THE EFFECT OF EXERCISE AND DIETARY CHOLESTEROL ON

CHOLESTEROL SYNTHESIS IN THE HAMSTER

by

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ABSTRACT

Physical inactivity and elevated plasma cholesterol are well characterized risk factors for the development of coronary heart disease (CHD). Consequently, manipulation of exercise intensity and dietary cholesterol may favourably alter lipid metabolism to reduce this risk. The present study examined both independent and interactive effects of exercise and dietary cholesterol on in vivo hepatic and intestinal cholesterol synthesis as well as plasma total and high density lipoprotein (HDL) cholesterol levels in hamsters. Male Syrian hamsters were randomized into one of i) low dietary cholesterol (0.03% w/w) sedentary (LC-S), ii) low cholesterol exercise (LC-E), iii) high cholesterol (0.12% w/w) sedentary (HC-S) or iv) high cholesterol exercise (HC-E) groups. Exercised hamsters were trained to run at increasing speeds on a motorized treadmill for 90 minutes daily over a two week period. Animals were subsequently run for 1 week at 70% of \( \text{VO}_2 \text{ max} \) for 90 minutes each day. Cholesterol synthesis was determined by measuring the rate of incorporation of \( ^3H \) into digitonin precipitable sterols in liver and small intestine over 2 hours following IP injection of \( ^3H_2O \). Plasma total cholesterol was significantly increased by dietary cholesterol in HC versus LC groups independent of an exercise lowering effect in HC-E animals. HDL cholesterol was also elevated in response to dietary cholesterol in HC groups, however LC-E hamsters had lower plasma HDL cholesterol than any other group through an interaction between exercise and diet. Incorporation of \( ^3H \) into liver cholesterol was increased in HC-S versus LC animals, whereas exercise lowered hepatic sterol synthesis in HC-E by an exercise and diet interaction. Although exercise did not affect intestinal cholesterol
synthesis, dietary cholesterol significantly decreased intestinal cholesterol synthesis in HC when compared with LC groups. Thus both plasma total cholesterol and small intestine responded characteristically to changes in dietary cholesterol levels, in opposition to liver cholesterol synthesis, which showed a compensatory increase to the attenuation of intestinal sterol synthesis. The exercise induced decrease evident in plasma cholesterol levels and hepatic cholesterol synthesis was not, however, observed in the small intestine. Therefore the independent response of liver and small intestine to exercise and dietary cholesterol level in this study indicate important differences in the manner through which these organs regulate whole body cholesterol balance.
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INTRODUCTION

Hypercholesterolemia is a major risk marker associated with the development of coronary heart disease (CHD), a disease responsible for up to 43% of all deaths in Canada (1). With such a large segment of the population affected by this insidious disease, it is essential that Canadians be informed of the risks related to CHD which are known to be associated with certain lifestyle practices. Epidemiological and experimental evidence suggest that exercise activity and dietary cholesterol intake are factors which affect cholesterol metabolism in the human (2-6).

Physical inactivity is generally considered to be a secondary risk factor in the development of CHD, with more active individuals tending to experience fewer and less severe clinical manifestations of the disease (7,8). Metabolic and physiological effects of long term exercise have received considerable attention in recent years due to the belief that such activity is beneficial in lowering elevated plasma cholesterol, a known risk marker for CHD. In contrast, it has been hypothesized that exercise promotes an increase in plasma high density lipoproteins (HDL) which may enhance the removal of cholesterol from peripheral tissues and decrease the formation of atherosclerotic lesions.

The way in which exercise protects against CHD is controversial, however, it is widely speculated that alterations in lipid metabolism are partly responsible.

Dietary cholesterol level is an additional factor regulating cholesterol metabolism and may therefore be important in the development of CHD. Dietary cholesterol has been shown to affect both plasma
cholesterol levels and endogenous hepatic and intestinal cholesterol synthesis (9,10).

Thus it was the aim of the present study to determine whether defined levels of exercise and dietary cholesterol independently, and/or through an interaction, influence;

i) plasma total cholesterol levels

ii) plasma high density lipoprotein cholesterol levels

iii) the rate of cholesterol synthesis in liver and small intestine
BACKGROUND AND LITERATURE REVIEW

i) Effects of Exercise on Lipids in Animals

The effects of exercise duration, frequency and intensity on plasma lipid concentrations are difficult to study with human subjects and generally involve time consuming and expensive experiments (11). As well, many direct and indirect confounding factors can alter plasma cholesterol and lipoprotein levels in humans. In contrast, animal experimentation allows greater external control of variables difficult to manipulate in humans. For example, exact amounts and composition of food ingested, activity levels, age, and life-span patterns of behavioral and physiological change can be monitored. In addition, whole organ and tissue samples are easily obtained for both qualitative and quantitative analyses. Laboratory animals can therefore serve as more useful investigative models in studies where stringent variable control and use of invasive preparatory techniques make human experimentation undesirable.

Papodoulos et al. (12) and Pels et al. (11) found that rats exercised for 4 and 12 weeks, respectively, had significantly lower plasma total cholesterol levels than their sedentary counterparts. Unfortunately, these studies were designed to detect only changes in plasma cholesterol and measurement of the factors responsible for these changes, such as cholesterol synthetic rate, were not performed.

A third study by Takashi et al. (13) used the conversion of [14]C-3 hydroxy 3 methylglutaryl Coenzyme A (HMG CoA) to [14]C-mevalonate by HMG CoA reductase to measure hepatic cholesterol synthesis. Plasma cholesterol levels were also determined. Compared to the sedentary control group,
exercising rats were found to have a significantly higher HMG CoA reductase activity and hepatic sterol synthetic rate. Plasma cholesterol was significantly lower in the active group. Several problems were evident with this study that may make interpretation of results difficult.

Firstly, limitations existed with the use of [14]C-mevalonate as a tool in the measurement of cholesterol synthesis. For example, the assumption was made that no dilution of the labelled mevalonate by endogenous mevalonate would occur. More importantly, it is possible that the formation of mevalonic acid is limited by HMG CoA reductase availability, in which case partial inhibition of HMG CoA condensing enzyme might occur with excess mevalonate present (14). Thus, use of [14]C-mevalonate in this study may not have been a sensitive indicator of changes in enzyme activity occurring at an earlier step in the pathway. Although inhibition of mevalonate to squalene is an additional possible control point, it is secondary to the primary inhibition of mevalonate synthesis per se (15).

Secondly, this study presented data on cholesterol synthesis in the exercise group from very early timepoints 20-30 minutes after [14]C-mevalonate injection. In fact, from examination of the data, it appeared as though incorporation decreased after 30 minutes in exercised rats versus controls. Since bar graphs alone were presented for HMG CoA reductase activity, it is difficult to ascertain whether or not similar trends in the two groups were occurring.

Lastly, it has been observed that in rats fed ad libitum, hepatic hepatic cholesterol synthetic activity exhibits a diurnal rhythm with a maximum of activity at midnight and a nadir at noon (16). No reference was made in this study (13) as to the exact time of sacrifice, and whether or
not the diurnal cycle was considered. Therefore, consistent data may not have been collected. As well, animals of both sedentary and exercise groups were maintained on an ad libitum feeding regimen. The fact that the exercised group was reported to have higher energy and total cholesterol intakes may have rendered the results uninterpretable.

Very few well controlled experiments exist describing the combined effects of dietary cholesterol and exercise on lipid metabolism. Clarke et al. (17) conducted a study designed to measure the interactive effects of diet and exercise on blood levels of low density lipoprotein, triglyceride, high density lipoprotein, and total plasma cholesterol. Although no independent exercise effects were observed on any of the blood lipid measurements, an interaction was seen in high cholesterol-fed exercised rats. This group had lower plasma cholesterol only if they were exercised, when compared with sedentary rats fed a low cholesterol diet. A similar trend was observed in plasma HDL cholesterol levels. Better control on food intakes and energy expenditure between exercise and sedentary groups would have improved experimental design. Moreover, neither the rate of cholesterol synthesis nor the activity of HMG CoA reductase were measured. Thus, although results of this study are suggestive of diet- and exercise-induced alterations in lipid metabolism, further study of the mechanism underlying these results would be informative.

ii) Use of the Hamster as an Animal Model

There is increasing evidence that the hamster, in comparison to other species, more closely resembles the human with regard to cholesterol
metabolism and therefore is a preferable investigative model. Spady and Dietschy (18) found that in rats, whole body synthesis of cholesterol occurs at much higher rates (12 mg/day per 100 g body weight) than either in the hamster (2.5 mg/day per 100 g body weight) or in man (1 mg/day per 100 g body weight). As well, rats have a higher proportion of cholesterol synthesized in the liver versus the intestine compared to hamsters. It is thought that the rat, with higher endogenous sterol synthetic rates, more readily adapts to changes in whole body cholesterol flux than either the hamster or man (19) by more efficiently converting excess cholesterol into bile acid (20). The hamster has proved to be a good model in which to study sterol metabolism, since hamster plasma LDL concentrations respond to alterations in dietary lipid intake in a manner virtually identical to humans (21). Exercise effects in hamsters are also similar to those observed in humans, where physical activity reduces serum triglyceride levels and body fat content, and has variable effects on serum cholesterol levels. Furthermore, discontinuation of chronic exercise in hamsters causes compensatory gains of body weight and body fat in a manner similar to that of humans (22). Thus, the hamster would appear to be a suitable model for both the dietary cholesterol and exercise components of the present study.

iv) Effects of Exercise on Lipid Metabolism in Hamsters

Tsai et al. (22) examined the effects of voluntary disc running at about 22,000 revolutions per day in female hamsters over a 35 day period. No significant difference was found in plasma cholesterol between sedentary (229 mg/dl) and exercised (220 mg/dl) animals. Fatty acid
synthesis, as measured with the use of tritiated water, was found to be significantly higher in liver of the exercised group. Measurement of cholesterol synthetic rates via incorporation of tritiated water was not performed. Certain control and design problems of the study make interpretation of these results difficult.

Failure to pair-feed exercised animals to the level of food consumed by the sedentary group resulted in higher food intakes in exercised animals. This may in turn have resulted in higher cholesterol intake and total plasma cholesterol levels. Jeske et al. (23) have suggested that substrate availability may be rate limiting for cholesterol synthesis. Increased fatty acid synthesis may also have been a result of greater food intake in the exercised hamsters in comparison to controls. Moreover, the application of the tritiated water methodology may have had limitations in the study by Tsai et al. (22). Dietschy and Spady (24) have shown that a 20 minute equilibration period is the minimum required for mixing of plasma and intracellular water after an injection of tritiated water. Consequently, the assumption in this study that equilibration had occurred 20 minutes after an intraperitoneal injection was questionable. Thus the specific activity of plasma may have been overestimated. Nevertheless relative changes in fatty acid synthesis should have been detected. A shorter time interval used for the equilibration is known to have a quantitative effect that should be measured and corrected for. In a separate study, Tsai et al. (25) measured only total serum cholesterol and found it to be decreased from 221 to 198 mg/dl in sedentary versus exercised hamsters. This change, however was not statistically significant. The experimental design was similar to the above, except that no radioisotopes were used. Neither experiment included measurement of
exercise intensity which makes comparison to other studies difficult.

iv) Energy Metabolism During Exercise

The manner by which cholesterol synthetic rate is altered by exercise is unknown, however it may be speculated that changes in the availability of precursor acetyl subunits play a role in this process. The tricarboxylic acid (TCA) cycle is a final common pathway for the oxidation of energy substrates (26). The importance of acetyl CoA in metabolism stems from the fact that it is the common end product of pyruvate decarboxylation, fatty acid oxidation, and amino acid catabolism (27) and initiates the primary step in the TCA pathway.

In the resting state, muscle derives approximately 90% of its energy from fatty acid oxidation, whereas glucose uptake by muscle represents only 10% of oxygen consumed (28). During exercise, however, substrate utilization for energy is altered in order to meet increased cellular demands for glucose, which may originate from sources including liver and muscle glycogen, lactic acid, pyruvate, aspartate, glutamate and alanine (26). During the first 5-10 minutes of exercise, muscle glycogen is the major fuel utilized (28). As exercise continues, blood flow to muscles increases and blood borne substrates, provided by hepatic glycogen stores and gluconeogenic pathways become increasingly important sources of energy. Blood glucose uptake increases 25-30 fold above basal levels in a one hour exercise bout with the increment being a function of intensity and duration of exercise. This glucose may contribute to 30-50% of oxidative metabolism in the muscle (28).

As exercise continues over 1-4 hours, the availability of muscle
glycogen progressively declines and is accompanied by a rise of up to 70% in uptake of free fatty acids (28). The mechanism for the shift in substrate utilization is believed to be linked to the ADP/ATP ratio. This ratio determines the rate of oxygen uptake, with a lower ratio of ATP to ADP stimulating an increase in cellular respiration (29). The effect of this will be to increase utilization of NADH for alternative reductive pathways, such as gluconeogenesis (26). In early exercise, before the attainment of steady state, the normal rate of ATP formation by oxidative phosphorylation and glycolysis is less than ATP hydrolysis by muscle. This results in an acceleration of glycolysis due to an increase in phosphofructokinase activity (26).

