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PLASMA LIPIDS AND PLASMA TRIGLYCERIDE CLEARANCE IN ENDURANCE TRAINED ATHLETES

The University of Arizona PH.D. 1981

PLASMA LIPIDS AND PLASMA TRIGLYCERIDE CLEARANCE IN ENDURANCE TRAINED ATHLETES

by

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A Dissertation Submitted to the Faculty of the

COMMITTEE ON ANIMAL PHYSIOLOGY (GRADUATE)

In partial fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Thomas Charles Rotkis

entitled Plasma Lipids and Plasma Triglyceride Clearance in Endurance Trained Athletes

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

James H. Wilmore	29 June 1981
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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

James H. Wilmore	8 May 1981
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ACKNOWLEDGMENTS

While none of these studies would have been possible without the enthusiastic participation of the subjects, there were others who helped get me through. I would like to thank Drs. Steve Goldman and Pete Farrell for striking the spark, Dr. Eddie Coyle and Mike Joyner for fanning the flame, and Drs. Jack Wilmore and Fred Roby for tending the blaze. Additionally, I appreciate the freedom that Drs. Benjamin Burrows and Sammy Campbell gave me to pursue my interests and the encouragement of Dr. Tom Wegner. Finally, I want to thank my wife for her support.

Portions of this work were supported by National Research Award 07243 and the Zuckerman Fund.

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ABSTRACT

The purpose of this series of studies was to investigate the plasma lipid profile and the ability to clear intravascular triglycerides (TG) in endurance trained athletes. Four studies were undertaken. Study I was a cross sectional determination of the relationship between cholesterol fractions and weekly running mileage. Study II used a training program to prospectively examine the changes in total and HDL-cholesterol (HDL-C) and to compare these to changes in body composition. Study III employed an intravenous fat tolerance test (IVFTT) and a post-heparin TG clearance test to indirectly assess lipoprotein lipase activity in three groups with varied training and performance backgrounds. Finally, Study IV examined the effects of a 12-week training program on TG clearance in previously untrained subjects.

In Study I there were significant correlations between HDL-C and percent body fat ($r=-0.36$, $p<.001$) and miles run per week ($r=0.50$, $p<.001$), the latter relationship remaining significant when statistically adjusted for age, alcohol consumption, or relative body composition. In Study II as the mean weekly running mileage increased to 44.9 miles, HDL-C increased by 5.0 mg/dl ($p<.01$), and total cholesterol remained unchanged. All components of body composition changed significantly, but only the change in lean weight significantly correlated with the change in HDL-C ($r=0.46$, $p<.025$).

In Study III the rate of Intralipid clearance correlated with fat weight ($r=-0.66$, $p<.001$), fasting TG ($r=-0.39$, $p<.05$), and $\dot{V}O_2$ max ($r=0.64$, $p<.001$). The heparin-induced fractional clearance of TG's correlated to $\dot{V}O_2$ max ($r=-0.51$, $p<.01$) and fat weight ($r=0.47$, $p<.01$). While the runners and the untrained subjects were indistinguishable in body composition and lipid profile, when divided into groups, the elite runners were leaner, had higher HDL-C ($p<.05$), and were able to clear Intralipid ($p<.01$) and plasma TG's ($p<.05$) faster than either of the other groups. Following the training period of Study IV, there was a trend towards a more favorable lipid profile and enhanced TG clearance, but only $\dot{V}O_2$ max ($p<.05$) and the post-heparin fractional clearance of TG's ($p<.05$) improved significantly.

These data suggest that endurance trained subjects have higher HDL-C, lower TG, and an enhanced capacity to clear the intravascular compartment of TG's. These changes cannot be explained solely by the body composition or diets of the athletes and must be due in part to changes evoked by their chronic training.

CHAPTER 1

INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the United States and a major health problem in most other industrialized nations. Although much attention has been directed to managing and treating patients with this disease, until recently far less effort has been expended in trying to prevent its development. Currently identified major risk factors for CHD include hypertension, hypercholesterolemia, diabetes mellitus, smoking, and male gender, with obesity, hyperuricemia, personality type (Type A/B), and family history having a lower predictive value. The role of exercise in this scheme has not been clearly defined both because of conflicting results from epidemiologic studies (1,2,3,4,5,6,7) as well as the inability to demonstrate a mechanism through which it could exert its influence. Additionally, highly active people typically modify their lifestyle in other ways which then makes it difficult to isolate those effects due strictly to exercise. An increased interest in, and an understanding of, cholesterol metabolism and the sub-fractions of cholesterol have provided new information about the relationship between exercise and CHD. It now appears that prolonged endurance training can have a profound effect on lipid metabolism resulting in an altered lipid profile and a favorable change in other cardiovascular risk factors.

Review of Literature

Lipoproteins

There are two major classes of lipids; cholesterol and triglycerides. Cholesterol is an essential component of cell walls, membranes, and many hormones, while triglycerides provide an efficient form of energy storage. However, excesses of cholesterol, may cause damage, scarring, and narrowing or occlusion of the arterial channel. If this occurs in the coronary circulation it can lead to angina or myocardial infarction, while in other arterial beds it can lead to stroke or claudication. Whether elevated triglyceride levels have an atherogenic potential independent of cholesterol has not been decided. Two large studies, The Western Collaborative Group Study (8) and the Stockholm Prospective Study (9) have arrived at different conclusions. The former study found that when the data for hypertriglyceridemia was adjusted for plasma cholesterol, there was no correlation between triglyceride levels and CHD , while the Stockholm group found a strong independent relationship between elevated triglycerides and CHD.

Cholesterol and triglycerides are relatively insoluble in plasma, therefore, they must be complexed with proteins and phospholipids before they can be transported through the circulation. These lipoprotein particles can be divided into four major classes based on their ultra-centrifugation proterties; chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Chylomicrons and VLDL are very rich in triglycerides (approximately 85% and 60% respectively). Chylomicrons are

derived from dietary fat and enter the general circulation from the portal circulation. VLDL are made in the liver and are produced when the caloric intake is high. Both chylomicrons and VLDL transport triglycerides to the periphery where the triglycerides are either immediately metabolized or deposited as high energy stores in adipose tissue or muscle.

Of the four classes of lipoproteins the LDL's have the largest concentration of cholesterol (45%). Their function is to transport cholesterol to the peripheral tissues (10), and they probably have a role in the control of the rate of peripheral synthesis of cholesterol (11). Goldstein and Brown have shown that there are specific membrane binding sites for LDL on peripheral tissues (12). The internalization of the LDL-receptor complex then inhibits the ability of the cell to produce cholesterol through a suppression of 3-hydroxy-3-methylglutamyl coenzyme-A (HMG-COA) reductase by the cholesterol component of the LDL (11). This interaction permits the body to regulate the size of the total body cholesterol pool by allowing the peripheral production to adjust to dietary and hepatic supply.

Finally, HDL's are produced by intestinal secretion and as a by-product of the metabolism of the triglyceride rich VLDL's and chylomicrons. Although the plasma concentration of HDL is the lowest of the lipoproteins, they may be the most important in terms of the development of CHD. This is due to their apparent ability to remove cholesterol from the peripheral tissue and carry it to the liver for metabolism and excretion (12). While the traditional concept of the

lipoproteins has been one of rigid compartmentalization, the emerging view is one of a dynamic equilibrium between all of the classes with an active exchange of both lipid and protein components.

