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The Effects of Diet and Exercise on Lipoprotein Metabolism

by

Sheila A. Bannerman

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Master of Science
in
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Winnipeg, Manitoba

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THE EFFECTS OF DIET AND EXERCISE ON LIPOPROTEIN METABOLISM

BY

SHIELA A. BANNERMAN

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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ABSTRACT

Adult male rats were fed either a high fat or a basal diet ad libitum. Half of the animals from each dietary group were submitted to an exercise program consisting of 2 hours of swimming, on alternate days for either 4 (phase I) or 10 (phase II) weeks. Body weight and food intake data were measured weekly, and serum cholesterol levels as well as body composition were measured at the end of the treatment.

Fat fed animals weighed more than basal fed controls and ate significantly more food. Serum total cholesterol was greater in fat fed animals primarily due to higher levels of low density lipoprotein cholesterol (LDL-C). When compared to basal fed controls, fat fed animals had significantly more body fat.

Exercised animals weighed less than their sedentary counterparts, yet food intake was similar in the 2 groups. Total circulating cholesterol was lower in exercised animals due to reduced levels of LDL-C. Exercise alone induced an increase in the HDL-C/total-C ratio in both phases of the study. This difference was primarily due to the reduction of total serum cholesterol associated with exercise. Carcass analysis revealed significantly lower levels of body fat in exercised as compared to sedentary animals.

In general, phase II had a greater effect on the variables studied than did phase I, indicating that duration is an important consideration for diet and exercise studies. These results indicate that diet and exercise have independent effects on growth, serum cholesterol and body composition.

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Dedicated to the memory of my dad

Douglas J. Bannerman

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REVIEW OF LITERATURE

1.1 INTRODUCTION

Coronary heart disease (CHD) is characterized by the formation of atherosclerotic lesions in the arteries that supply blood to the heart (Gordon et al, 1977). Cross sectional and longitudinal studies in man, as well as experiments in animals, provide evidence for a relationship between serum lipid and lipoprotein levels and the incidence of CHD. It has been suggested that levels of individual lipoproteins or combinations of lipids and lipoprotein cholesterol may be better risk predictors than is the level of serum lipid alone (Gordon et al, 1977;Kannell et al, 1979a). Both diet and physical activity have been shown to alter lipoprotein levels, thus the relationship between diet, exercise and CHD has become the focus of recent research in the area.

Lipoproteins are classified according to their size and density (Taskier, 1971). There are four main types of lipoproteins, these are chylomicrons, the very-low-density lipoproteins (VLDL), the low-density lipoproteins (LDL), and the high density lipoproteins (HDL). Each lipoprotein varies in the amount of cholesterol transported through the plasma.

present knowledge suggests that LDL and HDL act antagonistically in relation to CHD. Elevated levels of low-density lipoprotein cholesterol (LDL-C) have been positively correlated to the development of atherosclerosis and thus have been implicated as a risk factor in the development of atherosclerosis (Castelli et al, 1977a;Miller and Miller, 1975). Elevated levels of high density lipoprotein cholesterol (HDL-C) have been negatively linked to atherosclerosis and therefore have become known as a "protective factor" in the fight against CHD (Miller, 1979;Shepherd et al, 1980). While the action of very low density lipoprotein cholesterol (VLDL-C) is unclear, it has been implicated as a positive risk factor particularly when associated with increased levels of LDL-C (Carlson and Botliger, 1972). Therefore, elevated levels of VLDL-C are suspected to contribute to the risk of developing CHD.

Several factors appear to influence the concentration of the various lipoprotein cholesterols. Decreased levels of HDL-C have been correlated with cigarette smoking (Criqui et al, 1980;Stamford et al, 1984a), obesity (Contaldo et al, 1980), as well as elevated triglyceride levels (Carlson and Botliger, 1972; Castelli et al, 1977b). All of these factors have been previously correlated with the risk of developing cardiovascular disease (Marx and Kolata, 1978). On the other hand, moderate amounts of alcohol have been shown to increase HDL-C (Castelli et al, 1977b; Stamford et al

1984b). Physical activity has been correlated with increased HDL-C levels, as well as decreased LDL-C levels, VLDL-C levels and total cholesterol levels (Morris et al, 1973;Paffenbarger and Hale, 1975; Kruisi et al, 1984). Thus exercise is seen as a potential negative risk factor in CHD. A causal relationship however remains to be established. Dietary intake causes various perturbations on plasma lipoprotein levels, and the interaction of diet and exercise as well as the independent effect of these two variables on lipid and lipoprotein metabolism provide the focus of this research.

1.2 CHOLESTEROL METABOLISM

Cholesterol is transported in the blood in association with three major groups of lipoproteins. Low density lipoproteins (LDL) carry the majority of cholesterol, 150 mg/dl or 65% of total plasma cholesterol. High density lipoproteins (HDL) and very low density lipoproteins (VLDL) transport similar amounts of cholesterol, 45 mg/dl and 40 mg/dl respectively. These values correspond to those of normal adult humans (Thompson and Bortz, 1978).

The structural, functional and metabolic interrelationships of LDL, VLDL and chylomicrons have been examined extensively, however until recently, little was known about HDL and its relationship to the other three classes of lipoproteins (Levy et al, 1980).

Chylomicrons are composed primarily of triglyceride (TG), originate in the intestine and are derived from exogenous fat. Chylomicrons disappear rapidly from the bloodstream with a half-life of about 10 minutes (Gurr and James, 1975). VLDL is the main carrier of exogenous TG and originate in the liver and small intestine. VLDL is thought to be catabolized in the same way as chylomicrons, although this point is not absolutely proven; the half life is 6-12 hours. VLDL is catabolized to IDL (an intermediate) which is further degraded to LDL (Levy et al, 1980).

In the LDL fraction the proportion of TG decreases, while the amount of protein increases. These components represent 10% and 20-25% of the LDL fraction respectively. LDL, like VLDL are made primarily in the liver, although the intestine is capable of some synthesis (Gurr and James, 1975).

VLDL and chylomicrons are a source of HDL components. It has been shown that lipolysis of chylomicrons or VLDL by the enzyme lipoprotein lipase (LPL) results in the formation of HDL particles (Levy et al, 1980). It is hypothesized that such a particle resembles HDL3 and that by continued acquisition of cholesterol it becomes HDL2 (Durrington, 1980). HDL3 particles are smaller and denser than the HDL2 particles, and contain more protein and less lipid relative to HDL2. The major apoproteins of HDL, AI and AII differ structurally and immunologically. Apoprotein AI has repeatedly been documented as an essential cofactor for lecithin

cholesterol acyl transferase (LCAT) activity. As well, it has remarkable detergent properties which may have significance in the uptake of cholesterol from cell membranes (Small et al, 1977). No specific function for AII has emerged, although it is thought to be related to the structure of HDL. HDL2 is richer in AI than AII and thus a greater plasma concentration of HDL2 is thought to be physiologically significant (Chang et al, 1979). Reductions in the plasma level of HDL2, especially if resulting in a decreased HDL2/HDL3 ratio, are considered to be unfavorable since it is thought that HDL2 is the subfraction with "antiatherogenic" properties (Gonen et al, 1981).

HDL particles are secreted by the liver, intestines, and possibly other tissues. It has become apparent that cholesterol esters play a vital role in HDL structure. The presence of cholesterol esters and their insertion into the HDL structure induces the characteristic spherical conformation of HDL. Without cholesterol esters, HDL particles are disc shaped. Transformation of this discoidal form involves the action of the enzyme lecithin cholesterol acyl transferase (LCAT), which catalyzes the conversion of cholesterol to cholesterol esters, and allows HDL to take on a spherical form making it metabolically active (Gurr and James, 1975).

HDL formation is increased during physiological conditions of increased catabolism of VLDL or chylomicrons, when associated with normal or increased levels of lipoprotein

lipase (LPL). Formation of HDL will be reduced when there is a decreased catabolism of VLDL and/or decreased levels of LPL (Witzum and Schonfeld, 1979). Thus, LPL plays an integral role in the metabolism of chylomicrons and VLDL has an important role in controlling the level of HDL in the blood.

Little is known about the metabolic fate of HDL. It has been postulated that HDL may play an important role in cholesterol efflux from the tissues, therefore reducing the amount stored there (Glomset, 1968). Another theory suggests that HDL, or at least some of its components may competitively interfere with the uptake of LDL by the tissue (Carew et al, 1976). Although these theories are by no means established, they are felt to provide plausible explanations for the possible protective role of HDL in atherogenesis (Levy and Rifkind, 1980).

1.3 DIET AND SERUM LIPOPROTEINS

Cardiovascular disease is the leading cause of death in North America. Major risk factors for the disease include obesity, elevated TG levels and elevated serum cholesterol levels (Marx and Kolata, 1978). Evidence of a relationship between diet and serum lipid and lipoprotein levels stems from numerous epidemiological reports as well as experimental studies in both animals and humans. Much of the research has shown that the ingestion of saturated fats leads to an elevation of plasma cholesterol whereas polyunsaturated fats produce the opposite effect (Shepherd et al, 1978).

The typical North American consumes approximately 42% of his energy as fat, with a polyunsaturated to saturated fatty acid ratio (P/S ratio) of 0.3-0.4:1, and a cholesterol content equal to 600-700 mg/day. Current recommendations suggest that dietary cholesterol be reduced to 300 mg/day, and the P/S ratio increased to 1-1.5:1 (Krause and Mahan, 1979).

1.3.1 Dietary Fat

Studies on the effects of dietary fat on cholesterol metabolism have involved the substitution of saturated fat with polyunsaturated fat, thus altering the P/S ratio (Kris-Etherton et al, 1984;Becker et al, 1983). Though controversy exists as to the effects of this type of dietary alteration on serum lipoprotein levels, the key factor appears to be the degree to which the P/S ratio is altered.

Schwandt, Janetschek and Weisweller (1982) examined the effect of a diet high in polyunsaturated fats and low in cholesterol on lipoprotein cholesterol metabolism. Thirty male normolipidemic subjects were fed either a moderately modified fat diet (P/S ratio 1.0, cholesterol content 250 mg/day) or an isocaloric control diet (P/S ratio 0.3, cholesterol content 250 mg/day) for 3 months each in a cross-over design. The polyunsaturated fat diet reduced LDL-C by 19% and 13%, respectively, in both groups. The cross-over design of this study demonstrates that similar concentrations of serum cholesterol and LDL-C were achieved in both

groups after 3 months of the test diet. This cholesterol lowering effect, as well as its complete reversal, had already occurred after 4 weeks on the diets. There was no change in HDL-C levels, thus a decrease was noted for the LDL-C/HDL-C ratio. Considering the atherogenic properties of the LDL-C fraction, this decrease was felt to be of significance (Schwandt et al, 1982). These results are in accordance with those of Schaefer et al (1981), who found a 16% decrease in LDL-C and no change in HDL-C in a group of 11 subjects fed a diet containing a P/S ratio of 2.0 and 250- 300 mg of cholesterol per day.

Shepherd and coworkers (1978) investigated the effects of a saturated fat diet (P/S ratio = 0.25, cholesterol intake 400 mg/day) versus a polyunsaturated fat (PUFA) diet (P/S ratio = 4.0, cholesterol intake 400 mg/day) in 4 healthy adult males. Each subject was studied twice for a period of 5 weeks, all subjects were subjected to both diets. When compared with the saturated fat diet, the PUFA diet decreased total cholesterol (24%), but also decreased HDL-C (33%). Analysis of the HDL subfraction by rate zonal ultracentrifugation indicated that the HDL2/HDL3 ratio fell by 28% on the PUFA diet.

Shepherds' study has been criticized as being extreme, with excessive amounts of polyunsaturated fatty acids, as well as being out of keeping with normal dietary recommendations (Durrington, 1980). Shepards' P/S ratio of 4.0 was

used to represent the PUFA diet, whereas a P/S of 1.0 and 1.5 were used in studies by Schwandt et al (1982) and Schafer et al (1981) respectively. Diets high in PUFA, such as these, are widely recommended for the general public as a health promotion effort. Therefore, there is an important need to define the quantitative and qualitative effects of these alterations in dietary fat on plasma HDL concentration and composition (Witzum and Schonfeld, 1979). Possibly, one can speculate that some PUFA is health promoting, while too much can be considered detrimental due to its negative effects on cholesterol metabolism.

1.3.2 Dietary Carbohydrate

It has been recommended that total fat intake be reduced to 30-35% of total calories and have a P/S ratio of 1-1.5:1 (Krause and Mahan, 1979). This decrease in total fat should be compensated for by an increased carbohydrate consumption (Committee on Nutrition, 1978). Since the average protein intake is 17%, the recommended carbohydrate content constitutes 50-55% of total calories. These dietary recommendations are based on the premise that an increased consumption of carbohydrate will not increase the risk of developing coronary heart disease (Coulston et al, 1983).

Several groups of researchers have demonstrated that carbohydrate rich diets result in decreased serum HDL-C levels, as well as increased serum TG concentration (Schonfeld

et al, 1976; Gonen et al, 1981; Coulston et al, 1983; Kashyap et al, 1982). High levels of serum TG as well as low levels of HDL-C are considered to be negative factors in relation to atherosclerosis (Hurter, 1975; Miller and Miller, 1975). These findings have led to questions concerning the wisdom of the current recommendations regarding dietary carbohydrate (Coulston et al, 1983).

Schonfeld et al (1976), reported on changes in fasting plasma lipids of young adults after they had been fed an 80% carbohydrate and 20% protein, formula diet for a period of 4-5 days. The high carbohydrate diet significantly altered the lipoprotein profile. Endogenous fat synthesis was stimulated resulting in increased serum levels of VLDL-C and VLDL-TG. As well, HDL became TG rich while HDL-C and LDL-C decreased.

Gonen and coworkers (1981) confirmed and extended the results of Schonfeld et al (1976). Eleven healthy normolipidemic subjects were fed a liquid formula diet containing 85% carbohydrate, 15% protein and supplemented with vitamins and minerals, for 7 days. Following a 12 hour fast, plasma lipoprotein profiles were determined. Serum levels of VLDL-C and VLDL-TG were increased as a result of the diet, while mean serum levels of LDL-C and HDL-C decreased significantly. There was a significant decrease seen in the HDL2/HDL3 ratio, due to a consistent reduction in the concentration of the HDL2 fraction. Reductions in the plasma level of HDL2

are considered to be unfavorable since it is thought that HDL2 is the subfraction with "antiatherogenic properties" (Gonen et al, 1981).

Coulston and coworkers (1983) criticized the results of both Schonfeld et al (1976) and Gonen et al (1981). They suggested that both studies may be misleading, since they reflect extreme situations (80% and 85% carbohydrate respectively), use formula diets not mixed diets, and have very short experimental periods. Coulston and coworkers (1983) recently incorporated 2 levels of dietary carbohydrate (40% and 60% of calories) into diets composed of conventional foods, in order to document the effects on various serum lipid and lipoprotein levels. Eleven normolipidemic volunteers were randomly assigned to either the "control" or "high carbohydrate" diets. The diets were consumed for 10 days and then dietary treatment was reversed so that all subjects were studied on both diets. The control diet was designed to approximate the average U.S. diet (Food consumption survey, 1980) and contained 40%, 19% and 41% of total calories as carbohydrate, protein and fat respectively. The high carbohydrate diet consisted of 60% carbohydrate (22-25% sucrose), 19% protein and 21% fat. Fasting blood samples indicated that TG concentration was significantly elevated on the 60% carbohydrate diet while HDL-C was significantly decreased. Plasma cholesterol concentration did not change significantly therefore there was a significant decrease in the HDL-C/Total-C ratio.

Kashyap et al (1982) reported similar findings. Nine healthy men received either a high (65% of calories) or low (15% of calories) carbohydrate diet for 3 weeks, in a cross-over design. An initial increase in serum TG was observed but considered transient, since a decline toward baseline levels was seen by the third week. This initial rise was felt to be the result of increased lipogenesis due to glucose oxidation. Previous research has shown that increased serum TG levels resulting from high carbohydrate diets are transitory in nature and will return to normal following 2-3 months on the diet (Antonis and Bersohn, 1961). The levels of LDL-C and HDL-C decreased due to diet, with the HDL2 fraction drastically reduced, resulting in a decreased HDL2/HDL3 ratio.

