ACUTE HIGH DENSITY LIPOPROTEIN CHANGES WITH EXERCISE

ACUTE HIGH DENSITY LIPOPROTEIN CHANGES

WITH EXERCISE

AT DIFFERENT INTENSITIES

BY

AUDREY L. HICKS

A THESIS

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree

Master of Science

McMaster University

July 1983

MASTER OF SCIENCE (1983)

School of Physical Education and Athletics

MCMASTER UNIVERSITY, Hamilton, Ontario

TITLE: Acute High Density Lipoprotein Changes With Exercise At Different Intensities

AUTHOR: Audrey L. Hicks, B.P.E.

SUPERVISORS: Dr. J.D. MacDougall
Dr. T.J. Muckle

NUMBER OF PAGES: iv, 89

ABSTRACT

ACUTE HIGH DENSITY LIPOPROTEIN CHANGES WITH EXERCISE AT DIFFERENT INTENSITIES

It is known that endurance-trained athletes possess higher levels of high density lipoprotein cholesterol (HDL-C) than sedentary controls, and it has been shown in previous studies that acute exercise may elevate these levels even further. The purpose of this study was to investigate the acute exercise response of the plasma HDL's and to determine if the magnitude of the acute response would be affected by the intensity of the exercise. Twelve men (19-41 yrs) ran an equivalent distance (9-12 km) on a treadmill on two separate occasions. On one occasion the exercise was performed at a speed corresponding to 60% of the subject's VO_2 max, and on the other occasion at a speed corresponding to 90% of VO_2 max. Changes in total cholesterol (TC), triglycerides (TG), HDL-C, HDL Apoprotein A (HDL-A), HDL Saturation (HDL-C/HDL-A), lactate (LA) and free fatty acids (FFA) were measured, and all values were corrected for changes in plasma volume. There were significant increases ($p<.01$) in HDL-C, HDL-A, and HDL saturation with exercise at both intensities, but greater increases in HDL-C (25% vs 14%) and HDL-A (18% vs 8%) were observed with the higher intensity exercise. Plasma FFA and TG were no different between conditions, although LA

concentration rose significantly during the high intensity exercise. The results indicate that increases in HDL components can occur with a relatively moderate exercise session, and that these increases are directly related to the intensity of the exercise.

ACKNOWLEDGMENTS

I would like to acknowledge the much appreciated contributions of Drs. J.D. MacDougall and T.J. Muckle, who supervised all phases of this project. In addition, I would like to acknowledge the technicians at Chedoke laboratory for their assistance in conducting the cholesterol and triglyceride assays. This project could not have been completed without the use of facilities at Chedoke laboratory, and Dr. Muckle and the laboratory staff deserve a special thank you for making these facilities available to me. I would also like to acknowledge the technical assistance provided by the Physiology Laboratory at Waterloo University for the free fatty acid assay. Finally, a grateful thank you to all subjects who volunteered to participate in the study - I hope your bruises have healed!

TABLE OF CONTENTS

		Page
CHAPTER I - INTRODUCTION		1
I.1.	Description of High Density Lipoproteins	1
I.2.	Anti-atherogenic Role of High Density Lipoproteins	2
I.3.	Factors Affecting High Density Lipoprotein Concentration	3
I.4.	Relationship Between Exercise and High Density Lipoproteins	3
I.5.	Possible Mechanisms Linking Exercise With Changes in HDL	7
I.6.	Hypothesis	8
CHAPTER II - REVIEW OF LITERATURE		
II.1.	Lipids	10
II.1(1).	Glycerides	11
II.1(2).	Cholesterol	11
II.2.	Normal Lipid Transport	12
II.2(1).	Chylomicrons	14
II.2(2).	Very Low Density Lipoproteins (VLDL)	14
II.2(3).	Low Density Lipoproteins (LDL)	15
II.2(4).	High Density Lipoproteins (HDL)	15

		Page
II.3(1).	Origin of High Density Lipoproteins	16
II.3(2).	Concept of HDL Saturation	18
II.3(3).	Relationship Between HDL and Coronary Heart Disease	19
II.3(4).	Factors Affecting Plasma HDL Concentration	22
II.4.	The Influence of Physical Activity on The Plasma Lipids	27
II.5.	Relationship Between Exercise and High Density Lipoproteins	29
II.5(1).	Cross-Sectional Studies	29
II.5(2).	Longitudinal Studies	32
II.5(3).	Acute Changes in HDL After a Single Exposure to Exercise	35
II.5(4).	Possible Mechanisms Behind the Exercise-Related Changes in HDL	38
II.6.	Substrate Utilization in Exercise	41
II.7.	Plasma Volume Changes in Response To Exercise	44
II.8(1).	Measurement of HDL Cholesterol	45
II.8(2).	Measurement of HDL Apoprotein A	46
II.9.	Diurnal Variation in Plasma Lipids and Lipoproteins	46

Page

CHAPTER III - METHOD

III.1. Experimental Design 48
III.2. Subjects 48
III.3. Exercise Procedures 50
III.4. Biochemical Analysis 51
III.4(1). Analysis of Plasma Triglycerides, Total Cholesterol, and HDL Cholesterol 52
III.4(2). Analysis of HDL Apoprotein A 52
III.4(3). Determination of HDL Saturation 53
III.4(4). Analysis of Lactic Acid and FFA 53
III.5. Statistical Analysis 54

CHAPTER IV - RESULTS

IV.1. Hematocrit 55
IV.2. Total Cholesterol and Triglycerides 55
IV.3. HDL-C and HDL-A 58
IV.4. HDL Saturation 63
IV.5. Lactic Acid and FFA 63
IV.6. Oxygen Uptakes and Respiratory Quotients 65

CHAPTER V - DISCUSSION AND CONCLUSIONS 66
REFERENCES 77

APPENDIX:

1. Total Amount of Exercise Performed By Each Subject
2. Description of Subjects in Pilot Study
3. HDL Characteristics of Pilot Study Subjects
4. Acute HDL Changes After a 9-12 km Run
5. Changes in Saturation With Acute Exercise
6. Changes in LDL-C With Acute Exercise
7. Relationship Between VO_2 Max and Percent Increase in HDL-C After Exercise
8. Anova Tables and F-Ratios

LIST OF TABLES AND ILLUSTRATIONS

TABLES:

Page		
22	1.	Factors Affecting HDL Concentration
49	2.	Description of Subjects
56	3.	Changes in Dependent Variables With Low Intensity Exercise
56	4.	Changes in Dependent Variables With High Intensity Exercise
64	5.	Relative Oxygen Uptake and Respiratory Exchange Ratio During Exercise

ILLUSTRATIONS:

Page		
57	1.	Changes in Total Cholesterol and Triglycerides With Acute Exercise
59	2.	Changes in HDL-C and HDL-A With Acute Exercise
60	3.	Change in Saturation With Acute Exercise
61	4.	Changes in Lactate and FFA With Acute Exercise
62	5.	Relationship Between Baseline HDL-C and Percent Increase in HDL-C With Acute Exercise

CHAPTER I

INTRODUCTION

I.1. Description of High-Density Lipoproteins

Cholesterol and triglycerides are transported in the plasma by specific complexes called lipoproteins. There are four classes of lipoproteins, the very low density lipoproteins (VLDL), the low density lipoproteins (LDL), the high density lipoproteins (HDL), and the chylomicrons, each differing in their specific composition and relative densities.

The high density lipoproteins are lipid-protein complexes which can be defined by a density of 1.063 - 1.21 gm/ml, and a size of 75-100 Å (Tall & Small, 1978; Levy & Rifkind, 1980; Mahley, 1982). Compared to the other lipoprotein classes, the HDL's have a greater protein content and less lipid, which is reflected by their greater density. About 50% by weight of HDL is protein, 30% phospholipid, 5% triglyceride, and 20% cholesterol (Fredrickson, et al, 1967; Tall & Small, 1978; Levy & Rifkind, 1980). Approximately 90% of the protein component is represented by the A apoproteins (AI and AII), which serve to solubilize the normally insoluble cholesterol and

phospholipids so that they can be transported (Tall & Small, 1978; Levy & Rifkind, 1980). The ratio between the cholesterol and protein content in HDL is not invariant, as it has been demonstrated that HDL can solubilize considerable amounts of exogenous cholesterol in addition to its original cholesterol content (Hsia, et al, 1975). Therefore, the degree of saturation of the HDL complex with cholesterol can be described by the ratio of HDL cholesterol (HDL-C) to HDL Apoprotein A (HDL-A).

I.2. Anti-atherogenic Role of High Density Lipoproteins

As early as 1951, Barr and co-workers discovered a negative relationship between the incidence of coronary heart disease (CHD) and the concentrations of HDL-C and HDL-A. A great deal of epidemiological evidence has accumulated since then, supporting this relationship (Miller & Miller, 1975; Gordon, et al, 1977; Pearson, et al, 1979). The suggested mechanism behind this relationship is that HDL-A, in combination with the enzyme lecithin: cholesterol acyltransferase (LCAT), removes free cholesterol from peripheral tissues and artery walls and converts it to cholesterol ester which is then transported by the HDL particle system to the liver for further catabolism and excretion (Glomset, 1970; Miller & Miller, 1975; Tall &

Small, 1978). This unique function of HDL has been referred to as reverse cholesterol transport.

I.3 Factors Affecting High Density Lipoprotein Concentration

In light of the consistent epidemiological evidence demonstrating an association between HDL concentration and risk of CHD, recent interest has focussed on those factors which can influence the plasma concentrations of HDL. Cigarette smoking appears to have an inverse relationship with HDL-C (Enger, et al, 1977; Morrison, et al, 1979), as does increased intake of dietary carbohydrates (Ernst, et al, 1980; Krauss, et al, 1982). On the other hand, moderate alcohol intake, female sex, oral contraceptives, and endurance exercise are all positively associated with concentrations of HDL-C (Wood, et al, 1976; Gordon, et al, 1977; Enger, et al, 1977; Morrison, et al, 1979; Ernst, et al, 1980; Hartung, et al, 1980; Beaglehole, et al, 1980). The relationship between physical exercise and HDL concentration will be examined in the present investigation.

I.4. Relationship Between Exercise and High Density Lipoproteins

Reports from numerous cross-sectional studies have revealed high HDL-C concentrations in endurance-trained

athletes when compared with sedentary controls (Wood, et al, 1976; Enger, et al, 1977; Lehtonen & Viikari, 1978; Hartung, et al, 1980). The evidence is not so clear with respect to the effect of exercise on the protein component in HDL. Higher levels of HDL-AI have been reported in endurance athletes (Lehtonen, et al, 1979; Krauss, et al, 1977) but there is almost no data available on the effect of exercise on the total apoprotein A content in HDL. Results from a recent pilot study revealed no significant difference in HDL-A between male distance runners and sedentary controls (Appendix 3).

Results from longitudinal training studies have been quite inconsistent, possibly due to differences in the intensity and duration of the training programs, and the relatively small sample sizes used. Increases in HDL-C after relatively moderate training programs have been reported several times (Erkelens, et al, 1978; Huttunen, et al, 1979; Peltonen, et al, 1981; Hartung, et al, 1981; Myhre, et al, 1981), whereas no change in HDL-C after similar training programs have also been reported (Lipson, et al, 1980; Allison, et al, 1981; Dufaux, et al, 1982a). A possible contributor to these discrepancies might be the lack of control for initial HDL-C concentration, before training begins. It may be that the ability of exercise to

increase HDL-C levels depends on the initial level, a possibility that will be explored in the present investigation.

Reports of changes in HDL-A after chronic training have been equally conflicting. Both increases in HDL-AI (Kiens, et al, 1980) and no change in HDL-AI or HDL-AII (Huttunen, et al, 1979) have been reported after moderate conditioning.

The effects of a single exercise session on HDL concentrations has been explored in several studies. Significant increases in HDL-C have been found immediately after a 42 km marathon (Thompson, et al, 1980), 3 hours of continuous running (Dufaux, et al, 1982a), and a 70 km cross-country ski race (Enger, et al, 1980). Conversely, no change in HDL-C was found after 30 minutes of cycling at an intensity corresponding to 65% of $\dot{V}O_2$ max (Cullinane, et al, 1980). Only one of these studies also measured HDL-AI, which was found to be elevated after completion of a marathon (Thompson, et al, 1980).

Evidently, the intensity and/or duration of the exercise could be important factors determining both whether an acute response will occur, and how long it will last. The pilot study (Appendix 4) revealed that even a routine training run (9-12 km completed in 45-60 min) can

significantly elevate both HDL-C and the HDL saturation (HDL-C/HDL-A), in the absence of any plasma volume shifts. Pre-exercise values in this particular study were re-established within 18 hours.

Much of the literature seems to suggest a dose-response relationship between the amount of exercise training and the degree of change in the plasma lipoproteins, as long as the exercise is of the endurance variety.

A positive relationship between distance run per week and HDL-C levels has been reported in two different studies (Lehtonen & Viikari, 1978; Hartung, et al, 1980), which seems to imply that duration of exercise may be the important factor. However, Huttunen and co-workers (1979) found a greater increase in HDL-C after a moderate (60% $\dot{V}O_2$ max) exercise training program versus a relatively mild (30% $\dot{V}O_2$ max) program, with duration being kept constant, which suggests intensity to be a key factor as well. In a training program that varied both the intensity and the duration, a greater increase in HDL-C was found after the low intensity (70-80% $\dot{V}O_2$ max), long duration period than after the high intensity (80-95% $\dot{V}O_2$ max), short duration period (Myhre, et al, 1981), which implicates both intensity and duration to be involved in the exercise-related increases in HDL-C. These factors will be explored further in the present study.

I.5. Possible Mechanisms Linking Exercise with Changes in HDL

While the exact mechanisms behind the exercise-related increases in HDL-C and/or HDL-A are unknown, some relationships between HDL and certain enzymes have been identified. Increased levels of lipoprotein lipase (LPL), the enzyme which catalyzes the hydrolysis of triglycerides in the VLDL and chylomicrons, have been reported in endurance athletes versus sedentary controls (Nikkila, et al, 1978; Krauss, et al, 1979; Peltonen, et al, 1981). The catabolism of VLDL and chylomicron triglyceride by LPL not only releases free fatty acids, but also the surface components of these lipoproteins (unesterified cholesterol, phospholipids, and A and C apoproteins) can now enter the plasma pool as HDL precursors. These precursor particles are then transformed into spherical HDL by the LCAT reaction previously mentioned (Tall & Small, 1978). It has also been suggested that during the transformation of these precursor particles into spherical HDL there is likely a large influx of cholesterol into the HDL fraction, since the relative deficiency of cholesterol in the HDL precursors would result in a chemical gradient favouring the movement of cholesterol into HDL (Tall & Small, 1978).

Therefore, if LPL activity is indeed important in the exercise-related increases in HDL-C, one should see a positive relationship between the breakdown of serum triglyceride (or the level of plasma free fatty acids) and the level of HDL-C, which has been supported in the literature (Wood, et al, 1976; Sauer, et al, 1980). However, the activity of LCAT must be an important part of this mechanism as well, since LCAT transforms the HDL precursors into cholesterol ester, which is then transported inside the HDL particle. Increases in LCAT have been reported after seven weeks of exercise conditioning, which supports the involvement of this enzyme (Lopez, et al, 1974).

As the intensity of exercise increases, the proportion of energy supplied by free fatty acids (FFA) decreases, due to increased utilization of carbohydrates (Fox, 1979). Therefore, if one supports the activity of LPL as being an important factor in inducing the higher HDL-C concentrations in endurance athletes, one should find the greatest acute increase in HDL-C at the exercise intensity in which FFA turnover is maximum, ie., when LPL activity is the greatest.

1.6 Hypothesis

The hypothesis for this study is that prolonged sub-maximal exercise, through the activity of LPL and LCAT,

results in an increase in the cholesterol content in HDL, which is proportional to the degree of utilization of FFA as as a fuel source. Furthermore, it is being proposed that the acute increases in HDL-C immediately following endurance exercise are most likely the result of a greater influx of cholesterol into the existing HDL particle system, and not the result of an increase in total HDL macromolecule concentration. Consequently, it is expected that increased saturations of HDL-A with cholesterol will be found after an endurance exercise session.