The constituents of HDL are derived from a variety of sources, and each component has its own plasma half-life. Therefore, the plasma concentration of HDL is the sum of a series of dynamic processes rather than the result of the turnover of a single unit. The major factors contributing to plasma HDL levels that have been identified are the rate of splanchnic secretion of nascent HDL, the rate of intravascular catabolism of chylomicrons and VLDL's, and the rate of catabolism of the various apoproteins, particularly apo A-I (14). Perturbation of any of these factors will result in a change in the plasma concentration of HDL; however, current knowledge is inadequate to explain all of the changes observed in HDL levels.

Newly formed HDL particles are produced either by secretion from the liver and the intestinal mucosa or by the intravascular turnover of lipoproteins. Those synthesized in the liver and the intestinal mucosa are secreted into the circulation low in apo A-I, apo C-II, and cholesterol ester, which they pick up after they enter the circulation (15). The intracellular synthetic site is the smooth endoplasmic reticulum (SER), and agents which increase the activity of the SER, such as ethanol (16), chlorinated hydrocarbons (17), and phenytoin (18), also cause a non-specific increase in HDL's. Another portion of the HDL's are derived from the catabolism of circulating triglyceride-rich chylomicrons and VLDL's. This is illustrated by the reciprocal relationship seen between HDL and the plasma triglyceride

concentration in conditions such as weight loss, high carbohydrate diets, and physical training. The precise mechanism by which the new HDL is formed within the plasma compartment is not well formulated. It is thought that this may occur either by budding of the particles from the surface of other lipoproteins, by coalescence of catabolic fragments from chylomicrons and VLDL's, or by nascent HDL's combining with these catabolic fragments (14).

The plasma concentration, integrity, and function of HDL's may depend in large part on apo A-I. In Tangier disease the patients do not make apo A-I, and there is a complete absence of circulating HDL and an accumulation of cholesterol esters in the reticuloendothelial system (19). In contrast to this, estrogens have been shown to stimulate the production of apo A-I (20), and this may be the primary factor responsible for the higher HDL levels in women.

The combination of low HDL and high LDL levels in patients with CHD has been noted since the early 1950's (21). However, since high levels of cholesterol were believed to be atherogenic and HDL-cholesterol constituted such a small fraction of the total cholesterol, HDL received little attention. The situation began to change in 1975, after Miller and Miller (22) proposed an anti-atherogenic role for HDL. Subsequent work has firmly established an association between HDL and CHD. In one cross sectional study done in five communities, in every locale HDL-cholesterol concentration was lower in subjects with CHD than in the remainder of the population (23). In a prospective study in Tromso, Norway (24), it was found that low HDL levels preceded the manifestation of CHD and that the association was independent of

other coronary risk factors. Additionally, HDL-cholesterol was three times as effective as LDL-cholesterol in distinguishing between CHD cases and control subjects. And in the Framingham study (25) HDL-cholesterol was found to be the most potent lipid risk factor for CHD in both sexes.

Endurance trained athletes have been noted for having high HDL-cholesterol levels (1,4,26); consequently, the relationship between endurance training and HDL-cholesterol received renewed attention following the reports of a negative association between HDL-cholesterol and CHD. In two cross sectional studies done at Stanford (27,28) active groups were compared to age- and sex-matched control groups without any attempt to control for other factors such as adiposity or smoking. When middle-aged male and female runners and young, elite, long distance runners were compared to their respective control groups, the active groups exhibited significantly higher HDL-cholesterol concentrations. Similar results have been found in tennis players (29), mountaineers (30), Norwegian skiers (31), and Finnish lumberjacks (32) and runners (33). While it is premature to assume that the elevated HDL-cholesterol in athletes is due to their training and not some other associated factor, there is a common pattern of change in plasma lipoprotein concentration that runs through all of these reports.

Role of Lipoprotein Lipase

Only a select group of peripheral tissues (adipose, lactating mammary gland, lung, skeletal muscle, and cardiac muscle) are capable of clearing triglycerides from chylomicrons and VLDL in the circulation (34). This capacity requires the presence of an endothelial bound enzyme, lipoprotein lipase (LPL). LPL is secreted by the specific tissue and then adheres to proteoglycans on the surface of the adjacent endothelial cells (35). As the proteoglycans diffuse across the surface of the cells, they eventually carry the attached enzyme to the luminal surface where it is exposed to the circulating lipoproteins. An additional co-factor, apoprotein C-II (apo C-II), is necessary for activation of the LPL, and increases the rate of the reaction without altering the enzyme-substrate affinity (34). While both VLDL and chylomicrons are poor in apo C-II when they are secreted into the plasma, they rapidly acquire the co-factor from their interaction with circulating HDL (34). In the presence of apo C-II the LPL hydrolyzes triglycerides releasing free fatty acids, which then diffuse across the endothelium and into the adjoining cells (36). The localization of the LPL to specific endothelial sites allows the triglycerides to be directed to metabolically active tissues or to storage sites. This control is made even finer since the rate of secretion of the LPL varies with each tissue and with changes in diet. High carbohydrate diets have been shown to increase the activity of LPL and the uptake of triglycerides by adipose tissues (37), and prolonged, heavy exercise in well-trained subjects increases LPL activity in skeletal muscle (38).

The LPL associated with each tissue varies in its catalytic rate at saturation and the apparent Michaelis constant (34). Cardiac muscle, which is capable of deriving a high percentage of its energy needs from lipolysis, produces LPL with a low Michaelis constant. Consequently, the enzyme is saturated even when the plasma triglyceride concentration is low. As a result, the rate of fatty acid uptake is dependent on the concentration of LPL on the capillary endothelium over a wide range of triglceride concentrations. This allows for a constant supply of fatty acids to the myocardium. The LPL associated with adipose tissue and skeletal muscle, on the other hand, has a relatively high Michaelis constant (39). Therefore, at physiologic levels of plasma triglycerides, the LPL is not saturated and the rate of clearance will be a function of the plasma triglyceride concentration. Thus, at low concentrations the myocardium will have a relatively greater access to the circulating pool than will other tissues, while when caloric intake is high and triglyceride levels are elevated, the adipocyte will have a greater capacity to extract and store this substrate. The variations in the enzyme activity in individual tissues may be more important in determining the pattern of distribution of triglyceride uptake, while the quantitiy of LPL available at the capillary endothelium may determine the overall rate at which triglycerides are cleared from the intravascular compartment.

Role of Heparin

Heparin is a naturally occurring mucopolysaccharide that is best known for its anti-coagulative properties; however, it also has the ability to accelerate the clearance of triglycerides from the plasma. The precise mechanism by which heparin acts has not been clarified. It is known that it releases the endothelial bound LPL into the general circulation along with certain other endothelial bound lipases probably of hepatic origin (40). While the triglyceride lipases released from the liver can make a substantial contribution to post-heparin lipase activity (PHLA), this class of enzymes is believed not to be important in the elimination of circulating triglycerides (41). It has been proposed that LPL is attached to the capillary endothelium by a proteoglycan, heparan-sulfate (35). The release mechanism probably involves competition between heparin and the membrane-bound heparan-sulfate for a binding site of the LPL, with the heparin-enzyme complex being carried away by the circulation. Although the free enzyme has the same kinetic properties, being released into the general circulation presents it to the total circulating triglyceride pool, thereby accelerating triglyceride clearance (42).