None of the research cited offer conclusions as to the mechanism(s) responsible for the decreases seen in HDL-C. Blum et al (1977) however, concluded that high carbohydrate diets resulted in a decreased synthesis of HDL. Gonen et al (1981) speculate that below a certain level of fat intake, production of those apoproteins associated with HDL is low resulting in reduced serum HDL levels. Further, Gonen (1981) postulates that low levels of LDL seen following high carbohydrate diets could somehow be connected with the diet induced impaired HDL production.

Thus, a lack of conclusive evidence regarding the metabolic implications of high carbohydrate diets confronts the

researcher. The type of carbohydrate ingested has been felt to be of some significance as was indicated in the Lipid Research Clinic Study (Ernst et al, 1980). There was some suggestion that sucrose may have a greater influence than starch on HDL-C levels, however this is largely speculation.

1.3.3 Dietary Cholesterol

The strong correlation between elevated plasma cholesterol levels and CHD has led to several metabolic studies relating plasma cholesterol levels in adult humans, to dietary cholesterol (Mattson et al, 1972;Quintao et al, 1971). More recent studies suggest that the cholesterol level of the various lipoproteins (HDL, LDL and VLDL) in serum play an important role in the development of CHD and are more meaningful indices of risk than is the level of total serum cholesterol alone (Gordon et al, 1977; Mahley et al, 1978). The prevalence and incidence of CHD are inversely correlated with HDL-C (Gordon et al, 1977) while elevated levels of LDL-C have been positively correlated to the development of CHD (Castelli et al, 1977; Miller and Miller, 1975). Thus, HDL and LDL act antagonistically in relation to CHD.

Mahley and Holcombe (1977) investigated the effects of cholesterol feeding in the rat. Osborne-Mendel rats weighing 200-300 g were fed a powdered diet containing 5% lard and 1% cholesterol, while control rats were fed a commercial pelleted lab chow consisting of 5% lard, 5% corn oil and no

added cholesterol (Ralston Purina). Rats were on the diets for 2-4 weeks prior to lipoprotein analysis. The cholesterol-lard fed rats developed a hypercholesterolemia that ranged from 300-600 mg/dl, compared to a plasma cholesterol of less than 80 mg/dl for control rats.

Alterations in the type and distribution of the plasma lipoproteins in cholesterol-lard fed rats were noted. Increases in the cholesterol content of VLDL and LDL were seen while a decrease was noted for HDL-C. The appearance of a qualitatively different lipoprotein fraction was determined by agarose electrophoresis, and has become known as cholesterol induced lipoprotein (HDLc). HDLc floats in the same density range as HDL, however it is smaller and richer in cholesterol than is the "typical HDL" (Mahley et al, 1978). The HDLc is metabolized in a manner similar to LDL, in that both bind to the same high affinity cell-surface receptors, whereas HDL do not (Goldstein and Brown, 1974).

A variable hypercholesterolemia, 300-600 mg/dl, is not limited to the rat species. Calvert and Scott (1974) reported a similar variability in the response of female pigs to cholesterol feeding. A diet containing 1% cholesterol induced moderate hypercholestremia in pigs following 8 weeks of feeding. The HDL-C concentration was generally higher in the cholesterol fed animals, however no clear-cut trend was present. LDL-C levels increased in the cholesterol fed animals and VLDL-C increased rapidly in one animal but not in

others. The authors speculated that irregular changes in serum lipoproteins may have been related to fluctuations in fat absorption due to periodic diarrhea. A panic reaction on the part of the pig prior to bleeding was also cited as a possible influence.

A more recent study by Clark, North and Harrold (1983) also investigated cholesterol feeding, again in the rat. When rats were fed a low fat diet (5% lard-corn oil mix, P/S ratio 0.3) and a low fat-high cholesterol diet (0.5%) there were no significant differences observed in HDL-C or total cholesterol level. This is a marked contrast to Mahley and Holcombe's observation of hypercholestremia on high cholesterol diets.

Since considerable variation exists in the response to cholesterol feeding in animal species of a homogeneous genetic background (Mahley and Holcombe, 1977; Calvert and Scott, 1974; Clark et al, 1983), it is not surprising to find similar variation in heterogeneous human studies. Tan and coworkers (1980) investigated the effects of a high cholesterol, high saturated fat diet (1028 mg cholesterol and P/S 0.14) on serum lipoproteins in 6 normolipidemic subjects. The subjects were initially placed on a low cholesterol polyunsaturated fat rich diet (100 mg and P/S 1.6) for 1 week and were then switched to the high cholesterol diet for 3 weeks. The high saturated fat diet resulted in a hypercholestremia which amounted to a 23% increase in total

cholesterol. Increases in VLDL-C and LDL-C accounted for approximately 75% of the total increase, the remainder due to increases in HDL-C.

Observational epidemiological studies have established that the higher the plasma total or LDL cholesterol level, the greater the risk that CHD will develop (Gordon et al, 1977). The Lipid Research Clinics Study (1984) showed that reducing total cholesterol by lowering LDL-C levels can diminish the incidence of CHD morbidity and mortality in men at high risk because of elevated LDL-C levels. A seven year study showed a 19% lower incidence of CHD among men who had been treated with cholestyramine (a cholesterol lowering drug) which reduced plasma total cholesterol and LDL-C by 8% and 12% respectively. Small increases in HDL-C were also seen, independently accounting for a 2% reduction in CHD risk.

Finally it is important to note that HDLc, the qualitatively different lipoprotein fraction noted by Mahley and Holcombe (1977) in rats, has also been shown to exist in humans. Mahley and coworkers (1978) investigated cholesterol feeding in eleven healthy adults. In study I, six subjects consumed 4-6 eggs/day for 4 weeks, while in study II, five subjects gradually increased their egg consumption over an 18 week period (1,2 and 3 eggs/day for the first, second and third 6-week period). Following study I, 3 of the 6 subjects showed increased serum cholesterol levels (20-25%).

In study II, again 3 subjects had significantly higher serum cholesterol levels (1-6%). Irrespective of whether total serum cholesterol changes during the course of the diet, the presence of a larger, more cholesterol rich fraction was consistently noted and was again identified as HDLc, giving further evidence to the existence of this qualitatively different HDL particle.

The precise role of HDLc is currently unknown, and it remains to be seen whether it is associated with an increased risk for the development of atherosclerosis (Durrington,1980).

1.4 EXERCISE AND SERUM LIPOPROTEINS

Epidemiological data suggest that the incidence of CHD is reduced among persons who are physically active (Morris et al, 1973; Paffenbarger and Hale, 1975). This reduction is associated with exercise induced alterations in fat metabolism. Elevated TG levels are often associated with CHD (Hurter et al, 1972) and these levels are lower in persons who engage in regular physical activity compared to sedentary individuals (Lehtonen and Viikari, 1978a,b; Enger et al, 1977; Haskell et al, 1980). As well, a classic paper by Miller and Miller (1975) suggested that low levels of plasma HDL-C are strongly associated with increased risk of CHD in man. It has also been observed that HDL-C levels in men performing regular exercise are higher than their more sed-

entary peers (Wood et al, 1976), and that HDL-C levels increase when men or women participate in a regular exercise program (Lopez et al, 1974).

1.4.1 Cross-Sectional Studies

Studies by Lehtonen and Viikari (1978 a,b) are but two of the many reports that provide evidence of a positive association between an active lifestyle and a high concentration of HDL-C. In their first study, plasma lipids from twenty-three regularly training men (mean age=44, average exercise 83 km running or skiing weekly) were compared to a sedentary control group of 15 healthy men (mean age=47) who did not participate in any regular exercise. Exercise increased the serum HDL-C and FFA concentration and decreased the TG levels significantly. The ratio of HDL-C/total-C increased solely due to the increase in HDL-C, total serum cholesterol remained unchanged. There was a positive correlation between the HDL-C concentration and the amount of weekly exercise.

In their second study, Lehtonen and Viikari (1978b), extended their results to occupational activity. Fasting blood was drawn from 12 lumberjacks (mean age=42) and the lipoprotein profile compared to a group of 15 electricians (mean age=46). All subjects were considered to have a normal diet and low to moderate alcohol consumption, based on the results of a questionnaire. The lumberjacks, whose oc-

cupational physical activity is considered vigorous, had significant greater levels of HDL-C and lower levels of TG than the more sedentary electricians. Again, there were no differences in levels of total cholesterol between the 2 groups, however the HDL-C/total-C ratio increased with exercise.

These studies and other cross sectional studies (Woods and Haskell, 1979) support the theory that physical activity increases the level of HDL-C in humans. Haskell and coworkers (1980) have criticized many of these studies, citing small sample size and lack of control within these studies for other factors associated with changes in HDL-C. They suggested that obesity, cigarette smoking and alcohol intake must be controlled because it is unlikely that they are distributed evenly among exercise and non-exercise subjects.

During a large scale study, known as the Lipid Research Clinics Program Prevalence Study, plasma lipids, lipoprotein determinations and treadmill exercise testing was performed on 2319 men and 2067 women (Haskell et al, 1980). All subjects were 20 years of age or older and were randomly selected from population surveys by nine clinics in Canada and the U.S.A.. The treadmill exercise test procedure was based on a modified Bruce protocol where subjects exercised until they reached 85-90% of their age predicted maximal heart rate. Dietary recalls were completed and the results were correlated with plasma lipoprotein determinations as well as performance on the treadmill test.

Neither treadmill exercise test duration nor heart rate response was significantly related to HDL-C levels for either men or women. However those participants who reported being physically active generally had higher HDL levels than those who reported no physical activity. When HDL-C was adjusted for age, body mass index, alcohol and cigarette use as well as inter clinic population variation, more active men and women had higher HDL-C levels than their sedentary counterparts. It was concluded that although HDL levels did not correlate with exercise tolerance, positive correlations were found between HDL and physical activity, independent of other factors which influence HDL (Haskell et al, 1980). Thus cross-sectional research supports the existance of a positive relationship between the level of HDL-C and the level of physical activity.

1.4.2 Longitudinal Studies

Cross-sectional studies which involve exercise and lipoprotein analysis have been criticized on the basis of a potential self- selection effect (Williams et al, 1982). It is suggested that subjects chosen are often athletes who have participated in their field for a number of years, and thus may have initially higher levels of HDL-C as well as lower TG concentrations. Longitudinal studies are more meaningful than cross sectional because the possibility of self selection is reduced and each participant acts as his

own control (Wood and Haskell, 1979). As well, longitudinal studies provide a better representation of the actual metabolic adaptations taking place.

The effects of moderate physical exercise on serum lipoproteins has been studied by Huttunen et al (1979). One hundred sedentary men (age 40-50 years) were randomly assigned to either an exercise or a control group. The control group was advised to maintain their previous exercise habits. The exercise group participated in a 4 month program that consisted of 3-4 weekly sessions involving walking, jogging, swimming, or cycling. HDL-C increased significantly in the exercise group following training while serum TG decreased. It is interesting to note that there was a decrease in serum cholesterol and LDL-C in both the exercise and control group which was attributed to seasonal trends typical for the population during spring months (Aromma et al, 1975). The experimental results shown here are in agreement with the thesis that moderate physical activity produces elevations in serum HDL-C concentration.

Nye and coworkers (1981) were unable to show a significant change in the HDL-C level of sedentary men taking part in a moderate intensity calisthenics program. However, the training period was somewhat shorter, and the subject population considerably smaller than that used by Huttunen et al (1979). Nye examined the change in serum lipoproteins in 17 men (age 30-45), who took part in a calisthenics program

twice weekly for 10 weeks. Although no change was seen in the total HDL-C concentration, a significant decrease in LDL-C was observed. It is interesting to note that although there was no change in total HDL-C, a significant change occurred in the concentration of the HDL subcomponents. HDL2 concentration increased while there was a decrease in the HDL3 component. This may be of significance, since it has been suggested that HDL2 is the lipoprotein fraction with "antiatherogenic" properties (Shepherd et al, 1980).

In an earlier study of still shorter duration, Lopez et al (1974) investigated the effects of a 7 week training program on serum lipoprotein levels in 13 young medical students. The exercise protocol consisted of four 30-minute sessions of intense physical exercise (jogging, bicycling and calisthenics) per week. The results showed a significant decrease in serum TG, VLDL-C and LDL-C levels, as well as a concomitant increase in HDL-C levels. In comparing the results of Nye et al (1981) to those of Lopez et al (1974) there are some obvious discrepancies. Lopez utilized a shorter training period than did Nye, yet increases in HDL-C levels were found in the former but not in the latter study. This discrepancy suggests the mechanism of increasing HDL-C with exercise is very complex.

Williams and coworkers (1982) showed that the amount of exercise (as reflected by number of miles run) as well as the duration of training are related to changes in serum li-

poproteins. Thus, Lopez et al (1974), whose protocol involved 4-30 minute exercise sessions per week may have induced a change in serum HDL-C levels as a result of a higher level of exercise intensity in comparison to Nye et al (1981) whose protocol involved 2-30 to 45 minute exercise sessions per week.

A recent study by Lipson et al (1980) confounds the issue. Five women and 5 men (age 19-22) participated in the study. For 6 weeks the subjects exercised on a treadmill for 30 minutes per day at an exercise intensity of 70% of V02 max (maximal aerobic power). The subjects diet was monitered throughout the experiment and total calorie content was adjusted daily to maintain constant body weight. Lipson reported a significant decrease in total plasma cholesterol, but no change in plasma TG, VLDL-C,LDL-C or HDL-C. The authors concluded that exercise conditioning did not elevate HDL-C levels, and that previous experiments showing elevations in HDL-C may well be due to alterations in body weight (Gordon et al, 1977). It should be noted that there was a small but significant weight loss during the experiment and that the intensity and duration of the exercise performed may have been insufficient to produce changes in the HDL-C level. Compounding these problems in experimental design was the small heterogeneous sample.

The relationship between body weight, exercise and HDL-C is by no means completely understood. Evidence from both

cross-sectional (Lehtonen and Viikari, 1978a,b; Haskell et al, 1980), and longitudinal (Huttunen et al, 1979; Nye et al, 1981; Lopez et al, 1974) studies indicate that endurance activities increase the plasma concentration of HDL-C. Moreover, population studies indicate that lean individuals have higher HDL-C levels than those who are more obese (Huttunen et al, 1979; Kannel et al, 1979b). Since weight loss is associated with exercise training, it is important to determine to what extent the HDL-C concentration of physically active people is attributable to their relative leanness.

Williams and associates (1983) examined the interaction between weight loss and exercise induced increases in plasma HDL-C. Eighty-one healthy but sedentary males age 30-55 were assigned to either a moderate running program or as sedentary controls in this one year study. Weight loss was strongly associated with increases in HDL-C in the exercise group, however weight changes in the absence of an exercise program produced no change in HDL-C levels. It appears that the metabolic consequence of the weight loss in the two groups was different.

This observation is supported by Huttunen and coworkers (1979) who, as previously cited, investigated the effects of an aerobic exercise program on a group of previously sedentary men. The success of the program was corroborated by the increase in vO2 max in the training group, but not the control group. An increase in HDL-C was noted for the exer-

cise group with no change seen in the sedentary group. A small but significant weight loss was evident in both groups upon completion of the program. To further investigate the relationship between changes in body weight and serum lipids, a separate calculation was made for the lipid levels of those subjects in the exercise group who maintained their body weight within 1 kg during the program. A highly significant increase in HDL-C despite the constant body weight was noted and in fact the absolute increase in HDL-C was slightly higher in this group than for the exercise group as a whole. On the other hand, the concentration of HDL did not change significantly in those sedentary subjects who lost more than 1 kg during the trial. These results clearly demonstrate that weight reduction is not the cause of the increase in HDL-C induced by physical activity.

1.5 DIET AND EXERCISE

Most training studies have considered but not controlled for the independent influence of diet on serum lipoproteins. Rats are a good model for training and diet studies concerned with lipoprotein metabolism since they show alterations in serum lipids and tissue lipases as a result of both treatments (Narayan et al, 1975; Wisenberg et al, 1975). A specific change in HDL-C as a result of training has never been shown in experiments with rats, thus it is not known if training induces a change in HDL-C similar to that seen in humans (Kimball et al, 1983).

A recent study by Kimball and coworkers (1983) investigated the independent effects of aerobic training and diet on various lipoprotein cholesterol levels and on tissue lipase activity. Forty-two male rats were assigned to either an exercise or control group and further split into a high carbohydrate or high fat treatment. Carbohydrate feeding significantly lowered all lipoprotein cholesterol concentrations as well as altering tissue enzyme levels.