The source of cytosolic acetyl CoA is of great importance in cholesterol formation since the initial biosynthetic steps occur in the cytosol. Citrate is likely the primary immediate precursor of cytosolic acetyl CoA and therefore supplies acetyl units for the first step in cholesterol synthesis (30). Mitochondrial membranes are not readily permeable to free acetyl CoA, a barrier that is normally overcome by citrate, which carries acetyl units across the inner mitochondrial membrane (IMM) to the cytosol in the following reaction (26):

\[(\text{IMM})\text{ Acetyl CoA} + \text{OAA} \rightarrow \text{Citrate} \rightarrow \text{Acetyl CoA} + \text{ADP} + \text{OAA} / \text{ATP/CoA}\]

Acetyl CoA may originate indirectly from the oxidation of glucose, fatty acids and protein, or directly from acetate and ketone bodies (30). Acetyl CoA formed during fatty acid oxidation enters the TCA cycle only if fat and carbohydrate degradation are in balance. If there is inadequate oxaloacetate (OAA) present to condense with acetyl CoA entering
the pathway, oxidation of pyruvate to acetyl CoA will not occur, and pyruvate will be directly converted to OAA instead. Oxaloacetate needs are met by direct conversion from pyruvate, itself generated from glycolytic and gluconeogenic-derived glucose (27). Thus although an increase in activity of the TCA cycle is seen during exercise, the availability of acetyl units for biosynthetic reactions from glucose and fatty acid oxidation is reduced. Excess acetyl CoA units produced during fatty acid oxidation are simultaneously shunted to the formation of ketone bodies, acetoacetate and D-3-hydroxybutyrate in the mitochondrial matrix. Although able to freely permeate the mitochondrial membrane, acetoacetate has not been considered a likely precursor for cytoplasmic acetyl CoA in liver because of low acetoacetyl CoA synthetase activity. Only extrahepatic tissues such as cardiac muscle, adipose tissue and brain have the ability to metabolize cytoplasmic acetoacetate by acetoacetyl CoA synthetase followed by thiolase, to yield two acetyl CoA units (27). It has been suggested that in the process of lipid biosyntheses, fatty acid synthesis predominates over cholesterol synthesis (31). Thus, whether the body was in a positive energy state, with relatively little ketone body formation, or negative energy state with increased ketone production, acetoacetate would provide only a limited amount of acetyl CoA for hepatic cholesterol synthesis. The lack of acetyl units for sterol biosynthesis is further substantiated by the fact that high levels of cyclic AMP present in a low energy state cause the liver to be more efficient in the synthesis of glucose and oxidation of fatty acids, and less so in the synthesis of fat, which also requires acetyl units (27). This is particularly true in exercising individuals (32). In addition, cholesterol synthesis may be affected by the availability of NADPH which facilitates HMG CoA conversion
to mevalonate, since two major suppliers of NADPH; pentose phosphate pathway and malic enzyme, are inhibited under conditions of a high ADP/ATP ratio as is seen in exercise (27).

v) Substrate Utilization During Post Exercise Recovery

Metabolic events occurring in the post-exercise recovery (PER) or repletion phase would be anticipated to exert a similar effect on acetyl CoA availability as the exercise period by altering cholesterol precursor availability.

During the recovery phase, oxidative and glycolytic activity remains accelerated as long as AMP and ADP are elevated in the tissues (27). Although muscle activity has ceased, catabolic processes must function to regenerate the deficit of high energy compounds generated by liver and muscle during previous periods of physical activity. Increased lactate production and accelerated oxygen uptake above resting levels are characteristic of this period (33). Lactate production aids in repletion of hepatic and muscle glycogen stores. It has been found that less than 18% of lactate contributes to glycogen synthesis during the latter stages of recovery with the majority of blood glucose for glycogen synthesis supplied by the gluconeogenic precursors pyruvate, alanine and glycerol (34). Krzentowski et al. (35) found that during PER, humans given an oral glucose load after 3 hr at 50% \( VO_2 \) max on a treadmill, showed the following differences in glucose metabolism as compared to non-exercising controls. Splanchnic output of glucose, muscle glycogen content and lipid oxidation were increased. In addition, a greater proportion of glucose was used for muscle, and not hepatic, glycogen repletion. It was hypothesized
that glucose-6-phosphate was used preferentially as a precursor for muscle glycogen synthesis, causing it to be shunted away from acetyl CoA formation. Others have reported an increase in activity of glycogen synthetase during this period (37).

Holm et al. (36) saw an increase in catecholamine production which persisted for up to 4 hours after exercise, the early phase of PER in humans exercising at 70% \( \dot{V}O_2 \) max for one hour. Catecholamines serve to activate glycogen synthase kinase in muscle and liver which inhibits glycogen formation (38). The main function then of catacholamines is to enhance catabolic processes which provide precursors for this "rebuilding" phase. A decrease in alanine was also observed 40 minutes into PER, which correlated with an increased fractional extraction of alanine and an increase in gluconeogenesis (38). Moreover, plasma triglyceride levels decreased, possibly due to increased muscle uptake for resynthesis of intramuscular lipid pool.

Bielinski et al. (33) observed that postmeal, post-exercise fat oxidation increased from 27-38\% of total energy expenditure over non-exercised controls. This was accompanied by a significant increase in carbohydrate storage and a decrease in fat storage, possibly due to decreased availability of acetyl CoA. Finally, it has been postulated that energy is required for stimulation of body protein turnover, which is more pronounced in PER than during exercise (39).

Others have observed very low plasma insulin levels and elevated blood glucagon during a 4 hour PER period in rats (38). The effects of insulin and glucagon are discussed elsewhere.

In summary, the PER phase is necessary for replenishment of muscle/liver glycogen and fat, processes which decrease the availability
of the primary cholesterol precursor, acetyl CoA.

vi) Effect of Dietary Cholesterol on Plasma Cholesterol in Humans

Epidemiological studies and clinical trials have shown convincing associations between serum cholesterol levels greater than 220 mg/dl and increased cardiovascular disease (CVD) incidence (9,10). It is from such studies that the risk of CVD from hypercholesterolemia, which tends to increase 1% for every 1 mg/dl rise in plasma cholesterol (40), has been established. Current evidence suggests that lowering blood cholesterol will in fact reduce the rate of CHD by slowing the progression of atherosclerotic lesions or even shrinking them (41).

It is apparent from epidemiological studies of various populations that a threshold level for cholesterol may exist whereby only very low (<100 mg/day) or very high (>1000 mg/day) cholesterol intakes will affect plasma levels (42,43) with intermediary intake having little or no effect (44). More carefully controlled metabolic studies have shown, however, that plasma cholesterol will rise in humans about 10 mg/dl for every 100 mg cholesterol/1000 Calories (40). This direct response exists when cholesterol intakes are 500 mg/day or less, although it is not agreed upon whether the relationship is linear or curvilinear (40). The area remains controversial in humans since control of human subjects is difficult, and large variation occurs in response to dietary cholesterol among individuals.
vii) Effect of Dietary Cholesterol on Plasma Cholesterol In Animals

Experimental studies in animals are easily controlled and results have shown a more consistent relationship between plasma cholesterol levels and dietary cholesterol. Turley et al. (20) found that cholesterol feeding in female hamsters (0.12% w/w) significantly decreased liver cholesterol synthesis as measured by tritium incorporation and increased plasma cholesterol levels. Similar results were obtained by Spady et al. (18), who in addition observed a significant decrease in HMG CoA reductase activity following cholesterol feeding. It appears from these and other studies that dietary cholesterol feeding has a definite effect on plasma levels in animals. Thus studying the relationship between dietary and plasma cholesterol in animals may provide valuable information on the effect of diet on cholesterol metabolism.

viii) Regulation of Lipid Synthesis

In man, cholesterol is obtained either by absorption from the diet (300-500 mg/day) or synthesis, primarily in the liver and small intestine (700-800 mg/day) (30). The rate of cholesterol excretion through all pathways must equal the rate of accretion through absorption and synthesis (19). The way in which this balance is maintained is not entirely clear, however, regulation by feedback inhibition, diurnal variation and substrate availability have been suggested (30).

Regulation of liver cholesterol synthesis by feedback inhibition is well documented. It has been found that in both rats and hamsters,
cholesterol feeding suppresses cholesterol synthesis and HMG CoA reductase activity in the liver and increases serum cholesterol levels (45-46). The cholesterol effect on HMG CoA reductase may be mediated by changes in the fluidity of its supporting microsomal membrane. Cholesterol from the diet is thought to accumulate in microsomal membranes as cholesterol esters, which in turn affect normal membrane fluidity and HMG CoA reductase activity (30). Changes in the amount of HMG CoA reductase are partly responsible for changes in cholesterol synthetic rates (47).

Bile acids within the enterohepatic circulation can similarly inhibit cholesterol synthesis in both liver and intestine (48). Because of structural similarities, it has been hypothesized that the unconjugated bile acid deoxycholic acid competes with HMG CoA for binding sites and thus may competitively inhibit cholesterol synthesis in its early stages (30). In addition, the detergent properties of bile acids are known to non-specifically inhibit many enzymes (48), perhaps including HMG CoA reductase (48). Figure 1 illustrates the primary factors influencing cholesterol metabolism:

![Figure 1: Major Steps in the Synthesis of Cholesterol and the Biochemical Steps Where Metabolic Control Takes Place](image)

This simplified scheme shows only certain key steps in the biochemical sequence. The specific conversions indicated by numbers may represent a single enzymatic step (i.e. step 4), or a sequence of earlier enzymatic steps (i.e. step 6). The three sites at which feedback inhibition is thought to be mediated are shown by the hatched blocks—Step 4, the primary site of feedback inhibition, and Steps 5 and 6, the sites of secondary control.
Cholesterol formation in liver and small intestine undergoes circadian variation in rodents, with peak synthetic activity occurring at the midpoint of the dark cycle in *ad libitum* fed rats (16). This control is thought to be associated with changes in rates of synthesis of HMG CoA reductase, and thus, its activity (30). Evidence from diurnal variation studies suggests that in rodents, the peak endogenous synthetic rate occurring at mid-dark cycle (4-6 hours postprandial) is functioning to replace dietary cholesterol that has been converted primarily to bile acids and excreted to aid in the digestion and absorption of fat (49). One would expect that if dietary cholesterol were the sole regulator of cholesterol synthesis, animals in the fasted state would undergo increased synthesis as described above. Tracer studies however have shown that in 24-48 hour fasted rats, a reduction in both hepatic cholesterol synthetic rates, and HMG CoA reductase activity is observed (49). It has been suggested that the formation of mevalonic acid is limited by the quantity of substrate available for HMG CoA reductase, which would result in partial inhibition of HMG CoA condensing enzyme (30).

Insulin and glucagon may also play a role in the process of regulating cholesterol synthesis. *In vivo* tracer studies have shown insulin to increase hepatic cholesterolgenesis and glucagon to block this effect (50). These hormones are thought to control both the total amount of enzyme present and the proportion of enzyme at an active site (50). Insulin and glucagon are also known to have powerful effects on the availability of acetyl CoA. Insulin causes increased utilization of acetyl units for anabolic pathways, particularly lipid synthesis, whereas glucagon indirectly causes diversion of acetyl units into oxidative, glucogenic and ketogenic pathways (27). Since fasting causes a shift in
these hormones similar to that seen during and immediately following exercise, it is possible that cholesterol precursors are derived from the same metabolic pool as is used for energy production. Thus substrate availability as determined by energy demands may influence synthesis of cholesterol, in addition to the other discussed control factors.

ix) High Density Lipoprotein Metabolism

High density lipoproteins (HDL) serve a putative role as protective agents against premature development of atherosclerosis and coronary heart disease. High density lipoproteins can originate in the liver or intestine as discoidal nascent HDL, and both from surface remnants of triglyceride rich lipoproteins or phospholipid-apoprotein associations (spherical HDL) (51). High density lipoproteins exist in blood primarily as 40 Å HDL₂ and 60 Å HDL₃ subfractions. The HDL₂ component has 3-4 fold more cholesterol ester and triglyceride compared with HDL₃. Thus HDL₂ is considered to be an efficient lipid carrier and a better protective agent against coronary heart disease.

Two mechanisms for this protective effect have been proposed. Firstly, HDL is thought to be protective against atherosclerosis through its function in "reverse cholesterol transport", where extra-hepatic cholesterol from the periphery is esterified by HDL molecules via lecithin cholesterol acyl transferase (LCAT), and returned to the liver for utilization (52). During normal fat metabolism, discoidal HDL circulates in the plasma or lymph where it is transformed to spherical HDL by the influx of cholesterol across a chemical gradient mainly from very low density lipoproteins (VLDL) and chylomicron remnants (52). Spherical HDL
transports newly formed cholesterol esters back to the liver. In addition, some cholesterol ester from HDL is transferred to VLDL and LDL, and reciprocally, triglycerides are transferred from these lipoproteins to HDL. High density lipoproteins readily adapt to changes in lipid status in humans. For example, when endogenous lipoproteins are exposed to a large influx of dietary fat and cholesterol, HDL levels increase as part of a normal response to excess cholesterol in the blood. When regulation of cholesterol balance in normal individuals is achieved through HDL reverse cholesterol transport and other lipoproteins, formation of atheromas is not favoured. Conversely, it has been shown that individuals with absent plasma HDL show severe impairment of cholesterol ester and triglyceride transport (51). It has also been noted that low HDL is as predictive as high LDL for coronary heart disease (53). Thus important questions have been raised regarding potential benefit of elevated HDL levels. Firstly, do factors other than diet have the ability to independently raise plasma HDL levels? And more important, would these high levels significantly alter the rate at which cholesterol undergoes reverse cholesterol transport, and thus remove cholesterol from the periphery?