In 1943, Hahn was the first to note that heparin abolished alimentary lipemia (41). Subsequently, heparin has been used to investigate hypertriglyceridemia in a variety of groups under a number of experimental conditions, but these studies often yielded conflicting results (41). The reason for this is PHLA measures the activity of hepatic as well as peripheral lipase; whereas, plasma

triglyceride clearance depends only on the peripheral lipases. Consequently, any change in hepatic lipase activity will be reflected in PHLA, while it will have no effect on the clearance of plasma triglycerides. The availability of specific assays for hepatic and peripheral lipases have helped resolve many of the previous discrepancies, and the ability to separate the activities of the lipases make these assay techniques preferable to fractional clearance studies. However, fractional clearance studies are not without some use. Although no information is available on the effect of chronic exercise on hepatic lipases, it is known that skeletal muscle and adipose tissue LPL is high in well-trained athletes (43), and that it increases in skeletal muscle with training (44,45). If it is assumed that chronic exercise does not alter the activity of hepatic lipases, then the fractional clearance of plasma triglycerides following the administration of heparin may provide an index of the changes in peripheral lipases in athletes.

Clearance of Plasma Triglycerides

Plasma levels of triglycerides are determined by two factors; their rate of secretion into and their rate of clearance from the circulation. Therefore, if there are matched changes in both of these processes, plasma triglyceride levels will remain constant despite a markedly altered flux of triglycerides through the circulation. Conversely, alterations in only one of the two processes will result in a change in the triglyceride level. One extreme, hypertriglyceridemia, may play a role in the development of CHD (9). High levels

of triglycerides may be directly toxic to the arterial endothelium or may cause damage through other mechanisms. Boberg et al. (46) have suggested that most cases of hypertriglyceridemia are due to an impaired removal rather than to an over-production of triglycerides. Endurance trained athletes, however, are characterized by low levels of triglycerides. Although it has not been directly studied in man, the lower triglyceride levels appear to be due, at least in part, to an enhanced clearance capacity. Altekruse and Wilmore (25) found that a 10-week training program reduced fasting triglyceride levels. Additionally, when these same subjects were given an oral fat load following training, they had lower peak levels and a briefer period of lipemia when compared to their pre-training values. While this study suggests that exercise may lower triglyceride levels by improving plasma clearance mechanisms, Simonelli and Eaton have found that in rats whether hepatic production, peripheral clearance, or both are altered with chronic exercise depended on the hormonal state of the animal (47). Therefore, exercise may not only provide a modality for studying the mechanisms of lipid metabolism, but may also prove beneficial to patients with hypertriglyceridemia.

While an oral fat tolerance test is useful, it has a number of limitations; it requires six to eight hours to perform, and the rates of gastric emptying, intestinal fat absorbtion, and chylomicron formation may introduce sufficient variability to obscure changes in the rate of plasma triglyceride clearance. In order to be clinically useful, a fat tolerance test must be safe, brief, and representative of the normal triglyceride clearance mechanisms. In response to these

needs, Boberg, Carlson, and Hallberg (48) developed the intravenous fat tolerance test (IVFTT). This test consists of a bolus injection of a fat emulsion (Intralipid, Vibram, Sweden) with monitoring of its clearance from the plasma. Whereas most other fat emulsions are cleared by the reticuloendothelial system, Intralipid, like chylomicrons, is removed by lung tissue, adipose cells, skeletal muscle, and cardiac muscle (49). In vitro, it also has enzyme kinetics with post-heparin lipase similar to that of chylomicrons, and its plasma elimination kinetics in dogs and man are identical to those of chylomicrons (50,51,52). Furthermore, hydrolysis of chylomicron triglycerides requires that the LPL co-factor, apo C-II, be present on the surface of the chylomicron. This apoprotein is acquired by the chylomicrons after they are secreted into the circulation with HDL serving as the probable circulating reservoir (34). Carlson (53) has demonstrated that Intralipid incubated in vitro with VLDL-free serum also acquires this apoprotein. These results indicate that the triglycerides in Intralipid are cleared from the intravascular compartment in a similar manner to the triglycerides in chylomicrons.

Elimination of a single intravenous dose of Intralipid can be characterized by two rate constants (50). At high concentrations, the removal follows zero-order kinetics with an absolute quantity cleared from the plasma per minute (K1), while at lower concentrations the reaction becomes first order, i.e. a constant percent is removed per minute (K2). K1 has no correlation to K2 nor to serum triglyceride levels (48), and has little physiological application since plasma triglyceride levels are rarely in this range. K2, however, has been

shown to have an inverse relationship to serum triglycerides in numerous clinical studies (54,55,56). Additionally, patients with atherosclerotic heart disease, peripheral vascular disease (57,58) and a variety of hypertriglyceridemias (49), who characteristically have elevated triglyceride levels, all have reduced K2. Therefore, determination of K2 provides a tool for assessing the functional capacity of endothelial bound lipases to clear plasma triglycerides.

Purpose

Four different studies were conducted to examine several aspects of the relationship between endurance training and plasma lipid content and metabolism. Study I was a cross-sectional determination of the relationship between the cholesterol fractions and age, alcohol intake, body composition, and weekly running mileage. Study II was a seven month training study on 22 women comparing changes in body composition to the changes in total and HDL-cholesterol. Study III, using three groups that varied in training level, compared the ability of each group to clear the plasma of endogenous and exogenous fats as measured by an intravenous fat tolerance test (IVFTT) and the fractional clearance of triglycerides following a heparin injection. Finally, study IV examined the effects of a twelve week training program on lipid clearance in a group of untrained subjects.

CHAPTER 2

METHODOLOGY

Subjects

Participants in all of the studies were volunteers. Only those who were healthy, taking no medication known to alter lipid levels or metabolism, and who had not had a change in body weight over the preceeding six months were chosen to participate. The 109 subjects in the experimental groups in study I were men between the ages of 20 and 55 years who had maintained constant levels of running of at least 10 miles per week for the preceding year. The sample was divided into groups based on weekly running mileage; non-runners (NR); low mileage (L), i.e. 10-19 miles per week; intermediate mileage (I), i.e. 20-39 miles per week; and high mileage (H), i.e. 40 or more miles per week. The non-running comparison group consisted of university students who, although more physically active than normal individuals, had not participated in an exercise program requiring high aerobic fitness for at least one year. The 22 subjects in study II were women between the ages of 20 and 38 who had not been in a training program for at least a year, and who had normal menstrual histories. While most of these women had some experience with running, none of them was running more than 10 miles per week at the time they were selected for the study. Study III used men between the ages of 20 and 35 who were divided into three groups: runners (n=7), those who had been running

for at least one year and whose best competitive marathon was over 3 hours and 30 minutes; elite (n=7), those who had been running for at least one year and whose best competitive marathon was completed in less than 2 hours and 30 minutes; and untrained (n=14), those who had performed no previous endurance training. The latter untrained group, became the subjects for the training program of study IV. The procedures for each study had been approved by the University of Arizona Human Subjects Committee and were carefully reviewed with each subject. Written, informed consent was obtained.

Methods

Body composition was determined by estimation of body density using the hydrostatic weighing technique (59). Underwater weight was measured to within $\pm$ 0.01 kg using a Chatillon scale, with ten determinations conducted on each subject. The highest weight that was reproduced at least three times was used in the subsequent calculations. The underwater weight was corrected for residual volume using a nitrogen dilution technique (60). Relative body fat was then estimated from body density using the Siri equation (61).

Venous blood samples for lipid determinations were obtained either from an antecubital vein (study I) or through a venous catheter placed in a forearm vein (studies II, III, IV). On those days when blood samples were obtained the subjects were not allowed any vigorous exercise. In studies I, III, and IV the subjects were instructed to arrive at the laboratory following a 12 hour fast and

having abstained from all alcohol for at least 24 hours. In study II fasting triglyceride levels were not required. However, to control for the lipid fluctuations associated with the phases of the menstrual cycle (62), all samples were obtained during the follicular phase.