Treadmill training for 1 hour/day for 10 weeks did not significantly affect lipoprotein cholesterol concentration. Training resulted in reduced body weight gains. Problems in experimental designs and/or omissions in reporting data such as:

1. small sample size
2. basal diet contains only 1% corn oil while the American Institute of Nutrition (AIN) (1977) recommends 5% corn oil
3. extreme P/S ratio of 0.02 in the high fat diet exacerbates the concern over PUFA sufficiency
4. no data on food intake or body composition,

act to limit the value of this report.

A similar study by Deshaies et al (1983) does not support the results of Kimball et al (1983). Sixty female rats were assigned to either an exercise (2 hours of forced swimming) or sedentary control group and further split into a high

carbohydrate or a high fat treatment. The animals on the high fat diet were further subdivided into either ad libitum fed or pair fed on an energy basis with the chow fed animals.

The high fat diet raised total cholesterol levels in the sedentary ad libitum fed group, but not in the sedentary pair fed controls. The HDL-C was higher in the exercise group than in sedentary controls, however it was only significantly greater in the chow fed group. This lack of statistical significance may well be due to the small sample size (10 animals per treatment group) and/or the relatively short duration of the experiment (4 weeks). It appears that the high fat diet significantly increased total cholesterol and decreased the HDL-C/total-C ratio while exercise had an overall effect of increasing serum HDL-C thus increasing the HDL-C/total cholesterol ratio.

Sedentary animals on the high fat diet weighed significantly more than the chow fed controls, however exercise prevented weight gain. Both sedentary and exercise animals fed the chow diet had similar body weights. Examination of food intake data showed that rats fed the high fat diet ate slightly less than twice as many calories as did the chow fed control rats. Deshaies did not speculate on the reasons for such dramatic differences in the feed/gain ratio between dietary or exercise treatments.

Close examination of the limited diet composition data provided by Deshaies is most alarming. When the fat content of the diet was increased from 2.5% to 40% an increase in caloric content from 3500 kcal/kg to 5400 kcal/kg (calculated values), no specific modifications were made to the diet to ensure nutritional adequecy. The National Academy of Sciences suggest that the nutrient requirements for rats can be met by diets of different caloric value as long as a constant nutrient-to-calorie ratio is maintained (1978). Based on these recommendations, the Deshaies study appears to be deficient in vitamins, minerals and protein, for the high fat diet. These dietary deficiencies therefore limit the value of this report.

Deshaies, Vallerand and Bukowiecki (1983) report on serum lipids and lipoprotein cholesterol distribution of exercise trained female rats fed sucrose. The animals were fed ad libitum either laboratory chow alone, or chow and a 32% aqueous sucrose solution. Half of each dietary group was submitted to an exercise program consisting of a daily three-hour long swimming bout, 5 days a week for 5-7 weeks.

The sucrose-fed animals had higher total cholesterol levels than chow fed animals, yet this increase was partially prevented by exercise. Cholesterol in the lipoproteins of lower densities was increased significantly with sucrose feeding and exercise preventing these increases altogether. HDL was not affected by exercise in chow fed animals, how-

ever sucrose feeding increased HDL-C in sedentary animals and exercise prevented this increase.

As with the studies by Kimball et al (1983) and Deshaies et al (1983), the report by Deshaies, Valerand and Bukowiecki (1983) does not appear to follow the guidelines for diet formulation as recommended by the National Academy of Sciences (1978). A constant nutrient-to-calorie ratio must be maintained in diets of different caloric value in order to meet the nutrient requirements of the rat. Since it appears that the nutrient-to-calorie ratio was not maintained when changing from the chow diet to chow plus 32% aqueous sucrose diet, the value of this report seems questionable.

In summary, epidemological studies have suggested that physical activity is associated with elevated levels of serum HDL cholesterol and inversely related to the development of CHD. Studies in humans have not established a causal relationship. The conflicting results in clinical studies are difficult to explain, but may be due to confounding factors such as changes in diet, changes in body weight, seasonal variations in serum lipoprotein concentrations, and compliance with the exercise protocol. To overcome problems inherent in human studies, several researchers have developed exercise protocols for rats. However, there have been limited reorts in rats on the effects of both exercise training and diet on lipoprotein cholesterol levels. Problems in experimental design and/or ommissions in reporting data act to limit the value of these reports.

The proposed experiment is designed to investigate the independent effects of aerobic training and diet on various lipoprotein cholesterol levels and body composition in the male rat.

Chapter II

MATERIALS AND METHODS

2.1 ANIMALS

One hundred and fifty weanling male Sprague Dawley rats were purchased from the University of Manitoba central breeding facility. The animals were received in two groups of 75 corresponding to phase I and phase II of the experiment. All animals were handled daily to avoid viciousness, and were weighed twice weekly until they reached a mean weight of 330 g . From each group of 75 rats, animals of extreme weights were removed, as well as any animals that appeared unhealthy. From the remaining group, 60 animals were randomly assigned to the treatment groups.

2.2 EXPERIMENTAL DESIGN

The experimental protocol consisted of 2 phases, Phase I (4 week duration) and Phase II (10 week duration), with Phase I preceeding Phase II. Animals were randomly assigned to either a basal or a high fat diet, then further subdivided into exercise and sedentary groups. Each of the 4 groups thus formed contained 15 animals (Table 1).

TABLE 1

Experimental Design

	Phase I(4 weeks)	Phase II(10 weeks)
Sedentary Control Group		
Basal Diet	15 animals	15 animals
Fat Diet	15	15
Exercise Group		
Basal Diet	15	15
Fat Diet	15	15
	60 animals	60 animals

n=120 animals

2.3 HOUSING AND MANAGEMENT OF ANIMALS

All animals were housed in individual cages (Zoology Animal Holding Facility, University of Manitoba). The room temperature was maintained at 21°C with a 14-10 hour light-dark cycle. Throughout the experiment, food and water were given ad libitum.

2.4 DIET FORMULATION

Diets were formulated according to the National Research Council's guidelines set forth in the Nutrient Requirements of Laboratory Animals (1978). Modifications were made for the high fat diet to ensure a constant nutrient-to-calorie ratio (Farnworth, 1983) as can be seen in Table 2 . Two fat sources were chosen for diet formulation. Corn oil was included as a source of essential fatty acids. Lard was chosen as a source of saturated fatty acids and because of its

solid consistency at room temperature which allowed formulation of a paste type of a diet which was easily handled by the animals. The level of corn oil in the basal diet was maintained at 5%, in accordance with the recommendations of the American Institute of Nutrition (1977),to ensure that the diet be sufficient in essential fatty acids. The P/S ratio of the diets were 5.3 and .77 for carbohydrate and fat diets respectively. The specific diet composition is listed in Table 2 . See Appendix A for a detailed list of suppliers. Diets were prepared in 20 Kg. lots and stored at -26°C.

TABLE 2

Diet Composition (%)

	Basal Carbohyrate	High Fat
Corn Starch	30%	18%
Glucose	35%	20%
Casein	20%	25%
DL-Methionine	0.3%	0.4%
Corn oil	5%	7.5%
Lard	0%	17.5%
Fiber	5%	5%
AIN Mineral Mix	3.5%	5%
AIN Vitamin Mix	1.0%	1.5%
Choline bitartrate	0.2%	0.3%
Caloric density	3.8 kcal/g	4.8 kcal/g
Joule density	15.9 Joules/g	20.0 Joules/g
P/S ratio	5.3	.77

2.5 EXERCISE REGIME

The exercise program consisted of 2 hours of forced swimming, on alternate days, for a total of either 4 or 10 weeks. Animals were given a 12 day familiarization period which began with 15 minutes of swimming and was gradually increased until all animals were swimming for 2 hours. Animals swam in 6 cylindrical tanks (58 cm high, 137 cm diameter), 5 animals per tank. The water depth was 31 cm and water temperature was maintained within a range of 33±2°C.

2.6 FOOD CONSUMPTION/WEIGHT GAIN

During the pre-experimental period, all animals were fed a commercial laboratory diet (Ralston Purina Co, St Louis, Missouri). Upon switching to the experimental diets, a 3 day familiarization period was given to allow animals to become accustomed to the new diets. Changes in body weight were recorded during this period, but food consumption data was not kept. During the experimental period food cups were filled every other day and the food weight was recorded and tallied at the end of each week. Due to the fat content of the high fat diet, food which remained in the cups was discarded with every second feeding (4 days). Body weight data were recorded on a weekly basis.

2.7 TERMINATION OF EXPERIMENTAL ANIMALS

Exercised rats were terminated within 24 hours of their last exercise session. All animals were fasted overnight, approximately 12 hours, prior to termination. Each animal was weighed and then anesthetized with an intraperitoneal injection of sodium pentobarbitol (0.60 mg/kg body weight).

Approximately 5 mls of blood was obtained by cardiac puncture, placed in vacutainer tubes and allowed to coagulate. Animals were then killed by cervical dislocation and gastrointestinal (GI) tracts removed and weighed. Carcasses were frozen at -15°C until analyzed.

2.8 SEPARATION OF LIPOPROTEINS

Coagulated blood was centrifuged for 10-15 minutes, serum separated with a Pasteur pipette, transferred to a 9 ml. screw-top vial and refrigerated.

Lipoproteins were fractionated by ultracentrifugation (Beckman L5-50B ultracentrifuge), using rotor type 40.3 at 18°C and 34,000 rpm for 18 hours (Appendix B).

2.9 CHOLESTEROL DETERMINATION

A cholesterol colorimetric assay kit was used for cholesterol determination (Fisher Diagnostics, Nepean, Ontario), based on the method of Allain et al (1974) (Appendix C).

2.10 DETERMINATION OF BODY COMPOSITION

Carcasses were frozen at -15°C until analyses. Frozen carcasses were thawed in groups of 4, weighed and autoclaved for 6 hours and then reweighed to account for any moisture loss.

Whole carcasses were mixed with an equal weight of water and then homogenized in a Waring commercial blender. Immediately following homogenization, a 200 g sample was removed and frozen at -15°C. Frozen samples were freeze dried, then reweighed and ground in a Waring commercial blender. Moisture content was determined by calculating the moisture loss of the freeze dried sample and correcting for the weight of the animal before it was autoclaved.

2.10.1 Protein Determination

Determination of protein was done in duplicate, according to the Kjeldahl method, boric acid modification (Appendix D).

2.10.2 Fat Extraction

Two gram samples of the freeze dried carcasses were fat extracted in duplicate for 6 hours in a soxhlet extractor, using hexane as a solvent.

2.10.3 Ash Determination

Percent ash was calculated according to the following formula: 100-(%protein + %fat + %moisture).

2.11 STATISTICAL ANALYSIS

Data was analyzed using the Statistical Analysis System (SAS), 1982 version. A two way analysis of variance (ANOVA) was used to determine the independent effects of diet and training. Training effect refers to trained versus untrained (sedentary), and diet effect compares the high fat diet to the basal diet. A three way analysis of variance was performed to determine differences between phase I and phase II. Multivariate analysis of variance was used to determine differences for observations made every week (body weight, food intake, feed efficiency). If significant differences were found, univariate analysis was performed to determine specific differences.

Chapter III

RESULTS

3.1 ENERGY INTAKE/FEED EFFICIENCY

Cumulative food intake for Phase I indicates the presence of an interaction between diet and exercise (Table 3). During Phase I, sedentary animals consumed more diet than exercised animals, this difference was more pronounced for fat fed as compared to basal fed animals.

In Phase II, (Table 4) diet had a significant effect on cumulative food intake, with fat fed animals eating 13.6% (4439J) more than basal fed animals. Although sedentary animals tended to eat more than exercised animals, the differences were not significant. No interactions between diet and exercise were detected for Phase II

Cumulative feed efficiency data (Total weight gain (g)/Total food intake (J)) suggest independent training and diet effects for Phase I, with training having a greater effect on feed efficiency, than did diet (Table 3). Fat fed animals were 9.2% more efficient than basal fed animals. Sedentary animals were 43.9% more efficient than exercised animals.

Phase II showed similar trends, with respect to feed efficiency (Table 4). Fat fed animals were 16.2% more efficient than basal fed, and sedentary animals were 31.3% more efficient than were exercised animals.

Comparing Phase I and II, a significant interaction was noted between activity and Phase, which suggested that the effect of activity on feed efficiency differed in the 2 phases (Table 5). This apparent interaction is not surprising when one considers that sedentary animals in Phase I were 41.6% more efficient than exercised animals, while in Phase II the difference was 31.6% greater for sedentary animals. It appears from this data that the effect of activity on feed efficiency becomes less evident over time (see appendix E).

TABLE 3

THE EFFECT OF DIET AND EXERCISE ON BODY WT. (g), FOOD INTAKE (J) AND CUMULATIVE FEED EFFICIENCY (TOTAL WEIGHT GAIN (g)/TOTAL FOOD INTAKE (J)) (PHASE I)

	n	Body Weight Initial	Body Weight Final	Cumulative Food Intake (J)	Cumulative Feed Efficiency
Sedentary					
Basal	14	331.60 ± 6.88	449.29 ± 9.2	9890.44 ± 215	0.0122 ± 0.0005
Fat	15	339.27 ± 5.3	496.27 ± 7.4	11890.59 ± 231	0.0131 ± 0.0005
Exercise					
Basal	14	334.47 ± 6.1	399.93 ± 9.3	9596.30 ± 277	0.0066 ± 0.0004
Fat	15	354.53 ± 6.9	433.93 ± 8.3	10420.51 ± 215	0.0076 ± 0.0003
ANOVA (p)					
Training Effect		NS	0.0001	0.0004*	0.0001
Diet Effect		0.03	0.0001	0.0001*	0.01

mean ± SEM

* a significant interaction was noted for activity * diet ($p<0.01$)

TABLE 4

THE EFFECT OF DIET AND EXERCISE ON BODY WT. (g), FOOD INTAKE (J) AND CUMULATIVE FEED EFFICIENCY (TOTAL WEIGHT GAIN (g)/TOTAL FOOD INTAKE (J)) (PHASE II)

	n	Body Weight Initial	Body Weight Final	Cumulative Food Intake (J)	Cumulative Feed Efficiency
Sedentary					
Basal	10	328.86 ± 2.8	561.43 ± 10.2	27494.26 ± 553	0.0084 ± 0.0002
Fat	14	351.40 ± 2.7	672.67 ± 12.7	32542.81 ± 658	0.0098 ± 0.0002
Exercise					
Basal	13	321.13 ± 4.9	472.77 ± 10.4	27305.04 ± 579	0.0056 ± 0.0003
Fat	14	332.93 ± 4.1	550.36 ± 14.9	31134.69 ± 887	0.0069 ± 0.0003
ANOVA (p)					
Training Effect		0.0009	0.0001	NS	0.0001
Diet Effect		0.0001	0.0001	0.0001	0.0001

TABLE 5

CUMULATIVE FEED EFFICIENCY* (Phase I vs Phase II)

Interaction	*Total weight gain (g)/Total energy intake (J)
Diet * Phase	NS
Activity * Phase	0.0001
Activity * Diet	NS
Activity * Diet * Phase	NS

3.2 LIPOPROTEIN CHOLESTEROL

Both diet and exercise had independent effects on serum cholesterol levels. In Phase I, the level of total circulating cholesterol was less in basal fed than in fat fed animals, largely due to differences in LDL-C (Table 6). Neither HDL-C nor VLDL-C were influenced by diet in Phase I. Differences in the HDL-C/Total-C ratio further reflect differences in LDL-C, since fat fed animals had lower HDL-C/Total-C levels than basal fed animals.

Exercised animals had less total cholesterol than their sedentary counterparts, again due to differences in LDL-C. However, a training effect was noted for both HDL-C and VLDL-C, with HDL-C 8.4% (3.7 mg/dl) lower in exercised animals, and VLDL-C 39.2% (4.3 mg/dl) lower in exercised as compared to sedentary animals. The HDL-C/Total-C ratio was greater in exercised animals primarily due to lower levels of total cholesterol due to differences in LDL-C. The lipoprotein profiles of Phase II animals were similar to those observed in Phase I (Table 7). Basal fed animals exhibited lower levels of total cholesterol than did fat fed animals (30.7 mg/dl). However, in phase II, a significant diet effect was seen for both HDL-C and VLDL-C, yet not for LDL-C as was seen in Phase I. Fat fed animals had more HDL-C and VLDL-C than did basal fed animals. The HDL-C/Total-C ratio was not influenced by diet.