Based on this hypothesis, this study will attempt to answer the following questions:

(1) Will the acute exercise response of plasma HDL differ between a prolonged period of sub-maximal exercise (60-65% $\dot{V}O_2$ max) and a series of high intensity intervals (90-95% $\dot{V}O_2$ max), given that the same total amount of exercise is being done in the two conditions?

(2) Will the acute response occur independently of the initial baseline level of HDL-C?

CHAPTER II

REVIEW OF LITERATURE

II.1. Lipids

Lipids comprise a chemically heterogeneous group of substances which have the common property of being insoluble in water, but soluble in fat solvents, such as chloroform, alcohols, or hydrocarbons. Another common feature of most lipids is that they commonly exist in the form of esters of fatty acids. Based on these properties, three distinct classes of lipids can be identified: neutral lipids, phospholipids, and glycolipids.

The term neutral lipid is used to describe the group of compounds having non-polar esters of fatty acids with alcohol, cholesterol, glycerol, Vitamin A, and other long chain alcohols (Gurr & James, 1980). The glycerides and cholesterol are the most common neutral lipids found in man. The phospholipids comprise those compounds which contain mixed esters of fatty acids and phosphoric acid with either glycerol or sphingosine, with the group derived from glycerol being the most common (Gurr & James, 1980). Lecithin is the most abundant phospholipid in the plasma, and it is derived primarily from the liver (Masoro, 1968). The glycolipids are compounds which contain a sugar moiety, but have the solubility properties of a lipid, such as

cerebroside sulphates, glycosyl ceremides, and gangliosides (Masoro, 1968; Gurr & James, 1980).

II.1(1). Glycerides

Glycerides are the main components of natural fats and oils, and are comprised of fatty acid esters of glycerol. The most abundant glyceride in mammals are the triglycerides, in which there are fatty acyl groups esterified to each of the three alcohols of glycerol. The triglycerides are a very convenient storage form of fatty acids in adipose tissue which can be easily broken down for the purposes of energy production. This breakdown is catalyzed by hormonally-controlled lipases, which hydrolyze the bond between the glycerol and fatty acids. The triglycerides are the most variable of the plasma lipids, as their concentration can be dramatically affected by the ingestion of a fatty meal (Masoro, 1968; Gurr & James, 1980).

II.1(2). Cholesterol

Cholesterol is an important structural component of all cell membranes. It is the most abundant sterol found in mammals, and it can be present in either a free or esterified form. The metabolism of cholesterol esters involves enzyme systems in the liver, adrenal cortex, pancreas, intestine, and the blood plasma. The liver is a key organ in the regulation of the plasma cholesterol ester level, as it is capable of both synthesizing cholesterol esters from

fatty acids and free cholesterol, and also hydrolyzing these esters back to free cholesterol. The total plama cholesterol concentration (both free and esterified) in the normal adult male ranges from about 140 to 260 mg/100 ml plasma, with the greater proportion of this cholesterol being in the esterified form (Masoro, 1968).

Cholesterol is synthesized endogenously, primarily in the liver, and can also be obtained exogenously from the diet. The total cholesterol concentration in the blood however, remains relatively constant due to very sensitive regulatory mechanisms. Cholesterol is continuously being degraded and resynthesized in order to keep the total concentration in a relatively steady state. One of the main factors behind this natural homeostasis is man's limited capacity for absorbtion of cholesterol, thus, ingesting large amounts of cholesterol will increase the total plasma concentration by only a small extent. Additional factors which help in maintaining cholesterol equilibrium are the reduction of endogenously synthesized cholesterol in response to increased cholesterol absorbtion, and increased degradation of cholesterol into bile acids when increased amounts of cholesterol are absorbed (Masoro, 1968).

II.2. Normal Lipid Transport

The plasma lipids rarely exist in a 'free' form, rather, they are transported through the plasma in

combination with proteins as lipoproteins. The plasma lipoproteins are specific complexes of lipid and protein, comprised of an inner hydrophobic core of non-polar lipids surrounded by an outer coat of protein and polar lipids. Such a structure protects the hydrophobic non-polar lipids from direct exposure to the plasma during transport (McMurray, 1982).

The protein components of the plasma lipoproteins are represented by a family of peptides called apoproteins or apolipoproteins. These apoproteins play critical roles in both the maintenance of lipoprotein structure and in the regulation of their metabolism. Three distinct classes of apoproteins have been identified, Apo-A, Apo-B, and Apo-C, with the concentrations of these specific classes being different in the different lipoproteins. Further divisions of these classes into subclasses (eg. Apo-AI or Apo-AII), and other minor apoproteins (eg. Apo-D, Apo-E) have also been described.

The ratio of lipid to protein varies between the different classes of lipoproteins, as does the particular lipid or protein moiety. Four main classes of lipoproteins have been identified, based on their specific components, size, and relative densities. The structure of each of the lipoprotein classes however is basically similar, with non-polar lipids occupying the central hydrophobic core, surrounded by an outer interface of apoproteins and polar lipids (McMurray, 1982).

II.2(1). Chylomicrons

Chylomicrons are the largest of the blood-borne lipoproteins, having a diameter of 120-1100 nm (Miller, 1979). These particles have the highest content of lipid relative to protein, being composed primarily (90%) of triglyceride of exogenous origin. The remaining 10% of the chylomicron is comprised of 5% cholesterol, 4% phospholipid, and 1% protein (Miller, 1979; Levy & Rifkind, 1980; Rifkind, 1982). The relatively minute content of protein in the chylomicrons makes them the lightest of the four lipoprotein classes, having a density of less than 0.95 gm/ml (Rifkind, 1982). All three families of apoproteins (A, B, and C) have been detected in the protein component of chylomicrons, with apoprotein C being the most predominant (McMurray, 1982).

The chylomicrons transport dietary glycerides absorbed from the intestines in the plasma to the liver or adipose tissue, and thus, are normally absent in fasting plasma. With a half-life of only 10 minutes, chylomicrons rapidly disappear from the bloodstream, primarily due to hydrolysis of the triglyceride component by lipoprotein lipase (McMurray, 1982).

II.2(2). Very Low Density Lipoproteins (VLDL)

The predominant lipid in VLDL, like the chylomicrons, is triglyceride, but whereas the glyceride in chylomicrons is exogenous in origin, VLDL carry only endogenous triglyceride which is synthesized in the liver. Sixty-five percent

(by weight) of VLDL is composed of triglyceride, 13% cholesterol, 12% phospholipid, and 10% protein (Levy & Rifkind, 1980; Rifkind, 1982). The latter component contains equal portions of apoprotein B and C, and also small traces of apoprotein A. VLDL are smaller in diameter than the chylomicrons (30-90 nm) but they are slightly denser particles, in the range of 0.95-1.006 gm/ml (Miller, 1979).

II.2(3). Low Density Lipoproteins (LDL)

LDL particles are the major cholesterol-carrying vehicles in blood, accounting for 60-70% of the total plasma cholesterol (Rifkind, 1982). They are composed of 43% cholesterol, primarily in the esterified form, 22% phospholipid, 10% triglyceride, and 25% protein (Rifkind, 1982). The protein component is almost entirely in the form of apoprotein B, with some small traces of apoprotein C. LDL in man arises primarily from catabolism and modification of VLDL, but LDL are denser particles than VLDL, having a density in the range of 1.006-1.063 gm/ml (Rifkind, 1982).

II.2(4) High Density Lipoproteins (HDL)

HDL particles are the smallest but densest of the four lipoprotein classes, having a diameter of 75-100 Å and a density of 1.063-1.21 gm/ml (Tall & Small, 1978; Levy & Rifkind, 1980). They have the highest protein content relative to lipid, which is reflected by their greater density. Approximately 50% by weight of HDL is protein,

represented primarily (90%) by the A apoproteins. The ratio between apoprotein AI and AII in the HDL is approximately 3 : 1 (Tall & Small, 1978). The remaining few percent of the protein component is represented by apoprotein C. The HDL contains about 20% cholesterol, with a cholesterol ester : free cholesterol ratio of approximately 3 : 1 (Levy & Rifkind, 1980). HDL normally accounts for about 20-25% of the total plasma cholesterol. Phospholipid and triglyceride are the remaining components of the HDL particle, accounting for 30% and 5% of the total mass respectively (Levy & Rifkind, 1980).

The heterogeneity of HDL particles is well established, dating back to 1954 when Gofman and co-workers reported that HDL actually contained three components: HDL_1 with a density of 1.05 gm/ml; HDL_2 with a density of 1.075 gm/ml; and HDL_3 with a density of 1.145 gm/ml. The ratio of lipid to protein is greatest in the HDL_1 subclass and least in HDL_3. Considerable evidence exists which suggests that HDL_2 is the most variable of the three subclasses and that changes in total HDL concentration in both health and disease are due largely to changes in the HDL_2 subfraction (Gofman, et al, 1954; Krauss, et al, 1977; Anderson, et al, 1978; Shepherd, et al, 1980).

II.3(1) <u>Origin of High Density Lipoproteins</u>

There is good evidence to suggest that the spherical HDL particles in plasma are produced from precursor HDL

particles that have a different structure and chemical composition. These HDL precursors appear to arise from two sources: through secretion by the liver or small intestine, and through the lipolysis of triglyceride-rich lipoproteins, namely VLDL and chylomicrons (Nikkila, 1978a; Miller, 1979; Tall & Small, 1980).

The nascent form of HDL secreted by the liver is discoidal is shape, consisting of a bilayer of phospholipid and unesterified cholesterol, with apoprotein E as the main peptide and with virtually no cholesterol ester (Miller, 1979; Nicholl, et al, 1980; Tall & Small, 1980). The transformation of this nascent form of HDL to plasma HDL requires the action of two important enzymes, lipoprotein lipase (LPL) and lecithin: cholesterol acyltransferase (LCAT). LPL catalyses the catabolism of chylomicron and VLDL triglyceride and it is activated by apoprotein C-II. The products of this breakdown (fatty acids and glycerol) are rapidly removed and and the surface components that are left (phospholipids, free cholesterol, and apoproteins A and C) are transferred into the HDL fraction as HDL precursors. The Apo-A then activates the enzyme LCAT in the presence of free cholesterol to produce cholesterol esters. LCAT takes a fatty acid from lecithin (the phospholipid) and by transferring it to free cholesterol produces a cholesterol ester which is drawn inside the HDL particle away from the aqueous interface on the surface. The other bi-product of

this reaction, lysolecithin, is removed from the HDL particle surface by albumin (Glomset, 1968; Tall & Small, 1978).

As the LCAT reaction continues, the discoidal HDL particle gradually becomes a sphere, with a core of cholesterol ester. Some of the cholesterol ester produced is transferred back to the triglyceride-rich lipoproteins, probably in association with apoprotein E (Miller, 1979).

II.3(2). Concept of HDL Saturation

Human serum must have the capacity to solubilize additional cholesterol beyond its own cholesterol content if one is to accept the idea of reverse cholesterol transport. A study by Hsia and co-workers (1975) demonstrated that the cholesterol content of human serum is normally below the maximum capacity of the lipoproteins for binding and transporting cholesterol, and that specific subfractions in the VLDL and HDL are capable of solubilizing considerable amounts of additional cholesterol. It was also found that this capacity for additional cholesterol binding is reduced in patients with coronary heart disease, since these patients appear to be deficient in the specific HDL and VLDL subfractions which are capable of additional cholesterol binding (Hsia, et al, 1975). Jonas and co-workers (1978) also provided evidence that human HDL has a high capacity for binding excess cholesterol, and that the rate of exogenous cholesterol uptake is inversely related to the initial content of free cholesterol in HDL.

Therefore, it appears that the ratio between the cholesterol and apoprotein content in HDL (and VLDL) is not invariant, and that by determination of the ratio between HDL-C and HDL-A the degree of saturation of the HDL-cholesterol complex or, inversely, the cholesterol binding reserve, can be assessed. Furthermore, this cholesterol binding reserve is apparently deficient in patients with CHD (Hsia, et al, 1975), or on chronic hemodialysis who have developed severe atherosclerosis (Rapoport, et al, 1978).

II.3(3). Relationship Between HDL and Coronary Heart Disease

A relationship between HDL concentration and the incidence of CHD has been recognized for some time. As early as 1951, Barr and co-workers observed reduced levels of both HDL-C and HDL-A in the presence of atherosclerosis. Since then, various case-control studies in the USA (Castelli, et al, 1977; Pearson, et al, 1979), Scandinavia (Nikkila, 1953), and Israel (Goldbourt & Medallie, 1979; Brook, et al, 1982) have confirmed this relationship. Furthermore, several prospective studies have also given support to a concept of an anti-atherogenic role of HDL. In the Framingham study (Gordon, et al, 1977), a four-year follow-up of 2,815 men and women revealed that HDL-C was the most potent lipid risk factor studied for the development of CHD, showing a striking negative relationship in both men and women. Similarly, a 25-year follow-up in the Minnesota

Prospective Study (Keys, 1980) revealed that men who died from CHD had a mean HDL-C concentration that was 4.42 mg% lower than those who died from other causes.

The average HDL cholesterol level for adult males is approximately 45 mg% and 55 mg% for females (Gordon, et al, 1977; Keys, 1980). A significantly high risk of developing CHD is evident if one's HDL-C is 35 mg% or lower, and there appears to be an almost linear relationship between HDL-C concentration and risk of CHD in both men and women. Furthermore, Jenkins and associates (1978) found that the number and severity of atherosclerotic lesions in the coronary circulation in patients with documented CHD were inversely related to the concentration of HDL-C.

In addition to the low cholesterol content in HDL, the apoprotein composition also appears to be altered in patients with CHD. Barr and workers (1951) observed reduced levels of both HDL-C and HDL-A in their sample of CHD patients, whereas Rapoport and his collegues (1978) have reported abnormalities in the apoprotein C content in patients with atherosclerosis. Apoprotein C activates LPL, thus, any defect in this enzyme's activation will affect the catabolism of the triglyceride-rich lipoproteins, and subsequently, the availability of HDL precursors.

There have been two main mechanisms suggested to explain the apparent protective role of HDL in reducing the risk of CHD. The work of Miller & Miller (1975) revealed

that the HDL concentration is inversely related to the total cholesterol pool. In reference to the work of Glomset (1970), which described the role of LCAT, Miller and Miller (1975) proposed that the activation of LCAT by HDL-A removes free cholesterol from the peripheral tissues and artery walls and converts it to cholesterol ester. The cholesterol ester is then drawn inside the HDL particle and is transported to the liver for catabolism and excretion. This whole process has been referred to as reverse cholesterol transport.

A second mechanism explaining the anti-atherogenic role of HDL was proposed by Carew and associates in 1976. These authors suggested that HDL may block the entrance of LDL cholesterol into cells by competitively binding to the LDL receptor sites. By interfering with these specific binding sites on cell membranes, HDL can prevent the deposition of cholesterol esters which presumably would prevent the formation of atherosclerotic lesions.

Considerable evidence has accumulated suggesting that the protective effect of HDL with respect to atherosclerosis lies with only a specific HDL subclass. It appears that of the three subfactions, HDL_2 is the most variable, with its concentration differing more dramatically than total HDL in various pathologic and physiologic conditions. In fact, the high levels of HDL found in women

and athletes, and the low levels found in diabetics and cardiac patients seem to be almost entirely due to differences in the HDL_2 component (Krauss, et al, 1977; Shepherd, et al, 1980; Eder & Gidez, 1982). Anderson and collegues (1979) have even suggested that measuring HDL_2 is 1.5 times more sensitive than measurement of total HDL-C with respect to predicting risk from CHD. It appears therefore, that the HDL_2 subfraction is the major contributor to the anti-atherogenic role of plasma HDL.

II.3(4). Factors Affecting Plasma HDL Concentration

A number of factors have been identified as having either a direct or indirect association with plasma HDL-C concentrations. Some of these factors are listed in Table 1, and a brief discussion of each will follow.

