterol was not greater in the exercised group of pigs compared to the non-exercised pigs (49 mg/dl versus 46 mg/dl, respectively), the percentage of cholesterol in the HDL fraction was significantly greater (29.5% versus 22.2%).

Exercise also increased the ratio of HDL to LDL in this study. Hill et al. (147) showed that electrophoretic plasma lipoprotein profiles obtained in pigs are highly correlated with the percentages of lipoproteins obtained by ultracentrifugation. Thus in the exercised pigs in this experiment, not only was the percentage of

cholesterol in the HDL fraction increased but the concentration of high density lipoproteins in the plasma were also increased. Similar increased in plasma HDL levels after exercise in humans have been previously reported (97, 166).

Plasma molar LCAT activity was reduced in the exercised pigs compared to the non-exercised pigs after 10 weeks of exercise. Lopez et al. (152) reported that 7 weeks of intensive exercise by young males increased molar LCAT activity over pre-exercise values. Possibly the difference in molar LCAT activities between our experiment and that of Lopez et al. (152) relates to the difference in exercise intensity. Simko et al. (108) also reported an increase in fractional LCAT activity in rats undergoing one hour of swimming daily. Plasma LCAT activity is negatively correlated with unesterified cholesterol levels (107, 130). Since plasma unesterified or total cholesterol levels were not reported by Simko et al. (108), it is not possible to determine if molar LCAT activity was changed by exercise in their experiment. In our experiment both total and unesterified cholesterol levels were decreased by exercise. Wallentin (107, 130) has reported that changes in molar LCAT activity are positively correlated with changes in plasma unesterified cholesterol, phospholipid or triglyceride levels in the plasma. Since endogenous substrates are used to assay LCAT activity, changes in activity could be due to changes in enzyme activity or changes in substrate availability (37, 107). Possibly the decrease in unesterified cholesterol levels that was observed in our experiment was

responsible for the decrease in molar LCAT activity. While statistically significant changes in molar LCAT activity have been reported, the physiological significance of these changes remains to be determined.

The mechanism by which exercise alters plasma cholesterol levels is still speculative. Fukuda et al. (103) reported that bile acid excretion was greater in exercised rats than in sedentary, pair-fed, control rats. Bobek et al. (106) has reported that exercise increases the turnover of cholesterol in exercised rats. These parameters were not investigated in our experiment.

The results obtained in this experiment indicate that an aerobic exercise program can beneficially modify plasma cholesterol parameters in pigs. After 10 weeks of exercise plasma cholesterol levels were reduced, HDL cholesterol percent was increased and levels of plasma HDL were increased compared to non-exercised pigs. These results show that changes occurring in the exercised pigs are similar to changes that occur in humans undergoing an exercise program. Utilization of the pig could provide an excellent model to investigate not only changes that occur with exercise but also the mechanism behind these changes.

PART V

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

These experiments were conducted to investigate factors which may influence body composition, plasma cholesterol levels, including total, unesterified and HDL cholesterol levels, and plasma LCAT activity. The dietary treatments investigated were: 1) comparison of plant protein vs animal protein, 2) saturated fat vs polyunsaturated fat (P:S, 0.3 vs 3), and 3) presence or absence of dietary fiber. The effects of an aerobic exercise program on these same parameters in pigs were also studied. Two animal species were utilized in these experiments; the pig, because it is physiologically similar to humans and the chicken, because its plasma cholesterol levels increase to a much higher level than does the pig when similar diets are fed. Although specific dietary ingredients were varied, in general energy composition of the experimental diets was: 42% from fat, 40% from carbohydrate and 18% from protein. Depending on the experiment the diets contained between 0.05% to 0.1% cholesterol. The only exception was when a corn-soy, grower diet (low-fat, low-cholesterol) was fed as a reference in one experiment. Thus, the diets were similar in energy composition to those typically consumed in the United States.

Body composition was investigated after two treatments: 1) after a moderate exercise program in pigs and 2) after feeding dietary fiber to pigs for 14 weeks and to chickens for 5 weeks.