As was seen in Phase I, exercised animals were shown to have less total cholesterol than sedentary animals. Although a significant training effect was seen for all three subfractions, major differences were due to LDL-C. HDL-C was greater in exercised animals in phase II, while the LDL-C and VLDL-C were less in exercised as compared to sedentary animals. The HDL-C/Total-C ratio was greater for exercised animals than for sedentary, which agrees with the trend seen in Phase I. Changes in the HDL-C/Total-C ratio in Phase II are the result of lower levels of LDL-C and VLDL-C as well a greater HDL-C levels in exercised animals.

Comparing Phase I and II, significant interactions were detected between diet and phase, and between activity and phase with respect to total cholesterol. It is evident that both diet and training had a greater effect on total cholesterol in Phase II, than in Phase I . Considering diet alone, basal fed animals had 20.1% (30.6 mg/dl) less total cholesterol than fat fed animals in Phase II, while in Phase I, basal fed animals had only 10.0% (9.0 mg/dl) less total cholesterol than fat fed animals.

Training alone led to lower levels of total cholesterol in exercised animals which amounted to a difference of 30.9% (51.9 mg/dl) in Phase II, and 25.2% (24.5 mg/dl) in Phase I. HDL-C was influenced by both diet and exercise in Phase II, whereas only an exercise effect was seen in Phase I. Diet alone led to higher levels of HDL-C in fat fed animals in

Phase II, however no diet effect was found in Phase I. Since the effect of diet on HDL-C differs between the two phases, it is not surprising that we observed a significant interaction between diet and phase (Table 8).

Similarly, an interaction was found between activity and Phase for HDL-C. Exercise alone affected HDL-C, and elevated levels were seen for the exercise group as compared to sedentary animals in Phase II. In Phase I, exercised animals exhibited significantly lower levels of HDL-C than their sedentary counterparts. Again, the effect of exercise on HDL-C was not the same in the two phases, thus a significant interaction was detected.

For LDL-C, an interaction was noted between diet and activity when comparing the two phases. Thus, independent diet and exercise effects are no longer evident.

With respect to VLDL-C, fat fed animals had higher levels than basal fed in Phase II, while in Phase I there was no diet effect. Thus a significant interaction between diet and phase. In both phases, a significant training effect was seen for VLDL-C. However, in Phase I exercised animals had 39.2% (4.3 mg/dl) less VLDL than sedentary animals, while in Phase II, the exercise effect was considerably greater, with exercised animals having 75.2% (17.0 mg/dl) less VLDL-C than sedentary animals, which explains the interaction detected for activity and phase.

For HDL-C/Total-C, a significant interaction between activity and phase indicates that the effect of activity on HDL-C/Total-C differed in the 2 phases. This is justified by comparing the percentage change in HDL-C/Total-C in the 2 phases. In Phase II, exercised animals had a 44.8% (0.29) greater HDL-C/Total-C ratio than sedentary animals, while in Phase I the trend was similar however, differences due to exercise amounted to only 18.7% (0.10).

TABLE 6

THE EFFECT OF DIET AND EXERCISE ON SERUM TOTAL CHOLESTEROL, LIPOPROTEIN CHOLESTEROLS (MG/DL)*, AND HDL/TOTAL CHOLESTEROL RATIO* (PHASE I)

	n	Total Cholesterol	HDL	LDL	VLDL	HDL/Total
Sedentary						
Basal	14	89.49 ± 1.8	42.80 ± 1.0	36.42 ± 1.1	10.24 ± 0.92	0.480 ± 0.01
Fat	14	104.22 ± 2.2	44.76 ± 0.88	47.99 ± 2.0	11.47 ± 1.5	0.431 ± 0.01
Exercise						
Basal	14	70.86 ± 2.3	40.64 ± 1.1	24.23 ± 1.9	6.00 ± 0.8	0.579 ± 0.02
Fat	15	73.97 ± 3.6	39.60 ± 1.6	27.16 ± 2.1	7.20 ± 1.2	0.542 ± 0.01
ANOVA (p)						
Training Effect		0.0001	0.003	0.0001	0.0004	0.0001
Diet Effect		0.0019	NS	0.0005	NS	0.0006

*mean ± SEM

TABLE 7

THE EFFECT OF DIET AND EXERCISE ON SERUM TOTAL CHOLESTEROL, LIPOPROTEIN CHOLESTEROLS (MG/DL)*, AND HDL/TOTAL CHOLESTEROL RATIO* (PHASE II)

	n	Total Cholesterol	HDL	LDL	VLDL	HDL/Total
Sedentary						
Basal	10	144.95 ± 8.0	50.60 ± 4.7	67.72 ± 5.4	26.64 ± 2.1	0.346 ± 0.17
Fat	14	180.22 ± 10.3	65.60 ± 3.5	83.79 ± 7.2	30.83 ± 2.9	0.371 ± 0.19
Exercise						
Basal	13	99.12 ± 3.8	64.64 ± 3.8	33.91 ± 5.8	2.28 ± 0.5	0.662 ± 0.04
Fat	14	125.34 ± 5.8	79.32 ± 5.7	34.67 ± 4.2	11.99 ± 3.4	0.635 ± 0.04
ANOVA (p)						
Training Effect		0.0001	0.004	0.0001	0.0001	0.0001
Diet Effect		0.0002	0.0008	NS	0.002	NS

* mean ± SEM

TABLE 8

SERUM TOTAL CHOLESTEROL, LIPOPROTEIN CHOLESTEROLS (mg/dl) and HDL/TOTAL CHOLESTEROL

(Phase I vs. Phase II)

Interaction	Total Cholesterol	HDL	LDL	VLDL	HDL/Total
Diet * Phase	0.005	0.0007	NS	0.009	NS
Activity * Phase	0.0006	0.0002	0.0001	0.0002	0.0001
Activity * Diet	NS	NS	0.03	NS	NS
Activity * Diet * Phase	NS	NS	NS	NS	NS

3.3 BODY COMPOSITION

In Phase I, basal fed animals had lower body weights than fat fed animals (Table 3). Differences in carcass composition were primarily due to differences in the percentage of body fat within the various groups. Basal fed animals had 5.1% less lipid than fat fed animals. Diet did have a significant effect on carcass protein, although the difference was small, with fat fed animals having 1.5% less protein than basal fed. Moisture and ash were also affected by diet, with fat fed animals having 2.6% less moisture and 0.53% less ash.

Exercised animals weighed less than sedentary animals in Phase I, with differences in carcass composition primarily the result of differences in body fat in the various groups. Exercised animals had 4.5% less lipid than sedentary animals. As with diet, exercise had a slight but significant effect on carcass protein, with sedentary animals having 1.4% less protein than exercised animals. As well, carcass moisture was less for sedentary animals than for exercised animals (3.5%). No differences were noted for ash.

In Phase II, as in Phase I basal fed animals weighed significantly less than fat fed animals (Table 4), and differences in carcass composition were again due primarily to differences in body fat (Table 10). Basal fed animals had 9.7% less carcass fat than did fat fed animals. Fat fed an-

imals had 2.7% less protein than basal fed animals, and moisture was found to be 6.5% less in fat fed animals than basal fed. No differences in ash due to diet were detected.

Exercise had a significant effect on all components of carcass composition, the greatest change being seen in the percentage of body fat. As in Phase I, exercised animals had less fat than sedentary animals, differences which amounted to 8.2%. Exercised animals also had 2.1% more protein, 5.3% more moisture and 0.78% more ash than sedentary animals following similar dietary regimes.

In comparing the 2 phases, the effect of diet on carcass protein differed (Table 11). In Phase I, fat fed animals had 1.5% less carcass protein, while in Phase II, a difference of 2.7% was seen, with fat fed animals still having less than basal fed.

The effect of diet on carcass fat was greater in Phase II than in Phase I, as indicated by differences of 5.1% and 9.7% for Phase I and II respectively, with basal fed having less fat than fat fed animals in both cases.

For exercise alone, a comparison of the 2 phases indicates that only for carcass lipid are the values for the 2 phases significantly different. In Phase I, exercised animals had 4.5% less lipid than sedentary animals, while in Phase II differences amounted to 8.2% with exercise still lower than sedentary.

TABLE 9

THE EFFECT OF DIET AND EXERCISE ON BODY COMPOSITION* (PHASE I)

	n	Protein	Lipid	Moisture	Ash
Sedentary					
Basal	14	20.16 ± 0.26	15.52 ± 1.05	58.48 ± 0.94	5.00 ± 0.09
Fat	14	18.41 ± 0.44	22.39 ± 0.98	55.08 ± 0.92	4.20 ± 0.15
Exercise					
Basal	14	21.30 ± 0.16	12.72 ± 0.70	61.17 ± 0.53	4.79 ± 0.22
Fat	15	20.06 ± 0.24	16.13 ± 0.98	59.44 ± 0.80	4.52 ± 0.21
ANOVA (p)					
Training Effect		0.0001	0.0001	0.0001	NS
Diet Effect		0.0001	0.0001	0.0003	0.004

*mean percent ± SEM

TABLE 10

THE EFFECT OF DIET AND EXERCISE ON BODY COMPOSITION* (PHASE II)

	n	Protein	Lipid	Moisture	Ash
Sedentary					
Basal	14	19.58 ± 0.41	21.50 ± 0.96	55.15 ± 0.64	3.77 ± 0.20
Fat	15	16.53 ± 0.34	32.81 ± 1.22	47.72 ± 0.91	2.98 ± 0.24
Exercise					
Basal	13	21.36 ± 0.35	14.87 ± 1.26	59.49 ± 0.92	4.27 ± 0.28
Fat	14	18.96 ± 0.39	23.05 ± 1.25	53.95 ± 0.85	4.04 ± 0.28
ANOVA (p)					
Training Effect		0.0001	0.0001	0.0001	0.003
Diet Effect		0.0001	0.0001	0.0001	NS

*mean percent ± SEM

TABLE 11

PERCENT BODY COMPOSITION (Phase I vs. Phase II)

	Protein	Lipid	Moisture	Ash
Diet * Phase	0.01	0.008	0.003	NS
Activity * Phase	NS	0.01	NS	0.02
Activity * Diet	NS	0.01	NS	0.03
Activity * Diet * Phase	NS	NS	NS	NS

Chapter IV

DISCUSSION

Diet composition affects nutrient intake. In both phases of this study, fat fed animals consumed more total food energy than did basal fed controls. Diets rich in fat are more palatable to rats (Deshaies et al, 1983; Rolls et al, 1980) and these palatable, high-energy, high-fat diets have been shown to produce obesity (Sclafani and Springer, 1976). The maxim, that the rat consumes food to satisfy its need for calories (Farnworth, 1983), is an oversimplification of the complex relationship between dietary energy and food intake. Rats are able to adjust food intake to compensate for the inclusion of non-nutritive bulk in their diet resulting in calorie dilution (Sibbald et al, 1956,1957). However, the regulatory response to high energy diets does not appear to be precise (Hoffman-Goetz and MacDonald, 1983).

Fat fed animals gained significantly more weight than did their basal fed controls. The palatable, high energy diet was formulated to maintain a constant nutrient-to-calorie ratio (National Academy of Sciences, 1978) and should be described as a nutrient dense diet, not simply a caloric dense diet. Therefore the increased feed efficiency observed in the high fat fed groups was a reflection of the difference

in total nutrient consumption between the two diet treatment groups.

The literature on the effects of high fat diets on food intake and growth in rats is confusing due to a number of poorly controlled experiments recently published. Reports that high fat diets do not affect food intake (Medeiros et al, 1984), growth (Kimball et al, 1983) and may decrease feed efficiency (Bazzare, 1984) may well be incorrect because of basic problems in diet formulation. For example, the physical consistency of Medeiros' high fat diet, semi liquid at room temperature, would make normal eating impossible. Kimball's diet is clearly essential fatty acid deficient. However, the best example of inadequete diet formulation is found in a report by Bazzarre. When the dietary fat content was increased from 4.5% to 60% of the diet, no attempt was made to maintain the nutrient-to-calorie ratio for protein, vitamins or minerals.

It has been demonstrated that rats on ad libitum feeding regimes consume significantly more energy as a high-fat nutrient dense diet than as a commercial chow diet (Pitts and Bull, 1977; Richard et al, 1982; Deshaies et al, 1983). The enhanced nutrient intake stimulated growth, body fat deposition and results in an increased feed efficiency.

In the current study, exercise training appeared to reduce food intake, however this effect may well be associated

with the onset of the exercise regime and was only significant in phase I. This trend was consistent with previous findings, where an acute treadmill exercise program was shown to reduce voluntary food intake (Nance et al, 1977; Applegate et al, 1982).

Exercised animals weighed significantly less than sedentary animals in both phases of the study. This is likely due to differences in energy expenditure in the two groups, and may also reflect the trend of reduced food intake in exercised animals.

The measurement of feed efficiency estimates the effect of dietary treatment and exercise on energy utilization, and its impact on growth as measured by weight gain per unit nutrient consumed. In the current study, fat fed animals were more efficient than basal fed animals, while exercised animals were shown to be considerably less efficient than their sedentary controls. Phase II animals were less efficient than phase I animals for each of the 4 treatment groups. This phase difference was not surprising when one considers the age of the animals. Growth curves of male rats show a maximum growth rate up to approximately 60 days of age, at which time the growth rate begins to subside (National Academy of Sciences, 1978). Thus, less energy would be directed toward growth and more energy expended in body maintenance over time, in all treatment groups. Exercised animals have a significantly greater energy expenditure than

do the sedentary controls, therefore, they would be less efficient. In comparing the two phases, we observed that exercise had significantly less of an effect on feed efficiency by the end of phase II. It is probable that exercised animals became more efficient swimmers over time, thus expending less energy during exercise.

Fat fed animals exhibited significantly higher levels of total serum cholesterol than did basal fed animals for both phases of the study. Moderate increases in serum cholesterol have been found in humans (Schwandt et al, 1982; Schonfeld, 1981; Shephard et al, 1978), rats (Deshaies et al, 1983; Glueck et al, 1979) and pigs (Calvert and Scott, 1974) following a fat rich diet. The effect of diet was considerably reduced in exercised animals as compared to their sedentary controls. It has been well documented that exercise reduces serum cholesterol in humans (Lipson et al, 1980; Lopez et al, 1974; Huttunen et al, 1979) however the literature for animal studies is not clear.

The type and duration of exercise has been shown to be an important consideration in determining if the exercise regime will have any effect on serum cholesterol (Woods et al, 1979). Deshaies et al (1983) found no significant change in total serum cholesterol following 4 weeks of swimming in female rats. Similarly, Kimball et al (1983) found no change in the serum cholesterol of male rats who were treadmill exercised 1 hour/day for 10 weeks. The current study showed a

decrease in serum cholesterol of male rats after both 4 and 10 weeks of swimming.

Another consideration is the sex of the animal. Applegate et al, (1982) observed reduced total serum cholesterol in male but not female rats following treadmill training indicating a sexually dimorphic effect.

Finally, a natural variation in the hypercholestremic response has been shown to exist in rats, further complicating the interpretation of results. Mahley and Holcombe (1977) found that cholesterol feeding induced a variable hypercholestremia ranging from 300 - 600 mg/dl. The current study confirms this variation in observations, thus a change (or lack of) in total serum cholesterol could in fact be masked by the variability of the results collected.

Comparing total serum cholesterol levels in phase I and phase II, there is a significant increase associated with the older phase II rats. This increase is believed to be a natural age-related change. Dupont et al (1980) reported a substantial progressive increase in the total serum cholesterol in rats of 3,6,12 and 18 months of age. Kritchevsky et al (1980) as well as Carlile and Lacko, (1981) indicated increased cholesterol levels in older animals.

It is evident that serum cholesterol was influenced by both diet and exercise. While all serum cholesterol fractions were affected, the most striking alterations occurred

in the LDL-C fraction. Fat feeding appeared to increase LDL-C but this was only of statistical significance in phase I. Koh et al (1982) noted increased LDL-C in rats fed saturated fat rich diets. Epidemological studies have established that the higher the plasma total or LDL cholesterol level, the greater the risk that CHD will develop (Gordon and Castelli, 1977). Thus, our results are in keeping with the thesis that diets high in saturated fat increase the risk of CHD by increasing LDL-C. Exercised animals were shown to have less LDL-C than their sedentary counterparts. Several researchers have shown that LDL-C levels are lower in individuals who are trained as compared to sedentary individuals (Wood et al, 1977; Lopez et al, 1974).