TABLE 1

Factors Associated With High HDL-C Levels	Factors Associated With Low HDL-C Levels
Female Gender	Male Gender
Familial Hyperalpha-lipoproteinemia	Familial LCAT Deficiency
Estrogenic Hormones	High-Carbohydrate Diet
Alcohol	Cigarette Smoking
High Physical Activity	Beta-blocking Drugs
Terbutaline	Insulin Deficiency
	Obesity

II.3(4)a. Gender

Gender is a major factor which has been shown to have an effect on plasma HDL concentrations. At all ages

following puberty, women have higher HDL cholesterol levels than males (Gordon, et al, 1977; Nikkila, 1978a; Beaglehole, et al, 1980). The hormone estrogen appears to play a role in this relationship, since exogenous administration of estrogens to males has been shown to cause increases in HDL concentration to the levels normally found in women (Krauss, et al, 1979). Additionally, Nikkila (1978a) suggested that part of the sex difference in plasma HDL-C concentrations may be explained by the higher activity of adipose tissue LPL that has been observed in women versus men.

II.3(4)b. Genetics

Genetics have been shown to play a significant role in regulating HDL levels, especially in disease states. Tangier disease is a rare genetic disorder in which the concentration of HDL is dramatically reduced, whereas familial hyperalphalipoproteinemia is characterized by elevated HDL-C levels, predominantly in the HDL_2 subclass (Tall & Small, 1980; Krauss, 1982). Familial LCAT deficiency is another inherited condition in which the absence of LCAT prevents the esterification of cholesterol. The plasma from patients with this disease contains discoidal nascent HDL particles which are rich in unesterified cholesterol but poor in cholesterol ester (Miller, 1979).

Although it is unknown whether the genetic influences controlling HDL levels in disease states are related to those in the general population, twin and family studies

have provided some evidence for the presence of genetic regulation of HDL levels. Concordance studies in 40 pairs of male adult twins suggested that both HDL-C and HDL-A are under some degree of genetic control (Berg, 1978).

II.3(4)c. Body Fat

Concentrations of HDL cholesterol are lower in obese individuals than in non-obese controls (Castelli, et al, 1977). These depressed HDL levels are usually accompanied by elevated plasma triglyceride concentrations. Studies of changes in HDL after weight reduction have revealed conflicting results. Both increases in HDL-C (Wilson & Lees, 1972) and no change in HDL-C (Widholm, et al, 1978) have been reported after gross weight reduction in obese subjects.

II.3(4)d. Diet

Several dietary components have been shown to have an effect on HDL concentration. Increases in carbohydrate intake appear to be negatively associated with HDL-C levels (Wilson & Lees, 1972; Ernst, et al, 1980), whereas increased dietary cholesterol has been positively associated with HDL-C concentration, although this relationship is not always evident (Tan & Dickenson, 1977; Mahley, et al, 1978). A strong positive association between alcohol intake and HDL-C levels has been reported several times (Yano, et al, 1977; Morrison, et al, 1979; Ernst, et al, 1980). Increased adipose tissue LPL activity and/or accelerated hepatic synthesis of nascent HDL have been suggested as possible mechanisms behind the alcohol-induced increases in HDL-C (Nikkila, 1978a).

II.3(4)e. Drugs

The use of oral contraceptives appears to have a positive relationship with HDL-C levels, but this association seems to be dependent on the relative estrogen and progestogen potency. Bradley, et al (1978) reported elevated HDL-C levels only in those women receiving exogenous estrogens, in fact, decreased HDL-C levels were found in women taking only progestogens.

A negative relationship between some beta-blocking drugs, such as propranolol, and HDL-C concentrations has been reported (England, et al, 1980; Wallace, et al, 1980). Conversely, it has recently been shown that terbutaline, a beta-adrenergic agonist, is associated with significant increases in HDL-C concentrations (Hooper, et al, 1981).

II.3(4)f. Smoking

A negative relationship between cigarette smoking and HDL-C concentration has been consistently reported in a number of studies, and has also been shown to be a dose-dependent relationship (Bradley, et al, 1978; Morrison, et al, 1979; Crique, et al, 1980). However, this relationship was not confirmed in a Scandinavian population study (Erikssen, & Enger, 1978).

II.3(4)g. Physical Activity

A number of cross-sectional studies have found high levels of endurance-type exercise to be positively associated with HDL cholesterol and other HDL measures (Wood, et

al, 1976; Enger, et al, 1977; Krauss, et al, 1977; Lehtonen & Viikari, 1978; Lehtonen, et al, 1979; Hartung, et al, 1980). This relationship has also held true is some (Erkelens, et al, 1978; Peltonen, et al, 1981), but not all (Lipson, et al, 1980; Dufaux, et al, 1982a) longitudinal training studies. Discrepancies in the literature reporting on the relationship between exercise and HDL are possibly due to lack of control for intensity, duration, and amount of exercise, plus, any other confounding factors, such as diet and cigarette smoking, which are also known to affect HDL concentration.

II.3(4)h. Disease

Diabetes has received a great deal of research attention with respect to its relationship with HDL levels. A negative relationship between diabetes and HDL-C levels has been observed in some, but not all, diabetic populations (Nikkila, 1978). Insulin plays a role in this relationship, but the heterogeneity of the diabetic population makes interpretation of this role difficult. It appears that insulin-deficient diabetics have both sub-normal HDL-C levels and low LPL activities, whereas patients who have been successfully treated with insulin for over 5 years have normal values for HDL-C and LPL activity (Nikkila, 1978; Eder, et al, 1979). Thus, depressed levels of HDL-C in diabetic patients are likely the result of an inefficient metabolism of VLDL and chylomicrons due to low activities of LPL.

II.4. The Influence of Physical Activity on the Plasma Lipids

Before discussing the research dealing with the relationship between exercise and high density lipoproteins, a brief summary of the effects of physical activity on the major plasma lipids will be presented.

The observation of lower concentrations of fasting plasma triglycerides in physically active subjects has been a consistent finding in several cross-sectional studies (Hurter, et al, 1972; Lehtonen & Viikari, 1978; Hartung, et al, 1980). This appears to be a dose-response relationship, as Hartung and co-workers (1980) observed fasting triglyceride concentrations of 154 mg%, 105 mg%, and 77 mg% in inactive controls, joggers, and marathoners respectively. Since VLDL carries the majority of endogenous triglyceride, the concentration of this lipoprotein is also lower in physically active individuals (Wood, et al, 1977).

Similarly, a common observation from longitudinal training studies has been a decrease in plasma triglycerides after training (Lopez, et al, 1974; Farrell & Barboriak, 1980; Kiens, et al, 1980; Lipson, et al, 1980). However, a number of studies have failed to demonstrate this decrease (Altekruse & Wilmore, 1973; Ratcliff, et al, 1978; Hartung, et al, 1981). It appears that the effect of chronic exercise conditioning in terms of lowering plasma triglycerides is dependent on both the initial triglyceride

level, and the intensity of the training program. People whose initial plasma triglycerides are high will more likely show a beneficial decrease in these levels after a vigorous training program than if they initially had low triglyceride levels (Wood & Haskell, 1979).

The effect of exercise on total plasma cholesterol is not so clear. A few cross-sectional studies have reported lower total cholesterol concentrations in physically trained subjects versus sedentary controls (Wood, et al, 1976; Hartung, et al, 1980; Vodak, et al, 1980). However, about as many studies have shown no difference in total cholesterol between athletes and controls (Hurter, et al, 1972; Epstein, et al, 1976; Enger, et al, 1977; Lehtonen & Viikari, 1978).

Reports from longitudinal studies have also yielded conflicting results with respect to changes in total cholesterol before and after training. Some studies have demonstrated decreased total cholesterol concentrations after a long-term physical conditioning program (Altekruse & Wilmore, 1973; Lopez, et al, 1974; Kiens, et al, 1980), but just as many have reported no change in total cholesterol after conditioning (Farrell & Barboriak, 1980; Hartung, et al, 1981; Myhre, et al, 1981). This inconsistency is perhaps not surprising, since total cholesterol change reflects the resultant of changes in HDL, LDL, and VLDL, and each of these may show exercise-induced alterations which differ not only in degree but also in direction.

II.5. Relationship Between Exercise and High Density Lipoproteins

The relationship between physical activity and HDL levels has received a great deal of research attention ever since the suggestion was made that high concentrations of HDL may offer some protection against CHD. Reports from various cross-sectional and longitudinal studies have revealed that endurance-trained athletes possess a characteristic lipoprotein profile which appears to occur independently of the effect of other factors which are known to influence HDL concentration.

II.5(1). Cross-Sectional Studies

As early as 1964, Carlson and Mossfeldt reported that male Swedish skiers had higher HDL cholesterol concentrations than the general population. Numerous cross-sectional studies since then have also found higher HDL-C levels in various groups of physically active individuals, such as runners (Wood, et al, 1977; Lehtonen & Viikari, 1978; Hartung, et al, 1980), cross-country skiers (Lehtonen & Viikari, 1978), tennis players (Vodak, et al, 1980), and soccer players (Lehtonen & Viikari, 1980).

The concentrations of apoproteins AI and AII have also been investigated in endurance-trained athletes. Krauss and co-workers (1977) found no difference in Apo-AII between runners and controls, however, higher concentrations of Apo-AI in athletes have been reported in a number of

studies (Krauss, et al, 1977; Lehtonen, et al, 1979; Miller, et al, 1979). This seems to be a significant finding, since one of the main functions of Apo-AI is to activate LCAT which esterifies cholesterol and thus facilitates its transport in HDL back to the liver (Glomset, 1970; Fielding, et al, 1972).

The effect of exercise on major enzymes involved in lipoprotein metabolism has also been investigated. Endurance-trained athletes have been found to have increased activities of lipoprotein lipase (LPL) in both adipose tissue and skeletal muscle (Nikkila, et al, 1978c; Taskinen, et al, 1980). These enzyme changes appear to be dependent on the type of training employed, since the LPL activity in sprinters, who train more anaerobically than endurance athletes, has been found to be no different than that of sedentary controls (Nikkila, et al, 1978c).

Miller and co-workers (1979) observed a strong correlation ($r=0.81$, $p<0.01$) between maximal aerobic capacity and HDL-C in eleven males who participated in varying degrees of habitual physical activity. They did not find a significant correlation between maximal aerobic capacity and the concentration of HDL-AI. However, after calculating the ratio of HDL-C to HDL-AI in each subject, the strongest correlation ($r=0.88$, $p<0.001$) was found between this ratio and maximal aerobic capacity. This ratio of cholesterol to Apo-AI in HDL has been suggested by Cheung and Albers (1977) to be directly related to the ratio of HDL_2 to HDL_3,

providing evidence that the higher HDL-C levels and higher ratios of cholesterol to protein observed in both athletes and women are due primarily to differences in the relative proportions of the HDL subclasses, rather than differences in actual HDL structure.

There is no doubt that the type of physical activity one engages in is an important factor determining whether the lipoprotein profile will differ from that of the average sedentary control. Most studies which have demonstrated the typical high HDL-C levels have been based on a sample of athletes who engage in prolonged endurance-type training, however, relatively little is known about the effects of short duration, high intensity training on plasma lipoprotein concentrations. Lopez and associates (1980) reported a significant increase in HDL-C in 14 males after 12 weeks of resistive (anaerobic) training. On the other hand, the HDL-C concentrations of 8 sprinters, whose training consisted primarily of short duration exercises like weight-lifting, were no different than those of sedentary controls in a study conducted by Nikkila, et al (1978). In another study which compared the lipid profiles of soccer players, ice hockey players, and sedentary controls, the lowest HDL-C concentrations were found in the ice hockey players, whose training consisted primarily of anaerobic exercise (Lehtonen & Viikari, 1980). Evidently, more research is needed in the area of anaerobic training in

order to understand its effect, if any, on plasma lipoprotein metabolism.

II.5(2). Longitudinal Studies

Changes in HDL concentration have been assessed during numerous types of exercise conditioning. However, a problem in comparing the results of these studies is the wide range in the intensity and duration of the different training programs, and the relatively small sample sizes employed. Increases in HDL-C have been reported after relatively moderate training programs, such as 3-4 months of mild to moderate exercise, 3 times per week (Huttunen, et al, 1979; Peltonen, et al, 1981). On the other hand, no changes in HDL-C after similar training programs were observed by Lipson, et al, 1980, and Allison, et al, 1981.

Intensity of the exercise in a training program appears to be an important factor, since Myhre and associates (1981) observed a greater increase in HDL-C after the low intensity (70-80% $\dot{V}O_2$ max), long duration portion of their training program than after the high intensity (80-95% $\dot{V}O_2$ max), short duration period. However, Huttunen and co-workers (1979) observed a greater increase in HDL-C after moderate (60% $\dot{V}O_2$ max) exercise conditioning versus relatively mild (30% $\dot{V}O_2$ max) conditioning, with duration being kept constant. Therefore, it appears that the greatest increase in HDL-C will occur when the exercise intensity is in the range of 60-80% of $\dot{V}O_2$ max.

Another study, while failing to demonstrate an absolute increase in HDL-C after a 10-week moderate conditioning program, did show a significant rise in the HDL_2 subfraction, with corresponding decreases in HDL_3 (Nye, et al, 1981). Therefore, even a very moderate activity program may have beneficial effects on the distribution of the HDL subfractions, in the absence of any absolute change in HDL-C concentration.

Changes in the major HDL apoproteins after chronic exercise has received far less attention. After a 12-week moderate intenstiy training program, Kiens and workers (1980) reported significant increases in both HDL-C and HDL-AI in their experimental group. These parallel increases in HDL-C and HDL-AI, with no change in the ratio, led the authors to believe that their subjects had actually increased their HDL macromolecule concentration. In direct contrast to this, Huttunen and associates (1979) reported no changes in HDL-AI after a 4-month moderate conditioning program. In this study, there was a significant increase in HDL-C after the training, but the lack of any change in HDL-AI resulted in an increase in the HDL-C to HDL-AI ratio.

Much more dramatic changes in HDL-AI were reported by Nestel and collegues (1979) in 13 male mountaineers after 8 weeks of intensive mountain climbing. These authors found that after only 3 weeks into the climb, the HDL-AI concentration had almost doubled, and persisted at this elevated level for the remaining period of the climb. However, there

is the possibility that other factors might also have contributed to this dramatic increase, such as chronic hypoxia and changes in plasma volume.

The effect of chronic exercise on some of the major enzymes involved in lipoprotein metabolism has also received some attention. A 56% increase in adipose tissue LPL activity was reported by Peltonen and workers (1981) after 15 weeks of moderate physical training, but the significance of this finding is debatable since similar increases were observed in a control group. Costill and associates (1979) reported elevated skeletal muscle LPL activity in juvenile diabetics after approximately 4 months of physical training.

Only one study has investigated the possible role that the enzyme LCAT might play in the exercise-mediated changes in HDL after training. Lopez and associates (1974) reported increased LCAT activity in four subjects following 7 weeks of physical conditioning.

There are only a few reports on the effect of exercise conditioning on HDL concentration in people with coronary heart disease. There is evidence that a moderate conditioning program of 3 months duration is sufficient to significantly elevate HDL-C in middle-aged cardiac patients (Erkelens, et al, 1978; Streja & Mymin, 1979; Hartung, et al, 1981). Two of these studies (Erkelens, et al, 1978; Hartung, et al, 1981) reported that an increase in the HDL-C/total cholesterol ratio accompanied this increase in HDL-C, and in a cross-sectional analysis, Erkelens and

co-workers (1978) reported that HDL-C was significantly higher in exercising versus non-exercising survivors of myocardial infarction. Nevertheless, whether or not an alteration in the lipoprotein profile through physical conditioning is a useful and safe means of secondary prevention of CHD remains to be determined.

II.5(3). Acute Changes in HDL After a Single Exposure To Exercise

It is not known whether a single exposure to exercise will acutely elevate HDL-C, or if the influence of exercise is more chronic in nature which takes several months to develop. It is also not known what quantity and intensity of exercise is necessary to induce a change in HDL concentration, and how long any such changes remain after the exercise is completed. The discrepancies in the literature can be explained, at least in part, by both inconsistencies in the intensity and duration of the single exercise session, and the lack of control for plasma volume shifts.