In studies II, III, and IV the maximum oxygen consumption ($\dot{V}O_2$ max) was determined on two separate occasions, and the mean value was used. Expired gases were measured continuously using a Beckman Metabolic Measurement Cart. Heart rate was monitored with a three lead chest electrode system (CM-5 configuration). A motor driven treadmill was used and the protocol was adapted to the ability of the individual in a manner similar to that described by Costill and Fox (63). The point of volitional fatigue was reached between 8 and 12 minutes, and in all cases an R value of 1.1 or greater, a heart rate greater than 175 beats per minute, or a plateauing in the oxygen consumption with increasing workload was obtained.

Intravenous Fat Tolerance Test (IVFTT)

An intravenous fat tolerance test (IVFTT) as described by Boberg et al. (48) was performed on all subjects in studies III and IV. For these studies a 10% fat emulsion (Intralipid, Vibram, Sweden) derived from soy beans was used. It is comprised of 10% soy bean oil, 1.2% egg yolk phospholipids, 2.25% glycerin, and pyrogen-free water and has an osmolarity of approximately 280 mosm/l (64). The neutral triglycerides are predominantly unsaturated fatty acids. The major component fatty acids are linoleic (54%), oleic (26%), palmitic (9%), and linolenic (8%)(64). The emulsified fat particles are

approximately 0.5 microns in diameter. The test was always performed in the morning following an overnight fast and prior to any strenuous exercise. A 19-guage indwelling cathether was inserted into an antebrachial vein and was used for blood sampling and for the injection of the fat emulsion. The catheter was attached to a 10-ml syringe by way of a 3-way stopcock. Following either the injection of the fat emulsion or blood sampling, and at periodic intervals, the catheter was flushed clear with normal saline. Following the withdrawal of 15 ml of blood for baseline studies, a single dose of fat emulsion (1 ml of 10% Intralipid per kilogram body weight) warmed to near body temperature, was injected as an intravenous bolus as rapidly as possible. In all instances this was accomplished in less than 60 seconds. At 5, 10, 15, 20, 25, 30, and 40 minutes following the injection a 7 ml blood sample was obtained. The samples were allowed to clot, were centrifuged for 7 minutes, and the plasma was pipetted into a separate container. Thirty-six IVFTT's were performed during these studies. On occasion a subject described a sensation of cold in his arm during the injection of the fat emulsion, but there were no major or other minor reactions noted. At the Karolinska Institute in Sweden over 2100 IVFTT's have been done without a major complication (49), which suggests that this is a safe procedure.

A standard curve was produced with known dilutions of the 10% fat emulsion. The emulsion was diluted with saline to produce standard solutions of 0.60%, 0.30 %, 0.25%, 0.20 %, 0.15%, 0.10% , and 0.05%. Fifty microliters of each standard was added to 5 ml of saline and prepared in quadruplicate. The samples were read in a Beckman

Model 35 spectrophotometer at a wavelength of 340 nm. The machine was zeroed against a saline blank before and after measurements were taken for each standard curve. The standard curve that was obtained was linear and passed through the origin. The concentration of the fat emulsion in the plasma could be determined from the standard curve.

The plasma samples obtained from the IVFTT were handled in a manner similar to the standard solutions. Fifty microliters of each sample were pipetted into 5 ml of saline, and samples were prepared in triplicate. Each sample was then read in duplicate in the spectrophotometer at 340 nm. Prior to the calculation of the emulsion concentration, each of the readings was corrected for the absorbance of the plasma (the value at time 0). Previous studies (49) have indicated that there is no resecretion of the emulsion fat during the first 50 minutes following an intravenous injection; consequently, the endogeneous plasma triglyceride levels may be assumed to be constant over the time interval of the test. Rossner (49) and Boberg et al. (48) have determined that such a fat emulsion is eliminated from the plasma by first-order kinetics, i.e. a constant per cent of the emulsion is cleared from the plasma per unit time. Linear regressions were performed on log-transformed data collected on each individual, the slopes of those regressions (K2) being equivalent to the exponential rate constant. These values were used to characterize the fat clearance or fat tolerance.

Fractional Clearance of Triglycerides with Heparin

In studies III and IV the post-heparin lipase activity (PHLA) was determined in the morning following an over nightfast. A 21-guage needle was inserted into an antecubital vein, a 7 ml baseline blood sample was withdrawn, and then 1000 units (1 ml) of heparin were given intravenously. Fifteen minutes later another 7 ml venous sample was drawn from the other arm. PHLA was characterized by the fractional clearance of the plasma triglycerides.

The samples were centrifuged, the plasma was pipetted into another container, and was stored at -20^{o}C until they were analyzed. Rossner has shown that when either the stock Intralipid solution or plasma samples containing the emulsion are stored for up to two weeks, there is no change in either the slope of the standard curves or in the calculated values of K2 (65). All other lipid determinations were made by the clinical laboratory at the Arizona Health Sciences Center. Total cholesterol was measured directly by the enzymatic method using a commercial kit (Dow Chemical) and cholesterol standards (Dow Chemical Diagnostics). HDL-cholesterol was assayed by the enzymatic method after precipation of lower density lipoproteins by heparin-manganese chloride (66). Triglycerides were measured by an enzymatic technique (67). The non-HDL-cholesterol in study I was calculated as the difference between total cholesterol and HDL-cholesterol.

Training Protocols

While studies I and III were cross-sectional in nature, studies II and IV had training phases. In study II the programs were individualized. Each woman was instructed to run five or six days per week and to slowly increase her total weekly mileage without regard to speed. In most weeks there was a single long run which accounted for approximately 25% of the total weekly mileage. The women were encouraged to constantly increase the distance they ran in order to acquire an adequate mileage background to complete a marathon. As a result, for any individual there was little change in the average training pace as the mileage increased throughout the study. Baseline testing was done at the beginning of the study and repeat measurements were made when the individual had increased her average weekly mileage by 30 miles per week (delta-30) for a least two weeks. Both the baseline and the delta-30 profiles were obtained during the follicular phase of the menstrual cycle. The untrained subjects from study III were placed on a training program for study IV. At the beginning of the study none of the subjects was running more than 5 miles per week. They were instructed to run five or six days per week without regard to speed with a goal of increasing to 25 miles per week by the end of the 12 week study.

Statistical Analysis

A standard statistical package (SPSS) was employed to analyze the data using zero order correlations, linear and multiple regression analysis, and analysis of variance (ANOVA) where applicable. When

significance was determined by ANOVA, Dunn's multiple comparison procedure was used to assess the difference between means. Values of $p<.05$ were considered significant. Data in the text, tables, and figures are presented as mean $\pm$ SD.

CHAPTER 3

Results

Study I

None of the subjects was taking medication known to alter cholesterol levels, and in general the dietary habits were similar amongst the groups (Table 1). The correlations between HDL-cholesterol and alcohol consumption, parental history of CHD, and non-HDL cholesterol were not significant (Table 2). However, a significant inverse correlation was obtained for the runners between HDL-cholesterol and per cent body fat ($r=-0.36$, $p<.001$), and a significant positive correlation between HDL-cholesterol and miles run per week ($r=0.50$, $p<.001$). The correlation between HDL-cholesterol and miles run per week remained significant when either age ($r=0.49$, $p<.001$), alcohol consumption ($r=0.50$, $p<.001$), or per cent body fat ($r=0.41$, $p<.001$) were controlled in a multiple regression analysis.

Both total cholesterol and non-HDL cholesterol were significantly correlated with age ($r=0.23$, $p<.02$ and $r=0.26$, $p<.01$ respectively) and per cent body fat ($r=0.30$, $p<.001$, and $r=0.48$, $p<.001$ respectively). There was a low correlation between miles run per week and non-HDL cholesterol ($r=-0.29$, $p<.01$), but this became insignificant when adjusted for per cent body fat ($r=0.05$) in a multiple regression analysis.