The serum profile of VLDL-C was similar to that of LDL-C. That is, VLDL-C was greater in animals who had been fat fed compared controls and was less in exercised animals than their sedentary counterparts. The metabolism of VLDL-C is related to that of LDL-C since VLDL are degraded to LDL in the bloodstream (Gurr and James, 1975). Thus it not surprising that both VLDL-C and LDL-C have been co-implicated as positive risk factors in the pathogenesis of CHD (Carlson and Botlinger, 1972). The recent LRC study (1984) showed that reducing serum cholesterol by lowering LDL-C can diminish the incidence of CHD mortality and morbidity in men at high risk because of elevated LDL-C levels. This provides strong evidence for a causal role for these lipids in the development of CHD.

Diet had no influence on HDL-C in phase I, however in phase II fat fed animals had more HDL-C than control animals. Diets rich in fat have been shown to increase total cholesterol, HDL-C and LDL-C in humans (Lukaski et al, 1984). Exercised animals were shown to have less HDL-C than their sedentary counterparts in phase I of the study. Although this change reached statistical significance, it was relatively small and may not be physiologically significant. More important was the fact that both total cholesterol and LDL-C decreased substantially in exercised animals in both phases. The LRC study (1984) determined that a 19% lower incidence of CHD in cholestyramine-treated men was accompanied by mean decreases of 8% and 12% in plasma total cholesterol and LDL-C, respectively. Small increases in HDL-C independently accounted for a 2% reduction in CHD risk. Thus major reductions in CHD incidence come from reductions in total cholesterol and LDL-C rather than increases in HDL-C.

In phase II, exercised animals had more HDL-C than their sedentary counterparts, a change that was not observed in phase I. Williams et al (1982) suggested that a "dose-response" relationship existed between exercise and the change in the plasma concentration of HDL-C in humans. They observed that HDL-C levels did not begin to change until a threshold exercise level was maintained for at least 9 months. One may speculate that 4 weeks is not long enough to see an increase in HDL-C, however after 10 weeks the increase in HDL-C becomes more evident.

As in phase I, phase II exercised animals had substanitally less total cholesterol and LDL-C than their sedentary controls. Thus, our findings support the theory that exercise training has a beneficial effect on the risk factors associated with CHD, namely lowering serum cholesterol and LDL-C.

The ratio of HDL-C /Total-C may well be a more important indicator of risk than is the level of HDL-C alone, as was shown in the LRC study (1984). A decrease in HDL-C /Total-C as caused by increased total serum cholesterol and a concomitant decrease in HDL-C have been positively related to atherogenesis (Zilversmit, 1973). In phase I of the current study, fat feeding significantly reduced HDL-C /Total-C by increasing levels of total circulating cholesterol. Similar results have been reported by Zilversmit (1973) and by Deshaies et al (1983).

No significant diet effect was seen in phase II, the HDL-C / Total-C ratio increased in sedentary fat fed animals and decreased in exercised fat fed animals. This antagonistic change appears responsible for masking any significant change. The increase in HDL-C /Total-C seen in fat fed sedentary animals is due to increases in both HDL-C and total cholesterol. The increase in HDL-C cannot been seen as beneficial and rather reflects the large increase in total circulating cholesterol. Diets rich in fat have previously been shown to increase both total cholesterol and HDL-C in humans (Lukaski, 1984).

Exercise alone induced an increase in the HDL-C/ Total-C ratio in both phases of the study. This was primarily due to the reduction of total cholesterol observed in both phases of the experiment. However, the HDL-C /Total-C ratio was significantly greater in phase II, (10 weeks) than in phase I, (4 weeks), for exercised animals indicating a time response. This reflects the contribution of increased HDL-C to the HDL-C /Total-C ratio in phase II, and further exemplifies the "dose-response" relationship between duration of exercise and change in HDL-C seen by Williams et al (1982).

Cross sectional studies have shown that physically active persons have higher levels of HDL-C than their sedentary counterparts (Lehtonen and Viikari, 1978a,b; Woods and Haskell, 1979). Several researchers have shown that exercise training leads to increases in HDL-C, decreases in total cholesterol and/or increases in the HDL-C /Total-C ratios in humans (Lopez et al, 1974; Huttunen et al, 1979) as well as rats (Deshaies et al, 1983). Our results showed that exercise increased HDL-C after 10 weeks yet not after 4 weeks. As well, the HDL-C /Total-C ratio was shown to increase in all exercised animals partially due to increases in HDL-C as well as decreases in total cholesterol. Considering the negative relationship which exists between HDL-C and incidence of CHD (Miller and Miller, 1975), these results are significant.

The mechanism by which exercise increases HDL-C is not completely understood. One suggested metabolic adapation is an increase in the activity of the enzyme lipoprotein lipase (LPL) following exercise training. Nikkila et al (1978), showed that long distance runners have greater LPL activity in muscle and adipose tissue than do sedentary men. Studies in rats have determined that exercise training increases the activity of LPL in skeletal muscle (Borensztan et al, 1975) as well as in cardiac muscle (Fukuda et al, 1979). The lipolysis of chylomicrons or VLDL by LPL results in the formation of HDL (Levy et al, 1980). Increased levels of LPL leads to increased formation of HDL, thus LPL plays an integral role in controlling the level of HDL in the blood (Witzum and Schonfeld, 1979). Considering the relationship between LPL activity and HDL formation, it is reasonable to speculate that exercise training may increase HDL by increasing LPL activity.

The plasma enzyme lecithin:cholesterol acyl transferase (LCAT) is important in the formation of functional HDL particles (Gurr and James, 1975). The activity of LCAT and the level of its activating protein, apo- AI (in HDL), have been shown to be greater in physically active persons than in sedentary persons (Woods and Haskell, 1979). The effects of training on this enzyme may contribute to the increase in HDL-C and HDL-C /Total-C ratio seen following exercise training.

Diet and exercise were shown to have independent influences on body composition. The major change observed was in the percent carcass lipid of animals in the various treatment groups. Fat fed animals had more body fat than basal fed controls. This effect was more pronounced in phase II, which was of 10 week duration. Previous researchers have observed diet induced changes in body composition. Farnworth and Kramer (1983) noted increases in the percent body lipid of rats fed a saturated fat diet as compared to animals fed an unsaturated fat diet. Although the energy content of the diets was similar, the nutritive value differed due to differences in constituent fatty acids. Thus, animal growth and body composition can be influenced by altering only the fatty acid content of the diet. Bazzare (1984) reported that male rats consuming a diet containing 60% fat for 6 weeks had more body fat than did control animals.

The increase in adiposity seen in fat fed animals reflects increased fat deposition in adipose cells, but may also reflect an increase in cell size and/or number. Plucinski et al (1984) noted a higher percentage of body fat in mice fed a palatable high energy "cafeteria" diet. This increased adiposity was shown to reflect an increase in the relative size and number of adipose cells. Another study by Medeiros et al (1984) reported that fat rich diets caused significant increases in adipose cell size as well as lipid contained within the cells of rats.

Exercised animals had less body fat than their sedentary controls. As with diet, changes in body fat due to exercise were more pronounced in phase II, which may indicate that the 10 week study was of greater significance in terms of improving the body composition profile. Several researchers have demonstrated the positive effects of exercise in reducing body fatness. Appleton et al (1984) found that male runners had less body fat than controls following 30 days of treadmill exercise. Pitts et al (1984) reported lower body fat levels in exercised rats, however the extent of this response was dependent on other factors such as the nature of the diet employed, the type and duration of the exercise regime as well as the sex of the animals.

Other animal studies have demonstrated that exercise training reduces body fatness by decreasing adipose cell size and lipid content through increased lipolytic activity of cells (Bukowiecki et al, 1980; Askew et al, 1975). It is theorized that the enhancement of adipocyte lipolytic capacity is an adaptive phenomenon characterizing a physiological situation where energy expenditure exceeds energy gain, such as exercise (Bukiowecki et al, 1980).

Diet and exercise were shown to have an independent effect on percent carcass protein, for both phases of the study. These changes are not felt to be of importance, and rather reflect a decrease in percent lipid. When protein is expressed as grams tissue, the difference between groups is

very small. Applegate et al (1982) found similar increases in the percentage of protein in treadmill trained rats but concluded that these changes were a reflection of decreased body fatness, since there were no differences when protein was expressed as grams of tissue.

Oscai et al (1973) found that 6 hours/day of forced swimming for 21 weeks induced increases in the lean body mass of female rats. Perhaps a more severe exercise protocol would have induced more substantial and thus more meaningful increases in lean body mass. However, the reduced adiposity seen in exercised animals is felt to be important considering that adiposity correlates directly with plasma TG levels and plasma TG levels correlate negatively with HDL-C levels (Wood et al, 1976). Thus reduced adiposity as induced by exercise as well as a basal diet in this study, may be seen as beneficial considering the possible relationship between HDL-C and decreased body fatness.

4.1 SUMMARY AND CONCLUSION

Diet and exercise have independent effects on growth, serum cholesterol and body composition. Animals fed a fat rich diet weighed more than basal fed animals and ate significantly more food. Serum total cholesterol was greater in fat fed animals primarily due to higher levels of LDL-C. When compared to basal fed controls, fat fed animals had significantly more body fat.

Exercised animals weighed less than their sedentary controls even though food intake was similar in the 2 groups. Total circulating cholesterol was lower in exercised animals due to reduced levels of LDL-C. Exercise alone induced an increase in HDL-C/total-C ratio in both phases of the study. This difference was primarily due to the reduction of total serum cholesterol associated with exercise. An increase in the HDL-C/total-C ratio as caused by decreased total serum cholesterol and a concomitant increase in HDL-C have been negatively related to atherogenesis (Zilversmit, 1973). Carcass analysis revealed significantly lower levels of body fat in exercised as compared to sedentary animals.

Generally speaking, phase II had a greater effect on the variables studied than did phase I, indicating that time is an important consideration for diet and exercise studies.

Our results confirm and extend the results of previous studies which implicate high saturated fat diets (Schwandt,

1982; Schafer et al, 1981) as well as low levels of physical activity (Morris et al, 1973; Paffenbarger and Hale, 1975) as risk factors for CHD. The major contributing factor is elevated levels of serum cholesterol, primarily due to increased LDL-C. Elevated levels of HDL-C correlate negatively with CHD and therefore are seen as a "protective factor" against CHD (Shepherd et al, 1980). Although little change was seen in HDL in the current study, previous research has shown that changes may occur in the HDL subfractions (HDL2 and HDL3) which may preclude a change in total HDL (Nye et al, 1981). Increases in the plasma level of HDL2, are considered to be favorable since it is thought that HDL2 is the subfraction with "antiatherogenic properties" (Gonen et al, 1981). More precise data on HDL2 and HDL3 may provide important information on CHD prevention. Further investigations are needed to elucidate the metabolic changes taking place.

Diet and exercise had independent influences on the variables studied, however results with rats must be cautiously interpreted. Although these results cannot be extrapolated to the human population, they provide important groundwork for future research.

REFERENCES

Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. 1974. Enzymatic Determination of total serum cholesterol. Clin Chem 20:470.

American Association of Cereal Chemists . 1962. Crude Protein-Kjeldahl method, boric acid modification. Method 46-12.

Antonis A, Bersohn I. 1961. The influence of diet on serum triglycerides in South African White and Bantu prisoners. Lancet i:3.

American Institute of Nutrition. 1977. Report of the AIN Ad Hoc Committee on standards for nutritional studies. J Nutr. 107:1340.

Applegate EA, Upton DE, Stern JS. 1982. Food intake, body composition and blood lipids following treadmill exercise in male and female rats. Physiol Behav. 28:917.

Aromma A, Bjorksten F, Eriksson AW, Maatela J, Kirjarinta M, Fellman J, Tammenin M. 1975. Serum cholesterol and triglyeride concentration of Finns and Finnish Laps. Acta Med Scand 198:13.

Askew EW, Barakat H, Huhl GL, Dohm GL. 1975. Response of lipogenesis and fatty acid synthetase to physical training and exhaustive exercise in rats. Lipids. 10:491.

Bazzarre TL. 1984. The effects of diet and exercise on feed intake, body weight, body fat and growth hormone in male weanling rats. Nutr Rep Int. 29:997.

Becker N, Ellingworth DR, Alaupovic PA, Connor WE, Sandberg EE. 1983. Effects of saturated, monounsaturated, and w-6 polyunsaturated fatty acids on plasma lipids, lipoproteins and apolipoproteins in humans. Am J Clin Nutr. 37:355.

Blum CB, Levy RI, Eisenberg S, Hall M, Goebel RH, Berman M. 1977. High density lipoprotein metabolism in man. J Clin Invest. 60:795.

Bukiowiecki J, Lupien J, Follea N, Paradis A, Richard D, LeBlanc J. 1980. Mechanism of enhanced lipolysis in adipose tissue of exercise trained rats. Am J Physiol. 239 (Endocrinol Metab. 2):E422.

Calvert GD, Scott PJ. 1974. Serum lipoproteins in pigs on high cholesterol, high triglyceride diets. Atherosclerosis 19:4851.

Carew T, Koschinsky T, Hayes S, Steinberg D. 1976. A mechanism by which HDL may slow the atherogenic process. Lancet. i:1315.

Carlson LA, Botlinger LE. 1972. Ischemic heart disease in relation to fasting values of plasma triglycerides and cholesterol (Stockholm prospective study). Lancet i: 865.

Castelli WP, Doyle JT, Gordon T. 1977. Alcohol and blood lipids. Lancet. ii:153.

Castelli WP. 1977. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulation 55:767.

Cheung MC, Albers JJ. 1977. The measurement of apoprotein AI and AII levels in men and women by immunoassay. J Clin Invest. 60:43.

Clark JM, North BB, Harold RL. 1983. The effects of dietary cholesterol and exercise on blood lipid levels in rats. Nutr Rep Int. 28:51.

Committee on Nutrition, American Heart Association. 1978. Circulation. 58:762A.

Contaldo F, Strazzullo P, Postiglone A, Riccardi G, Patti L, DiBiase G, Mancini M. 1980. Plasma high density lipoprotein in severe obesity after stable weight loss. Atherosclerosis. 37:163.

Coulston AM, Liu GC, Reaven GM. 1983. Plasma glucose, insulin and lipid responses to high carbohydrate, low fat diets in normal humans. Metabolism. 32:52.

Criqui MH, Wallace RB, Heiss G, Mishkel M, Schonfeld C, Jones G. 1980. Cigarette smoking and plasma high density lipoprotein cholesterol. (The Lipids Research Clinics Program Prevalence Study). Circulation. 62(suppl iv): 70.

Deshaies Y, LeBlanc J, Richard D. 1983. Influence of a palatable, high fat diet and exercise training on the high density lipoprotein to total cholesterol ratio in the rat. Metabolism. 32:62.

Deshaies Y, Vallerand AL, Bukiowiecki LJ. 1983. Serum lipids and cholesterol distribution in lipoproteins of exercise trained female rats fed sucrose. Life Sciences. 33:75.

Dupont J, Mathias M, Spindler AA, Janson P. 1980. Dietary fat saturation and cholesterol turnover in aging rats. Age. 3:19.

Durrington P. 1980. HDL-C: Methods and clinical significance. CRC (Critical Reviews in Clinical Laboratory Science). 18:31.

Enger SC, Herbjornsen K, Erikssen J, Fretland A. 1977. High density lipoproteins and physical activity: the influence of physical activity, age and smoking on HDL-cholesterol and the HDL/total cholesterol ratio. Scand J Clin Lab Invest. 37:251.

Ernst N, Fisher M, Smith W, Gordon T, Rifkind B, Little J, Mishkel M, Williams O. 1980. The association of plasma high density lipoprotein cholesterol with dietary intake and alcohol consumption. The Lipids Research Clinics Program Prevalence Study. Circulation. 62(Suppl iv):41.

Farnworth ER. 1983. The composition of diets used in rapeseed oil feeding trials. In: High and low Erucic acid rapeseed oils. Kramer J, Sauer F. and Pidgen W. (ED). Academic Press.

Farnworth ER, Kramer JK. 1983. The effect of dietary fatty acids on growth and carcass composition in the rat. Nutr Rep Int. 27:799.

Fukuda N. 1979. IV. Effects of exercise on plasma and liver lipids of rats. Nutr Metab. 23:256.

Glomset J. 1968. The plasma lecithin:cholesterol acyl transferase reaction. J Lipid Res. 9:155.

Glueck CJ. 1979. Dietary fat and atheroslcerosis. Am J Clin Nutr 32:2703.

Goldstein JL, Brown MS. 1974. Binding and degradation of low density lipoprotein by cultural human fibroblasts. J Biol Chem. 249: 5153.