Significant increases in HDL-C have been found immediately after a 42 km marathon (Thompson, et al, 1980), 3 hours of continuous running (Dufaux, et al, 1982a), and a 70 km cross-country ski race (Enger, et al, 1980). The degree of this increase however, is inconsistent. Enger et al (1980) reported a 12% increase in HDL-C immediately following a 70 km cross-country ski race in 20 well-trained men, with accompanying decreases in LDL-C and triglycerides. Thompson et al (1980) however, observed only a 6% increase

in HDL-C in 12 men immediately after completing a 42 km marathon, but their values were not corrected for the slight hemodilution which was evident after the race. A small increase in HDL-AI immediately following the marathon was also reported in this particular study, baseline levels being re-established within one hour post-exercise. An approximate 8% increase in HDL-C after 3 hours of continuous running was reported by Dufaux and co-workers (1982a) in their sample of 15 untrained men. No values were reported for plasma volume shifts in this particular study.

Both the intensity and duration of the exercise appear to affect this acute response since no change in HDL-C was found after 30 minutes of cycling on a bicycle ergometer at an intensity of 65% $\dot{V}O_2$ max (Cullinane, et al, 1980), nor after a 20 km run in which the intensity was not controlled (Taskinen, et al, 1980).

Reports on the duration of the acute exercise response are quite inconsistent. In Enger's (1980) study of the cross-country skiers, a 4-day follow-up revealed a very gradual decline in HDL-C, with concentrations still significantly higher than pre-race values 4 days after the race. Conversely, pre-exercise HDL-C levels were re-established within 24 hours following the 3 hours of continuous running in Dufaux's (1982) study. Thompson's (1980) data on the marathoners is more difficult to interpret. Pre-exercise

HDL-C concentrations appeared to be re-established within 4 hours after completion of the marathon, but they were significantly higher again 18 hours post-exercise. Despite the added complication of the expanded plasma volume in this particular study, it appeared as if the acute response only lasted a few days at the most.

Dressendorfer and workers (1982) found changes in HDL-C during a 20-day road race (500 km) which supported the contention that the acute response is short-lived. HDL-C concentrations increased during the days of running, but a 3-day rest period in the middle of the race reversed these changes.

Evidently, the intensity and/or duration of the exercise are important factors determining both whether an acute response will occur, and how long it will last. Furthermore, control for any acute changes in blood plasma volume is essential before attempting to assess the magnitude of any change in the lipoprotein profile as a result of a single exercise session.

In view of previous work by Nikkila and co-workers (1978c) which had demonstrated higher skeletal muscle and adipose tissue LPL activity in trained endurance athletes, Taskinen and associates (1980) conducted a study to determine if LPL activity would increase acutely following a single exercise session. Tissue biopsies and venous blood samples were taken from 10 trained male distance runners

before and after a 20 km run. A two-fold increase in skeletal muscle LPL activity was reported, accompanied by a smaller increase in adipose tissue LPL activity. The dramatic increase in muscle LPL activity suggests that the intramuscular triglycerides must be undergoing a very rapid turnover, and that circulating VLDL triglycerides must supply free fatty acids for both oxidation and restoration of muscle triglycerides during and after a prolonged exercise session. Surprisingly, no significant change in any of the lipoproteins was observed after the exercise (Taskinen, et al, 1980).

A recent study by Kantor and associates (1983) also provides evidence that acute exercise can result in increased LPL activity. These authors reported a two-fold increase in LPL activity one day after completion of a 42 km marathon, which was also accompanied by a 9% increase in HDL-C and a 39% decrease in triglycerides. It was suggested that the increase in LPL activity mediated both the fall in triglycerides and the increase in HDL cholesterol (Kantor, et al, 1983).

II.5(4). Possible Mechanisms Behind the Exercise-Related Changes in HDL

While searching for underlying mechanisms responsible for the exercise-related increases in HDL, it is important to control for the possible influence of factors other than physical activity which could also be influencing HDL concentration. For example, athletes tend to differ

from non-athletes not only in activity level, but also in diet and relative body weight, two factors which are known to exert specific effects on HDL concentration. Furthermore, the possibility can not be ruled out that the same factors which may pre-dispose an individual for a high exercise capacity may also pre-dispose one for high HDL levels.

A few studies have addressed these possibilities. In a cross-sectional investigation of marathon runners, joggers, and sedentary controls, Hartung and workers (1980) reported no difference in dietary habits between the three groups, although HDL-C was still found to be positively related with activity level. Similarly, Lehtonen and Viikari (1978) found no difference in relative weights between two groups of subjects differing in activity level, but significantly higher HDL-C levels were observed in the trained group. Thus, it does appear that physical activity per se plays a specific role in raising HDL-C concentrations.

Although the precise mechanisms behind the exercise-mediated increases in HDL are unknown, evidence suggests that changes in LPL activity play an important role in changing the concentration of HDL. In support of this association between HDL concentration and LPL activity are the consistent observations of a negative relationship between VLDL and HDL concentration (Altekruse & Wilmore, 1973; Lopez, et al, 1974; Nikkila, 1978b). Catabolism of VLDL and chylomicron triglyceride by LPL not only releases

free fatty acids for oxidation, but also the surface components of these lipoproteins (unesterified cholesterol, phospholipids, and A and C apoproteins) to enter the plasma pool as HDL precursors. Transformation of these precursors into spherical HDL follows upon esterification of the free cholesterol by LCAT, an enzyme which is activated by apoprotein AI (Tall & Small, 1978).

Tall and Small (1978) have also proposed a mechanism which might explain the higher cholesterol content in the HDL of endurance-trained athletes. They suggest that during the transformation of HDL precursors into spherical HDL there is likely a large influx of cholesterol into the HDL fraction, since the relative deficiency of cholesterol in the precursor particles would result in a chemical gradient favouring the movement of cholesterol into HDL. Since endurance-trained athletes have higher LPL activities (Nikkila, 1978c) and thus a faster rate of catabolism of VLDL and chylomicrons, one would assume that they would also have a more active transformation of HDL precursors into spherical HDL, and thus, a greater influx of cholesterol into the HDL particle.

The consistent observation of a negative relationship between the concentration of serum triglycerides and the levels of HDL-C provides further support for the role played by LPL in mediating the exercise-related increases in HDL (Hurter, et al, 1972; Wood, et al, 1976; Lehtonen & Viikari, 1978). Evidence suggests that endurance athletes

have a more active adipose tissue lipolysis than their sedentary counterparts even in the resting state, which suggests that physical training may enhance one's capacity to mobilize and utilize fat as a fuel source (Hurter, et al, 1972; Astrand & Rodahl, 1977).

Whether or not changes in LCAT activity also play a role in the exercise-related increases in HDL concentration is difficult to determine. Only one study is available which has measured LCAT in subjects before and after exercise conditioning (Lopez, et al, 1974), but the results did demonstrate a slight increase in the activity of this enzyme in 4 subjects after 7 weeks of training.

II.6. Substrate Utilization in Exercise

Of the three available fuel sources (protein, fat, carbohydrate), carbohydrate and fat serve as the two major fuels during exercise. Proteins have previously been considered as not being particularly important for providing energy during exercise, but recently it has been suggested that protein catabolism might contribute more to exercise metabolism than had been assumed. It now appears that during exercise in which glycogen stores are depleted, protein may contribute 10-15% to the total energy supply (Lemon & Mullin, 1980; Rennie, et al, 1980).

Nevertheless, the predominant foodstuffs used for ATP production during exercise are fat and carbohydrate. The

percentage participation of these two fuels in energy metabolism is determined by a variety of factors, such as the intensity and duration of the exercise, and the diet (Astrand & Rodahl, 1977; Fox, 1979).

Muscle glycogen and blood glucose are the two forms of carbohydrate that are used as metabolic fuel, both serving as substrates for the glycolytic pathway. Free fatty acids (FFA) are the usable form of fats, and working muscles rely on intramuscular triglycerides and plasma FFA for this energy source (Fox, 1979). The respiratory exchange ratio ($R = \dot{V}CO_2/\dot{V}O_2$) has been suggested as reflecting a good estimate of the relative proportions of fat and carbohydrate oxidation in the exercising muscles under both normal (Essen, et al, 1977) and extreme (Jansson, 1982) dietary conditions. An R value of 1.0 suggests exclusive utilization of carbohydrates as a fuel source, whereas an R value of 0.71 suggests that lipids are being oxidized exclusively (Matthews & Fox, 1976). Any value between these two extremes will give an indication of the relative proportion of fat and carbohydrate being utilized. An R value greater than 1.0 is an indication of hyperventilation, in which more CO_2 is being blown off than oxygen is being consumed.

Given normal dietary conditions, the choice of fuel for the exercising muscles is primarily determined by the relative intensity of the activity in relation to the individual's maximal oxygen uptake, since fat utilization

depends on an adequate supply of oxygen (Astrand & Rodahl, 1977). At high exercise intensities, increases in anaerobic glycolysis must accompany aerobic metabolism in order to sustain adequate levels of ATP regeneration. The acceleration in glycolysis leads to an increased rate of lactic acid production and this in turn suppresses FFA mobilization (Issekutz, et al, 1975; Paul, 1975). It has been suggested that there is an increased re-esterification of FFA in the presence of lactate, which would explain why FFA mobilization appears to be inhibited once lactate starts accumulating (Issekutz, et al, 1975).

During prolonged sub-maximal bouts of exercise in which lactate levels are not elevated, plasma FFA concentrations gradually increase as the exercise progresses (Nagle, et al, 1970; Pruett, 1970; Jones, et al, 1980). At intensities in which lactate concentrations start increasing, the plasma FFA concentration drops. The degree of decline in FFA levels appears to be a direct function of the degree of rise in lactate (Pruett, 1970).

After completion of exercise, there is a dramatic increase in plasma FFA levels to above those seen before or during exercise. Friedberg and associates (1960) ascribed this rise in FFA during recovery from exercise to a decreased removal of plasma FFA by the muscle while the rate of FFA mobilization remains at an accelerated rate. However, Hagenfeldt and Wahren (1975) concluded that the

post-exercise rise in plasma FFA was more the result of an increase in the rate of FFA release from adipose tissue than a decreased removal from the plasma.

The duration of this post-exercise increase in plasma FFA is unclear, but seems to depend on the severity of the exercise. Plasma FFA concentrations have been shown to remain elevated for about one hour after completion of 40 minutes of cycle ergometry at an intensity corresponding to 57% $\dot{V}O_2$ max (Hagenfeldt & Wahren, 1975), whereas they remained elevated for more than 5 hours after completion of two exercise bouts to exhaustion at an intensity of 85-90% $\dot{V}O_2$ max (Pruett, 1970). Thus, it appears that FFA mobilization during and after exercise depends more upon the intensity of the exercise than the total energy expenditure.

II.7. Plasma Volume Changes in Response To Exercise

In quantifying acute changes in the concentrations of the plasma lipids in response to exercise, correction must be made for any changes in plasma volume which might also have occurred. Such changes are already recognized, and whether an acute hemoconcentration or hemodilution occurs appears to depend upon the severity of the exercise bout. A prolonged period of sub-maximal exercise (30-65% $\dot{V}O_2$ max) results in an increase in total plasma protein content, causing an increase in plasma volume, or hemodilution (Beaumont, et al, 1972). Conversely, bouts of

higher intensity exercise result in a reduction of plasma protein content, causing a decrease in plasma volume, or hemoconcentration (Nylin, 1947; Beaumont, et al, 1972; Costill & Fink, 1974). The hemoconcentration associated with a loss in plasma protein content is due to the decreased osmotic pressure which accompanies decreased plasma protein concentration. This in turn causes a loss of fluid from the capillary and thus, a hemoconcentration. The opposite occurs with an increase in plasma protein concentration.

II.8(1). <u>Measurement of HDL Cholesterol</u>

Ultracentrifugation is a traditional method used for measurement of HDL. The cholesterol content in HDL is measured in the plasma fraction of density greater than 1.063 gm/ml (Warnick & Albers, 1978; Eder, 1982). This procedure, however, is technically difficult and time consuming, requiring ultracentrifugation for approximately 24 hours.

A more practical and therefore widely used method for determining HDL-C is to measure the cholesterol content of the supernatant following precipitation of the apo-B-containing lipoproteins, namely LDL and VLDL (Ishikawa, et al, 1976; Warnick & Albers, 1978). The most reliable procedure for precipitating LDL and VLDL from plasma or serum uses heparin and manganese chloride in concentrations of 1.2 - 2.0 mg% and 0.046 - 0.092M

respectively (Ishikawa, et al, 1976; Warnick & Albers, 1978). The cholesterol content in the supernatant is then enzymatically assayed by means of a standard cholesterol assay.

II.8(2). Measurement of HDL Apoprotein A

There are several immunological methods available for estimation of HDL-A, including electroimmunoassay (Farish, et al, 1975; Curry, et al, 1976; Kostner, 1979), radial immunodiffusion (Albers, et al, 1977; Cheung & Albers, 1977), and radioimmunoassay (Fainaru, et al, 1975; Karlin, et al, 1976; Schonfeld, et al, 1977). These techniques, although precise, are relatively expensive and, being manual to a degree, are tedious as well.

II.9. Diurnal Variation in Plasma Lipids and Lipoproteins

Blood levels of both HDL cholesterol and HDL Apoprotein A appear not to be affected by recent food intake. Numerous studies have failed to find any significant difference between fasting and non-fasting levels of either HDL-C or HDL-A (Farish, et al, 1975; Henderson, et al, 1980; Booth & Lacey, 1982). Similarly, evidence indicates that total cholesterol concentration is also quite stable during the course of a day, and that any small fluctuations that do occur do not appear to be related to meals, activity, or time of day (Shapiro, et al, 1959; Henderson, et al, 1980; Booth & Lacey, 1982). Triglyceride concentrations, on the other hand, can vary markedly with recent

food intake. The lowest levels are usually found after fasting overnight, and the highest levels after eating a fatty meal (Havel, 1957; Henderson, et al, 1980).

CHAPTER III

METHOD

III.1. Experimental Design

It is known that physical activity is associated with high levels of HDL cholesterol. This study was designed to test the hypothesis that acute increases in plasma high density lipoprotein cholesterol with exercise would be affected by the intensity of the exercise. It was hypothesized that lower intensity exercise, where lipids would be the major fuel source, would result in greater changes in HDL-C than high intensity exercise, where the major fuel source would be carbohydrate.

Twelve subjects ran on a treadmill on two separate occasions. The total amount of exercise (ie. the equivalent distances which were run) was kept the same on each occasion, but the intensity of the exercise was different. Acute changes in the plasma lipids and lipoproteins were analyzed both within and between the two exercise conditions.

III.2. Subjects

Twelve men, aged 19-41 years, participated in the study. All were above average fitness, with about half

TABLE 2

DESCRIPTION OF SUBJECTS

SUBJECT	AGE (yrs)	WEIGHT (kg)	VO_2 max (ml/kg/min)
A.S.	23	68	57.7
T.R.	19	66	65.3
J.K.	29	70	53.0
C.H.	26	70	56.2
S.L.	24	66	70.2
G.J.	24	80	59.0
J.C.	31	78	63.7
C.W.	34	64	73.2
M.O.	20	73	72.6
D.E.	27	62	80.6
N.O.	41	72	55.8
D.M.	40	80	51.8
$\bar{X} \pm SE$	28.2 ±2.2	70.7 ±1.8	63.3 ±2.8

being very well trained. A description of the subjects' age, weight, and maximal oxygen uptake is provided in Table 2.

III.3. Exercise Procedures

Data was collected on each subject on three separate occasions. In the first occasion, maximal oxygen uptake was directly determined from a graded treadmill run to exhaustion, utilizing a protocol recommended for the testing of athletes (Thoden, et al, 1983). Minute ventilation was constantly recorded by a Hewlett-Packard 4000 VR digital pneumotachometer, and expired CO_2 and O_2 concentrations were measured by a Hewlett-Packard Capnometer (Model 47210A) and a Goddard Rapox oxygen analyzer. All data were continuously recorded on a four channel physiograph (PMP-4B).