Table 1

Subjects' Characteristics for Study I

Characteristic	Non-Runners	Runners
Number of Subjects	19	90
Age, years	23.5±2	38.1±10
Relative Body Fat, %	14±6	16±6
Distance/week, miles	0	34±22
Years of Running	0	4.4±4.2
Total Cholesterol, mg/dl	182±32	210±37
HDL-Cholesterol, mg/dl	34±7	54±10
N-HDL-Cholesterol, mg/dl	147±30	156±37

Data presented as mean ± S.D.

Table 2

Correlations between Cholesterol and its Fractions, and the Subjects' Characteristics for Study I

Characteristics	HDL Cholesterol	N-HDL Cholesterol	Total Cholesterol
Cholesterol	.17	.96*	---
N-HDL-C	.17	---	.96*
Miles Run Per Week	.50***	-.29*	-.15
Age	-.10	.26**	.23*
Relative Body Fat	-.36***	.48***	.38***

*p<.05

**p<.01

***p<.001

When the sample was divided into groups based on weekly running mileage (Table 3), there were no inter-group differences in alcohol consumption, or parental history of CHD. However, the non-runners were younger than the runners (23.5 and 38.1 years respectively), and within the runners there was a significantly lower relative body fat in the higher mileage groups ($p<.01$). Additionally, there was a non-significant decreasing trend in both total cholesterol and non-HDL cholesterol values, and a significant increase in HDL-cholesterol ($p<.001$) with increases in running mileage.

Study II

While none of the women were running more than 10 miles per week when they were selected for the study, they were running a mean of 13.5 miles/week (range 0 to 24 miles/week) when the baseline determinations were made. Because of the varying running abilities of the subjects, injuries, and the need to time the sampling with a specific phase of the menstrual cycle, the second measurements were made between four and seven months after training began, at that point where the woman had attained delta-30 for two consecutive weeks. At that time they had increased their mean weekly mileage to 44.9 miles (range 30 to 60 miles). All components of body composition showed small but statistically significant changes (Table 4). Mean total body weight, fat weight, and relative body fat decreased (0.89 kg, 1.69 kg, and 2.58% respectively), while mean lean weight increased 0.80 kg . Mean HDL-cholesterol increased 5.0 mg/dl ($p<0.01$). Mean total cholesterol and the ratio of total cholesterol to

Table 3

Group Characteristics when Divided by Running Mileage, Study I

Groups*	N	Relative Fat	HDL-Chol mg/dl	N-HDL-Chol mg/dl	Total Chol mg/dl
Non-Runners	19	14±6	34±7	147±30	182±32
Low	28	19±5	47±7	170±37	217±37
Intermediate	30	17±6	53±9	158±41	211±43
High	32	13±4	60±9	143±28	203±31
Total Group	109	16±6	50±12	155±36	205±38

*See text for explanation of groups
Data presented as mean ± S.D.

Table 4

Body Composition and Cholesterol Values of Women Runners, Study II

	Baseline	Delta-30	Change
Total Weight, kg	57.1±1.4	56.2±1.3	-0.9*
Lean Weight, kg	42.3±1.0	43.0±0.9	0.8*
Fat Weight, kg	14.9±0.7	13.2±0.7	-1.7**
Relative Body Fat,%	25.8±4.0	23.2±4.0	-2.6**
Total Cholesterol, mg/dl	174.7±6.2	181.0±4.7	6.3
HDL-Cholesterol, mg/dl	53.5±2.3	58.5±2.3	5.0*
Total Cholesterol/HDL-C	3.4±0.1	3.2±0.1	-0.2

* $p<0.01$
** $p<0.005$

HDL-cholesterol were not significantly altered. The only positive correlation between changes in body composition and plasma lipids were between the increase in lean weight and the increase in HDL-cholesterol ($r=0.46$, $p<.025$).

Study III

When all of the subjects in study III were evaluated as a single group, there were no significant correlations between any component of body composition and either total cholesterol, HDL-cholesterol, or triglyceride levels, while $\dot{V}O_2$ max showed a positive correlation with HDL-cholesterol ($r=0.39$, $p<.05$)(Table 5). Although the ability to clear exogenously administered triglycerides, as measured by K2, exhibited no significant correlation with HDL-cholesterol, there were significant negative correlations with fat weight ($r=-0.66$, $p<.001$), total cholesterol ($r=-0.42$, $p<.03$), and triglyceride levels ($r=-0.39$, $p<.05$), and a significant positive correlation with $\dot{V}O_2$ max ($r=0.64$, $p<.001$). The correlation between K2 and total cholesterol became insignificant ($r=0.27$, p=NS) when triglycerides were held statistically constant using partial correlation analysis. The fractional clearance of endogenous triglycerides expressed as the per cent change in triglycerides was significantly correlated only to $\dot{V}O_2$ max and fat weight ($r=-0.51$, $p<.01$; and $r=0.47$, $p<.01$ respectively) and unrelated to other lipids or measures of body composition.

Table 5

Correlations between Lipids, Clearance Factors, and Body Composition for Study III

	$\dot{V}O_2$	Tot Wt	Fat Wt	Lean Wt	T Chol	HDL-C	Trig	K2
TC	-.17**	.20	.28					
HDL-C	.39*	-.33	-.13	-.31	.10			
TRIG	-.18	.30	.22	.18	.55**	-.16		
K2	.64***	-.42*	-.66***	.05	-.42*	.21	-.39*	
FC	-.51**	.39*	.47**	.08	-.16	-.10	-.06	-.26

*p<.05
**p<.01
***p<.001

When the subjects were divided into the three groups based on their running experience, there were no inter-group differences in age or lean body weight, but the elite runners were significantly leaner ($p<.05$)(Table 6). As would be anticipated from the experimental design there were highly significant inter-group differences in the mean weekly training mileage ($p<.01$) and in the mean aerobic capacity ($p<.01$). The lipid profiles of the groups were similar with no inter-group differences in either total cholesterol or triglyceride levels. The HDL-cholesterol of the elite runners was significantly greater than that of either the runners or the untrained subjects ($p<.05$). The greatest differences between the groups occurred in their abilities to clear an exogenous triglyceride load. While the runners and the untrained subjects were indistinguishable, the elite runners had both a lower peak concentration of triglyceride (versus runners, $p<.05$; versus untrained, $p<.001$) as well as a more rapid clearance (K2)(for both groups, $p<.01$). This same pattern was seen with the fractional clearance of triglycerides following the heparin injection. The untrained subjects and the runners were able to clear a similar fraction of their triglycerides in 15 minutes ($14 \pm 4\%$ and $11 \pm 3\%$, respectively); however, the elite runners were superior to either group ($27 \pm 4\%$, $p<.05$).