A second way in which HDL may decrease risk of cardiovascular disease is by directly retarding atheromatous lesion growth (51). Normally low density lipoprotein (LDL) is taken up into the cell through receptor-binding and lysosome internalization, followed by a release of cholesterol into the cell lumen by hydrolysis of constituent LDL cholesterol esters. The resultant free cholesterol in turn inhibits endogenous cholesterol synthesis via inhibition of HMG CoA reductase (53). As LDL concentrations increase, saturation kinetics occur, decreasing the number of available LDL binding sites and causing LDL particles to accumulate in the plasma.
Normally, regulation of plasma LDL is achieved in part, by enhanced HDL removal of cholesterol from the cell lumen, which in turn causes an influx of LDL to cells across a favourable concentration gradient. Experimental evidence suggests that in addition to its normal function of reverse cholesterol transport, HDL and in particular HDL$_2$, protects against formation of plaque and subsequent atherogenesis by reducing cellular LDL cholesterol uptake. Cell culture studies have shown that molar ratios of HDL:LDL in excess of 5:1 inhibit uptake and subsequent degradation of LDL into cells of cultured fibroblasts (53), vascular endothelial cells (53) and smooth muscle tissue (54).

Various factors are thought to raise HDL cholesterol levels. Chronic alcohol consumption causes an increase in VLDL flux and is associated with increased HDL concentration and a lower risk of cardiovascular disease (53). Similarly physical exercise has been shown to elevate HDL although the consistency and mechanism of this response remains controversial. It has been hypothesized that endurance athletes have elevated levels of HDL cholesterol and apoproteins (AI, AII) in part because of reduced HDL apoprotein catabolism as well as an increased ability to clear triglycerides from skeletal muscle (55). The increased triglyceride clearance most likely results from the exercise-induced rise in lipoprotein lipase (LPL), catalyzing the hydrolysis of triglycerides in VLDL and chylomicrons, the breakdown products of which enter the plasma pool as HDL precursors to form spherical HDL.

Mechanistically, HDL is believed to compete with LDL for cellular receptor binding sites, which in turn decreases surface binding of LDL and its consequent uptake, degradation and contribution to net cell cholesterol content (51). Furthermore, and in contrast to LDL, HDL has a
slower rate of internalization thereby decreasing cellular accumulation of cholesterol. Thus, in smooth muscles and vascular endothelia, where high rates of LDL uptake and initiation and progression of atherosclerotic lesions are known to occur (54), HDL may be of some benefit in reducing the risk of atherosclerosis by decreasing substrate supply for plaque formation. Caution must be exercised in interpreting these observations however, as it is unknown whether decreased cellular uptake of LDL, particularly in extrahepatic tissues, can regulate whole body cholesterol balance. For example, endogenous synthesis may increase in response to decreased LDL uptake and counteract any net decrease in cell cholesterol due to LDL alone.

In summary, the level of HDL in the blood may be inversely related to the risk of CHD. The positive effects of HDL might be mediated by enhanced reverse cholesterol transport, or inhibition of cellular LDL uptake. Studying the response of HDL levels to exercise may be useful in furthering our knowledge of CHD risk factors.
RATIONALE

Plasma cholesterol concentration is independently influenced by both exercise activity and the level of cholesterol in the diet. Studies suggest that exercise may decrease plasma cholesterol level while dietary cholesterol has the opposite effect. However, the mechanism by which these effects occur is not understood. A possible mechanism through which these effects on plasma cholesterol are mediated is by altering the rate of endogenous cholesterol synthesis. Exercise may alter the availability of acetyl CoA subunits necessary for the initial stages of sterol synthesis, thereby decreasing its rate of formation. In contrast, it is believed that dietary cholesterol exerts negative feedback inhibition on both hepatic and intestinal rates of sterol synthesis.

Therefore the purpose of this study was to determine whether exercise activity and dietary cholesterol feeding, independently, or through an interaction, influence; plasma total cholesterol levels, HDL cholesterol levels and synthesis of cholesterol in liver and intestine, the organs largely responsible for cholesterol production.

Findings from this study will further our knowledge of control mechanisms of plasma cholesterol and cholesterol synthesis, via exercise activity and dietary cholesterol level.
MATERIALS AND METHODS

i) Methodological Considerations

a) Determination of Maximal Oxygen Consumption in Hamsters

It is well known that endurance exercise enhances the respiratory capacity of muscle by inducing enzymatic changes that increase the oxidation of pyruvate, fatty acids and ketones (29). This rise in muscle respiratory capacity results from an increase in both the composition and total number of mitochondria present in muscle tissue (29). Endurance is a function of relative work rate (56) which is reflected in the ability of muscle mitochondria to utilize oxygen (32). Comparison of trained and untrained individuals exercising at the same relative work rate has shown that trained individuals have metabolic responses to exercise that are different from those of their untrained counterparts. For example, depletion of muscle and liver glycogen stores is slower, and a greater reliance on fat oxidation for generation of energy occurs in trained subjects (29). Although there exists some inter-individual variability, uptake of oxygen reaches a reasonably constant rate given a specified training schedule. Thus in an exercise study it is desirable to have all subjects working at the same capacity if variables other than oxygen consumption, but which may be related to the changes in oxidative capacity, are to be measured. Such control would eliminate potential confounding effects of training state.

A pilot study was therefore performed to determine maximal oxygen consumption (\(\dot{V}O_2\) max), to determine the ideal length of time needed for
the training phase in order to achieve a stable $V_{O2 \max}$ across the exercise group and to determine the caloric expenditure associated with exercise of defined work load and duration. The work load and duration of exercise was manipulated, together with projected food intakes, to achieve a constant body weight in the exercise group.

Hamsters of similar age and weight to those in the present study were run daily at the beginning of the dark cycle at as high a speed as possible until they could no longer run. The running apparatus consisted of a freely spinning exercise wheel positioned on a motorized treadmill which was enclosed in an air-tight chamber. Figure 1 illustrates the equipment used to determine oxygen consumption of a hamster during exercise (see Appendix A for details).

![Diagram of equipment used to determine oxygen consumption of a hamster during exercise](image)

Figure 1. Samples of post-exchange air were analyzed with a single channel paramagnetic oxygen analyzer (i) (model 211, Westinghouse, Pittsburgh, PA), and oxygen concentration differences between room and exchanged air were recorded (ii). Oxygen consumption, calculated as the product of air flow rate (1.23 L/min) through the air tight exercise chamber multiplied by the oxygen concentration difference between room and chamber post-exchange air, was used to
b) Measurement of Cholesterol Synthesis

Regulatory mechanisms of cholesterol synthesis are commonly measured indirectly by i) determining the activity of microsomal HMG CoA reductase or directly by ii) measuring incorporation of labelled precursor such as [14]C-acetate or tritium (³H) from tritiated water ³H₂O into cholesterol (23-24). Since accuracy in quantitating these rates is desirable, the method of choice should be consistent and reliable.

Measurement of HMG CoA reductase activity alone is often used to assess relative differences of synthetic rates in a given tissue preparation (24). Good technique during microsome preparation is necessary to avoid underestimation of absolute rates of cholesterol synthesis.

The labelling of precursor is also simple but is subject to intracellular dilution. For example, [14]C-acetate is taken up by the cell and converted to [14]C-acetyl CoA in the mitochondrial matrix. It cannot, however, be assumed that this will be the sole source of acetyl CoA for sterol synthesis, since fatty acid and glucose oxidation may contribute acetyl units which would dilute this labelled pool and its specific radioactivity (23,24). The magnitude of dilution varies both in different organs or in the same organ under different metabolic conditions, and therefore represents an unreliable method.

The use of tritiated water in measuring cholesterol synthesis is considered a better method in comparison to the above described technique. Incorporation of tritiated water into cholesterol circumvents dilution problems associated with carbon labelled substrates. The method is based on the assumption that ³H atoms from ³H₂O are incorporated into stable, non-exchangeable positions in the sterol molecule. The dilution effect is
avoided by use of high specific radioactivity, since relatively little unlabelled water is generated metabolically within, or between cells (23).

A limitation with the tritiated water technique arises in the estimation of $^3$H incorporated into each carbon atom of the cholesterol molecule, or $^3$H/C ratio. Eighteen acetyl CoA units containing 36 carbon atoms are needed to synthesize one cholesterol molecule, having 27 carbon and 46 hydrogen atoms. Seven of these hydrogen atoms come directly from water and 15 from NADPH produced in reductive biosynthetic pathways (24). However, the degree of equilibration of NADPH with tritium is questionable. With high enrichment levels of tritium, the assumption is generally made that the reductive H of NADPH is fully equilibrated with $^3$H$_2$O and that 22 ug atoms of tritium are incorporated into each umole of cholesterol, giving an incorporation ratio of 0.81 (24). Use of tritiated water as a label solves dilution problems associated with carbon labelled substrates and was the method of choice for the present experiment.

Cholesterol synthetic activity may be expressed in different ways, depending on the intention of the experiment. Typical expressions include; i) incorporation of radioactive material into cholesterol per organ or ii) per gram tissue or iii) as mg of cholesterol synthesized per mg tissue cholesterol, or as fractional synthetic rate (FSR).

ii) Experimental Design

To allow simultaneous assessment of dietary cholesterol and exercise on parameters of lipid metabolism in hamsters, a two by two factorial experiment was designed. Because of limited space on the exercise equipment, division of the study into two separate experiments was
required. The two experimental study groups differed only on the basis of diet, and will subsequently referred to as; Low cholesterol diet (LC), and High cholesterol diet (HC). Thus for each of the two consecutive study periods, hamsters were allocated into either exercise (E) or sedentary (S) groups within each of the diet treatment groups. Figure 3 illustrates the general experimental design used in this study.

FIGURE 3. Experimental Design. Factors altered were exercise and level of dietary cholesterol
iii) Animals - Diet and Exercise Procedures

a) Diet

On day one of each of two four week studies, Syrian hamsters (male, 50 days old, 105.9 ± 6 grams (± S.D)) obtained from Charles River Laboratories Inc., Montreal, were randomized into either sedentary or exercise groups containing 12 animals each. Animals were individually housed in stainless steel cages and exposed to 12 hour light/dark cycling for two weeks prior to, and during the study period. All hamsters had free access to water and were fed ground Purina Laboratory Rodent Diet (5001) to which 5% corn oil had been added. Cholesterol concentration of the diet was 0.03% and 0.12% (w/w) for LC and HC respectively (see Appendix B for complete diet composition). Exercising animals were presented with an amount of food identical to the averaged previous day's intake of ad libitum fed sedentary controls. Food cups were removed from all hamsters during the daily exercise period.

b) Exercise

Exercised hamsters were run simultaneously at the beginning of the dark cycle for 90 minutes daily on a motorized treadmill. Animals were subjected to a 2 phase schedule over the experimental period. During the initial training phase running speed was increased by 2.5 meters/minute/day for two weeks to ensure adaptation to the apparatus and attainment of a constant VO₂ max. Phase two consisted of high intensity running at 70% VO₂ max at approximately 35 meters/minute,
following a 5 minute warm-up. During this phase animals were run daily at the same time for a minimum of one week. Both exercise and non-exercise groups were exposed to infra-red lighting during exercise so as to not perturb the normal diurnal light cycle. The following diagram illustrates the daily feeding and exercise schedule:

![Daily feeding and exercise schedule diagram]

**FIGURE 4.** Daily feeding and exercise schedule.

c) Entry/Exit Points of Study

Although both exercise and sedentary groups entered the study on the same day, exit dates were staggered in order to test individual animals at similar times during a defined period of the light/dark cycle. Specifically, cholesterol synthesis in hamsters has been shown to be maximal both at the mid-point of the dark cycle and 4-6 hours post-feeding (49,57). Given this time frame, only 4-6 animals could be killed per day. Therefore, commencing on day 22 of each study, following the two week training phase and a minimum of one week intense exercise, 2 pairs of animals were sacrificed per day as subsequently described, with one hamster from each of exercise and sedentary groups forming the pair.
iv) Measurement of Cholesterol Synthesis and Plasma Cholesterol