During the second and third visits to the laboratory, subjects performed two different exercise tasks. Task one was a continuous run on the treadmill at a speed calculated to correspond to 60% of the subject's maximum $\dot{V}O_2$ (McMiken & Daniels, 1976). This continuous run lasted between 54.5 and 59 minutes, with each subject completing a distance equivalent to 9 - 12 km.

The second exercise conditon involved running on the treadmill at a speed calculated to correspond to 90% of the subject's $\dot{V}O_2$ max (McMiken & Daniels, 1976). This was done in the form of one-minute exercise intervals alternating with one-minute rest periods, and was continued until

the same equivalent distance was completed as in the first conditon. On four occasions during task two, subjects maintained this pace for 3 minutes to ensure steady state conditions for measurement of oxygen uptake. The total exercise time for this task ranged between 36 - 38 minutes.

Oxygen uptake was directly measured every ten minutes in both exercise conditions, with ventilation and expired gas concentrations being monitored for at least two minutes to ensure a steady state condition. Heart rate was monitored continuously throughout each exercise condition.

The order of the two exercise conditions was randomized between subjects, and the time of day that the exercise was done was kept consistent within subjects.

III.4. Biochemical Analysis

Venous blood samples (49 ml total) were taken by venepuncture from the antecubital vein at four times during both the continuous and interval exercise sessions: 1) immediately before the exercise, 2) halfway through the exercise, 3) immediately following the exercise, and 4) at the end of a 20 minute rest period following the exercise. For sampling during the continuous run, exercise was interrupted for 60-90 seconds and immediately resumed. Blood was collected into 7 ml Vacutainers containing EDTA, and for lactate analysis it was also collected into Vacutainers containing Sodium Fluoride and Potassium

Oxalate. All samples were immediately refrigerated at 4°C. Before centrifugation, hematocrit was measured in duplicate by a microcapillary tube technique.

A 1 ml aliquot of plasma from each blood sample was treated with heparin and manganese chloride, according to the procedure recommended by the Lipids Research Clinics (1974) N.I.H. publication to produce the supernatant for the HDL analysis. For reasons of technical difficulty, measurement of the HDL subfractions was not done. All plasma samples and supernatants were stored at -70°C and were analyzed within 4 months.

III.4(1). <u>Analysis of Plasma Triglycerides, Total Cholesterol, and HDL Cholesterol</u>

Plasma triglycerides, total cholesterol, and HDL cholesterol were quantified enzymatically by a Centrifugal Fast Analyzer, using standard assay kits obtained from Boehringer (Mannheim, Germany). The fast analyzer used was a "Centrifichem" system no. 400 (Union Carbide Corporation, Pleasantville, N.Y.) spectrophotometric analyzer which rapidly performs determinations on blanks, standards, and controls, and computes the relative concentrations in terms of optical density, with digital print-out. Coefficients of variation of cholesterol, triglycerides, and HDL-C analysis were 4.4%, 5.88%, and 3.3% respectively, as determined in preliminary studies.

III.4(2). <u>Analysis of HDL Apoprotein A</u>

Measurement of HDL-A was done by a Centrifugal Fast

Analyzer Kinetic Nephelometric Immunoassay. The fast analyzer measured the rate of change in optical density as a function of the rate of antigen-antibody interaction. The actual concentration of HDL-A was determined by comparison with a standard calibration curve of normal human serum at appropriate dilutions. Rabbit antisera, specific to Apoprotein A, was used as the antibody, and was diluted in a phosphate buffer (ph=7.5, I=.07M) with polyethyleneglycol (PEG). The antisera dilution chosen was that which yielded the fastest reaction rate with the particular antigen concentration and thus, specific precautions were taken to avoid situations of antigen or antibody excess (Sternberg, 1977).

Interassay variation in measurement of either HDL-A or HDL-C was avoided by analyzing all samples from an individual subject in a single autoanalyzer run, and each sample was measured in duplicate. Within-run coefficient of variation for HDL-A analysis was previously determined to be 2.2%.

III.4(3). Determination of HDL Saturation

The saturation of HDL with cholesterol was calculated as being the ratio of the concentration of HDL-C to the concentration of HDL-A, expressed as a percent.

III.4(4). Analysis of Lactic Acid and FFA

Lactic acid was measured spectrophotometrically at 350 nm, incorportating the procedure outlined in the Sigma

Technical Bulletin (1968), using a Turner Model 350 spectrophotometer.

The concentration of free fatty acids in the plasma was also measured spectrophotometrically at 550 nm, using the colorimetric procedure described by Duncombe (1963). All samples were done in duplicate. This portion of the analysis was performed by the author utilizing the laboratory facilities at the University of Waterloo.

III.5. Statistical Analysis

Results were analyzed using a subjects (12) by condition (2) by time (4) repeated measures analysis of variance design. Post-hoc Tukey A tests were then used to test selected differences between variables.

Correlations were calculated by the Pearson product-moment formula.

CHAPTER IV

RESULTS

The acute responses of all the dependent variables for both exercise conditions are presented in Tables 3 and 4. The total amount of exercise performed by each subject can be found in Appendix 1.

IV.1. Hematocrit

Hematocrit decreased slightly during the low intensity exercise, but not until 20 minutes post-exercise was this decrease significant ($p<.05$). Conversely, there was a significant increase in hematocrit at both sampling times during the high intensity exercise ($p<.01$), suggesting a slight hemoconcentration. Pre-exercise hematocrit values in this condition were re-established by 20 minutes post-exercise. To control for these small changes in plasma volume, the concentrations of the plasma lipids and metabolites were adjusted according to the percent change in plasma volume from the baseline measurement in each subject.

IV.2. Total Cholesterol and Triglycerides

The acute exercise response of total cholesterol concentration is illustrated in Figure 1(a). There was a

Tables 3 and 4:

Changes in hematocrit, total cholesterol, triglycerides, HDL-C, HDL-A, Saturation, HDL-C/total cholesterol, lactic acid, and FFA with exercise at low and high intensities (N=12). All concentrations are corrected for changes in plasma volume.

TABLE 3

CHANGES IN DEPENDENT VARIABLES WITH LOW INTENSITY EXERCISE
60% VO_2 MAX ($\bar{X}$±SE)

VARIABLE	PRE-EXERCISE	MID-POINT	POST-EXERCISE	20 MIN. POST-EXER.
HEMATOCRIT (%)	44.5±0.75	44.1±0.73	44.25±0.71	43.5±0.9
TOTAL CHOLESTEROL (mg %)	171.2±8.8	174.4±9.7	175.1±9.2	174.7±10.5
TRIGLYCERIDES (mg %)	104.4±19.8	109.11±16.6	110.2±18.5	92.1±15.1
HDL-C (mg %)	48.4±4.1	51.9±4.1	54.3±4.1	54.8±4.7
HDL-A (mg %)	104.3±5.4	113.4±6.4	112.2±5.5	113.0±6.2
SATURATION (%)	46.0±2.4	45.4±1.5	48.1±2.1	48.1±2.2
HDL-C/TOTAL CHOL. (%)	28.8±2.5	30.5±2.5	31.8±2.5	32.3±2.8
LACTIC ACID (mg %)	9.94±1.8	13.43±2.7	11.7±1.6	
FFA (mmoles/l)	0.64±0.07	0.65±0.09	0.75±0.06	1.04±0.12

TABLE 4

CHANGES IN DEPENDENT VARIABLES WITH HIGH INTENSITY EXERCISE
90% VO_2 MAX ($\bar{X}$±SE)

VARIABLE	PRE-EXERCISE	MID-POINT	POST-EXERCISE	20 MIN. POST-EXER.
HEMATOCRIT (%)	44.0±0.77	46.0±0.72	45.6±0.57	43.7±0.53
TOTAL CHOLESTEROL (mg %)	173.0±9.7	185.9±11.4	184.9±10.0	180.4±10.3
TRIGLYCERIDES (mg %)	96.6±15.0	100.2±13.1	96.7±10.1	81.7±7.4
HDL-C (mg %)	47.2±3.5	55.3±4.4	58.3±3.9	54.5±4.2
HDL-A (mg %)	104.3±5.3	118.3±5.6	121.7±5.3	117.1±6.0
SATURATION (%)	44.8±1.4	46.2±2.0	47.6±1.8	46.1±1.6
HDL-C/TOTAL CHOL. (%)	28.2±2.5	30.9±2.8	32.2±2.3	30.6±2.7
LACTIC ACID (mg %)	9.9±1.8	42.3±3.5	40.2±5.1	
FFA (mmoles/l)	0.63±0.08	0.54±0.07	0.72±0.08	1.0±0.16

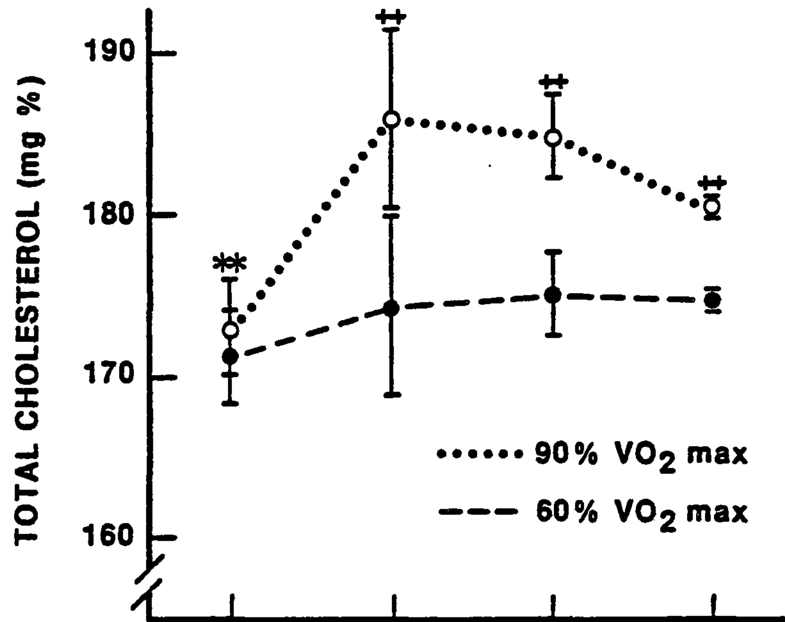
Figure 1:

Mean data for total plasma cholesterol and triglyceride changes with exercise at low and high intensities (N=12). The variance portrays the standard error of the mean difference between conditions.

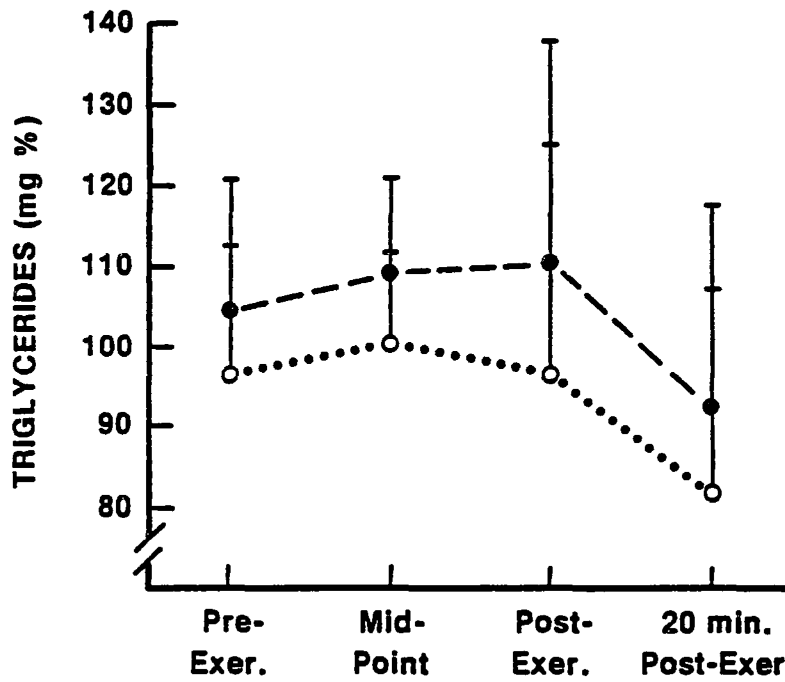
FIGURE 1

CHANGES IN TOTAL CHOLESTEROL AND TRIGLYCERIDES WITH ACUTE EXERCISE ($\bar{X} \pm SEM\Delta$)

(a)



(b)



Between-condition differences (++p < .01)
Within-condition differences (**p < .05)

significant interaction ($p<.05$) between the exercise condition and the time of sampling, indicating that the degree of change in total cholesterol was affected by the particular exercise intensity. No significant change in total cholesterol occurred in the low intensity exercise, whereas in the high intensity exercise the total cholesterol was significantly higher than the pre-exercise level at all time points.

The acute change in plasma triglycerides is shown in Figure 1(b). There was a significant change in triglyceride concentration over time ($p<.05$), but there was no effect of exercise intensity on the acute response. Post-hoc analysis revealed that the triglyceride concentration at 20 minutes post-exercise was significantly lower ($p<.05$) than half-way through the exercise.

IV.3. HDL-C and HDL-A

The pre-exercise concentrations of HDL-C and HDL-A did not differ between exercise conditions. Figures 2(a) and 2(b) illustrate the acute changes in HDL-C and HDL-A. There were significant increases in both of these variables under both exercise conditions ($p<.01$). The degree of this increase however, was much greater in the high intensity exercise, both at the mid-point and immediately post-exercise sampling times ($p<.01$). At 20 minutes post-exercise, HDL-C was again equivalent between the two

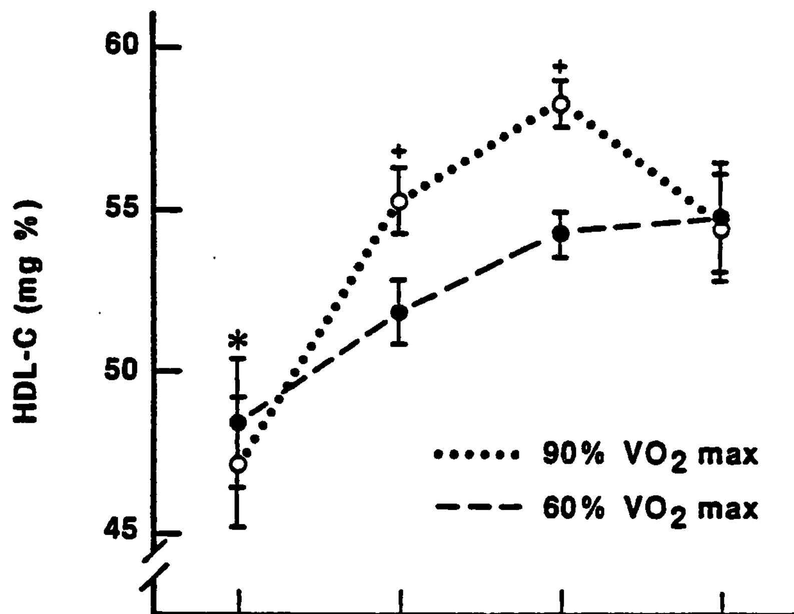
Figure 2:

Mean data for HDL-C and HDL-A changes with exercise at low and high intensities (N=12). The variance portrays the standard error of the mean difference between conditions.