Study IV

During the twelve weeks of the endurance training program the subjects increased their mean weekly mileage from 3.5 miles (range 0 to 5 miles) to 25.7 miles (range 20 to 30 miles), and improved their

Table 6

Subjects' Characteristics for Study III

	Untrained	Runner	Elite
Number of Subjects	14	7	7
Age, years	29.6±0.8	32.3±1.7	25.3±1.7
Weekly Mileage	6±2	33±5	73±7
Pace, min/mile	--	8.1±0.3	6.3±0.1
Total Body Weight, kg	75.2±1.9	78.6±3.9	66.3±2.6
Lean Weight, kg	60.2±1.2	65.4±4.0	61.6±2.4
Fat Weight, kg	15.0±1.2	13.2±2.4	4.7±0.6
Percent fat, %	19±1	17±3	7±1
$\dot{V}O_2$ Max, mg/kg-min^{-1}	39.9±1.2	46.1±2.3	63.3±2.1
Total Cholesterol, mg/dl	172±13	162±16	146±8
HDL-cholesterol, mg/dl	45±3	46±4	56±2
LDL-cholesterol, mg/dl	109±11	104±13	79±7
TC/HDL-C	4.0±0.4	3.6±0.4	2.6±0.2
K2, %/min	4.0±0.4	5.9±1.0	9.2±1.3
Peak Intralipid TG, mg/dl	127±6	104±9	77±3
Triglycerides T=0, mg/dl	90±21	63±10	54±2
Triglycerides T=15, mg/dl	76±17	56±8	40±3
Triglyceride clearance, %	14±4	11±3	27±4

Data presented as mean ± S.D.

mean $\dot{V}O_2$ max by 4.6 ml/kg-min^{-1} ($p<.05$)(Table 7). They exhibited small, non-significant changes in their body composition and lipid profiles. Whereas, there was no improvement in their ability to clear the exogenous triglyceride load (K2), there was an increase in their fractional clearance of triglycerides following heparin administration ($p<.05$). This change was independent of changes in body composition and changes in all cholesterol fractions; however, it was highly correlated with the change in fasting triglyceride levels ($r=-0.82$, $p<.02$). Although the lack of significant change in the clearance of exogenous fats was not expected, the higher fractional clearance of endogenous triglycerides implies that changes in the plasma lipid clearing ability did occur.

Table 7

Changes in the Subjects' Characteristics with Training, Study IV

Factor	Initial	Final	Change
Weight, kg	74.6±5.1	73.5±4.6	-1.1
Lean Weight, kg	58.8±2.3	58.8±2.3	-0.1
Fat weight, kg	15.8±3.1	14.8±2.9	-1.0
$\dot{V}O_2$ Max, ml/kg-min^{-1}	36.6±11.0	41.2±8.4	4.6*
Total Cholesterol, mg/dl	180±56	192±47	11.6
HDL-Cholesterol, mg/dl	48±5	49±4	1.3
TC/HDL-C	3.9±1.3	3.9±0.7	0.0
Triglycerides, mg/dl	65±22	56±13	-9.1
K2, %/min	4.0±0.4	4.1±0.7	0.1
Peak Intralipid, mg/dl	108±12	86±7	-42.5
Triglyceride clearance, %	24±20	45±18	21*

Data presented as mean ± S.D.
Significance $p<.05$

CHAPTER 4

DISCUSSION

Study I

The results from study I confirm previous work which indicates that people who engage in regular endurance exercise have higher HDL-cholesterol levels than inactive normals (1,6,27,31). This study also shows that there is a positive correlation between the number of miles run per week and the plasma HDL-cholesterol concentration. However, mileage alone was not a good predictor of an individual's HDL-cholesterol level. While the HDL-cholesterol levels may be raised by exercise, the final level will depend on the pre-training baseline and the individual's response to the stimulus of chronic exercise. Additionally, while Lehtonen and Viikari have suggested that there may be a mileage threshold which must be achieved before changes in HDL-cholesterol are seen (68), this was not evident in this study. Furthermore, several recent studies have shown increases in HDL-cholesterol levels following short term, low-intensity training (69,70), even when the level of exercise was insufficient to cause a cardiovascular training effect (70). While these exercise programs only raised HDL-cholesterol from the sub-normal to the normal range, rather than to supra-normal levels, they do suggest that an individual's plasma HDL-cholesterol will increase concomitant with an increase in chronic exercise.

From the Framingham data, it can be assumed that any increase in HDL-cholesterol level, even within the normal range, will have a favorable impact on an individual's risk for developing CHD (25). It is also important to note that high mileage training, which would be out of the reach of most people, does not seem to be required to achieve these increases in HDL-cholesterol. Rather, as discussed below, the important determinants may be the duration and the relative intensity of the exercise. Whether HDL-cholesterol continues to increase as the mean weekly running mileage increases cannot be determined from these data. However, based on information obtained on a group of elite distance runners (71), it appears that there is a point beyond which further increases in running have little effect on HDL-cholesterol.

When the subjects were divided into groups based on weekly running mileage, the groups were similar with respect to diet, daily alcohol consumption, and parental history of CHD, but had a significant difference in HDL-cholesterol levels. Because of the large variability within, and the over-lap between the training groups, only those subjects who were running more than 40 miles per week had HDL-cholesterol levels that did not over-lap those of the non-runners. These values are consistent with the findings of Lehtonen et al. on another group of endurance trained athletes (68). Since training intensity and duration alter substrate utilization and may therefore alter the rate of turnover of intravascular triglycerides, the average running pace and the number of hours spent

running each week were also evaluated. Neither, however, provided a substantially higher correlation with HDL-cholesterol ($r=-0.33$, $p<.05$ and $r=0.52$, $p<.001$ respectively) than did mean weekly running mileage.

Although total cholesterol levels tend to increase with age, HDL-cholesterol has been shown to be stable in males between the ages of 20 and 60 (72). Therefore, while the age difference between the runners and the non-runners probably explains the lower total cholesterol levels in the non-runners, it should have had no effect on the HDL-cholesterol results. However, the advantages of selecting such a control group were two-fold. First, the non-runners were very lean with relative weights similar to those of the elite runners. Consequently, the low HDL-cholesterol levels in the non-runners imply that being lean is not sufficient, by itself, to cause an increase in HDL-cholesterol. Secondly, while the higher mileage runners certainly had larger daily energy expenditures than the non-runners, most of the non-runners, through participation in recreational sports, probably were as active as the lower mileage runners. These sports are characterized by intermittent as opposed to continuous activity as is found in running; consequently, the important difference between the non-runners and the lower mileage groups may have been the type rather than the amount of daily exercise. That the non-runners did not demonstrate comparable changes in HDL-cholesterol is therefore important. Nikkila et al. (43) have found that intermittent work, even if it is of a high intensity such as sprint training, will not

elevate HDL-cholesterol concentrations. Therefore, the higher HDL-cholesterol values seen in the runners appear to be related to the continuous nature of their training and not merely to their being active or lean.

Study II

The results of study II are consistent with the hypothesis that dynamic exercise itself is an important factor in determining plasma HDL-cholesterol concentrations independent of the body composition changes that accompany it. The mean baseline values of 25.8 % body fat, 53.3 mg/dl HDL-cholesterol, and 175 mg/dl total cholesterol are representative of untrained women in this age range (3,73). None of the subjects was taking medications known to alter HDL-cholesterol, had any unusual dietary habits, had a mean intake of more than two ounces of alcohol per day, or altered their diet during the study other than to increase the daily caloric intake. There were statistically significant changes in lean weight, fat weight, relative body composition, and HDL-cholesterol (Table 4). Moreover, the increases in HDL-cholesterol might have been even greater if the women had done no running before the initial set of testing; however, they were running a mean of 13.5 miles/week prior to beginning the study. The increase in the mean HDL-cholesterol level of 5.0 mg/dl was significantly correlated only to the increase in lean weight. It has been suggested that the elevated HDL-cholesterol levels of athletes may be attributed to their relative leanness, their diets or alcohol intake, or the weight losses that are commonly associated with

training (3,27,74,75,76). However, none of these possibilities adequately explains these results. Studies utilizing sedentary populations have found a negative correlation between relative body weight and HDL-cholesterol levels, but this was not the case in this study nor has it been characteristic of other active groups (27,77). HDL-cholesterol may increase with weight loss, but in a recent study conducted on severely obese individuals, Streja et al. (78) reported that this only occurred when there was a 7.3% or greater decrease in body weight. Thus, the small weight loss in these women (1.6% of body weight) is unlikely to account for the changes in HDL-cholesterol. As suggested by Adner and Castelli (79), a more important factor may have been the increase in lean weight (0.8 kg). This was the only change in body composition that was significantly correlated with the increase in HDL-cholesterol. Havel, et al. (80) have found that during prolonged, moderate intensity, exercise plasma free fatty acids (FFA) were responsible for approximately 50% of all the fat oxidized. Hydrolysis of circulating triglycerides by endothelial bound LPL accounted for an additional 12% of the fat oxidized. However, during the recovery period following exercise, the muscles extracted substantially larger amounts of FFA than were oxidized, implying that the muscles were replenishing depleted intra-muscular triglyceride stores. Both of these mechanisms increase the turnover of circulating triglycerides, are dependent on the lean muscle mass, and may explain the correlation between the changes in lean weight and the increases in HDL-cholesterol.