Both pairs of animals were fasted overnight prior to their final day in the study. Following normal routine, exercising animals were returned to their cages after 90 minutes of running and given free access to water. One half hour later, the first of four hamsters to be studied received a bolus of 50% glucose solution (1g/kg) via gastric intubation. The remaining 3 hamsters were similarly intubated at 1/2 hour intervals. Following a 3 1/2 hour rest the first hamster received an intra-peritoneal (IP) injection of approximately 30 mCi tritiated water, followed again by the remaining animals at 1/2 hour intervals. Two hours after injection animals were lightly anesthetized with ether (USP for anesthesia) and exsanguinated. It should be noted that the order of the 4 animals being sacrificed changed daily to avoid potential confounding of results by temporal differences from the time of exercise activity. During exsanguination approximately 4 ml of blood were withdrawn from the heart and centrifuged at 1500 RPM for 20 minutes. Plasma was removed for specific activity, plasma total and HDL-cholesterol determinations. Liver and small intestine were immediately removed, rinsed with saline, weighed, and frozen in liquid nitrogen before storage at -70°C.

v) Laboratory Analyses

a) HDL-Cholesterol Determination

Precipitation of HDL-cholesterol was performed immediately following animal sacrifice, using a modified dextran sulphate method (58). HDL in
plasma is often expressed as the proportion of the lipoprotein that is cholesterol, usually around 15%. Expression of HDL concentration in plasma by its cholesterol content has been established as a standard method for HDL determination. Thus, following centrifugation, 200 μl plasma samples were combined with 20 μl reagent (1 ml heparin, 2 ml magnesium chloride, 1 ml 0.15M sodium chloride), shaken for 10 minutes and centrifuged at 3000 rpm for 20 minutes. The resulting supernatant was removed and stored at -70°C until analyzed for total HDL cholesterol content as described below.

b) Plasma Specific Activity

Following storage at -70°C, 100 μl samples of plasma were diluted 1000 times with distilled water, and 100 μl of the resulting solution mixed with 10 ml scintillation fluid to determine disintegrations per minute. Specific activity was used to calculate cholesterol synthetic rate, and indirectly, percentage body fat (see page 34).

c) Cholesterol Determination

Cholesterol concentration of plasma total, HDL, and tissue cholesterol were quantitated in vitro using an enzymatic kit (Biopacific Diagnostics Inc.) (59). Previously frozen plasma samples and free cholesterol isolated from tissue and dissolved in isopropanol, were assayed in duplicate. Ten μl of sample was combined with 1 ml cholesterol reagent and incubated 5 minutes at 37°C. Colorimetric analysis was used to determine concentration of cholesterol relative to internal reference standards by spectrophotometry at 505 nm (Coleman Spectrophotometer,
d) Tissue Preparation

Liver (1.0 g) and intestine (0.5 g) samples were saponified in duplicate to liberate free and esterified cholesterol. For liver, 2 ml 30% potassium hydroxide (KOH) in water were added and the mixture heated to 80-90 °C. Two ml ethanol (EtOH) were then added and the sample heated for 2 hours at 90 °C. For intestine, the procedure was identical except the volumes of KOH and ETOH were halved. Standards containing 100 μl [14]C-cholesterol and two ml 150 mg/dl unlabelled free cholesterol were run in triplicate with each tissue batch. Two ml 30% KOH were added to these standards which were then analyzed as described above.

Saponified tissue was twice extracted to remove lipids and free cholesterol. Liver and internal standards were extracted after the addition of 2 ml methanol (MeOH) and 12 ml hexane:chloroform (4:1 v/v). Samples were shaken 10 minutes and following addition of 1 ml H₂O, were further shaken for 10 minutes and centrifuged at 2300 rpm for 10 minutes. The resultant supernatant was retained and solvent removed by drying under N₂ gas. Intestine samples followed the same procedure except that 3 ml MeOH were added initially.

e) Isolation of Free Cholesterol

The method of Sperry (60) was used to precipitate free cholesterol from sample extracts. The procedure was initiated by the addition to each sample of 4 ml acetone/EtOH (1:1 v/v), 1 ml digitonin solution (2% in 80%
EtOH) and 1 ml distilled water. The samples were covered and allowed to settle overnight at 23°C in screw top tubes. Centrifugation (15 minutes, 2300 RPM) and removal of supernatant followed. The remaining digitonin complex was washed successively 3 times with 1.5 ml of first 80% EtOH and second 1.5 ml ether, dried completely under N₂ gas, and heated for 1 hour at 110°C. To remove cholesterol from the digitonide complex, 0.3 ml pyridine was added and the precipitate dissolved by gentle heating. Samples were again washed 3 times with ether, only this time the supernatants were pooled and kept after each centrifugation. The ether was removed via N₂ stream over heat as before. All samples were placed in vacuum over sulphuric acid for 24 hours. Isopropanol was subsequently added in appropriate amounts to dilute the sample to within detectable concentration range for cholesterol. One half the sample was combined with 10 ml scintillation cocktail and counted for ten minutes. Synthetic rates were determined as described in "Calculations" section.

vi) Calculations

a) Cholesterol Determination

Cholesterol values were expressed either as a concentration (plasma total and HDL) or absolute value (sample and total tissue values) and were derived from the following equation:

\[
\text{concentration} = \frac{\text{optical density}_{\text{test sample}} \times \text{[standard]}}{\text{optical density}_{\text{standard}}} \times \text{volume dilution factor}
\]

\[
= \text{mg/dl}
\]

\[
\text{absolute} = \text{mg/unit tissue}
\]
b) Fractional Synthetic Rate

The expression of tissue cholesterol synthesis both as the radioactivity per gram of tissue and as a fraction of total tissue cholesterol were determined, to allow differential determination of results and comparison to current literature. DPM/g tissue per hr were calculated, knowing the incubation time and total tissue weight. DPM were calculated using the channels ratio method for counting efficiency on a Nuclear Chicago Isocap 300 Instrument and a quench curve specific to the solvents and solutes used was constructed. Since the plasma specific activity (DPM/mL plasma) was known and assumed to be fully equilibrated with tritiated water, the amount of tritiated water incorporated per gram of tissue per hour was calculated as follows:

\[
\frac{\text{DPM/g tissue.hr}}{\text{DPM/mL plasma (or water)}} = \frac{\text{mL } ^3\text{H}_2\text{O /gram tissue.hr}}{9} \tag{1}
\]

Dividing this number by \(18 \times 10^9\) (mL water per nmol water)

\[
= \frac{\text{nmol } ^3\text{H}_2\text{O /gram tissue.hr}}{\text{mg cholesterol/hr}} \tag{2}
\]

In order to express this rate as a fraction of the total cholesterol pool, mg cholesterol/g tissue was needed. Dividing equation (1) by (2) gave;

\[
\frac{\text{nmol } ^3\text{H}_2\text{O/g.hr}}{\text{mg cholesterol per hr}} \tag{3}
\]

The incorporation of the \(^3\text{H}/\text{C}\) ratio as determined by the method of
Dietschy et al. (24) occurred at this point since it was known theoretically how much cholesterol a certain amount of tritiated water represents (22 ug atoms of $^3$H for each umol of cholesterol). This number was divided by two as each water molecule contains two hydrogens atoms. Thus, carrying equation (2);

$$\frac{\text{nmol H}_2\text{O}}{\text{mg cholesterol per hr}} \times 386 \text{ ng/nmol}$$

Thus, carrying equation (2);

$$11 \text{ nmol}^3\text{H} \times \frac{\text{nmol cholesterol}}{\text{nmol cholesterol}}$$

The units (g cholesterol/mg cholesterol per hour) were adjusted and the result expressed as that fraction of the total cholesterol pool synthesized via tritium incorporation, or;

$$\frac{\text{mg cholesterol (synthesized)}}{\text{mg cholesterol (total)}} \times 100$$

$$= \text{FSR (%) }$$

c) Body Composition

Body water volume of each animal was determined from the plasma water $^3$H$_2$O activity at time of sacrifice. It was assumed that at 120 minutes the injected tritiated water had equilibrated across the body water compartment. Lean body mass was calculated as (body water volume/0.73). Corrections were made for the exchange of hydrogens of body water with those of body proteins and carbohydrates (61,62) and for the presence of solutes in plasma (62). Body fat (%) was calculated as (body weight - lean body mass) assuming a two pool model.
vii) Statistical Analyses

A two factor, two way analysis of variance (ANOVA) with fixed effects was used to determine the effect of exercise, dietary cholesterol and their interaction on each of plasma total cholesterol and HDL-cholesterol, and cholesterol synthesis. A one factor ANOVA was used to check for differences in starting body weights between all exercise and sedentary animals of LC and HC diets. All calculations were completed with the assistance of Lotus "Symphony" (63) and CSS.STAT (64) computer packages.

All results were obtained using a two factor analysis of variance, testing not only for independent diet and exercise effects, but also for their interaction. Therefore, the format subsequently used to describe these results is as follows:

i) if no interaction existed, the grand means for combined diet and/or exercise groups will be presented along with the probability ("p") value

ii) if an interaction was observed, the overall significance level ("p") of both diet and exercise effects, and their interaction will be presented. In addition, individual group means and their relationship to one another as determined by "Tukey's" post hoc test, will be discussed.

Anova tables and grand means for diet and exercise groups are presented in Appendices C, D, and E.
RESULTS:

i) Food Intake, Body Weights and Percentage Body Fat

Figures 5A and 5B illustrate average daily food consumption (x ± SD) of sedentary and exercised animals fed low and high cholesterol diets, respectively. All four study groups showed a gradual decrease in food intake over the experimental period. Statistical comparison was made between sedentary and exercised animals both within and between diet groups on days 1, 15 and 21. Results showed that exercise significantly decreased food consumption (p < 0.05) on days 1, 15 and 21 regardless of the diet treatment. Although not significant, exercised animals fed the low cholesterol diet consumed slightly less food than exercised animals consuming the high cholesterol diet. These relationships between food consumption and exercise activity appeared to be consistent over the experimental period and food consumption was therefore included as a covariable during subsequent statistical manipulation of variables.

Body weights of sedentary and exercised hamsters consuming low and high cholesterol diets are shown in Figures 6A and 6B respectively. Starting body weights of animals were not different between the four treatment groups due to systematic randomization at study onset. Sedentary animals of both dietary treatment groups showed relatively constant increases in body weight during the 21 day study period. In contrast, exercised hamsters initially showed similar rates of weight gain compared with sedentary groups, followed by a progressive reduction in rate of weight gain at about day 9 as exercise intensity
FIGURE 5A. Food Intake of Hamsters Fed Low Cholesterol Diets

Data are group means ± S.D.
FIGURE 5B. Food Intake of Hamsters Fed High Cholesterol Diets

Data are group means ± S.D.
FIGURE 6A. Body Weights of Hamsters Fed Low Cholesterol Diets

Data are group means ± SD.
FIGURE 6B. Body Weights of Hamsters Fed High Cholesterol Diets

Data are group means ± SD.
increased. The pattern of weight change of individual animals within exercised groups showed considerable variation compared to their respective sedentary controls such that on day 21 the variance in body weights of the exercise groups was much greater than that of the sedentary groups. Low and high cholesterol-fed exercising hamsters had significantly reduced body weights on day 21 in comparison to their sedentary counterparts (p < 0.05). The latter observation was due to independent effects of both diet (p < 0.001) and exercise (p < 0.001). Greater body weight on study day 21 was observed in exercising hamsters consuming low cholesterol diets compared to those exercised and fed high cholesterol diets although their food consumption was slightly lower on average than the latter group. However, weight gain relative to body weight on day 1 (final body weight - initial body weight) of exercised hamsters was similar between the two groups on average, and caused by an independent diet (p < 0.001) and exercise (p < 0.001) effect in comparison to respective sedentary counterparts.