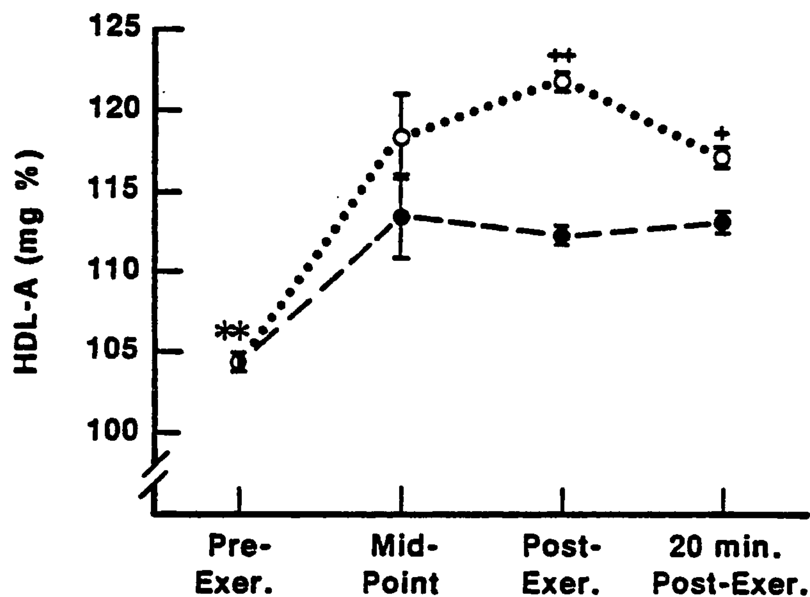
FIGURE 2

CHANGES IN HDL-C AND HDL-A WITH ACUTE EXERCISE ($\bar{X}$±SEMΔ)

(a)



(b)



Between-condition differences (+p < .05; ++p < .01)
Within-condition differences (*p < .05; **p < .01)

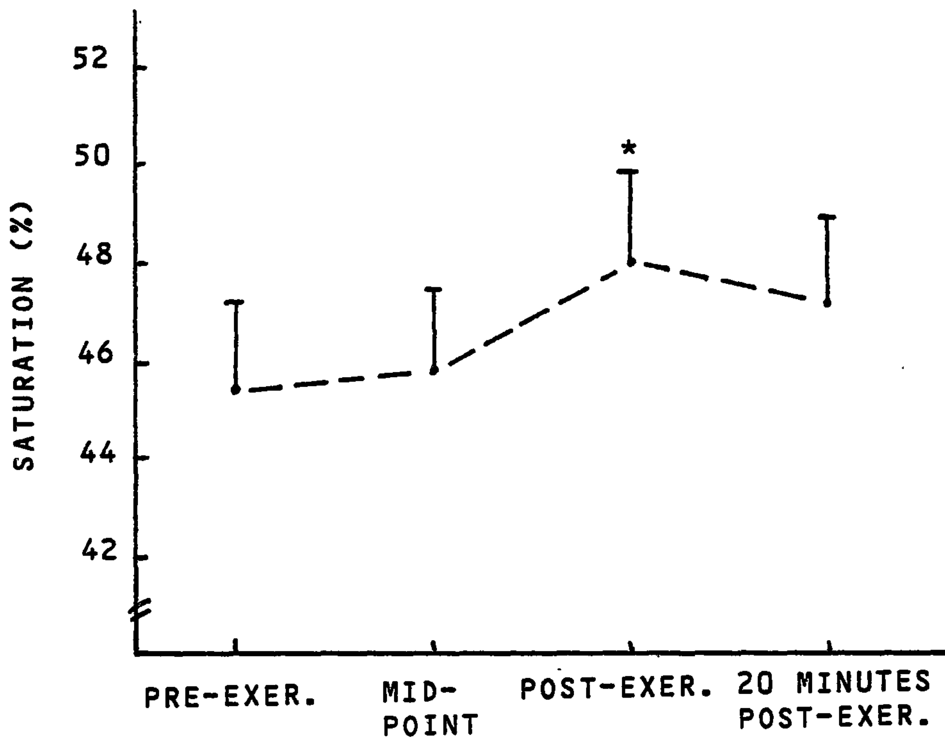
Figure 3:

Mean data for changes in HDL saturation with exercise (N=12). Values have been collapsed across exercise condition.

FIGURE 3

CHANGE IN SATURATION WITH ACUTE EXERCISE

($\overline{X}$ + SE)



* Variable significantly higher than two previous sampling times ($p<.05$).

Figure 4:

Mean data for changes in plasma lactate and FFA with exercise at low and high intensities (N=12). The variance portrays the standard error of the mean difference between conditions.

FIGURE 4

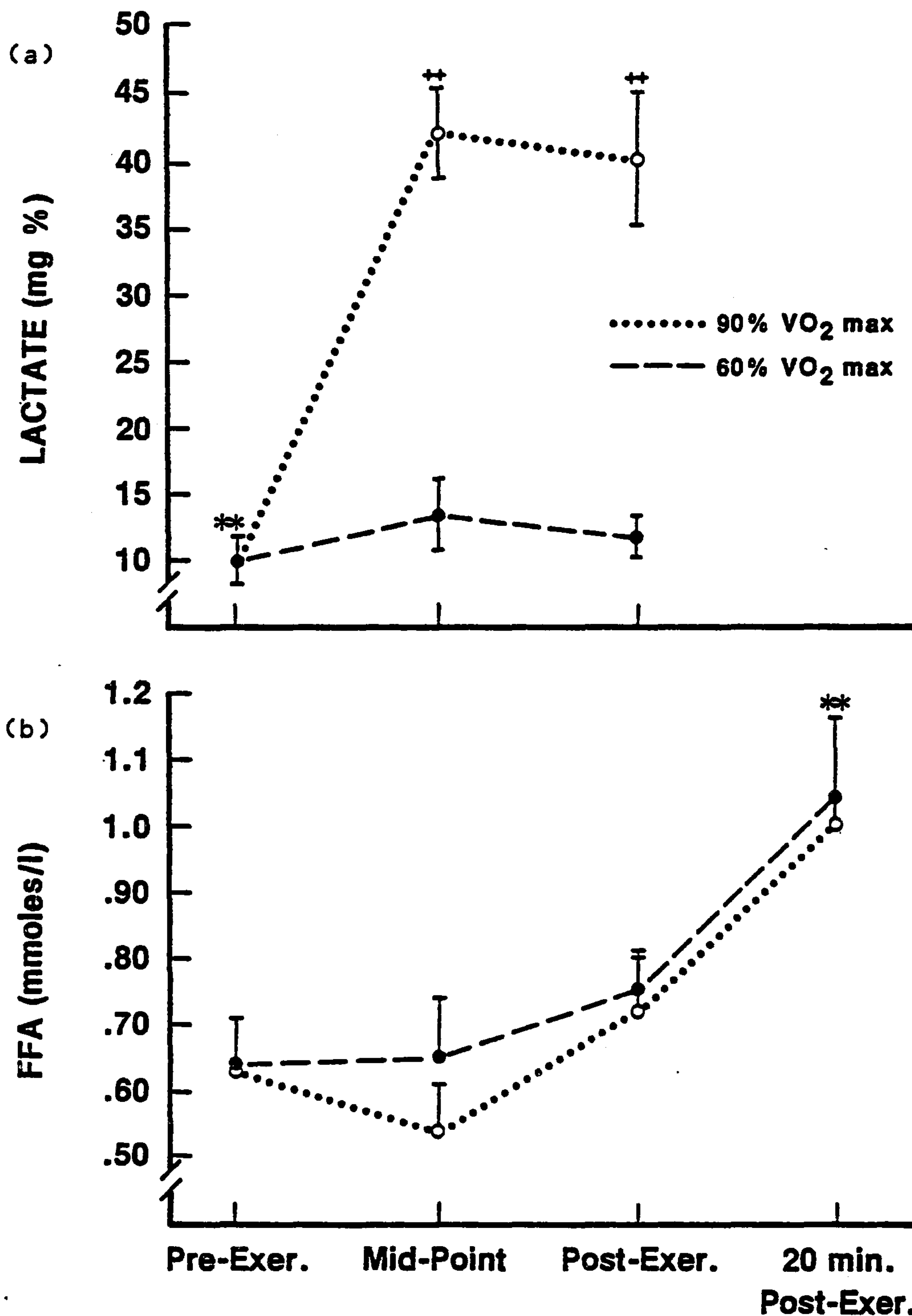


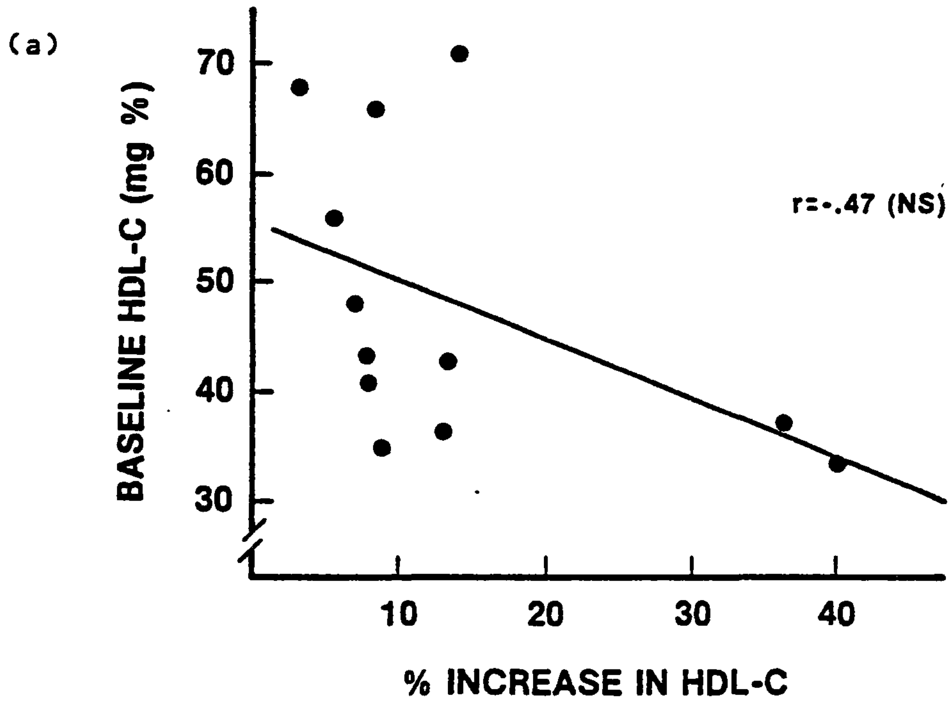
Figure 5:

Correlation between baseline concentration of HDL-C and percent increase in HDL-C after exercise at low and high intensities (N=12).

FIGURE 5

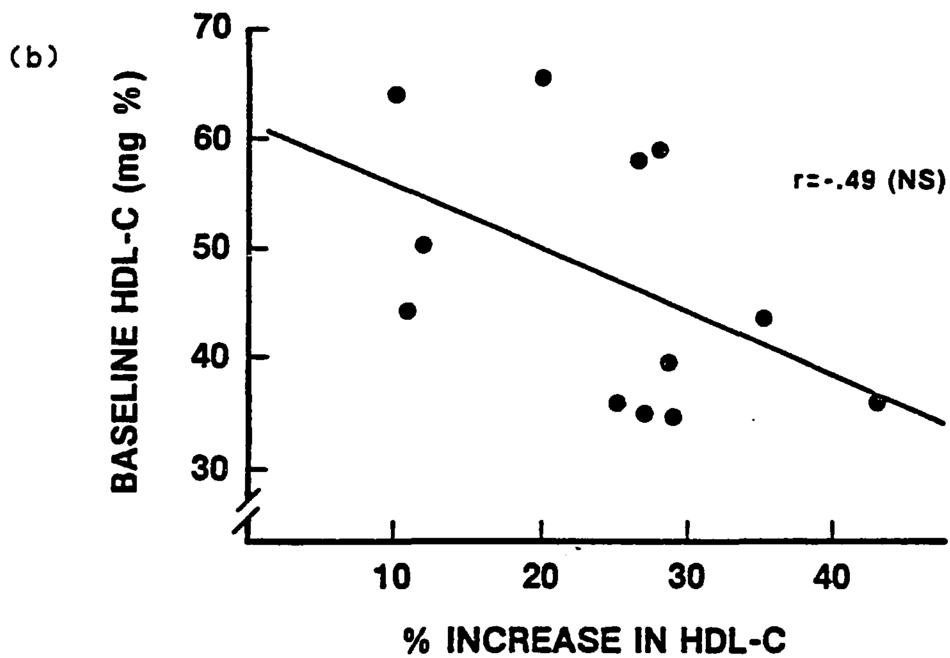
RELATIONSHIP BETWEEN BASELINE HDL-C AND PERCENT INCREASE IN HDL-C AFTER CONTINUOUS EXERCISE AT 60% VO_2 MAX

(a)



RELATIONSHIP BETWEEN BASELINE HDL-C AND PERCENT INCREASE IN HDL-C AFTER INTERVAL EXERCISE AT 90% VO_2 MAX

(b)



conditions, but HDL-A was still significantly higher in the high intensity condition at this time ($p<.05$).

Figures 5(a) and 5(b) illustrate the relationship between the pre-exercise level of HDL-C and the percent increase following exercise. There was no significant relationship between baseline concentration and relative increase in either exercise condition.

IV.4. HDL Saturation

If the data is collapsed across exercise condition, a significant change in saturation over time ($p<.01$) was evident, and post-hoc analysis revealed that the saturation was significantly higher immediately post-exercise than at either the mid-point or pre-exercise sampling times ($p<.05$). This is illustrated in Figure 3. There was no difference in saturation between or within the two exercise intensities, possibly due to the large variability between subjects (See Appendix 5).

IV.5. Lactic Acid and FFA

There was a very significant effect of exercise intensity on the acute response of lactic acid (Figure 4a). The low intensity exercise had no effect on the initial pre-exercise lactate concentration, whereas there was a dramatic increase in lactate with the high intensity exercise, reaching a concentration of 42 mg% in the mid-exercise blood sample ($p<.01$).

TABLE 5

RELATIVE OXYGEN UPTAKE (% of max) AND RESPIRATORY EXCHANGE RATIO DURING EXERCISE

SUBJECT	LOW INTENSITY		HIGH INTENSITY	
	VO_2	R	VO_2	R
A.S.	56	.91	87	1.0
T.R.	60	.84	90	.92
J.K.	79	.79	98	1.0
C.H.	64	.93	95	.98
S.L.	57	.83	86	.92
G.J.	62	.85	90	.90
J.C.	56	.86	88	.92
C.W.	58	.83	91	.93
M.O.	61	.80	90	.97
D.E.	57	.90	94	.94
N.O.	62	.79	97	.96
D.M.	57	.84	83	.97
$\bar{X} \pm SE$	61% ± 1.9	.85 ±.01	91% ±1.4	.95 ±.01

Results are averages of measurements made at 10-minute intervals.

The concentration of free fatty acids (FFA) did not show any significant changes either during or immediately after exercise at either intensity (Figure 4b). There appeared to be a slight drop in FFA at the half-way point during the high intensity exercise, but this decrease was no significant. There was a significant increase in FFA concentration during the recovery period ($p<.01$), for at 20 minutes post-exercise FFA concentration was significantly higher than all other sampling times under both exercise conditions.

IV.6. Oxygen Uptakes and Respiratory Exchange Ratios

The actual measured oxygen uptakes and respiratory exchange ratio for each subject are presented in Table 5. The average VO_2's for the low and high intensity exercise conditions were 61% and 91% of $\dot{V}O_2$ max respectively, and the corresponding R values were .85 and .95 respectively.

CHAPTER V

DISCUSSION AND CONCLUSIONS

This study was designed to determine whether or not the acute response of the plasma high density lipoproteins to a single exercise session would be affected by the metabolic turnover of FFA as a fuel source, or, in other words, whether it would be a function of the intensity of the exercise. Since previous studies have reported a positive relationship between the total number of miles run per week and the concentration of HDL-C (Lehtonen & Viikari, 1978; Hartung, et al, 1980), in the present study the total amount of exercise performed by each subject was kept constant (ie. equivalent caloric expenditure) and only the intensity of the exercise was manipulated.

Changes in Plasma Volume

Acute changes in plasma volume in response to exercise were estimated from changes in hematocrit. Hematocrit changes are often used to estimate plasma volume shifts, but Costill and Fink (1974) suggested that the use of this method makes three major assumptions: 1) that there is a constant volume of circulating red cells, 2) that the size of the red blood cell remains constant, and 3) that the ratio between the venous and whole-body hematocrit is

constant. Previous work by Nylin (1947) supports the first assumption, but Costill and Fink (1974) claim that it is inaccurate to make the next assumptions during exercise, since it possible that the red cells might shrink during exercise thereby changing the hematocrit measurement.