Gonen B, Patsch W, Kuisak I, Schonfeld G. 1981. The effects of short term feeding of a high carbohydrate diet on HDL subclasses in normal subjects. Metabolism. 30:1125.

Gordon T, Castelli W, Hjortlund M. 1977. High density lipoproteins as a protective factor against coronary heart disease. Am J Med 62:707.

Gotto A. 1983. HDL: Biochemical and metabolic factors. Am J Cardiol. 52:3A.

Gurr MI, James AT. 1975. Lipid Biochemistry. Chapman and Hall Co. London.

Haskell W, Taylor H, Wood P, Schrott H, Heiss G. 1980. Strenuous physical activity, treadmill exercise test performance and plasma high density lipoprotein cholesterol. The Lipid Research Clinics Program Prevalence Study. Circulation 62(suppl iv): 53.

Hoffman-Goetz L, MacDonald MA. 1983. Effect of treadmill exercise on food intake and body weight in lean and obese rats. Physiol Behav. 31:343.

Hurter R, Peyman M, Swale J, Barnett C. 1972. Some immediate and long term effects of exercise on the plasma lipids. Lancet i:671.

Huttunen J, Lansimies E, Voutilainen E, Ehnholm C, Hietanen E, Pentilla I, Siitonen O, Rauramaa R. 1979. Effect of moderate physical exercise on serum lipoproteins. A controlled clinical trail with special reference to serum high density lipoproteins. Circulation. 60:1220.

Kannell WB, Castelli WP, Gordon T. 1979a. Cholesterol in the production of atherosclerotic disease. Ann Intern Med. 90:35.

Kannel WB, Gordon T, Castelli WP. 1979b. Obesity, lipids and glucose intolerance. - The Framingham study. Am J Clin Nutr. 32:1238.

Kashyap ML, Barnhart RL, Srivastava LS, Perisuette G, Vink P, Allen C, Hogg E, Brady D, Glueck C, Jackson RL. 1982. Effects of dietary carbohydrate and fat on plasma lipoproteins and apolipoproteins C-II and C-III in healthy men. J Lipid Res. 23:877.

Kimball TJ, Childs MT, Applebaum-Bowden D, Sembrowich W. 1983. The effect of training and diet on lipoprotein cholesterol, tissue lipoprotein lipase and hepatic triglyceride lipase in rats. Metabolism. 32:497.

Koh ET, Chi MS, Chung K. 1982. Effects of dietary fats on lipid metabolism of nutritionally stressed rats. Metabolism. 26:457.

Krause MV, Mahan LK. 1979. Food, Nutrition and Diet Therapy. WB Saunders Co. Philadelphia.

Kris-Etherton PM, Ho CY, Fosmere MA. 1984. The effect of dietary fat saturation on plasma and hepatic lipoproteins in the rat. J Nutr. 114:1675.

Kritchevsky D, Scott DA, Malhotra S. 1980. Cholesterol synthesis in tissues of young and old Fischer-344 and Sprague Dawley rats. Exp Gerentology. 15: 369.

Kruisi T, Kostiainen E, Vartiainen E, Pitkanen L, Ehnholm C, Korhonen HT, Nissimen A, Puska P. 1984. Acute effects of marathon running on levels of serum lipoproteins and androgenic hormones in healthy males. Metabolism. 33:527.

Lacko AG, Carlile S, Kudchodkar BJ. 1984. Evaluation of lipid and lipoprotein metabolism in rats as a model for studying age-related physiological changes. J Gerontology. 39:513.

LaRosa JC, Cleary P, Meusing RA, Gorman P, Hellerstein HK, Naughton J. 1982. Effects of long-term moderate physical exercise on plasma lipoproteins. The National Exercise and Heart Disease Project. Arch Intern Med. 142:2269.

Lehtonen A, Viikari J. 1978a. The effect of vigorous physical activity at work on serum lipids with special reference to serum high-density lipoprotein cholesterol. Acta Physiol Scand. 104:117.

Lehtonen A, Viikari J. 1978b. Serum triglycerides and cholesterol and high density lipoprotein cholesterol in highly physically active men. Acta Med Scand. 204:111.

Levy R, Rifkind B. 1980. The structure, function and metabolism of HDL: a status report. Circulation. 62(suppl IV):iv4.

Lindgren, FT. 1975. Preparative ultracentrifugal laboratory procedures and suggestions for liporotein analysis. In Analysis of Lipids and Lipoproteins. Ed. EG Perkins, Illinois.

The Lipid Research Clinics Coronary Primary Prevention Trial results. 1984. I. Reduction in incidence of coronary heart disease. II. The relationship of reduction of incidence of coronary heart disease to cholesterol lowering. JAMA. 251:293.

Lipson LC, Bonow RO, Schaefer EJ, Brewer HB, Lindgren FT. 1980. Effects of exercise conditioning on plasma high density liporoteins and other lipoproteins. Atherosclerosis. 37:529.

Lopez SA, Vial R, Balart L, Arroyave G. 1974. Effect of exercise and physical fitness on serum lipids and lipoproteins. Atherosclerosis. 20:1.

Lukaski HC, Bolonchuk WW, Klevay LM, Mahalko JR, Milne, DB, and Sandstead HH. 1984. Influence of type and amount of dietary lipid on plasma lipid concentrations in the endurance athlete. Am J Clin Nutr. 39:35.

Mahley RW, Bersot TP, Innerarity TL, Lipson A, Margolis S. 1978. Alterations in human high-density lipoproteins, with or without increased plasma-cholesterol, induced by diets high in cholesterol. Lancet. i:807.

Mahley RW, Holcombe KS. 1977. Alterations of the plasma lipoproteins and apoproteins following cholesterol feeding in the rat. J Lipid Res. 18:314.

Mattson F, Erickson B, Klingsman A. 1972. Effects of dietary cholesterol on serum cholesterol in man. Am J Clin Nutr. 25: 589.

Mayer J, Marshall NB, Vitale JJ, Christensen JH, Moshaseki MB, Stare FJ. 1954. Exercise, food intake and body weight in normal rats and genetically obese mice. Am J Physiol. 177:544.

Marx JL, Kolata GB. 1978. Combatting the # 1 Killer. The Science report on Heart Research. AAAS Publication Washington p 76-90.

Miller GH, Miller NE. 1975. Plasma high density lipoprotein concentration and development of ishemic heart disease. Lancet. i:16.

Miller, NE. 1979. The evidence for antiatherogenicity of high density lipoprotein in man. Lipids. 13:914.

Medeiros L, Anderson S, Svacha A. 1984. Effect of diet on the number and size of adipose cells in rats. Nutr Rep Int. 29:823.

Morris JM, Chave SP, Adam C, Sivey C, Epstein L, Sheehan D. 1973. Vigorous exercise in leisure time and the incidence of cornonary heart disease. Lancet. i:333.

Morris JN, Everitt MG, Pollard R. 1980. Vigorous exercise in leisure time: protection against coronary heart disease. Lancet. 2:1207.

Nance DM, Bromley B, Barnard RJ, Gorski RA. 1977. Sexually dimorphic effects of forced exercise on food intake and body weight in the rat. Physiol Behav. 19: 155.

Narayan KA, McMullen JJ, Butler DP. 1975. Effect of exercise on tissue lipids and serum lipoproteins of rats fed two levels of fat. J Nutr. 105:581.

National Research Council, Nutrient Requirements of Laboratory Animals. 1978. 3rd Edition. Washington, DC.

Nationwide Food Consumption Survey 1977-1978. Food and nutrient intakes of individuals in one day in the United States, Spring 1977. USDA Washington, DC. Preliminary Report No. 2, September, 1980.

Nikkila EA, Taskinen MR, Rehunen S, Harkonen M. 1978. Lipoprotein lipase activity in adipose tissue and skeletal muscle of runners: relation to serum lipoproteins. Metabolism. 27:1661.

Nye ER, Carlson K, Kirstein P, Rossner S. 1981. Changes in high density lipoprotein subfractions and other liproteins induced by exercise. Clin Chim Acta. 113:51.

Oscai LB, Mole PA, Krusack LM, Holloszy JO. 1973. Detailed body composition analysis on female rats subjected to a program of swimming. J Nutr. 103: 412.

Paffenbarger RS, Hale WE. 1975. Work, activity and coronary heart mortality. N Engl J Med. 242:545.

Pitts GC. 1984. Body Composition in the rat: interactions of exercise, age, sex and diet. Am J Physiol (Reg Integ Comp Physiol 15): R495.

Pitts GC, Bull LS. 1977. Exercise, dietary obesity and growth in the rat. Am J Physiol. 232 (Regulatory Integrative Comp. Physiol 1):R38.

Plucinski TM, Bruner RK, Leatherwood JM, Eisen EJ. 1984. Effects of feeding pattern and dietary regimen on growth and adipose tissue cellularity in polygenic obese mice. J Anim Sci. 59:350.

Quintao E, Grundy S, Ahrens. 1971. Effects of dietary cholesterol on the regulation of total body cholesterol in man. J Lipid Res. 12:233.

Richard D, Labrie A, Lupien D, Tremblay A, Leblanc J. 1982. Role of exercise training in the prevention of hyperinsulinemia caused by a high energy diet. J Nutr. 112:1756.

Rogers QR Harper AE. 1965. Amino acid diets and maximal growth in the rat. J Nutr. 87:267.

Rolls BJ, Rowe EA, Turner R. 1980. Persistent obesity in rats following a period of consumption of a mixed, high energy diet. J Physiol. 298: 415.

Schafer EJ, Levy RI, Ernst ND, VanSaut FD, Brewster HB. 1981. The effects of a low cholesterol, high polyunsaturated fat and low fat diets on plasma lipid and lipoprotein cholesterol levels in normal and hypercholestremic subjects. Am J Clin Nutr. 34: 1758.

Schonfeld G, Weidman S, Witztum J, Bowen RW. 1976. Alterations in levels and interrelations of plasma apolipoproteins induced by diet. Metabolism. 25:261.

Schwandt P, Janetsckuk P, Weisweiler P. 1982. High density lipoproteins unaffected by dietary fat modification. Atherosclerosis. 44:9.

Sclafani A. Springer D. 1976. Dietary obesity in adult rats: similarity to hypothalmic and human obesity syndromes. Physiol Behav. 17:461.

Shepherd J, Packard CJ, Patsch J, Gotto CA, Taunton OD. 1978. Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoproteins and the metabolism of apolipoprotein A-1. J Clin Invest. 61:1582.

Shepherd J, Packard CJ, Stewart JM, Vahance BD, Lawrie TD, Morgan HG. 1980. The relationship between the cholesterol content and subfraction distribution of plasma high density lipoproteins. Clin Chem Acta. 101:57.

Sibbald IR, Berg RT, Bowland JP. 1956. Digestible energy in relation to food intake and nitrogen retention in the weanling rat. J Nutr. 59:385.

Sibbald IR, Bowland JP, Robblee AR, Berg RJ. 1957. Apparent digestible energy and nitrogen in the food of the weanling rat. J Nutr. 61:71.

Small DM. 1977. Salts in the blood: high density lipoprotein systems and cholesterol removal in liver and bile. In Falk symposium 23, Bianchi I, Gerok W, Sickinger K. Eds. MTP Press, Lancaster.

Stamford BA, Matter S, Fell RD, Sady S, Papanek P, Cresanta M. 1984a. Cigarette smoking, exercise and high density lipoprotein cholesterol. Atherosclerosis. 52:73.

Stamford BA, Matter S, Sady S, Cresanta M, Papanek P. 1984b. Cigarette smoking, physical activity, and alcohol consumption: relationship to blood lipids and lipoproteins in premenopausal women. Metabolism. 33:585.

Tan MH, Dickenson MA, Alders JJ, Havels RJ, Cheung MC, Vigne J. 1980. The effect of a high cholesterol and saturated fat diet on serum high density lipoprotein cholesterol, apoprotein A-1, and apoprotein E levels in normolipidemic humans. Am J Clin Nutr. 33:2559.

Taskier F. (ED). 1971 . Lipids, metabolism and diagnostic applications. Boehringer Mannheim Corp. New York .

Thompson P., Bortz W. Significance of High density lipoprotein cholesterol. 1978. J Am Ger Soc. 26:440.

Williams C. 1973. The use of titanium dioxide as a catalyst for large-scale Kjeldahl determination of the total nitrogen content of cereal grains. J Sci Fd Agric. 24:343.

Williams PT, Wood PD, Haskell WL, Vranizan KM. 1982. The effects of running mileage and duration on plasma lipoprotein levels. JAMA 247:2674.

Williams PT, Wood PD, Krauss RM, Haskell WL, Vranizan KM, Blair SN, Terry R, Farquhuar JW. 1983. Does weight loss cause the exercise induced increase in plasma high density lipoproteins. Atherosclerosis. 47:173.

Wisenberg CL, Hains KL. 1975. Effect of diet on lipoprotein lipase activity in the rat. J Nutr. 105:447.

Witzum J, Schonfeld G. 1979. High density lipoproteins. Diabetes. 28:326.

Wood PD, Haskell WL. 1979. The effect of exercise on plasma high density lipoproteins. Lipids. 14:417.

Wood PD, Haskell W, Klein H, Lewis S, Stern MP, Farquhar JW. 1976. The distribution of plasma lipoprotein in middle aged male runners. Metabolism. 25:1249.

Worthington RE, Miller J. 1983. High fat - high cholesterol diets reduce serum HDL cholesterol levels in rats. Nutr Rep Int. 28:1361.

Zilversmit DB. 1973. A proposal linking atherogenesis to the interaction of endothelial lipoprotein lipase with triglyceride rich lipoproteins. Circ Res. 33: 633.

Appendix A

NUTRIENT SUPPLIERS

CARBOHYDRATE

Corn Starch	Casco Brand, Canada Starch Co. Cardinal, Ontario K0E 1E0
Alphacel Non-Nutritive bulk	ICN Pharmaceuticals Inc. Life Sciences Group 26201 Mills Road Clevelend, Ohio 44128
Dextrose sugar	The R. Wine Baril Winnipeg, Manitoba

PROTEIN

Vitamin Free Casein "Vita free"	USB (United States Biochemicals) 21000 Miles Parkway Clevelend, Ohio 44128
DL-methionine	USB

FAT	
Mazola Corn Oil	Best Foods (Division of Canada Starch Inc.) CP 129 Station 'A' Montreal, Quebec H3C 1C5
TenderFlake Lard	Maple Leaf, Canada Packers
VITAMINS AND MINERALS	
AIN Vitamin Mixture 76	ICN Pharmaceuticals Inc.
AIN Mineral Mixture 76	ICN Pharmaceuticals Inc.
Choline Bitartrate	USB

Appendix B

SEPARATION OF LIPOPROTEINS

1. Preparation of serum (modified method of Lundgren (1975))

   a) each serum sample required 2 tubes:

      i) "Run 1" (density 1.0063 gm/ml)=0.6 ml serum + 1.8 ml NaCl (density 1.0063)

      ii) "Run 2" (density 1.0630 gm/ml)=0.6 ml serum + 1.8 ml NaCl (density 1.0819 gm/ml)

   b) all tubes were mixed on a Vortex mixer and placed in the ultracentrifuge head.

2. Separation of lipoprotein fractions

   a) lipoproteins were fractionated by ultracentrifugation (rotor type 40.3) at 18°C and 34,000 rpm (calculated 103,578 G) for 18 hours (Beckman L5-50B ultracentrifuge).

   b) Ultracentrifugation in a medium of density 1.0063 gm/ml separated the VLDL (top layer) from the HDL+LDL (bottom layer). Ultracentrifugation in a medium of density 1.0630 gm/ml separated the VLDL+LDL (top layer) from the HDL (bottom layer). Only the bottom layers are saved, thus ending up with an:

i) HDL and LDL fraction, as well as an

ii) HDL fraction, from each serum sample.

c) Using a syringe, 1.5 ml was removed in one smooth continuous motion from the top fraction of each tube. Care was taken to not disturb the bottom fraction, or touch the sides of the tube. The bottom fraction was then carefully transferred to a 1.0 ml volumetric tube with a Pasteur pipette. Tubes were then rinsed several times with 3-4 drops of distilled water, and the rinsings added to the volumetric tube to bring the volume up to 1.0 ml.