Percentage body fat of hamsters upon sacrifice is shown in Figure 7. No differences were observed between sedentary (20.8 ± 4.0%, X ± SEM) and exercised (20.6 ± 3.8%) hamsters fed low cholesterol diets. Similarly, percentage body fat of sedentary animals (25.9 ± 3.9%) consuming high cholesterol diets was not different from exercising hamsters (17.6 ± 2.9%) consuming high cholesterol diets.

ii) Organ Weights and Cholesterol Content

Table 1 contains values of liver and intestine weights and their total cholesterol content. Both dietary cholesterol (p < 0.001) and
Figure 7. Percent Body Fat of Hamsters

Data are group means ± SEM
### TABLE 1. Weights and Cholesterol Content of Liver and Intestine in Hamsters

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>Total Weight (g)</th>
<th>Total Cholesterol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Intestine</td>
</tr>
<tr>
<td>LOW CHOLESTEROL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sedentary</td>
<td>4.27 ±1.23</td>
<td>7.48 ±0.56</td>
</tr>
<tr>
<td></td>
<td>± 1.49 ±0.08</td>
<td>± 5.24 ±0.36</td>
</tr>
<tr>
<td>(n=12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>exercise</td>
<td>3.92 ±0.11</td>
<td>6.87 ±0.43</td>
</tr>
<tr>
<td></td>
<td>± 1.45 ±0.08</td>
<td>± 5.19 ±0.36</td>
</tr>
<tr>
<td>(n=11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIGH CHOLESTEROL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sedentary</td>
<td>5.24 ±0.14</td>
<td>24.62 ±2.95</td>
</tr>
<tr>
<td></td>
<td>± 1.51 ±0.03</td>
<td>± 6.57 ±0.37</td>
</tr>
<tr>
<td>(n=16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>exercise</td>
<td>4.63 ±0.15</td>
<td>22.37 ±1.70</td>
</tr>
<tr>
<td></td>
<td>± 1.55 ±0.29</td>
<td>± 6.46 ±0.49</td>
</tr>
<tr>
<td>(n=11)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **a** - significantly different from corresponding exercise group, \( p < 0.001 \)
- **b** - significantly different from high cholesterol-fed groups, \( p < 0.002 \)
- **c** - significantly different from high cholesterol-fed groups, \( p < 0.001 \)
- **d** - significantly different from high cholesterol-fed groups, \( p < 0.003 \)

Data are group means ± SEM
total cholesterol content. Both dietary cholesterol (p < 0.001) and exercise (P < 0.002) exerted independent effects on total liver weights. Specifically, among high cholesterol-fed animals, sedentary and exercise groups had significantly higher (p < 0.05) liver weights than those fed a low cholesterol diet. Independent of the latter effect, sedentary animals had overall higher liver weights (p < 0.05) than exercised animals, although this effect was largely due to exercise in the high cholesterol-fed group. Small intestine weights did not differ between any of the four experimental groups. When liver weight was normalized to total body weight, high cholesterol-fed animals again had a higher ratio of liver weight to body weight (0.041 + .002) (+ SEM) versus low cholesterol-fed hamsters (0.032 +.002). Total liver cholesterol content was not affected by exercise in either diet group, but was significantly higher (p < 0.001) in hamsters consuming the high cholesterol diet. Similarly, intestinal cholesterol content was increased (p < 0.002) by the high cholesterol diet, but was not affected by exercise.

iii) Plasma Cholesterol Levels

Plasma total and HDL cholesterol levels in hamsters are presented in Table 2. Plasma cholesterol was significantly and independently influenced by dietary cholesterol and physical activity. High cholesterol-fed animals had higher plasma total cholesterol (207.09 + 10.10 mg/dl) levels compared with low cholesterol-fed animals (108.84 + 5.17 mg/dl) (p < 0.001), regardless of the exercise state. Exercise was associated with a lowering of plasma cholesterol (p < 0.01) independent of diet treatment, however
### TABLE 2. Plasma Total and HDL Cholesterol of Hamsters

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>Plasma Total Cholesterol (mg/dl)</th>
<th>HDL-Cholesterol (mg/dl)</th>
<th>HDL:Total Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW CHOLESTEROL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sedentary</td>
<td>114.2 ± 4.12&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>89.7 ± 3.72&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>exercise</td>
<td>103.4 ± 6.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.9 ± 3.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HIGH CHOLESTEROL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sedentary</td>
<td>226.1 ± 9.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.9 ± 3.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.42</td>
</tr>
<tr>
<td>exercise</td>
<td>188.2 ± 10.42</td>
<td>84.5 ± 3.39</td>
<td>.45</td>
</tr>
</tbody>
</table>

<sup>a</sup> - significantly different from corresponding exercise group, p < 0.01

<sup>b</sup> - significantly different from high cholesterol fed groups, p < 0.001

<sup>c</sup> - significantly different from corresponding exercise group, p < 0.001

<sup>d</sup> - significantly different from high cholesterol fed groups, p < 0.004

Data are group means ± SEM (see Appendix C for sample size)
the larger decrease observed in high cholesterol-fed animals versus those consuming the low cholesterol diet might suggest an interactive effect \((p < 0.13)\) for this variable. HDL cholesterol, like plasma total cholesterol, did show an overall increase \((77.35 \pm 3.6\) to \(89.23 \pm 2.8\) mg/dl), \((p < 0.003)\) in response to dietary cholesterol and a decrease \((74.74 \pm 3.4\) from \(91.83 \pm 3.9\) mg/dl), \((p < 0.001)\) in response to exercise. More predominant however was the observed interaction \((p < 0.05)\) of these two factors on HDL cholesterol levels. Exercise appeared to lower HDL cholesterol significantly \((89.74 \pm 3.86\) to \(64.95 \pm 3.19\) mg/dl), \((p < 0.05)\) only if the low cholesterol diet was consumed. Consequently mean HDL cholesterol values for low \((89.74 \pm 3.86\) mg/dl) or high \((93.92 \pm 4.09\) mg/dl) cholesterol-fed sedentary animals were not different from animals in the high cholesterol exercise group \((84.54 \pm 3.56\) mg/dl).

iv) Cholesterol Synthetic Rate

Figure 8 shows the rate of incorporation of \(^3\)H\(_2\)O into newly synthesized cholesterol (nmol/hr.g) of liver and intestine in hamsters. Results indicate that although liver cholesterol synthesis was increased by dietary cholesterol \((356.64 \pm 41\) from \(240.15 \pm 28\) nmol/hr.g), \((p < 0.009)\) and decreased by exercise \((242.68 \pm 45\) from \(354.11 \pm 41\) nmol/hr.g), \((p < 0.02)\), it was significantly modified by the interaction of these two factors \((p < 0.013)\). Only when sedentary animals consumed high cholesterol diets did they have incorporation rates that were higher \((466.84 \pm 58\) nmol/hr.g) than high cholesterol exercise \((246.43 \pm 25\) nmol/hr.g), low cholesterol sedentary \((241.38 \pm 24\) nmol/hr.g), and low cholesterol exercise \((238.92 \pm 32\) nmol/hr.g), \((p < 0.05)\) groups. Thus exercise
FIGURE 8. Incorporation of $^3$H$_2$O Into Cholesterol in Liver and Intestine of Hamsters

Data are group means ± SEM
lowering of hepatic sterol synthesis was observed only in animals consuming high cholesterol diets (p < 0.05), even though the overall effect of adding cholesterol to the diet was to increase hepatic cholesterol synthesis as compared with low cholesterol-fed animals. In contrast to the liver, the cholesterol synthetic rate in the small intestine was affected differently by diet and exercise. Increasing dietary cholesterol significantly decreased intestinal sterol synthesis (768.20 ± 52 from 1162.47 ± 85 nmol/hr.g) while exercise had no effect (944.16 ± 52 vs 986.51 ± 71 nmol/hr.g), (p < 0.05).

Figure 9 illustrates the cumulative incorporation of $^3$H$_2$O into liver and intestine of hamsters. Animals fed the low cholesterol diet changed neither liver nor intestinal rates of cholesterol synthesis with exercise. Hence, the net amount of cholesterol synthesized in each group by liver and small intestine remained unchanged. In comparison to either of the low cholesterol-fed groups, high cholesterol-fed sedentary animals synthesized more cholesterol in liver, and less in intestine, parallelling the direction of change in synthetic rate observed in Figure 7. It appears as though the net increase in liver cholesterol production was larger in magnitude than the decrease in intestinal synthesis. As a result an increase in net cholesterol production in this group was evident. Exercise in the same group did not change intestinal sterol production, but decreased liver derived cholesterol to levels seen in the low cholesterol-fed group.

Figure 10 shows cholesterol synthesis in liver and intestine expressed as a fraction of the total cholesterol content within each respective tissue. High cholesterol-fed animals showed lower hepatic
FIGURE 9. Cumulative Incorporation of $^3\text{H}_2\text{O}$ Into Cholesterol of Total Liver and Intestine in Hamsters

Data are group means
FIGURE 10. Newly Synthesized Cholesterol Expressed as a Fraction of Total Cholesterol Content in Liver and Intestine of Hamsters

Data are group means
sterol synthesis (0.22 ± 0.37 mg/g), (p < 0.001) compared with low cholesterol-fed animals (1.36 ± 0.15 mg/mg tissue cholesterol). No effect of exercise in this group was observed on cholesterol synthesis. Diet similarly reduced intestinal cholesterol synthesis (6.30 ± 1.05 from 12.99 ± 1.39 mg/mg tissue cholesterol), (p < 0.001) but no effect of exercise was evident.

Covariate analysis of factors including body weight gain, food consumption and dietary fat content did not significantly influence the values obtained for synthetic rate (nmol/hr.g) between exercised and non-exercised groups. In addition, normalization of synthetic rate to lean body mass did not significantly alter the magnitude or direction of the values obtained for hepatic or intestinal sterol synthesis in Figure 8.
DISCUSSION:

i) Body Weights and Food Intake

Body weights of sedentary animals fed high and low cholesterol diets increased at a similar and constant rate even though food intake progressively declined over the study period. Similarly exercised animals, although consuming less food than their sedentary counterparts, gained weight at a relatively constant rate until the last week of study where body weights plateaued. These results were generally in agreement with previous data showing a constant increase in body weight with age, even though food intake tended to diminish (65). Although not measured in the present study, it has been suggested that in hamsters, both somatic growth and fat storage are responsible for this weight gain over time. It was not expected however that exercised animals would consume less food than sedentary groups.

Freely running hamsters normally consume more food compared with sedentary controls of similar age (66). Given this observation, it was thought that pair-feeding exercised animals to sedentary controls, combined with manipulation of energy expenditure, would result in relatively constant body weights of exercising hamsters over the study period, counteracting the normal rate of weight gain in sedentary animals. This design was used in order to eliminate any confounding by weight gain that might have clouded true exercise effects on cholesterol synthesis or other variables. Constant body weights, however, were not achieved in exercise groups until the last study week, when exercise intensity
highest. During the first study week increases in weight gain of exercised groups were similar to sedentary animals. One would have expected exercised hamsters, with lower food intakes and ramped levels of exercise intensity, to have immediately shown a deceleration in weight gain. It has been shown, however, that hamsters respond to disturbances in energy balance not by changing patterns of food ingestion, but by decreasing basal metabolic rate (BMR) (67). For example, an animal weighing 10% less than its pair-fed control would be expected to decrease metabolic rate by 25% (67). Thus exercising hamsters may have been able to compensate initially for increases in energy expenditure by lowering BMR. During the penultimate and final weeks of the study, BMR changes may not have been sufficient to overcome the energy deficit imposed by the intensity of the exercise regimen. BMR was not tested throughout the study to support this theory, however maintenance of consistent body weights was achieved on average, in exercised groups as originally intended.

A possible improvement in the study design would have been to have included an exercise group in which animals were allowed free access to food. Such a group would, in addition to providing information regarding the changes in lipid metabolism due to weight gain as described above, be more representative of a free-living human subject undergoing exercise. It became obvious that including an ad libitum fed exercise group would not have been different from the pair-fed regime used. This was because the exercise groups, although pair-fed, did not always ingest all their food as anticipated. In addition, the exercise group fed the low cholesterol diet gained less weight than the exercise group fed the high cholesterol diet, even though the latter group consumed more food on average. It is likely that the stress imposed by running, or perhaps
longer sleep periods and less feeding time in this group, caused a decrease in food intake by exercised animals. However, neither dietary fat nor total food intake differences between any experimental group exerted significant effects on measured parameters of lipid metabolism as tested with analysis of covariance procedures.

ii) Body Composition, Organ Weights and Organ Cholesterol Content

Percentage body fat was not different between sedentary and exercise groups, although a trend towards lower values was noted in the high cholesterol-fed exercise group. These results were in opposition to studies which showed a decrease in body fat of exercising hamsters relative to sedentary controls (65,66). Subjective visual examination at sacrifice suggested less fat was deposited in the viscera of exercised hamsters compared with sedentary counterparts. The indirect method of water dilution space used to determine percentage body fat is well proven (61,62). Thus, procedural problems may have been a cause for the variability in observed body fat within groups. It was noted on occasion that, following intraperitoneal injection, a drop of tritiated water would emerge from the injection site after removal of the needle. This would have the effect of increasing the apparent amount of unabsorbed label thus reducing the apparent body fat content. If such loss of label occurred in a non-random fashion, body composition differences between groups may have been obscured by the large variability.

Liver weights were increased in high cholesterol-fed animals, but decreased in exercised animals of either diet treatment. Cholesterol
content of liver was also increased in high cholesterol-fed animals, but was not affected by exercise in either the high or low cholesterol-fed groups.

The increased liver weights in high cholesterol-fed animals were likely due to accumulation of cholesterol in this tissue (71,72). Higher observed rates of hepatic cholesterol synthesis would also have contributed to the increase in tissue cholesterol and weight. It is not known whether greater fat deposition occurs in the liver when high cholesterol diets are consumed, but this may also have occurred. Prolonged exercise in excess of one hour causes depletion of liver glycogen stores (69,70). Moreover, exogenous glucose is taken up predominantly by muscle to replete its glycogen stores, rather than liver as in the resting state (70). Perhaps the decrease in liver weights of exercised hamsters was due to loss of glycogen and not to increased removal of exogenous cholesterol from this tissue.