Apparently, changes in hematocrit underestimate actual changes in plasma volume during exercise (Astrand & Saltin, 1964; Beaumont, et al, 1972). However, in the present study, the changes in hematocrit were small compared to the changes in the concentrations of the variables measured, in fact, the acute changes in HDL-C and HDL-A in both conditions were still significant if the concentrations were not corrected for changes in plasma volume.

Total Cholesterol and Triglycerides

The lack of any significant change in plasma total cholesterol after exercise at 60% $\dot{V}O_2$ max was not surprising, since similar findings have been reported after a 20 km run (Taskinen, et al, 1980), and after a marathon (Thompson, et al, 1980). However, the very significant increase in total cholesterol after the high intensity exercise was not expected, and has not been reported elsewhere in the literature. The mechanism behind this increase can only be speculated upon. Increases in catecholamine levels might have been involved, since large doses of epinephrine have been shown to increase total

cholesterol in dogs (Steinberg, 1963). Although not measured in the present study, it is known that catecholamine levels increase as the intensity of the exercise increases. Exercising at 90% of $\dot{V}O_2$ max has been reported to increase norepinephrine levels to almost 3 times the resting level (1.6 ug/l $\rightarrow$ 4.0 ug/l) while with prolonged exercise at 60% $\dot{V}O_2$ max the increase was only 40% (Hartley, 1975). An alternative mechanism might have been the increased cell breakdown which presumably would have occurred more in the high intensity exercise (Siegle, et al, 1980), thus releasing more cholesterol to the plasma pool from cell membranes.

The acute response of the plasma triglycerides was similar to what has been reported in other studies (Hurter, et al, 1972; Taskinen, et al, 1980; Thompson, et al, 1980), and in the pilot study. A significant decrease in plasma triglycerides in the hours following a bout of exercise has been reported several times (Enger, et al, 1980; Thompson, et al, 1980; Kantor, et al, 1983), and it is possible that this might also have been found in the present study if blood sampling had been continued, since there was already a decrease in triglycerides after 20 minutes of recovery from exercise.

Changes in HDL-C and HDL-A

Acute increases in HDL-C have been reported immediately following a 42 km marathon (Thompson, et al, 1980) and a 70 km cross-country ski race (Enger, et al, 1980).

Subjects in the present study, however, only ran 9-12 km, yet increases of similar magnitude were found. This reinforced results from the pilot study, in which significant increases in HDL-C were found after a similar run of 9-12 km (Appendix 4).

Although the increase in HDL-C was significant in both exercise conditions, the intensity of the exercise substantially affected the magnitude of the increase. The effect of varying exercise intensity has not been previously investigated in studies of the acute exercise response of plasma HDL, yet it appears to be a very significant factor and could possible explain why some studies have failed to find acute changes in HDL-C. Furthermore, it appears that this effect of intensity is very short-lived, since it disappeared within 20 minutes (See Figure 2a).

To answer the question whether the initial baseline level of HDL-C might be related to the magnitude of the acute response, the baseline levels were correlated with the percent increase following exercise (See Figures 5a and 5b). The pre-exercise concentration of HDL-C appeared to have no relationship with how much it increased with either exercise condition (r=-.47 for low intensity; r=-.49 for high intensity). There was a mean increase of 13.7% in the low intensity exercise, and 24.7% in the high intensity exercise, which corresponded to absolute increases of 5.9 mg% and 11.1 mg% respectively. Evidently, HDL-C rose by

approximately the same amount within each condition, independent of the initial level. Thus, the baseline level of HDL-C does not appear to be related to the magnitude of the acute response to exercise. Furthermore, the fitness level of an individual, as indicated by $\dot{V}O_2$ max, is also not related to the magnitude of the acute response of HDL-C to exercise (see Appendix 7).

Reports of acute changes in the major HDL apoprotein (HDL-A) after exercise are limited. Thompson and associates (1980) found a small increase in HDL-AI after the marathon (compared to the day before value), but this dropped to below pre-race levels in the hours following. The present study revealed significant increases in HDL-A under both exercise conditions, with intensity of exercise again appearing to be an important factor influencing the magnitude of this increase.

As explained previously, the HDL saturation was calculated as the ratio between the cholesterol and apo-A in the HDL. There was a large variability between subjects in both the magnitude and direction of acute changes in saturation, and there was no perceptible effect of exercise intensity on this response. However, if the data is collapsed across exercise condition, the post-exercise saturation was significantly higher than either the mid-point or pre-exercise time points. This indicates that the increase in HDL-C was greater than the increase in HDL-A, confirming the findings of the pilot study (Appendix 4). Therefore, while there might have been a small increase in

total HDL concentration, as evidenced by increases in both HDL-C and HDL-A, there also appeared to be a change in the composition of HDL. It is likely that the increase in saturation reflected an increase in cholesterol in the HDL_2 subfraction, as suggested by Cheung and Albers (1977). Analysis of the HDL subfractions with this type of acute exercise is something which should be investigated in the future.

Changes in Lactate and FFA

The acute effect of exercise on lactic acid concentration found in this study is similar to that previously reported. Exercise at intensities up to approximately 70% $\dot{V}O_2$ max has little effect on plasma lactic acid concentrations (Nagle, et al, 1970; Pruett, 1970), but as the intensity increases above this level, lactate concentrations rise markedly (Nagle, et al, 1970; Pruett, 1970). This increase in lactate is an indication of an accelerated contribution from anaerobic glycolysis, and thus, increased utilization of glycogen (Astrand & Rodahl, 1977). An increase in the respiratory exchange ratio from .85 in the low intensity exercise to .95 in the high intensity exercise occurred in the present study, which provides some support for the idea that the two exercise conditions were different enough in intensity to impose different metabolic demands in terms of fuel for energy production.

However, there was no discernible effect of the different exercise intensities on FFA mobilization, since there was no difference in plasma FFA concentrations between the two conditions. There was a significant rise in plasma FFA once exercise was completed, but the intensity of the exercise did not affect the degree of this increase. This sharp rise in FFA concentration during recovery from exercise has been reported several times (Hurter, et al, 1972; Jones, et al, 1980) and is thought to be due to a decreased uptake of FFA by the muscles while mobilization remains at an accelerated rate (Friedberg, et al, 1960).

Free fatty acid concentrations during the high intensity exercise were somewhat lower than during the low intensity exercise, but not significantly so. Apparently, the decline in pH due to the lactacidosis in the high intensity condition was not sufficient to inhibit FFA mobilization. Issekutz and co-workers (1975) suggested that lactate levels must rise by approximately 50 mg% before FFA mobilization will be suppressed in exercising dogs, and Jones et al (1980) observed a significant reduction in FFA turnover rates in exercising humans when lactate levels rose to 80 mg%.

Therefore, while the two exercise intensities had distinct effects on the acute responses of lactate, cholesterol, and HDL, they were not sufficiently different to affect the acute response of the plasma FFA. Perhaps if

the high intensity exercise had been performed at 95-100% of VO_2 max this difference might have occurred.

VO_2 Response

The measured oxygen uptakes in this study matched the expected $\dot{V}O_2$'s as determined by McMiken's and Daniel's (1976) formula very closely. The treadmill speeds employed were intended to impose a demand of 60% and 90% of each subject's $\dot{V}O_2$ max in the two exercise conditions, and the directly measured oxygen uptakes averaged 61% and 91%.

In the high intensity condition, $\dot{V}O_2$ and R were measured after 3 minutes of gas analysis to ensure steady state. This was considered to be a valid time point since Astrand and Rodahl (1977) suggested that even 2 minutes is long enough to achieve a steady state $\dot{V}O_2$ measurement during intermittent exercise with short rest periods. Similarly, Hagberg and associates (1978) have reported half-times of the $\dot{V}O_2$ response at these intensities to be well under one minute.

Conclusions

The hypothesis for the present study was that the acute increase in HDL-C in response to exercise would be proportional to the degree of utilization of free fatty acids as a fuel source. The results from this study neither supported nor disproved this hypothesis, since there was no difference in plasma FFA concentrations between the two

exercise conditions. However, plasma concentrations of FFA do not necessarily directly reflect FFA turnover, since mobilization and uptake of FFA could have been quite different between the two exercise conditions while plasma concentrations were the same. Future studies should address this question by directly measuring FFA turnover.

Nevertheless, the important finding in this study was that acute changes in HDL can occur after a relatively moderate bout of exercise, even though other studies have not always shown this (Cullinane, et al, 1980; Taskinen, et al, 1980). The greater increase in HDL-C with the higher intensity exercise is a finding which has not been previously reported. The investigation of Myhre, et al (1981), as summarized in Chapter II, revealed greater increases in HDL-C after low (70-80% $\dot{V}O_2$ max) versus high (80-95% $\dot{V}O_2$ max) intensity training programs. However, the total amount of training was not controlled between the two programs in that particular study, and thus, the separate effects of intensity versus duration of exercise could not be determined.

The mechanism(s) behind the greater increase in HDL-C with the higher intensity exercise in the present study can only be speculated upon. One factor might have been the increase in total cholesterol which also occurred with the high intensity exercise, thereby increasing the

amount of cholesterol in the plasma which could be carried by HDL. This could not have been the only mechanism however, since there was no difference in HDL-C between the two conditions after 20 minutes of recovery, while total cholesterol was still significantly higher in the high intensity condition at this time. The possibility that a change in LDL-C might have occurred was also looked into, but there were no significant changes in LDL-C either between or within exercise conditions, as estimated by the Friedwald (1966) formula (Appendix 6).

Results from this study provide only limited information on the duration of the acute exercise response of the plasma HDL. It does appear that the immediate acute response is the result of both the amount of exercise and the intensity at which it was performed. However, the separate effect of intensity seems to disappear after 20 minutes of recovery while the effect of the exercise per se appears to last much longer. A longer follow-up after the exercise would have provided more information regarding the duration of the acute response, but, based on the results from the pilot study it is likely that pre-exercise HDL levels would have been re-established within 18 hours.

This relatively short duration of the acute response of plasma HDL raises another point. Although a single bout of exercise can acutely elevate HDL levels, it can not explain entirely the higher fasting concentrations

of HDL-C that have consistently been observed in athletes versus controls. The effect of physical activity on plasma HDL concentrations appears to be the result of both an acute response to the last training bout and also a chronic exposure to exercise.

Whether the activity of LPL was greater in the high versus low intensity exercise is difficult to determine from the results of this study since there were no differences in plasma triglycerides or FFA between conditions. However, the effect of increased LPL activity on plasma concentrations of triglycerides takes several hours to occur, in fact, significant decreases in triglycerides are not usually found until several hours after an exercise bout (Enger, et al, 1980; Thompson, et al, 1980; Kantor, et al, 1983). This is something which should also be investigated in the future, through either direct measurement of LPL activity, or a longer follow-up of plasma lipid concentrations.

Summary

Acute increases in HDL-C, HDL-A, and HDL saturation were observed following a run of 9-12 km. The increases in HDL-C and HDL-A varied directly with the intensity of the exercise. Initial pre-exercise concentrations of HDL-C were not related to the magnitude of the acute response in either exercise condition.

REFERENCES

Albers, J.J., J.L. Adolphson, and W.R. Hazzard. "Radioimmunoassay of human plasma Lp(a) lipoprotein." J. Lipid Res. 18: 331-338, 1977.

Allison, T.G., R.M. Iammarino, K.F. Metz, G.S. Skinner, L.H. Kuller, and R.J. Robertson. "Failure of exercise to increase HDL-C." J. Card. Rehab. 1: 257-265, 1981.

Altekruse, E.B. and J.H. Wilmore. "Changes in blood chemistries following a controlled exercise program." J. Occup. Med. 15: 110-113, 1973.

Anderson, D.W., A.V. Nichols, and H.B. Brewer. "Ultracentrifugal characterization of the human plasma lipoprotein distribution." in Report of the High Density Lipoprotein Methodology Workshop Lippel, K. (ed) N.I.H. Publication, 1979, p290.

Anderson, D.W., Nichols, A.V., S.S. Pan, and F.T. Lindgren. "High density lipoprotein distribution. Resolution and determination of three major components in a normal population sample." Atherosclerosis 29: 161-179, 1978.

Astrand, P. and K. Rodahl. Textbook of Work Physiology Toronto: McGraw-Hill, Inc., 1977.

Astrand, P.O. and B. Saltin. "Plasma and red cell volume after prolonged severe exercise." J. Appl. Physiol. 19: 829-832, 1964.

Barr, D.P., E.M. Russ, and H.A. Eder. "Protein-lipid relationships in human plasma." Am. J. Med. 11: 480-492, 1951.

Beaglehole, R., D.C. Trost, I. Tamir, P. Kwiterovich, C.J. Glueck, W. Insull, and B. Christensen. "Plasma HDL cholesterol in children and young adults." Circulation 62(Suppl. 4): IV83-IV92, 1980.

Beaumont, W., J.E. Greenleaf, and L. Juhos. "Disproportional changes in hematocrit, plasma volume, and proteins during exercise and bed rest." J. Appl. Physiol. 33: 55-61, 1972.

Berg, K. "Genetic influence on variation in serum high density lipoprotein." in High Density Lipoproteins

and Atherosclerosis A.M. Gotto, N.E. Miller, and M.F. Oliver (eds) Amsterdam: Elsevier/North-Holland Biomedical Press, 1978, p. 207-211.

Booth, S. and R.W. Lacey. "Effect of recent food on estimation of high-density lipoprotein and total cholesterol in normal subjects." Ann. Clin. Biochem. 19: 176-181, 1982.

Bradley, D.D., J. Wingerd, D.B. Petitti, R.M. Krauss, and S. Kamcharan. "Serum high density lipoprotein cholesterol in women using oral contraceptives, estrogens, and progestins." New Eng. J. Med. 299: 17-20, 1978.

Brook, J.G., Aviram, M., A. Viener, and E. Shilansky. "HDL subfractions in normolipidemic patients with coronary atherosclerosis." Circulation 66: 923-926, 1982.

Carew, T.E., T. Koshinsky, S.B. Hayes, and D. Steinberg. "A mechanism by which high density lipoproteins may slow the atherogenic process." Lancet 1: 1315-1317, 1976.

Carlson, L.A. and F. Mossfeldt. "Acute effects of prolonged heavy exercise on the concentration of plasma lipids and lipoproteins in man." Acta. Physiol. Scand. 62: 51-59, 1964.

Castelli, W.P., J.T. Doyle, T. Gordon, C.G. Hames, M.C. Hjortland, S.B. Hulley, A. Kugan, and W.J. Zukel. "HDL cholesterol and other lipids in coronary heart disease." Circulation 55: 767-772, 1977.

Cheung, M.C. and J.J. Albers. "The measurement of apolipoprotein AI and AII levels in men and women by immunoassay." J. Clin. Invest, 60: 43-50, 1977.

Costill, D.L. and W.J. Fink. "Plasma volume changes following exercise and thermal dehydration." J. Appl. Physiol. 37: 521-525, 1974.

Costill, D.L., P. Cleary, .J. Fink, C. Foster, J.L. Ivy, and F. Witzmann. "Training adaptations in skeletal muscle of juvenile diabetics." Diabetes 28: 818-822, 1979.

Criqui, M.H., R.B. Wallace, and G. Heiss. "Cigarette smoking and plasma high density lipoprotein cholesterol." Circulation 62(Suppl. 4): 70-76, 1980.

Cullinane, E., B. Lazarus, P.D. Thompson, A. Saratelli, and P.N. Herbert. "Acute effects of a single exercise session on serum lipids in untrained man." Clin. Chim. Acta. 109: 341-344, 1981.

Curry, M.D., P. Alaupovic, and C.A. Suenram. "Determination of apolipoprotein A and its constitutive AI and AII polypeptides by separate electroimmunoassay." Clin. Chem. 22: 15-22, 1976.

Dressendorfer, R.H., C.E. Wade, C. Hornick, and G.C. Timmis. "HDL-C in marathon runners during a 20-day road race." JAMA 247: 1715-1717, 1982.

Dufaux, B., G. Assmann, and W. Hollman. "Plasma lipoproteins and physical activity: A review." Int. J. Sports Med. 3: 123-136, 1982b.