Appendix C

CHOLESTEROL DETERMINATION

1. Principle of method - Cholesterol was determined enzymatically using a colorimetric assay kit (Fisher Diagnostics), based on the method of Allain et al (1974). The principles of the reactions are summarized below:

cholesterol ester hydrolyzate

a) cholesterol esters $\rightleftharpoons$ cholesterol + fatty acids

cholesterol oxidase

b) cholesterol + 02 $\rightleftharpoons$ cholesten-3-one + H202

peroxidase

c) 2 H202 + 4aminoantipyrine + phenol $\rightleftharpoons$ quinoneimine + 4H20

*a chromagen with maximum absorbance at 505 nm.

The intensity of the color produced is directly proportional to the level of total cholesterol in the sample.

2. Procedure

a) 0.025 ml each of distilled water, of cholesterol standard and of samples to be assayed were pipetted into separate 4 ml test tubes.

b) 1.0 ml of cholesterol reagent was added to each tube and all tubes were mixed well (vortex).

c) All tubes were incubated in a 37°C waterbath for 15 minutes.

d) 1.5 ml of 0.89% saline solution was added to each tube and all tubes were mixed well.

e) Absorbance of standard and samples were read at 505 nm, employing the distilled water sample as the blank.

Appendix D

PROTEIN DETERMINATION

1. Principle (AACC, 1962; Williams, 1973):

a) Digestion - The digestion step serves to decompose the organic material. After the nitrogenous material is broken down the nitrogen is converted to ammonia which immediately reacts with sulfuric acid to form the salt, ammonia sulfate. At the end of digestion all nitrogen should be present in the form of ammonia ions.

$$\text{Protein} + H_2SO_4 \rightleftharpoons NH_3 + CO_2 + SO_2 + H_2O$$

$$2NH_3 + H_2SO_4 \rightleftharpoons (NH_4)_2SO_4$$

b) Distillation and Titration - The ammonium ions are held in the flask by the sulfuric acid. The sodium hydroxide neutrallizes the acid and an excess forms ammonium hydroxide from which ammonium is readily liberated by heat.

$$(NH_4)_2SO_4 + 2NaOH \rightleftharpoons 2NH_3 + Na_2SO_4 + 2H_2O$$

The ammonia boric acid complex is then titrated with standard acid (hydrochloric acid).

2. Procedure

a) 1500 gr of sample was weighed on to weighing paper and placed in kjeldahl flasks. All samples were done in duplicate.

b) 10.5 gr of titanium dioxide kjeldahl mixture was added.

c) 20 ml concentrated sulfuric acid was then added and samples were placed on a digestion rack for 60 minutes, rotating flasks at 15 minute intervals.

d) Samples were left to cool for 12 minutes then 290 ml water was added (50 ml was added, samples were swirled to dissolve salts, then an additional 240 ml water was added).

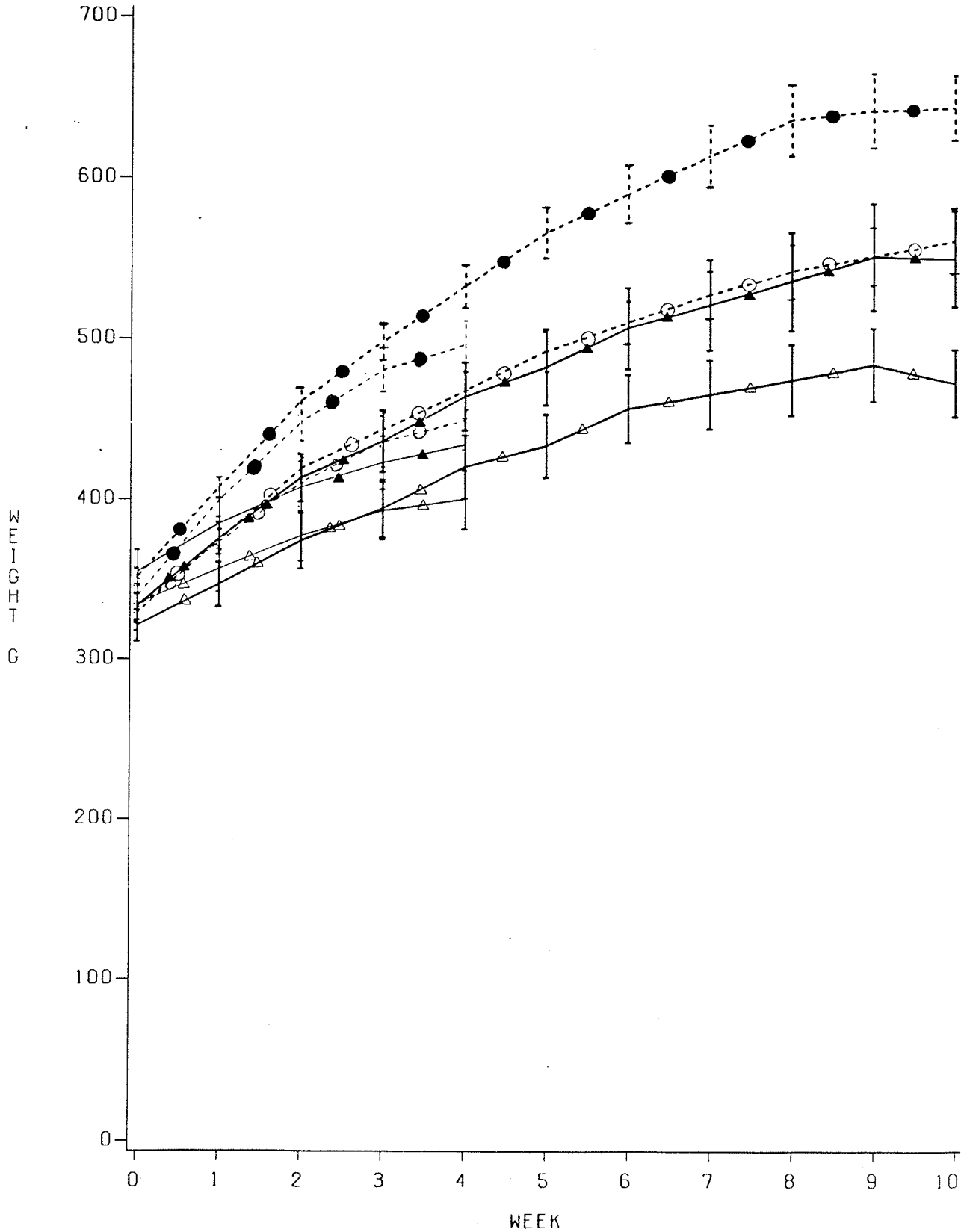
e) 50 ml of boric acid (with indicator) was placed in a 250 ml erlenmeyer flask and placed under condenser tips.

3. 60 ml of concentrated sodium hydroxide was then slowly added to kjeldahl flask, and flasks were attached to condenser apparatus and left to condense for 40 minutes. After 40 minutes, the ammonia will have been trapped within the boric acid.

a) After the condensing apparatus has been turned off, boric acid samples were left to stand for 10 minutes and were then titrated with hydrochloric acid (.1056 N).

BODY WEIGHT (G)

(PHASE I AND PHASE II)



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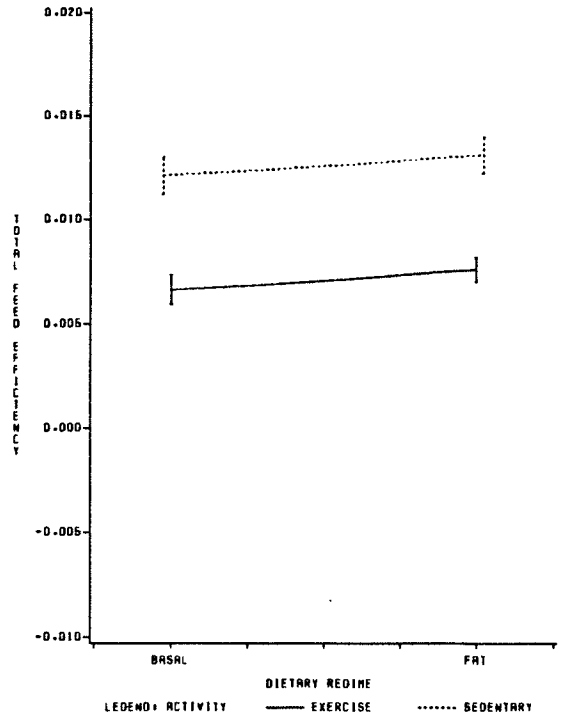

MEAN (O/J) ± 2 STANDARD ERROR OF THE MEAN (SEM)

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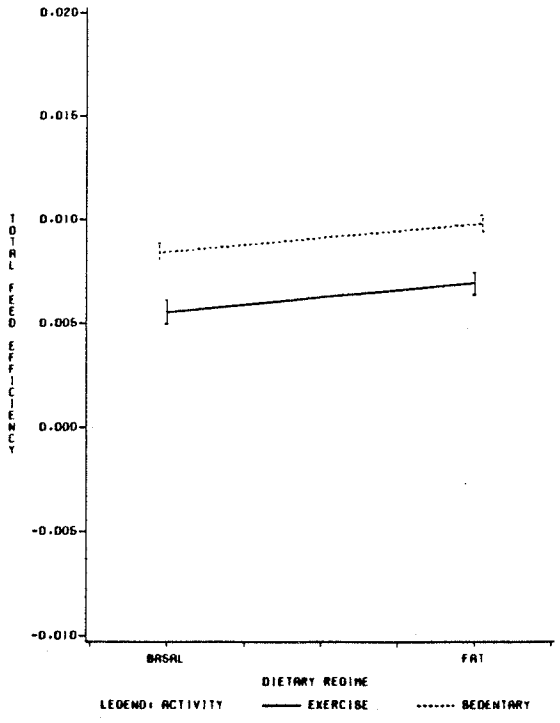

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TOTAL CHOLESTEROL
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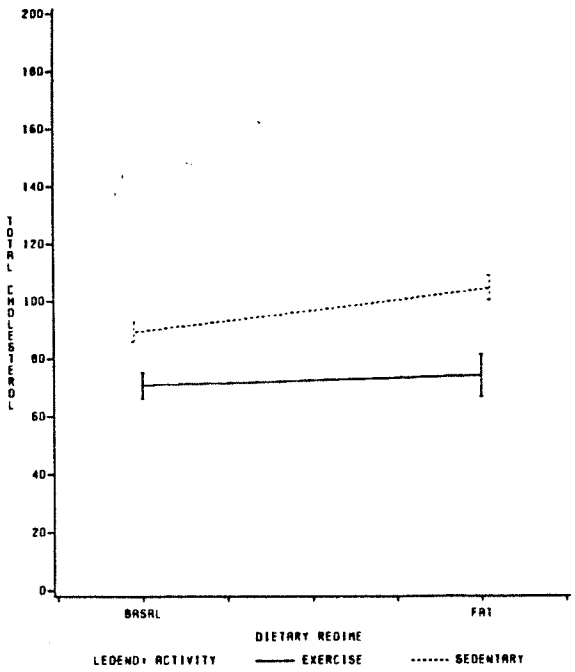

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TOTAL CHOLESTEROL
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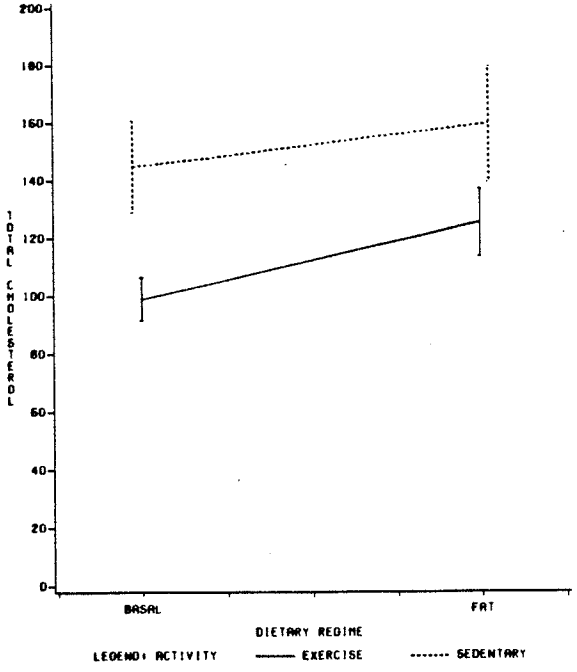

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HIGH DENSITY LIPOPROTEINS (HDL)
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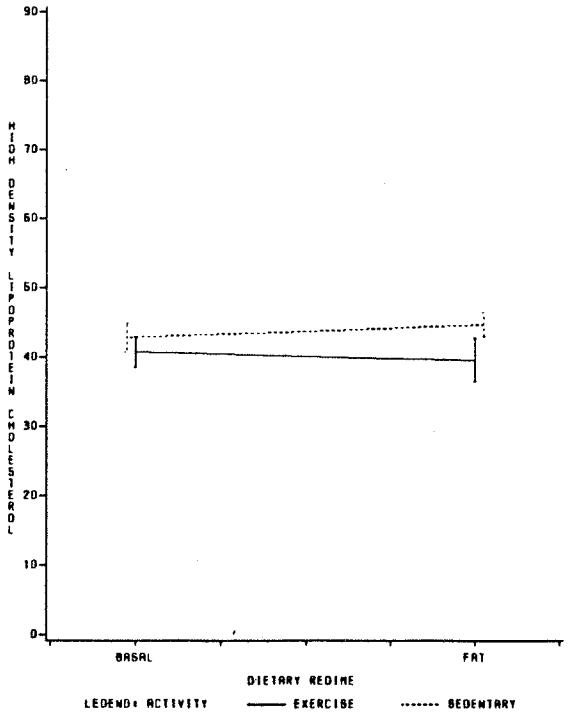

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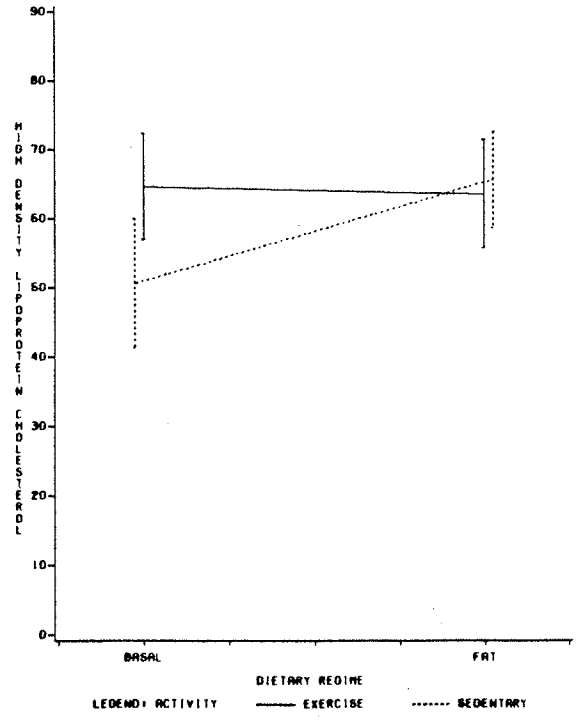

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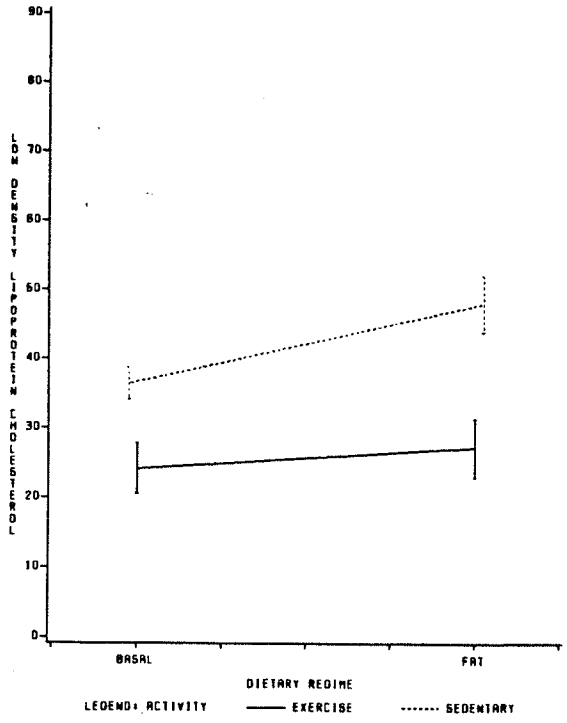