Study III

Study III was designed to examine the relationships between various body composition parameters, aerobic capacity, lipid levels, and the ability to clear endogenous and exogenous triglycerides. As in study II, the fasting lipid levels had no correlation to body composition or to one another, while HDL-cholesterol was significantly related only to $\dot{V}O_2$ max. This is further evidence that exercise is an important determinant of HDL-cholesterol independent of its effect on body composition. Since K2 is a measure of the ability to clear triglyceride from the intravascular compartment, the significant negative correlation with the triglyceride level ($r=-0.39$) was expected and has been previously reported (81). It would be anticipated that if the other factors that regulate plasma triglyceride levels remained constant, then an enhanced clearance capacity would result in lower plasma triglyceride concentrations. Also, a higher $\dot{V}O_2$ max indicates that the individual has a higher oxidative capacity. In the skeletal muscles this is manifested by a greater number of mitochondria, higher levels of oxidative enzymes, and an improved ability to utilize fat at any relative or absolute work level.

Work by other investigators suggest that exercise-induced changes in triglycerides may be the result of a more complex series of interactions (47,82). In the basal fasting state most of the circulating triglycerides are carried in the VLDL class of lipoproteins. Since the VLDL are of hepatic origin, fasting triglyceride levels reflect a balance between hepatic production and peripheral

disposal, and they are relatively independent of any acute dietary effects. Using Zucker rats, Simonelli and Eaton attempted to determine the effect of chronic exercise on this dynamic balance (47). Normal members of this species are thin and normolipemic. However, those that are homozygous for an autosomal recessive gene are obese and have hypertriglyceridemia, hypoglucagonemia, and insulin resistance. This constellation of findings is similar to the findings in patients with type IV hyperlipidemia. When the rats were placed on a chronic exercise program, the basal triglyceride levels dropped in both groups, but only the obese rats had an enhanced ability to clear Intralipid. In addition the obese rats had decreased plasma insulin and unchanged glucagon levels. The thin, normolipemic rats already had normal insulin levels before the training program, and the major change that accompanied their lowered triglyceride levels was a doubling in the basal glucagon levels. Therefore, while the effects were similar, the changes in the mechanisms controlling plasma triglyceride levels were very different in the two groups. In the obese rats this was due both to an enhanced clearance capability and to an insulin-mediated decrease in hepatic triglyceride production. In the thin, normolipemic rats the increase in glucagon, rather than any change in insulin, was responsible for the decreased hepatic triglyceride production. Glucagon has been shown to reduce the conversion of FFA to triglyceride by stimulating the synthesis of ketone bodies and thus modulating the availability of FFA substrate for lipid synthesis (83). It is apparent that both of these hormones play important roles in determining plasma triglyceride levels.

Consequently, Eaton et al. (82) have suggested that it may be more informative to use the molar ratio of insulin to glucagon to predict the results of their combined effects. In the previous study of Simonelli and Eaton, both groups of rats had a favorable decrease in the molar ratio despite the distinctly different pattern of the hormonal changes, and similar changes have been found in man following intensive physical exercise (44). These results also suggest that care must be taken when making comparisons of the lipid changes in dissimilar groups.

The correlation between K2 and $\dot{V}O_2$ max is probably due to the relationship that each of these factors has with LPL. As the major enzyme responsible for clearing circulating triglycerides, changes in the amount of endothelial-bound LPL should be reflected in corresponding changes in K2. However, since the IVFTT measures the LPL activity over the entire vascular bed, it is not possible to determine whether the observed changes in LPL occurred in the adipose tissue or in the skeletal muscle portion of the vascular bed. Nikkila et al. (43) estimated that endurance trained athletes had 2.3 times the total body LPL activity of normal subjects, and that these increased values reflected increases in both the adipose tissue and skeletal muscle LPL (2.5 and 1.7 times greater than normal respectively). It is not clear whether increased LPL activity in skeletal muscle would be of any advantage during exercise. As was noted previously, during exercise skeletal muscle derives approximately 12% of its energy needs by hydrolysis of circulating triglycerides, with FFA and intracellular triglyceride providing the

remainder of the lipid needs. Since these other sources are rarely depleted even during prolonged exercise, if the LPL-released FA merely spared these depots, there would be no apparent advantage. However, if the FA produced increases in the availability of FA for oxidation, thus reducing the need for carbohydrates, then it would be beneficial. These data do not allow insight into this question. An additional effect of the increased skeletal muscle LPL activity would be to increase the rate at which the skeletal muscle could replenish intracellular triglyceride stores following exercise. Because of the high Michaelis-Menton constant of skeletal muscle LPL, the rate of uptake of FA is dependent on the amount of endothelial bound LPL. Consequently, when this level increases FA will be taken up more rapidly and a relatively greater portion of the circulating triglycerides will be directed to the skeletal muscles.

Since the fractional clearance of triglycerides following heparin injection is another measure of LPL activity, it also was expected to correlate with body composition and $\dot{V}O_2$ max ($r=0.51$, $p<.01$ and $r=0.47$, $p<.01$ respectively). The non-significant correlation between K2 and fractional clearance ($r=-.26$) is consistent with work done by Wada et al. (81) and Huttunen et al. (84). In two large groups of healthy subjects with no known chronic illnesses, neither study found a significant correlation between K2 and triglyceride clearance. Neither group speculated as to why these two measurements of LPL did not correlate, but they did suggest that while K2 and fractional clearance were both closely related to triglyceride removal, they gave clinically important, though different,

information. It may be that the heparin-induced release of the hepatic lipases, which have been shown not to be important in the clearance of circulating triglycerides (85), is responsible for this discrepancy.

Study IV

The 13 % improvement in aerobic capacity and the 1.0 kilogram loss in fat weight in the subjects in study IV are consistent with the changes found in other studies of similar intensity and duration (86,87). While the change in the heparin-induced clearance of triglycerides was the only lipid measurement that reached statistical significance (increased clearance of 21%, $p<.05$), most of the other lipid measurements demonstrated favorable, although not statistically significant, trends, i.e., HDL-cholesterol increased, triglycerides decreased, K2 increased, and the peak level of the fat emulsion decreased. Since most biochemical processes are controlled by proteins, the time course of changes in these processes is related to the time course of the adaptive changes in intracellular proteins. Hickson et al. (88) have estimated that the adaptation to endurance exercise of the systems that limit maximum oxygen uptake is less than 11 days. It may be assumed that the proteins that comprise the plasma triglyceride clearance system respond in a similar time interval. Booth has suggested that when a large, stepwise increase in training is applied, there is an exponential increase in enzyme levels with a new steady state achieved in approximately five half-lives (89). If instead the same load is applied in an incremental manner, then the

rate of enzyme increase is nearly linear and the time to achieve the new steady state level is longer. Therefore, with the incremental increases in training distance throughout study IV, a steady state was never attained, and an assessment of the direction not the degree of change may give an indication of the effect of the program. Consequently, these findings are consistent with those of study III which showed that those who had a higher aerobic capacity had a greater ability to clear the intravascular compartment of triglycerides, and it suggests that these changes are associated with an endurance training program. It is anticipated that if the training had continued the fractional clearance and K2 would have shown larger, increases.