Although exercise has been shown in some experiments to alter body composition (161, 162), in this experiment the moderately exercised pigs had similar carcass fat and water percentages as the non-exercised pigs. As both groups of pigs were allowed unlimited access to food, food consumption was approximately 8% greater in the exercised pigs compared to the non-exercised pigs. While rather intensive exercise has been shown to depress appetite and result in less weight gain in exercised animals compared to non-exercised animals (162, 163), in this experiment the rapid growth of the pigs plus the moderate exercise regimen may have stimulated appetite; at least to compensate for the energy expended in the exercise.

A very interesting finding was that feeding rolled oats decreased the percentages of body fat in both pigs and chickens. Wheat bran, however, was without affect on body composition. The mechanism by which rolled oats lowers body fat is not clear. Wheat bran has been shown to increase the excretion of fecal lipids, but whether rolled oats also increases fecal lipids has not been investigated. Also, whether an increase in fecal lipids could alter body composition to the extent observed in this experiment is questionable; especially since the final body weight and food consumption were similar between treatments. Seemingly, if significant amounts of energy were being lost in the feces the body weights of the animals fed rolled oats would be less than those fed the other diets. Certainly an area that warrants further investigation is the mechanism by which rolled oats alter body composition.

These experiments have also shown that dietary changes or an aerobic exercise program can alter plasma total cholesterol levels, even when high-fat, high-cholesterol diets are fed. In pigs, plasma total cholesterol levels were reduced, compared to appropriate controls, when: plant protein replaced animal protein, the P:S ratio was increased from 0.3 to 3.0 and when the pigs underwent an aerobic exercise program. In chickens feeding rolled oats, but not wheat bran, reduced plasma cholesterol levels. When polyunsaturated fats were fed in diets with plant proteins, although the pigs were consuming approximately 4.5 g of cholesterol per day, their plasma cholesterol levels were similar to pigs consuming low fat diets without cholesterol. Since plasma cholesterol levels are one the major risk factors in the development of atherosclerosis in humans, reduction in plasma cholesterol levels would be beneficial. These experiments show that, in animals, dietary modifications or a relatively moderate exercise program can reduce plasma total cholesterol levels.

Recently, epidemiological studies have identified plasma HDL cholesterol concentrations as being important in the development of atherosclerosis (13-15). It is important to report both the absolute HDL cholesterol levels and the percentage of total cholesterol in the HDL fraction, as HDL cholesterol levels tend to correlate with total cholesterol levels (48, 53). In these experiments the percentage of total cholesterol in the HDL fraction was most significantly increased when dietary fiber was fed to either pigs or chickens, or when the pigs were exercised. While the protein source fed or the fat source fed both influenced the absolute

levels of HDL cholesterol neither treatment altered the percentage of cholesterol in the HDL fraction.

Whether dietary fiber or exercise increase HDL cholesterol levels by similar mechanisms remains to be determined. Both the feeding of dietary fiber and exercise increase plasma cholesterol turnover (93, 106), and both treatments have been shown to increase the excretion of fecal steroids (93, 103). Since HDL levels have been reported to be a substrate for synthesis of hepatic bile acid, it has been hypothesized that HDL cholesterol levels increase in response to the increased hepatic synthesis of bile acids (105). At least in exercise a more conceivable hypothesis is that the increased HDL cholesterol levels arise due to an increased turnover of VLDL. As VLDL remnants are produced, HDL has been postulated to facilitate the conversion of VLDL remnants to LDL by accepting unesterified cholesterol from the VLDL remnant (31). Thus, through this mechanism HDL cholesterol levels could be increased by exercise. Whether a similar mechanism occurs when different sources of dietary fibers are fed is unknown.

No consistent effects of treatments on plasma LCAT activity were observed. Molar LCAT activity was decreased in two treatments in which plasma unesterified cholesterol levels were decreased (increasing the P:S ratio and exercise). Wallentin (107) has reported that unesterified cholesterol levels are correlated with molar LCAT activity. But two other treatments, substituting plant protein for animal protein and feeding dietary fiber, resulted in decreases in plasma unesterified cholesterol levels and increases

in molar LCAT activity. The LCAT reaction is very complicated, involving many substrates. Since changes in LCAT activity could be due to either changes in actual enzyme activity or changes in the availability of endogenous substrates, it is unclear as to the actual physiological significance of changes in LCAT activity.

In summary these experiments have demonstrated that changes in dietary ingredients and an aerobic exercise program can produce beneficial changes in plasma cholesterol parameters in species with similar cholesterol and lipoprotein metabolism as man.

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