In contrast to liver, intestine weight was not increased in animals consuming the high cholesterol diet. The small intestine is a medium through which cholesterol enters the body and although this organ assists in regulation of whole body sterol balance, it is not a center for processing or storage of cholesterol. Lack of an exercise effect on intestine weight was consistent with the knowledge that intestine, unlike the liver, does not store glycogen and is not responsible for utilizing glycogen as an energy source to regulate energy balance during exercise. Even though total weight of the intestine did not change in high cholesterol-fed animals, cholesterol content was significantly elevated. In addition to absorption of exogenous free cholesterol, 7% of circulating LDL is taken up by the small intestine (19). Thus a transient increase of
cholesterol content by exogenous absorption, as well as a net increase in uptake of existing elevated plasma LDL levels may have caused the total cholesterol content of this tissue to rise without appreciably changing total tissue weight.

iii) Total Plasma Cholesterol

In the present study hamsters consuming a high cholesterol diet exhibited total plasma cholesterol levels 48% higher than those of low cholesterol-fed animals. While exercise had no effect on plasma total cholesterol in the latter group, a 15% decrease was seen in plasma total cholesterol in high cholesterol-fed exercised hamsters. These results were similar to other findings of either cholesterol induced increases (20,72,71) or exercise induced decreases (22,11,12) in total plasma cholesterol levels. Presently, no interactive effect between dietary cholesterol and exercise on this variable was observed.

Cholesterol feeding has been specifically shown to cause an increase in net intestinal cholesterol absorption, followed by a dose-dependent suppression in rates of receptor-mediated hepatic LDL clearance, and a reciprocal increase in plasma LDL concentration (21). In the hamster, approximately 45% of circulating cholesterol is carried in the LDL subfraction, while HDL carries nearly 50% (72). In the present experiment it was reasonable to assume that LDL was responsible for the observed increase in plasma cholesterol, since the magnitude of change of HDL would not have accounted for the large increase in total plasma cholesterol. HDL increased in plasma of high cholesterol-fed animals by 14% when compared
to the low cholesterol-fed group. This increase may have resulted from an increased production of HDL precursors, most likely nascent HDL from liver and intestine, and chylomicron or VLDL remnants. An increase in HDL production may reflect the body's attempt to regulate whole body cholesterol balance, since a primary function of HDL is to carry excess cholesterol from the periphery to the liver via "reverse cholesterol transport", previously described.

The direct effect of cholesterol feeding on total plasma cholesterol level may have been exacerbated by the addition of corn oil to the diet. Suppression of LDL receptor synthesis and intracellular cholesterol ester formation, caused by fat feeding, have been shown to elevate plasma LDL levels (21). Also, carcass cholesterol content increases in exercised versus sedentary animals (25). Thus reduction in plasma cholesterol might have been expected in the low cholesterol exercise group, if cellular demands for cholesterol were increased during exercise. However, only a trend towards lower plasma cholesterol was observed. As with hepatic sterol synthesis in the latter group, it is likely that plasma levels were maintained even though pools may have been depleted. It would have been useful to extend the duration of the study to evaluate the ability of balance mechanisms to preserve plasma cholesterol levels.

The exercise induced lowering of plasma cholesterol in high cholesterol-fed hamsters paralleled the decrease in hepatic synthetic rate observed within the same group. Although the magnitude of change in plasma cholesterol was less than synthetic rate (19% vs. 53%) the change in level might be explained by a reduction in synthesis. Limited substrate availability induced by exercise activity may result in proportionately less cholesterol synthesis by the liver. Even though dietary cholesterol
would be present in high enough quantities to counteract this effect, increased peripheral membrane and tissue demand for cholesterol, imposed by the exercise paradigm, may have increased its influx to these compartments. As a consequence the molar ratio of free cholesterol to cellular components, such as the phospholipid component of membranes, may have been reduced, causing an efflux of cholesterol from the plasma into the periphery.

iv) Plasma High Density Lipoprotein Cholesterol Levels

HDL cholesterol (HDL-C) concentration in plasma showed a net increase in animals fed the high cholesterol diet, compared to low cholesterol-fed animals, although the ratio of HDL:total plasma cholesterol decreased by 41% in the latter group. In addition, exercise caused an overall decrease in plasma HDL levels independent of diet. The main effect however, was the observed interaction between diet and exercise where exercise lowered HDL cholesterol only when animals consumed the low cholesterol diet. In no instance did exercise independently or through an interaction with dietary cholesterol, increase HDL cholesterol levels. Since exercise only slightly increased HDL cholesterol levels in high cholesterol-fed hamsters, the disproportionately large increase in plasma cholesterol levels was most likely responsible for the decreased ratio of HDL-C to total plasma cholesterol in this group.

Failure of exercise to directly increase plasma HDL-C was consistent with results seen in similar exercise studies. Tsai et al. (22) observed no difference in either HDL-C or HDL-C:total plasma cholesterol ratio in
hamsters run voluntarily for 30 days and fed low cholesterol diets. Similarly, exercise did not alter HDL-C:total plasma cholesterol ratio in exercised rats fed high or low cholesterol diets (73,17). Pels et al. found a significant decrease in HDL-C, but no change in HDL-C:total plasma cholesterol ratio in rats fed high cholesterol, high fat diets and trained to run at 70% and 85% of VO_{2} max in comparison to sedentary controls (11). These and other studies in rats (73,17) have consistently shown lower HDL-C in exercised animals even though the inter-study exercise conditions have varied considerably.

In the present study, the observed interactive effect of diet and exercise on HDL-C was intriguing, when one considers the independent effects of exercise and dietary cholesterol levels. If exercise were decreasing endogenous cholesterol synthesis by reducing substrate availability, then it is possible that exercising animals fed low cholesterol diets might be in negative cholesterol balance. Increased cellular demands for cholesterol would not be met by the low cholesterol content of the diet and consequently no compensation for a decrease in cholesterol synthesis could be achieved. The formation of HDL from VLDL and chylomicron remnants would be reduced under the pair-fed imposition of both energy and dietary cholesterol simply due to a decrease in their production. Thus the amount of excess circulating cholesterol would be less and the functional need for HDL to remove cholesterol attenuated. Furthermore, if HDL-C does slow LDL-mediated internalization of cholesterol into the cell, then its presence would not be desirable under conditions inducing cellular cholesterol deficit. Thus, although exercise did not independently increase HDL-C, the interaction between dietary cholesterol and exercise was significant. When considered together with
the observation that plasma total cholesterol was reduced with exercise,
this finding suggests that the changes in HDL cholesterol may be secondary
to changes in plasma cholesterol and may only reflect their direct
functional importance in sterol metabolism, not an independent effect of
exercise.

No difference in percentage body fat was noted between exercised and
sedentary hamsters. Therefore the effect of greater fat loss and increased
lipoprotein lipase activity often observed with exercise training and
thought to indirectly cause an increase in circulating HDL levels (55),
cannot be evaluated in the present study.

One might conclude from the present and aforementioned studies that
HDL production is not increased by physical exercise per se. It has been
shown that development of atherosclerotic lesions was attenuated in
exercised rats compared with sedentary controls even though average HDL-C
concentrations were lower and HDL-C:total plasma cholesterol remained
unchanged. These data suggest that although exercise may beneficially
retard lesion development, the effect may not be directly mediated by
changes in the level of HDL as previously suggested.

It was thought that the hamster would be a useful model with which
to study human cholesterol metabolism. There is increasing evidence that
the hamster is more suitable than the rat for studies of cholesterol
metabolism since endogenous synthetic rate (2.5 mg/day/100 g body weight)
resembles that of the human (1 mg/day/100 g body weight) more closely than
the rat (12 mg/day/100 mg body weight) (18).

Studies in humans have shown a dose-response relationship between the
amount of exercise training and degree of change of plasma HDL (74).
Unfortunately many of the studies in this area cannot distinguish between true chronic adaptive changes in lipoproteins from an acute or transient response, due to poor control on intensity, duration and type of exercise, state of training, dietary intakes and baseline measurements. Consequently the evidence for (75,76,77) and against (78,79,80) a positive effect of exercise on HDL in humans is controversial and difficult to evaluate. It might have been useful in the present study to have determined baseline HDL-C values for each hamster at the start of the study so as to have measured the magnitude of change in each animal. This approach might have eliminated some of the variability in absolute plasma HDL cholesterol levels measured. Human studies however have failed to show any significant correlations of baseline HDL-C with degree of HDL-C change (74). In addition, it is possible that the study was of insufficient duration for adaptive changes to have occurred. Many human studies that support an exercise-induced increase in HDL cholesterol examined exercising athletes over many years.

Thus these data indicate that exercise does not increase HDL-C levels independently in the hamster, an observation that may serve to dispute the notion that exercise induces changes in HDL that directly, or indirectly decrease the risk of cardiovascular disease. Hamsters did however appear to respond similarly to humans consuming high fat and cholesterol diets, by increasing slightly the production of HDL (75), although the percentage of dietary fat consumed in the human studies was greater than the present study.

v) Fractional Synthetic Rate of Cholesterol

Cholesterol synthesis expressed as that fraction of tissue
cholesterol which was newly synthesized showed a significant decrease in both liver and intestine in response to the high cholesterol diet, while exercise had no effect on this variable. Fractional synthetic rate provides a measure of how synthesis of cholesterol within a defined period relates to the freely exchangeable pool of cholesterol within specific tissues.

Previous studies have shown that hamsters respond to dietary cholesterol by increasing organ uptake of cholesterol, primarily as LDL, with consequent organ weight gains (81). The observed decrease in FSR of intestine was most likely due to both the reduction of endogenous cholesterol synthesis, as well as increased uptake of cholesterol into this organ. Since rates of LDL uptake were not measured, it is impossible to tell whether cholesterol uptake was larger in this organ than in liver. It is however reasonable to assume that the larger % increase (19 vs 2%) in organ weight of liver over intestine of high cholesterol-fed animals was due mainly to greater accumulation of free cholesterol in this tissue. Approximately 25-50% of liver weight gained could be directly attributable to an increase in cholesterol content. The magnitude of this increase must have been larger than the increase in liver synthetic rate, since the trend favoured a reduction in FSR in high cholesterol-fed animals. In addition, the observed decrease in average body weight of exercised animals in comparison to sedentary controls did not affect liver weight, supporting the idea that cholesterol was the primary contributor to increased liver weights in high cholesterol-fed animals.

In the hamster, the small intestine might be considered as more important compared with liver in regulating cholesterol balance on a low
cholesterol diet for several reasons. Firstly, basal synthetic rates in intestine were twice as high as those of liver, regardless of dietary cholesterol content. Improvement on techniques for evaluating cholesterol synthesis have indicated that a much larger proportion of whole body synthesis of cholesterol occurs in the small intestine, in both man and hamster (18), than previously believed. These findings are supported by the notion that the intestine is the site at which the body first contacts cholesterol and thus attempts to regulate its balance. The liver may be quantitatively less important when intake of cholesterol is low. In this situation, exercise apparently exerts no significant alteration of sterol synthesis in either organ.

vi) Hepatic and Intestinal Cholesterol Synthesis

Dietary cholesterol content and exercise activity were observed to cause an increase and decrease respectively in the average rate of hepatic cholesterol synthesis (nmol/hr.g) in hamsters. Specifically, dietary cholesterol increased hepatic synthesis in high cholesterol-fed animals, while exercise had the opposite effect. Exercised hamsters fed the low cholesterol diet however did not alter hepatic or intestinal cholesterol synthesis. Such inconsistency between group responses to dietary cholesterol and physical activity was explained by their significant interaction, through which the various diet and exercise levels exerted more detectable physiological effects in combination, than when considered alone.

The interactive effect of diet and exercise was most obvious in livers of high cholesterol-fed animals, where sterol synthesis was lowered
in response to exercise. Explained differently, only sedentary animals consuming a high cholesterol diet showed increased rates of tritium incorporation into liver tissue. Hamsters fed the low cholesterol diet did not alter hepatic sterol synthesis in response to exercise.

Very few studies have examined the effect of prolonged physical exercise on hepatic sterol synthesis in animals and it appears that an interaction between diet and exercise factors on this variable has not previously been tested. Takashi et al. (13) used [14]C-mevalonate to measure cholesterol synthesis in rats that were exercised at 60-75% \( \text{VO}_2 \text{max} \) for two weeks and fed ad libitum diets with no added cholesterol. A significant increase was observed in hepatic sterol synthesis of exercising rats in comparison to sedentary controls. This result was not consistent with those of exercising hamsters in the present study where no change in hepatic sterol synthesis occurred in response to exercise in the low cholesterol group. Valid comparison with the latter study becomes difficult for several reasons as stated in the Introduction. Hence the ability of a rat to adapt to disturbances in energy balance that might influence cholesterol formation make it an incomparable model to the hamster (20), which more closely resembles the human (18) in this regard.

vii) Possible Mechanisms

An explanation of the mechanisms of diet and exercise induced effects, independently and interactively, requires examination of factors which are primary regulators of body sterol balance. These include a) cholesterol absorption, b) endogenous sterol synthesis, c) bile acid
formation and d) hormonal control.

a) Intestinal Absorption and Cholesterol Homeostasis

Intestinal absorption of cholesterol was not measured in the present study, however it is unlikely that exercise caused a decrease in absorption of cholesterol from the small intestine of low cholesterol-fed animals. In general, the effect of intense physical activity over the long term would be to increase membrane turnover, particularly in the gastrointestinal tract (82) and thus the requirement for cholesterol. This would be expected to cause either an increase or no change, in absorption efficiency of cholesterol in the absence of any other exercise induced intestinal disturbance. If, however, an increase in absorption had occurred in low cholesterol exercised hamsters, the amount would have been small given the low cholesterol content of the diet, and probably not have inhibited hepatic or intestinal sterol synthesis significantly. In contrast, high cholesterol-fed animals should have responded differently to the large influx of cholesterol from the diet whether exercised or not.