When the subjects were divided into the running groups, the only significant differences between the runners and the untrained subjects were the average weekly mileage and the $\dot{V}O_2$ max. There are several possible explanations for this unanticipated result. Wada et al. have found that K2 is inversely correlated with age and triglyceride levels (81), and because of the similarity between these two groups it would be anticipated that they would at least have had similar baseline levels. Also, although all of the runners had been training for over a year and had been able to complete at least one marathon, their mean maximum aerobic capacity was relatively low (46.1 $ml/kg\text{-}min^{-1}$). Since training can only increase $\dot{V}O_2$ max by 15-30%, other factors, such as pre-training $\dot{V}O_2$ max and muscle fiber composition, are probably important in limiting an individual's maximum oxidative potential. Therefore, selecting a group of trained

runners, who had been unable to complete a marathon in less than 3 and a half hours, may also have resulted in the selection of a group that had a genetically determined low aerobic capacity, a relatively low percentage of slow twitch skeletal muscle fibers, and a relatively reduced capacity to extract triglycerides.

The elite runners on the other hand present the opposite situation. They differed significantly from both the runners and the untrained subjects in aerobic capacity, fat weight, and the ability to clear the plasma of both endogenous and exogenous triglycerides. The major body composition difference between the elite runners and the other groups was the elite runners' very low fat weight. Since there were no significant differences in lean weight between the training groups, this suggests that the observed differences in triglyceride clearance were associated with the amount of endothelial bound LPL in the skeletal muscle capillary bed. Therefore, it appears that in order to get enhanced plasma triglyceride clearance, it may not be sufficient alone to train, but it may also be important that the skeletal muscle have the capacity to increase its production of LPL.

There is no question that chronic exercise produces profound changes in multiple organ-systems. Whether these changes in and of themselves have a favorable effect on cardiovascular risk, as suggested by numerous epidemiological studies, remains to be determined. It appears that in order to be maximally effective an exercise should be performed on a regular basis and be a continuous, prolonged (20 minutes or greater) exercise of moderate intensity (90). The importance of this type of program, as opposed to an

intermittent one, may be that while both impose a load on the cardiovascular system, only the continuous program maintains a high metabolic demand over a period of time sufficient to stimulate the fat mobilization and utilization systems. Central to all of these changes may be the increases in skeletal muscle and adipose tissue LPL. Nikkila et al. (91) have found a highly significant positive correlation between LPL activity in adipose cells and HDL-cholesterol levels in normal human subjects. Whether the changes in skeletal muscle LPL play an important role in providing fatty acids for the exercising muscle is unclear. While the mean contribution of circulating triglycerides was less than 12% of the energy needs during exercise, one study had a range of 0 to 20% in four subjects (80). Since slow twitch fibers have both a greater oxidative potential, as well as higher LPL levels than fast twitch fibers (92), the discrepancy between the subjects may have been due to differing muscle fiber compositions. If so, subjects with a high percentage of slow twitch fibers would have less of a dependence on carbohydrate stores during prolonged exercise. Following exercise the relative increase in skeletal muscle LPL allows for a more rapid clearance of triglyceride from the intravascular compartment, and also directs a relatively greater proportion of the triglycerides to the skeletal muscle, presumably to replenish intramuscular lipid stores. The heightened metabolic demand and enhanced plasma clearance system result in a more rapid turnover in plasma triglycerides, and consequently, in the VLDL's and chylomicrons that are primarily

responsible for transporting them. The direct correlation between LPL and the rate of clearance of the triglyceride-rich lipoproteins from the intravascular compartment would then result in higher HDL concentrations. Therefore, chronic exercise leads to increased energy demands and to a higher oxidative capacity in the skeletal muscles. The needs for fatty acid substrate are met in part through an increased level of LPL in adipose tissue and skeletal muscle, which also results in lower basal triglyceride levels, higher HDL levels, and an improved capacity to clear the intravascular compartment of triglycerides. The beneficial effects of an increase in HDL levels relative to cardiovascular risk may only be secondary to the improved capacity of the skeletal muscle to utilize fat. The response to a given exercise stimulus may be further modified by the hormonal milieu and certain other factors such as the relative muscle fiber composition. It is attractive to speculate that the runners in study III, who required more than three and a half hours to complete the marathon, may not have been more proficient runners, despite their training, for the same reason that they did not have higher HDL levels, i.e. because they were unable to develop an efficient oxidative system. In addition it is also possible that since slow twitch fibers have a high oxidative capacity and produce relatively large amounts of LPL, they may have a greater capacity for generating HDL than fast twitch fibers. If this is true then skeletal muscle fiber composition, through its effect on HDL, may be a risk factor for the development of atherosclerotic vascular disease.

CHAPTER 5

SUMMARY

The purpose of this series of studies was to investigate the plasma lipid profile and the ability to clear intravascular triglycerides (TG) in endurance trained athletes. Four studies were undertaken. Study I was a cross sectional determination of the relationship between cholesterol fractions and weekly running mileage. Study II used a training program to prospectively examine the changes in total and HDL-cholesterol (HDL-C) and to compare these to changes in body composition. Study III employed an intravenous fat tolerance test (IVFTT) and a post-heparin TG clearance test to indirectly assess lipoprotein lipase activity in three groups with varied training and performance backgrounds. Finally, Study IV examined the effects of a 12-week training program on TG clearance in previously untrained subjects.

In Study I there were significant correlations between HDL-C and percent body fat ($r=-0.36$, $p<.001$) and miles run per week ($r=0.50$, $p<.001$), the latter relationship remaining significant when statistically adjusted for age, alcohol consumption, or relative body composition. In Study II as the mean weekly running mileage increased to 44.9 miles, HDL-C increased by 5.0 mg/dl ($p<.01$), and total cholesterol remained unchanged. All components of body composition changed significantly, but only the change in lean weight

significantly correlated with the change in HDL-C ($r=0.46$, $p<.025$). In Study III the rate of Intralipid clearance correlated with fat weight ($r=-0.66$, $p<.001$), fasting TG ($r=-0.39$, $p<.05$), and $\dot{V}O_2$ max ($r=0.64$, $p<.001$). The heparin-induced fractional clearance of TG's correlated to $\dot{V}O_2$ max ($r=-0.51$, $p<.01$) and fat weight ($r=0.47$, $p<.01$). While the runners and the untrained subjects were indistinguishable in body composition and lipid profile, when divided into groups, the elite runners were leaner, had higher HDL-C ($p<.05$), and were able to clear Intralipid ($p<.01$) and plasma TG's ($p<.05$) faster than either of the other groups. Following the training period of Study IV, there was a trend towards a more favorable lipid profile and enhanced TG clearance, but only $\dot{V}O_2$ max ($p<.05$) and the post-heparin fractional clearance of TG's ($p<.05$) improved significantly.

These data suggest that endurance trained subjects have higher HDL-C, lower TG, and an enhanced capacity to clear the intravascular compartment of TG's. These changes cannot be explained solely by the body composition or diets of the athletes and must be due in part to changes evoked by their chronic training.

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