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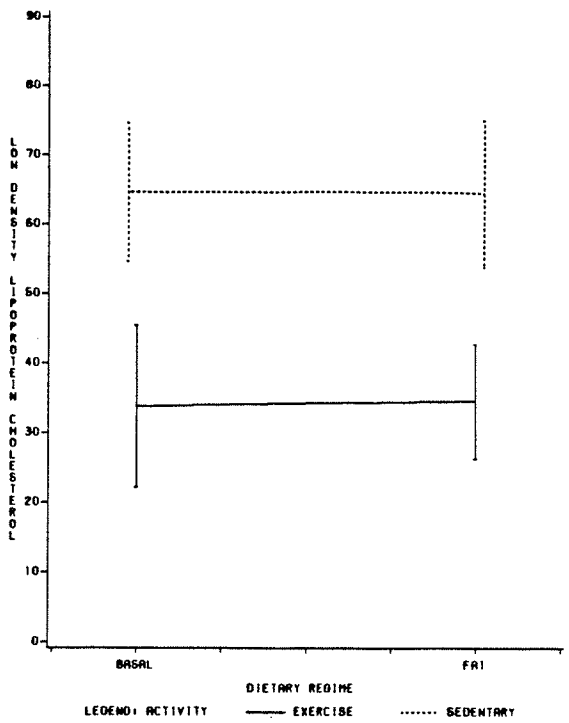

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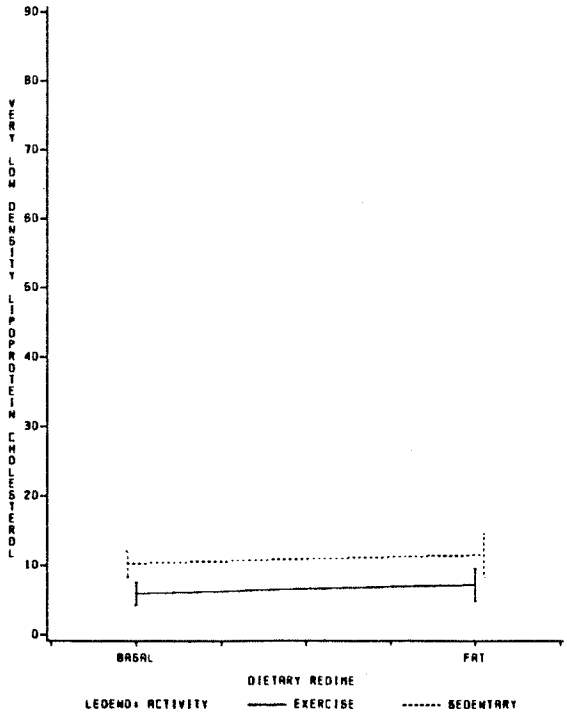

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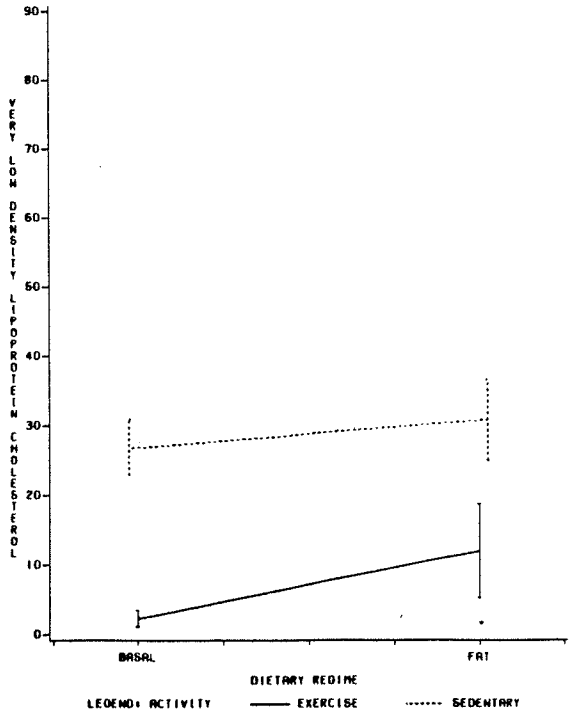

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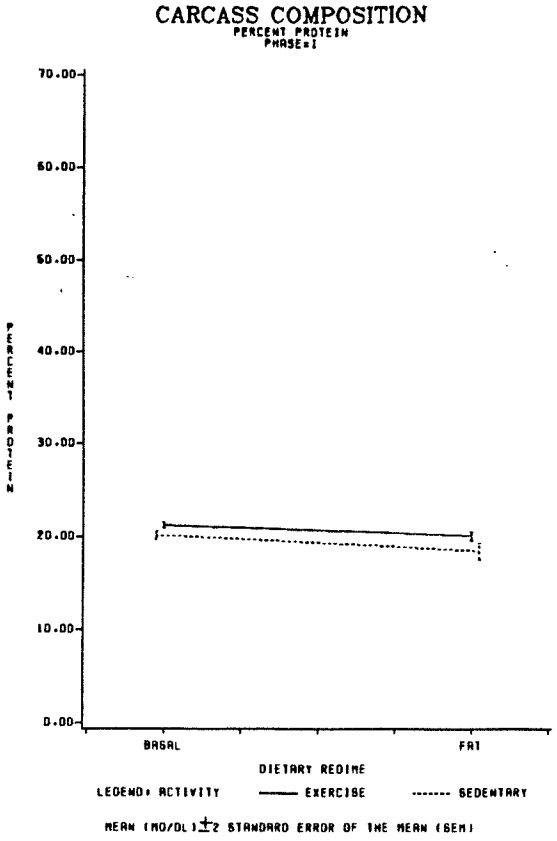
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PERCENT PROTEIN
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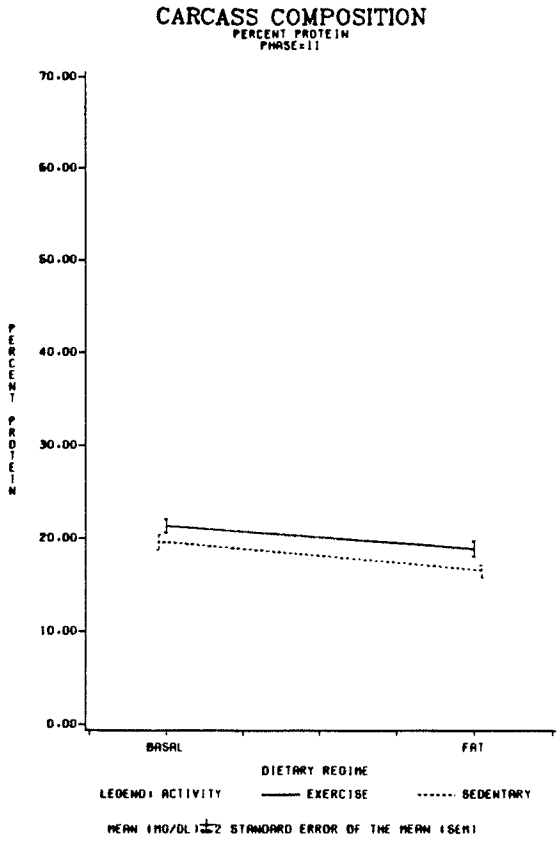
SEDENTARY
MEAN (MG/DL) ± 2 STANDARD ERROR OF THE MEAN (SEM)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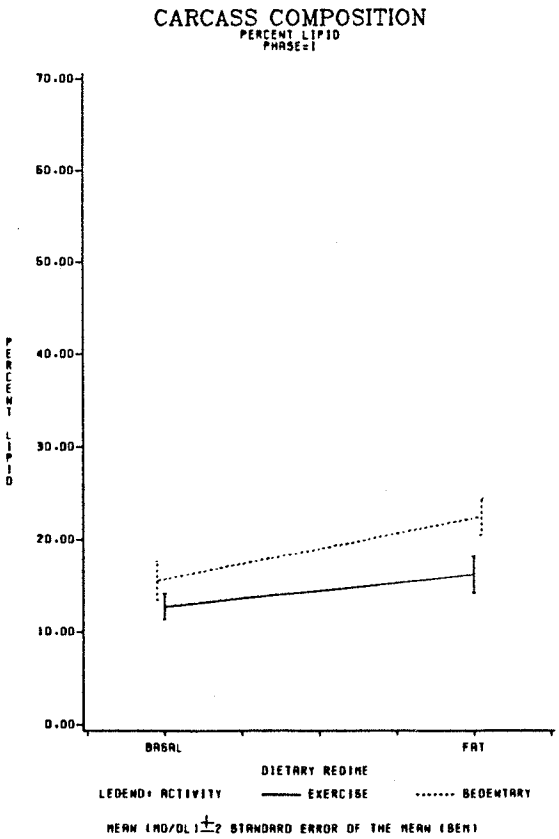
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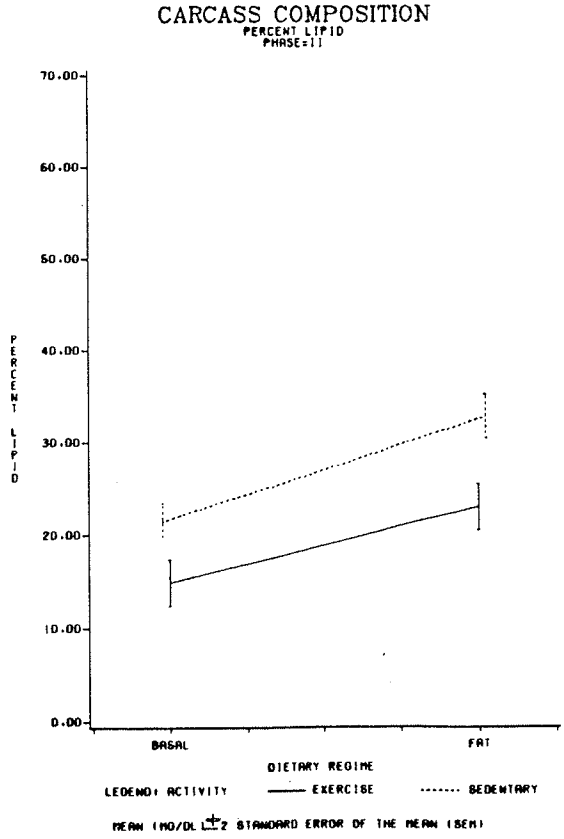
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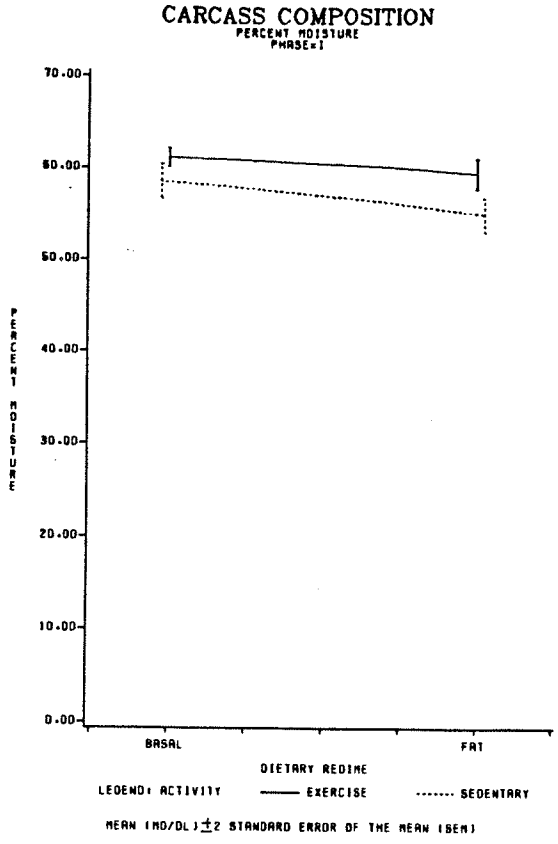
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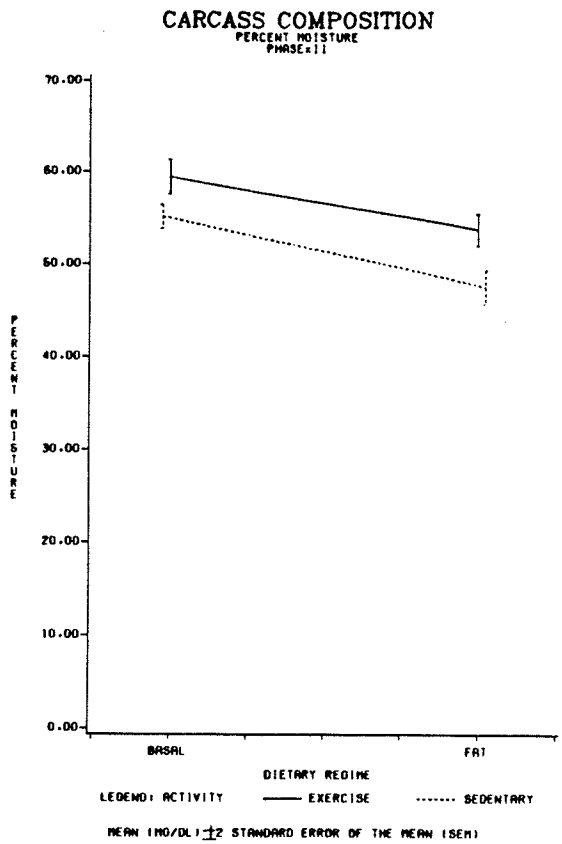
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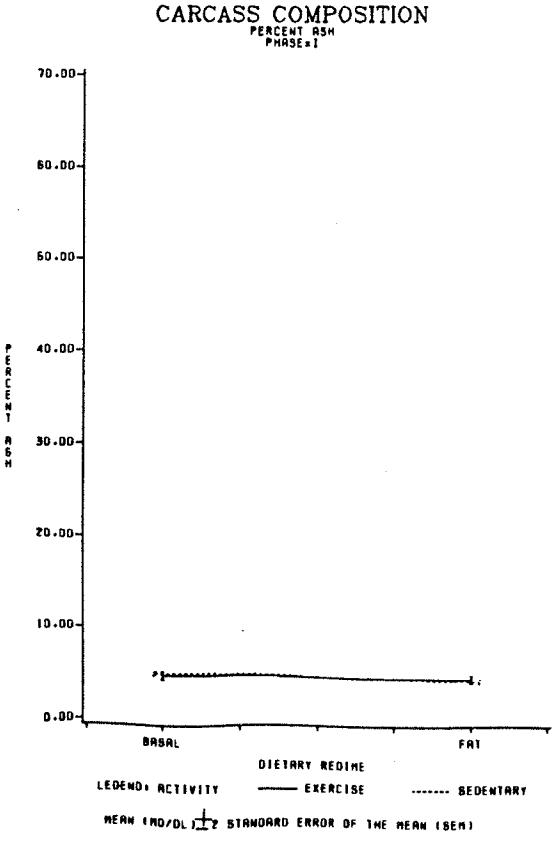
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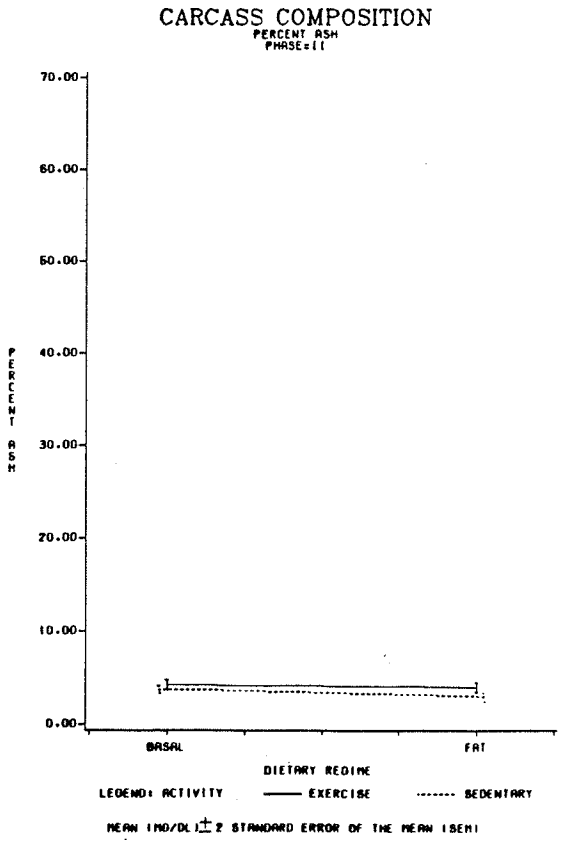
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MEAN (MG/DL) ± 2 STANDARD ERROR OF THE MEAN (SEM)