Dietary cholesterol is known to cause an increase in net absorption of cholesterol from the small intestine (19). Although the rate of absorption tends to plateau as dietary concentration increases, it has been shown that up to 40% of circulating cholesterol in the plasma can be from exogenous sources (83). The inhibition of hepatic and intestinal cholesterol synthesis in response to dietary cholesterol has been seen in the hamster (20) and other animals (48), although the magnitude of the response to dietary cholesterol is often less in the intestine than in the liver (48,84). In any event, the expected increase in absorption of
cholesterol due in large part to excess dietary cholesterol should have inhibited both hepatic and intestinal sterol synthesis in this group (20). Thus it was intriguing to find that only the small intestine decreased synthetic rate in response to dietary cholesterol, whereas increases were seen in liver.

Intestinal synthesis of cholesterol occurs primarily in endothelial crypt cells to replenish cell membranes of rapidly proliferating gastrointestinal tissue. Cell villi also synthesize cholesterol, most likely to stabilize chylomicrons and other lipoproteins during triglyceride absorption (85). Reduction of intestinal sterol synthesis occurs directly by feedback inhibition of HMG CoA reductase by the presence of free cholesterol from the diet and bile acids (85). The mechanism for decreased HMG-CoA reductase activity may include both an immediate inactivation of preformed enzyme and a longer term reduction of enzyme synthesis (86). As well, subsequent enzymes in the synthetic pathway between mevalonate and squalene may also be reduced with prolonged dietary cholesterol feeding (85). In the present experiment, feeding a high cholesterol diet most likely caused a net increase in cholesterol absorption and subsequent increase in the formation of chylomicron remnants, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and bile acids. These components inhibit HMG CoA reductase (30) and endogenous synthesis of cholesterol in the intestine.

b) Endogenous Cholesterol Synthesis

It has been established in hamsters that increases in the hepatic
cholesterol pool following cholesterol feeding result in compensatory regulation of body cholesterol balance in several ways. Firstly, hepatic and intestinal cholesterol synthesis is reduced. Secondly, secretion by liver of newly synthesized and absorbed cholesterol into bile or bile acids is increased. As well, suppression of hepatic sterol synthesis is thought to be stimulated by an increase in receptor-dependent LDL uptake (21,30). It was therefore surprising that an increase in hepatic synthesis was observed in response to dietary cholesterol. Other experimental manipulations in hamsters have elicited similar increases in hepatic sterol synthesis. It was found that triglyceride feeding in hamsters disrupted the classical metabolic responses to cholesterol feeding in the liver. LDL receptor activity, cholesterol ester formation and cholesterol synthesis were affected (21). For example, with cholesterol feeding, elevated saturated triglyceride intakes have been shown to increase rates of hepatic sterol synthesis in situations where receptor-dependent LDL transport was suppressed. Furthermore, this response was shown to increase as the ratio of cholesterol to fat in the diet increased, and more importantly, to be reversed if a diet low on these lipids, or with a lower ratio, was fed (21). Thus in the present study, moderately elevated fat intakes may explain why only high cholesterol-fed sedentary animals, with a higher ratio of cholesterol to fat exhibited elevated rates of liver sterol synthesis compared with low cholesterol groups.

The mechanism for this response is speculative. It has been suggested that saturated fat, being a poor substrate for esterification reactions, results in lower accumulation of intercellular cholesterol esters, causing a decrease in the synthesis of LDL receptors disproportionate to need (21). Normally, synthesis of the LDL receptor itself is under feedback
regulation so that its activity, hence the amount of cholesterol entering the cell, is inversely proportional to cellular cholesterol content (30). The ability of cells to accumulate cholesterol may be the essential element in regulating sterol synthesis. Thus, under the presently described condition, regulatory pools may react to decreased receptor synthesis and activity by compensatory increases in cellular rates of sterol synthesis in the face of elevated LDL cholesterol (21). In addition, high fat diets have been shown to elevate plasma cholesterol as dietary cholesterol content increases (87). Direct application of this mechanism to results of the present study however remains difficult since corn oil, a predominantly unsaturated fat, was consumed with two levels of dietary cholesterol. Unfortunately the effect of corn oil feeding in conjunction with cholesterol was not examined in the study by Spady et al. (21). As well, the level of fat in this study was moderately high (8%) but not as high as the former study (20%).

An alternative explanation for the large increase in hepatic sterol synthesis of high cholesterol-fed animals was that the liver may have responded to the large decrease observed in intestinal cholesterol output by increasing its own sterol synthesis, independent of normal regulatory processes. Surprisingly, the total amount of cholesterol synthesized in this group was 20% higher than any other group, even though intestinal synthesis was dramatically reduced. Such an overcompensation, with no obvious metabolic control, has been seen in obese hamsters, or those suffering from essential fatty acid deficiency, however the reason for this was unclear (88). Although animals in the present experiment were not obese or presumably not suffering from amino acid deficiency, one might
conclude that hamsters are generally susceptible to alterations in liver sterol synthesis under a variety of experimental conditions.

If the unexpected result in hepatic sterol synthesis was not physiological and instead caused by some unknown, uncontrolled variable, then one would have expected to see similar responses in the low and high cholesterol-fed sedentary groups. Since this was not observed, and since dietary cholesterol was the only altered variable between the two sedentary groups, it is possible that the ratio of dietary cholesterol and dietary fat, which increased on the high cholesterol diet, was a true cause for the difference.

In addition, it is conceivable that some of the experimental animals were unduly exposed to stress. Physiological stress is a condition known to cause an increase in hepatic cholesterol synthesis through the action of epinephrine (88) and may have been an overriding factor in causing uncharacteristic cholesterol synthesis in the liver. Sedentary animals fed the high cholesterol diet were handled a great deal on the last study day, and although the level of handling was not different from that received by the exercise group, sedentary animals were not handled nearly as much as those of the exercise group earlier during the experimental period. Thus excessive handling or disturbance of normal daily patterns may have caused the unexpected, and possibly transient response in livers of the sedentary, high cholesterol-fed group. Why low cholesterol-fed sedentary animals would not have similarly responded is not clear.

Elevated liver sterol synthesis in response to cholesterol feeding may well be explained by the high ratio of cholesterol to fat in the diet. However, elucidation of the interactive mechanism by which liver lowered cholesterol synthesis in high, but not low cholesterol-fed animals,
remains difficult. Understanding how a diet and exercise interaction produced this effect may be facilitated by first examining why exercise, in absence of dietary cholesterol effects, caused a fall in liver synthesis in the high cholesterol-fed group.

A possible mechanism by which exercise may reduce liver cholesterol synthesis is through limiting the availability of cholesterol precursor substrate during exercise. Formation of HMG CoA requires an adequate supply of precursor acetyl CoA in the cell cytosol, and therefore inhibition of this supply of acetyl CoA would depress the rate of sterol synthesis. Prolonged exercise, as described earlier, causes diversion of acetyl units into pathways of energy metabolism and fails to provide an abundance of this substrate for fatty acid or sterol synthesis. It has indeed been hypothesized that HMG CoA reductase, the rate-limiting enzyme for cholesterol synthesis, is not normally saturated with substrate in the cell. Consequently the activity of this enzyme may be sensitive to changes in the amount of acetyl CoA available for sterol synthesis (89). When metabolic changes occur during exercise which decrease acetyl CoA flux, the activity of HMG CoA reductase may also decrease, resulting in a change of similar magnitude and direction in cholesterol synthetic activity.

Furthermore, the source of acetyl CoA units could be an important determinant of sterol synthetic rate. When acetyl units are available in the cell cytosol, they are most likely derived from acetate by acetyl CoA synthetase (30). The latter is important since it has been shown that even this supply of acetyl CoA, considered secondary to citrate-derived acetyl units, is preferentially used for fatty acid synthesis and not cholesterologenesis (90). Figure 11 illustrates that cell preparations
exposed to (-)hydroxycitrate, an inhibitor of acetyl CoA transfer from the inner mitochondrial membrane to the cell cytosol via citrate, showed decreases in both fatty acid and cholesterol synthesis. When acetate, a potential acetyl CoA precursor, and (-)hydroxycitrate were added simultaneously (Table 3), both fatty acid and cholesterol synthesis increased, however the relative contribution of acetyl units to sterol synthesis was reduced (90).

Conversely, it has been shown that the high levels of citrate that are generated during situations of positive energy balance, co-ordinate glycolysis and lipogenesis by inhibiting phosphofructokinase and activating acetyl CoA carboxylase. It may be concluded from these observations that the source of acetyl CoA for sterol synthesis is highly dependent on the abundance of citrate in the cell. Prolonged exercise causes diversion of pyruvate, a potential precursor of cytosolic acetate, through the glycolytic pathway for the formation of inner mitochondrial acetyl CoA. This diversion is responsible, in part, for the increase in tricarboxylic acid cycling, and decreased availability of citrate during exercise. Thus lack of available citrate, and acetate, may have contributed to the presently observed decrease of hepatic sterol synthesis. The fact that cholesterol formation decreased in liver, but not intestine is consistent with the latter hypothesis, since the small intestine, in contrast to the liver, is not responsible for regulation of energy substrate metabolism during prolonged exercise.

In addition to the immediate metabolic changes associated with an acute bout of exercise, it is possible that long term enzymatic changes may also have been induced by exercise, resulting in depression of hepatic
FIGURE 11. Influence of (-)-hydroxycitrate on the rate of cholesterol and Fatty Acid Synthesis in Perfused Rat Liver

40 min after operation $^3$H$_2$O was added to the perfusion medium. 30 min thereafter a control liver sample was taken. Different amounts of (-)-hydroxycitrate were added and perfusion continued for another 60 min, after which 2 additional liver samples were taken. Values represent means, vertical bars: ± S.E.M.

TABLE 3. Influence of Acetate plus (-)-hydroxycitrate on cholesterol and Fatty Acid Synthesis in Perfused Rat Liver

<table>
<thead>
<tr>
<th>Series</th>
<th>Acetate (-)-Hydroxycitrate</th>
<th>Cholesterol Incorporation $^3$H$_2$O into g, wet wt./hour</th>
<th>Fatty acids Incorporation $^3$H$_2$O into g, wet wt./hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10 mM</td>
<td>2.27±1.02 (5)</td>
<td>25.90±1.54 (4)</td>
</tr>
<tr>
<td>B</td>
<td>10 mM</td>
<td>4.02±1.33 (6)</td>
<td>18.31±1.16 (5)</td>
</tr>
</tbody>
</table>

Series A: Experimental design as in fig. 3; after a 30 min perfusion period without addition (line 2) (-)-hydroxycitrate was added together with acetate. For further details, see 17. Series B: In a comparable set of experiments the influence of 10 mM acetate alone on lipid synthesis was determined. Means ± S.D. from two series of experiments are given. Number of measurements in brackets.

(Decker and Barth (90))
sterol synthesis. The mechanism of this adaptation might be similar to that seen during fasting, since the latter shows parallel physiological responses to exercise with respect to energy metabolism. Specifically, conversion of squalene to cholesterol has been shown to be reduced in 24-72 hour bouts of fasting in rats, due to decreased supply of substrate and consequent decreased activity of the enzymes in this pathway (30).

A difficulty of the substrate hypothesis arises when trying to explain, in isolation from other factors, why no drop in synthetic rate was seen in exercise versus sedentary low cholesterol-fed animals. If lack of available substrate for cholesterol formation alone could lower its synthetic rate, then one would have expected to see similar results in exercised animals, regardless of dietary cholesterol content. This is where the availability of dietary cholesterol may become rate limiting in combination with exercise, and explain through an interaction of these two variables the observed results. Lack of an exercise effect may have reflected an inability of cholesterol regulatory mechanisms to further suppress basal rates of cholesterol synthesis beyond "normal" rates, as they do with exposure to dietary cholesterol.

One reasonable explanation might be that exercise, in low cholesterol-fed animals, did indeed have the effect of lowering cholesterol synthesis, but since there was no appreciable excess of dietary cholesterol with which to compensate this effect, the liver and possibly intestine may have responded by trying to increase endogenous synthesis to replete body pools. It has been shown that exercise may affect cholesterol turnover rate, and lower the tissues' cholesterol pool (91). Thus the net effect of a compensatory increase in sterol synthesis, when combined with an exercise lowering effect would be no change in
cholesterol synthesis, which was the current observation.

One might also speculate that failure to decrease cholesterol synthesis in the tissues studied was elicited as a sparing effect on the existing low body cholesterol pool. Cytosolic acetyl CoA, through adaptation of rate-limiting enzyme activities, may have been shunted through pathways of sterol metabolism at a higher rate than normal, in an attempt to maintain basal rates of sterol production. In addition, there may have been some down regulation of hepatic LDL receptors, in an attempt to reduce the sensitivity of cholesterol feedback inhibition and preserve the rapidly depleting cholesterol pool. The exact mechanism of such a response however cannot be defined.

c) Bile Acid Synthesis and Homeostasis

The formation of bile acids serve as an important excretory pathway for cholesterol. An inverse relationship between 7α-C-hydroxylase, the rate limiting enzyme for bile acid formation, and the activity of HMG CoA reductase in the liver has been hypothesized. Hence when dietary cholesterol levels are high, HMG CoA reductase is inhibited, and 7α-C-hydroxylase facilitates formation of bile acids (92). Similarly, when bile acid production is low, an increase in hepatic sterol synthesis occurs. It therefore becomes difficult to explain on the basis of bile acid response to dietary cholesterol alone, why an increase in hepatic cholesterol synthesis was observed in animals presently fed the high cholesterol diet. The interaction between diet and exercise may better explain this occurrence. First, in low cholesterol-fed animals bile acid production
would be relatively low and produced from both endogenous and exogenous sources if needed for digestion and absorption of dietary fat. During exercise, the exercised group may have had increased cellular demands for cholesterol, a stimulatory response for hepatic sterol synthesis in addition to the direct effects of $7\text{C}$-hydroxylase. If, however, substrate availability were limiting in the formation of cholesterol, then no net change in synthesis would occur, as was observed. Unfortunately the converse argument for high cholesterol-fed animals, where elevated bile acid production would inhibit sterol synthesis along with dietary cholesterol, does not explain the observed increase in hepatic sterol synthesis of the sedentary group. Had the liver responded to the high cholesterol load by decreasing its endogenous synthesis, then further suppression of hepatic synthesis seen in the exercise group might have been attributed to physical activity through reduced substrate availability. One might speculate that in the present experiment bile acid feedback inhibition on hepatic sterol synthesis may have become uncoupled, however how this might occur is not clear.

It has been observed that alterations in hepatic synthesis in hamsters are not directly related to the cholesterol content of bile under certain experimental conditions (93). Thus the assumption that changes in bile acid cholesterol content preceeded any changes in sterol synthesis in the present study may be incorrect. It is possible, that liver cholesterol synthesis responded independently of bile acid formation, as well as dietary cholesterol level, as suggested earlier.

Both plasma cholesterol level and cholesterol synthesis changed in similar directions under the present diet and exercise manipulations. Parallel responses between hepatic cholesterol synthesis and plasma
cholesterol are not commonly reported. Decrease in hepatic synthesis generally occurs in response to high cholesterol feeding to maintain the balance between plasma cholesterol and normal body pools (94). This situation usually results in no change, or an increase, in plasma cholesterol depending on the level of cholesterol in the diet. Similarly, an increase in hepatic synthesis usually occurs in response to lack of dietary cholesterol, in an attempt to restore plasma cholesterol to normal values (48). Thus failure of the hamsters under the present experimental conditions to elicit the classical response of reduced liver cholesterol synthesis in the face of elevated plasma cholesterol suggests that high cholesterol and fat feeding acts independently of exercise on these parameters of lipid metabolism. This was suggested for bile acid control of sterol synthesis.

d) Hormonal Factors Controlling Cholesterol Synthesis

Among the factors which may have resulted in the observed responses of hepatic sterol synthesis in the present study are short term hormonal influences. Insulin is known to stimulate cholesterologenesis and may be partly responsible for maintaining the normal diurnal rhythm of HMG-CoA reductase (95,50). In contrast, glucagon inhibits cholesterol synthesis, an effect mediated by cyclic AMP, which diminishes the activity of HMG CoA reductase by enhancing reductase kinase activity (50). Insulin and glucagon are thought to control both the amount of enzyme present, as well as the proportion of the enzyme in the active state (50). It is well established that the hormonal response to exercise is characterized by a
fall in plasma insulin and rise in plasma glucagon (28,56). Both hormones are essential regulators of glucose metabolism and may be involved in the allocation of acetyl CoA units for use in energy purposes during exercise, instead of sterol synthesis. During post-exercise recovery, insulin rises rapidly to enhance precursor utilization for glycogen repletion (70). In contrast, glucagon remains high after exercise and maintains hepatic uptake of gluconeogenic precursors (35). Even though the relative concentrations of these hormones change, the net effect is to divert energy towards glycogen repletion via gluconeogenesis, which inhibit the transport of acetyl CoA into the cytosol for cholesterol synthesis.

In addition, chronic exercise may suppress the response of insulin to rising blood glucose. Since insulin exerts control over the activity of HMG CoA reductase and cholesterol synthesis, long term adaptive changes might serve to keep sterol synthesis chronically lower in exercising animals. In the present study however, the secular trend of this variable was not measured.

Growth hormone and epinephrine, two additional hormones known to be elevated during exercise (28), have been shown to stimulate cholesterol synthesis by stimulating HMG CoA reductase activity (95). Growth hormone may also increase hepatic synthesis of cholesterol by augmenting thyroid function (95). In hamsters, exercise has been shown to increase secretion of growth hormone and somatic growth (96). In this study, however, animals were allowed to run voluntarily and were fed ad libitum. It has since been shown that if exercising hamsters are pair-fed to sedentary controls, then the increase in somatic growth is prevented (97). Thus the effects of growth hormone on sterol synthesis in the present study were probably not significant.
Epinephrine is known to be elevated during both the exercise and post-exercise recovery periods (33). Although epinephrine is thought to stimulate cholesterol synthesis by increasing HMG CoA reductase activity (95), this effect in the present study was probably not large since a decrease in hepatic synthetic rate was observed. In fact, if the sedentary animals fed the high cholesterol diet experienced greater stress than the exercise group on the day of sacrifice, as previously suggested, then elevated epinephrine in the sedentary group might not have been different from that of the exercise group. If this were the case, then the effects of epinephrine on the sedentary or exercise groups would be relatively equal. One could then conclude that the increase in hepatic sterol synthesis in sedentary animals fed the high cholesterol diet was not due to stress. Since the hormone was not measured such a conclusion is only speculative.
GENERAL SUMMARY AND CONCLUSIONS

Results of the present study have shown that in hamsters, a significant interaction between dietary cholesterol and exercise activity influence parameters of plasma total cholesterol, HDL cholesterol and cholesterol synthesis.

Plasma total cholesterol was elevated by dietary cholesterol but also lowered in response to exercise, independent of diet. More important however, was the interactive effect between diet and exercise that reduced high plasma cholesterol levels in exercising animals fed high cholesterol diets. In contrast, even though HDL-C was lowered through an interaction of these two factors, a significant change occurred only in exercising hamsters fed low cholesterol diets.

Cholesterol synthesis in liver and small intestine did not respond similarly to changes in the level of dietary cholesterol and exercise in this experiment. Intestine synthetic rates were decreased only in response to diet, while hepatic synthetic rates showed an opposite increase. Moreover, a diet and exercise interaction produced a decrease in hepatic synthesis in hamsters fed high cholesterol diets.

In general, the liver appeared to respond independently, even uncharacteristically, to the change in body cholesterol balance imposed by a high cholesterol diet and prolonged exercise. Only under the latter conditions did exercise lower cholesterol synthesis. In addition, the mechanism of this response may not have been linked to the changes observed in HDL cholesterol levels.

Several important conclusions can be drawn from these study results; i) HDL cholesterol is not increased by exercise in the hamster
ii) Exercise may decrease precursor substrate availability and thus limit the rate at which endogenous cholesterol is synthesized

iii) The interaction between dietary cholesterol level and exercise activity that served to lower both hepatic cholesterol synthesis and plasma total cholesterol may be important only when high cholesterol, moderately high fat diets are consumed

iv) The liver responds to altered ratios of cholesterol to fat in the diet independently, or as a compensation to the reduction in cholesterol synthesis in the small intestine

In summary, if the hamster serves as a representative model of human cholesterol metabolism with regard to diet and exercise conditions, these findings suggest that there may be some benefit derived from exercise if a high cholesterol diet were habitually consumed. On the contrary, a combination of physical exercise and low dietary cholesterol does not appear to have significant effects on sterol metabolism, that would ultimately reduce the risk of cardiovascular disease. Intuitively, this is what one would expect, since low cholesterol consumption and physical activity are independent recommendations set forth to lessen the risk of CHD in Canadians.
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APPENDIX A

\( \dot{V}O_2 \) Max Measurement

Procedure:

Animals were run at minimal speed for two days prior to testing and allowed to familiarize themselves with the running apparatus. Treadmill speed on subsequent days was increased every 2 minutes after 5 minutes of warm-up, until the animals could no longer run. Mean \( \dot{V}O_2 \) consumed was calculated for the last 30 seconds of each 2 minute stage and plotted daily over a 2 week period. In addition, animals were considered to have reached a constant \( \dot{V}O_2 \) max when the speed/oxygen consumption curve no longer shifted to the right.

Results:

Average \( \dot{V}O_2 \) max of pilot hamsters was determined to be 10 ml \( O_2 / \) minute, or 76 ml \( O_2 / \) kg body weight/minute.
APPENDIX B

Diet Composition*

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<thead>
<tr>
<th>%</th>
<th>GROSS ENERGY</th>
<th>PHYSIOLOGICAL FUEL VALUE</th>
<th>PPM</th>
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<td>Arginine</td>
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<td>Leucine</td>
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<td>Lysine</td>
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<tr>
<td><strong>Cholesterol</strong></td>
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VITAMINS

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</tr>
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<td>Menadione</td>
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</tr>
<tr>
<td>Thiamine</td>
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</tr>
<tr>
<td>Riboflavin</td>
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</tr>
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<td>Niacin</td>
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</tr>
<tr>
<td>Pantothenic Acid</td>
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</tr>
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<td>Choline</td>
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<tr>
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<tr>
<td>Vitamin C</td>
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GROSS ENERGY 4.25 KCal/g
PHYSIOLOGICAL FUEL VALUE 3.30 KCal/g

VITAMINS

| Carotene | 4.50 |
| Menadione | -- |
| Thiamine | 17.70 |
| Riboflavin | 8.00 |
| Niacin | 95.0 |
| Pantothenic Acid | 24.0 |
| Choline | 22.5 (X100) |
| Folic Acid | 5.90 |
| Pyridoxine | 6.00 |
| Biotin | 0.07 |
| B12 | 22.0 |

| Vitamin A | 15.0 |
| Vitamin D | 4.5 |
| Vitamin E | 65.0 |
| Vitamin C | - |

FIBER (crude) 5.80
Neutral detergent 16.00
Ash 7.30
Calcium 1.00
Phosphorus 0.61
Magnesium 0.21
Sodium 0.40
Chlorine 0.50

| Iron | 198.0 |
| Zinc | 70.0  |
| Manganese | 64.3 |
| Copper | 18.0 |
| Cobalt | 0.6  |
| Iodine | 0.7  |
| Chromium | 1.8 |
| Selenium | 0.20 |

* as analyzed by Purina

** cholesterol content of Study "A"
Powdered free cholesterol was added to ground chow to increase the concentration to 0.12g/100g for Study "B".
APPENDIX C
ANOVA TABLES AND GRAND MEANS

TOTAL PLASMA CHOLESTEROL (mg/dl)

ANOVA TABLES

Total Plasma Cholesterol (mg/dl)

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<td>429.27</td>
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Means and Standard Deviations

Grand Means:

Low Cholesterol Diet = 108.84 (mg/dl)
High Cholesterol Diet = 202.89
Sedentary Animals = 170.13
Exercise Animals = 145.81

HDL CHOLESTEROL (mg/dl)

ANOVA TABLES

HDL Cholesterol (mg/dl)

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<td>86</td>
<td>8.103</td>
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Means and Standard Deviations

Grand Means:

Low Cholesterol Diet = 77.35 (mg/dl)
High Cholesterol Diet = 89.23
Sedentary Animals = 91.83
Exercise Animals = 74.74
APPENDIX D
ANOVA TABLES

INTESTINE FRACTIONAL SYNTHETIC RATE (FSR)

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<td>(A): diet</td>
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<td>(B): exercise</td>
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QUICK MEANS AND STANDARD DEVIATIONS

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</tr>
<tr>
<td>Entire sample</td>
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LIVER FRACTIONAL SYNTHETIC RATE (FSR)

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QUICK MEANS AND STANDARD DEVIATIONS

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## APPENDIX E
ANOVA TABLES AND GRAND MEANS

### INTESTINE SYNTHETIC RATE (Nmol/hr.g)

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<tr>
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**Grand Means:**
- Low Cholesterol Diet = 1162.46 (nmol/hr.g)
- High Cholesterol Diet = 766.20
- Sedentary Animals = 944.16
- Exercise Animals = 986.51

### LIVER SYNTHETIC RATE (Nmol/hr.g)

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<tr>
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**Grand Means:**
- Low Cholesterol Diet = 240.15 (nmol/hr.g)
- High Cholesterol Diet = 356.64
- Sedentary Animals = 354.11
- Exercise Animals = 242.68