Dufaux, B., G. Assmann, H. Schacton, and W. Hollmann. "The delayed effects of prolonged physical exercise and physical training on cholesterol level." Eur. J. Appl. Physiol. 48: 25-29, 1982a.

Duncombe, W.G. "The Colorimetric micro-determination of long-chain fatty acids." Biochem. J. 88: 7-10, 1963.

Eder, H.A. and L. Gidez. "The clinical significance of the plasma high density lipoproteins." Med. Clin N. Am. 66: 431-440, 1982.

Enger, S.C., K. Herbjørnsen, J. Erikssen, and A. Fretland. "HDL's and physical activity: The influence of physical exercise, age, and smoking on HDL-C and the HDL/total cholesterol ratio." Scand. J. Clin. Lab. Invest. 37: 251-255, 1977.

Enger, S., S.B. Strømme, and H.E. Refsum. "HDL-C, total cholesterol, and triglycerides in serum after a single exposure to prolonged heavy exercise." Scand. J. Clin. Lab. Invest. 40: 341-345, 1980.

England, J.D., L.A. Simons, J.C. Gibson. "The effect of metoprolol and antenol on plasma high density

lipoprotein levels in man." Clin. Exp. Pharmacol. Physiol. 7: 329-333, 1980.

Epstein, L., G.J. Miller, R.W. Stiff, and J.N. Morris. "Vigorous exercise in leisure time, coronary risk factors, and resting electrocardiogram in middle-aged male civil servants." Br. Heart. J. 38: 403-409, 1976.

Erikssen, J. and S.C. Enger. "The effect of smoking on selected coronary heart disease risk factors in middle-aged men." Acta. Med. Scand. 203: 27-30, 1978.

Erkelens, D.W., J.J. Albers, W.R. Hazzard, R.C. Frederick, and E.L. Bierman. "Moderate exercise increases HDL-C in MI survivors." Clin. Res. 26: 158A, 1978.

Ernst, N., M. Fisher, W. Smith, T. Gordon, B.M. Rifkind, J.A. Little, M.A. Mishkel, and O.D. Williams. "The association of plasma HDL cholesterol with dietary intake and alcohol consumption." Circulation 62(Suppl. 4): IV41-IV52, 1980.

Essen, B., L. Hagenfeldt, and L. Kaejser. "Utilization of blood-borne and intramuscular substrates during continuous and intermittent exercise in man." Journal of Physiology 265: 489-506, 1977.

Fainaru, M., M.C. Glangeaud, and S. Eisenberg. "Radioimmunoassay of human HDL apo-protein AI." Biochim. Biophys. Acta. 386: 432-445, 1975.

Farish, E., J. Shepherd, T. Lawrie, and H.G. Morgan. "Plasma apolipoprotein A levels in healthy human adults." Clin. Chim. Acta. 62: 97-101, 1975.

Farrell, P.A. and J. Barboriak. "The time course of alterations in plasma lipid and lipoprotein concentrations during eight weeks of endurance training." Atherosclerosis 37: 231-238, 1980.

Fielding, C.J., V.G. Shore, and P.E. Fielding. "A protein cofacator of lecithin: cholesterol acyltransferase." Biochem. Biophys. Res. Comm. 46: 1493-1498, 1972.

Fox, E.L. Sports Physiology Toronto: WB Saunders Company, 1979.

Friedberg, S.J., W.R. Harlan, D.L. Trout, and E.H. Estes. "The effect of exercise on the concentration and turnover of plasma nonesterified fatty acids." J. Clin. Invest. 39: 215-220, 1960.

Friedwald, W.I., R.I. Levy, and D.S. Fredrickson. "Estimation of the concentration of LDL-C in plasma, without the use of the preparative ultracentrifuge." Clin. Chem. 18: 499-502, 1972.

Glomset, J.A. "Physiological role of lecithin: cholesterol acyltransferase." Am. J. Clin. Nutr. 23: 1129-1136, 1970.

Glomset, J.A. "The plasma lecithin: cholesterol acyltransferase reaction." J. Lipid Res. 9: 155-166, 1968.

Gofman, J.W., O. Delalla, and F. Glazier. "The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis, and CHD." Plasma 2: 413, 1954.

Goldbourt, U. and J.H. Medallie. "High density lipoprotein cholesterol and the incidence of coronary heart disease in the Israel Ischemic Heart Disease Study." Am. J. Epidem. 109: 296-308, 1979.

Gordon, T., W.P. Castelli, M.C. Hjortland, W.B. Kannel, and T.R. Dawber. "HDL as a protective factor against coronary heart disease." Am. J. Med. 62: 707-713, 1977.

Gurr, M.I. and A.T. James. Lipid Biochemisty New York: Chapman & Hall, 1980.

Hagberg, J.M., F.J. Nagle, and J.L. Carlson. "Transient O_2 uptake response at the onset of exercise." J. Appl. Physiol. 44: 90-92, 1978.

Hagenfeldt, L. and J. Wahren. "Turnover of free fatty acid during recovery from exercise." J. Appl. Physiol. 39: 247-250, 1975.

Hartley, L.H. "Growth hormone and catecholamine response to exercise in relation to physical training." Med. Sci. Sports. 7: 34-36, 1975.

Hartung, G.H., J.P. Foreyt, R.E. Mitchell, I. Vlasek, and A.M. Gotto. "Relation of diet to HDL-C in

middle-aged marathon runners, joggers, and inactive men." New Eng. J. Med. 302: 357-361, 1980.

Hartung, G.H., W.G. Squires, and A.M. Gotto. "Effect of exercise training on plasma high-density lipoprotein cholesterol in coronary-disease patients." Am. Heart. J. 101: 181-184, 1981.

Havel, R.J. "Early effects of fat ingestion on lipids and lipoproteins of serum in man." J. Clin. Invest. 36: 848-854, 1957.

Henderson, L.O., A.L. Saritilli, E. LaGarde, P.N. Herbert, and R.S. Shulman. "Minimal within-day variation of HDL-cholesterol and apolipoprotein AI levels in normal subjects." J. Lipid Res. 21: 953-955

Hooper, P.L., W. Woo, L. Visconti, and D.R. Pathak. "Terbutaline raises high-density-lipoprotein cholesterol levels." New Eng. J. Med. 305: 1455-1457, 1981.

Hsia, S.L., C.H. Hennekens, Y. Chao, and W.B. Reader. "Decreased serum cholesterol-binding reserve in premature myocardial infarction." Lancet 2: 1000-1004, 1975.

Hurter, R., M.A. Peyman, J. Swale, and C. Barnett. "Some immediate and long-term effects of exercise on the plasma lipids." Lancet 2: 671-675, 1972.

Huttunen, J.K., E. Lansimies, E. Voutilainen, C. Ehnholm, E. Hietanen, I. Penttila, O. Siitonen, and R.Rauramaa. "Effect of moderate physical exercise on serum lipoproteins." Circulation 60: 1220-1229, 1979.

Ishikawa, T.T., J.B. Brazier, P.M. Steiner, L.E. Stewart, P.S. Gartside, and C.J. Glueck. "A study of the heparin-manganese chloride method for determination of plasma-lipoprotein cholesterol concentration." Lipids 11: 628-633, 1976.

Issekutz, B., W. Shaw, and T.B. Issekutz. "Effect of lactate on FFA and glycerol turnover in resting and exercising dogs." J. Appl. Physiol. 39: 349-353, 1975.

Jenkins, P.J., R.W. Harper, and P.J. Nestel. "Severity of coronary atherosclerosis related to lipoprotein concentration." Br. Med. J. 2: 388-391, 1978.

Jonas, A., L.K. Hesterberg, and S.M. Drengler. "Incorporation of excess cholesterol by high density serum lipoproteins." Biochim. Biophys. Acta. 528: 47-57, 1978.

Jones, N.L., G. Heigenhauser, A. Kuksis, C.G. Matsos, J.R. Sutton, and C.J. Toews. "Fat metabolism in heavy exercise." Clin. Sci. 59: 469-478, 1980.

Kantor, M.A., E.M. Cullinane, P.N. Herbert, and P.D. Thompson. "Acute increase in lipoprotein lipoprotein lipase following prolonged exercise." Med. Sci. Sports. 15: 125-126, 1983.

Karlin, J.B., D.J. Juhn, J.I. Starr, A.M. Scanu, and A.H. Rubenstein. "Measurement of human HDL apolipoprotein AI in serum by radioimmunoassay." J. Lipid Res. 17: 30-37, 1976.

Keys, A. "Alpha lipoprotein (HDL) cholesterol in the serum and the risk of coronary heart disease and death." Lancet 2: 603-606, 1980.

Kiens, B., I. Jorgensen, S. Lewis, G. Jensen, H. Lithell, B. Vessby, S. Hoc, and P. Schnohr. "Increased plasma HDL-cholesterol and apo AI in sedentary middle-aged men after physical conditioning." Eur. J. Clin. Invest. 10: 203-209, 1980.

Kostner, G.M., P. Avogaro, G.B. Bon, G. Cazzolato, and G.B. Quinci. "Determination of HDL's: Screening methods compared." Clin. Chem. 25: 939-942, 1979.

Krauss, R.M. "Regulation of high density lipoprotein levels." Med. Clin. N. Amer. 66: 403-430, 1982.

Krauss, R.M., F.T. Lindgren, J. Wingerd, D.D. Bradley, and S. Ramcharan. "Effects of estrogens and progestins on high density lipoproteins." Lipids 14: 113-118, 1979.

Krauss, R.M., F.T. Lindgren, P.D. Wood, W.L. Haskell, J.J. Albers, and M.C. Cheung. "Differential increases in plasma HDL subfractions and apolipoproteins (Apo-LP) in runners." Circulation 56(Suppl. 3): 4, 1977.

Lehtonen, A. and J. Viikari. "Serum triglycerides and cholesterol and serum HDL cholesterol in highly physically active men." Acta Med. Scand. 204: 111-114, 1978.

Lehtonen, A. and Viikari, J. "Serum lipids in soccer and ice-hockey players." Metabolism 29: 36-39, 1980.

Lehtonen, A., J. Viikari, and C. Ehnholm. "The effect of exercise on HDL apoproteins." Acta Physiol. Scand. 106: 487-488, 1979.

Lemon, P.W.R. and F.J. Nagle. "Effects of exercise on protein and amino acid metabolism." Med. Sci. Sports. 13: 141-149, 1981.

Lemon, P.W.R. and J.P. Mullin. "Effect of initial muscle glycogen levels on protein catabolism during exercise." J. Appl. Physiol. 48: 624-629, 1980.

Levy, R.I, and B.M. Rifkind. "The structure, function and metabolism of high density lipoproteins: a status report." Circulation 62(Suppl. 4): IV4-IV8, 1980.

Lipson, L.C., R.O. Bonow, E.J. Schaefer, H.B. Brewer, and F.T. Lindgren. "Effect of exercise conditioning on plasma HDL's and other lipoproteins." Atherosclerosis 37: 529-538, 1980.

Lopez, A., M.H. Stone, C. Johnson, and L. Bell. "Effect of resistive training on serum lipids and lipoproteins of middle age sedentary men." Am. J. Clin. Nutr. 33: 939, 1980.

Lopez, A., R. Vial, L. Balart, and G. Arroyave. "Effect of exercise and physical fitness on serum lipids and lipoproteins." Atherosclerosis 20: 1-9, 1974.

Mahley, R.W., T.L. Innerarity, T.P. Bersot, A. Lipson, and S. Margolis. "Alterations in human high-density lipoproteins with or without increased plasma cholesterol, induced by diets high in cholesterol." Lancet 2: 807-809, 1978.

Masoro, E.J. Physiological Chemistry of Lipids in Mammals Philadelphia: WB Saunders Company, 1968.

Mathews, D.K. and E.L. Fox. The Physiological Basis of Physical Education and Athletics Toronto: WB Saunders Company, 1976.

McMiken, D.F. and J.T. Daniels. "Aerobic requirements and maximum aerobic power in treadmill and track running." Med Sci Sports 8:14-17, 1976.

McMurray, W.C. A Synopsis of Human Biochemistry Philadelphia: Harper & Row Publishers, 1982.

Miller, G.J. and N.E. Miller. "Plasma HDL concentration and the development of ischemic heart disease." Lancet 1: 16-19, 1975.

Miller, N.E. "Plasma lipoproteins, lipid transport, and atherosclerosis: recent developments." J. Clin. Path. 32: 639-650, 1979.

Miller, N.E., S. Rao, B. Lewis, G. Bjorsvik, K. Myhre, and O.D. Mjos. "High density lipoprotein and physical activity." Lancet 1: 111, 1979.

Morrison, J.A., K. Kelly, M. Mellies, I. deGroot, P. Khoury, P.S. Gartside, and C.J. Glueck. "Cigarette smoking, alcohol intake, and oral contraceptives: Relationships to lipids and lipoproteins in adolescent school children." Metabolism 28: 1166-1170, 1979.

Myhre, K., O.D. Mjos, G. Bjorsvik, and S.B. Stromme. "Relationship of high density lipoprotein cholesterol concentration to the duration and intensity of endurance training." Scand. J. Clin. Lab. Invest. 41: 303-309, 1981.

Nagle, F., D. Robinhold, E. Howley, J. Daniels, and K. Stoedefalke. "Lactic acid accumulation during running at submaximal aerobic demands." Med. Sci. Sports 2: 181-186, 1970.

Nestel, P.J., M. Podkolinski, and N.H. Fidge. "Marked increase in high density lipoproteins in mountaineers." Atherosclerosis 34: 193-196, 1979.

Nicholl, A., N.E. Miller, and B. Lewis. "High density lipoprotein metabolism." Adv. Lipid Res. 17: 53-106, 1980.

Nikkila, E.A. "Metabolic and endocrine control of plasma high density lipoprotein concentration" in High Density Lipoproteins and Atherosclerosis A.M. Gotto, N.E. Miller, and M.F. Oliver (eds) Elsevier/ North Holland Biomedical Press, 1978a, p.177-192.

Nikkila, E.A. "Studies on the lipid-protein relationship in normal and pathologic sera and the effect of heparin

on serum lipoproteins." Scand. J. Clin. Lab. Invest. 5: 8, 1983.

Nikkila, E.A., M. Taskinen, and M. Kekki. "Relation of plasma high density lipoprotein cholesterol to lipoprotein lipase activity in adipose tissue and skeletal muscle of man." Atherosclerosis 29: 497-501, 1978b.

Nikkila, E.A., M. Taskinen, S. Rehunen, and M. Harkonen. "Lipoprotein lipase activity in adipose tissue and skeletal muscle of runners: Relation to serum lipoproteins." Metabolism 27: 1661-1671, 1978c.

Nye, E.R., K. Carlson, P. Kirstein, and S. Rossner. "Changes in high density Lipoprotein subfractions and other lipoproteins induced by exercise." Clin. Chim. Acta. 113: 51-57, 1981.

Nylin, G. "The effect of heavy muscular work on the volume of circulating red corpuscles in man." Am. J. Physiol. 149: 180-184, 1947.

Paul, P. and W.C. Holmes. "Free fatty acid and glucose metabolism during increased energy expenditure and after training." Med. Sci. Sports. 7: 176-181, 1975.

Pearson, T.A., B.H. Buckley, S.C. Achuff, P.O. Kwiterovich, and L. Gordis. "The association of low levels of HDL cholesterol and arteriographically defined coronary artery disease." Am. J. Epidem. 109: 285-294, 1979.

Peltonen, P., T. Marniemi, E. Hietanen, I. Vuori, and C. Ehnholm. "Changes in serum lipids, lipoproteins, and heparin-releasable lipolytic enzymes during moderate physical training in man: A longitudinal study." Metabolism 30: 518-526, 1981.

Pruett, E.D.R. "FFA mobilization during and after prolonged severe muscular work in men." J. Appl. Physiol. 29: 809-815, 1970.

Rapoport, T., M. Aviram, C. Chaimovitz, and J.G. Brook. "Defective high-density lipoprotein composition in patients on chronic hemodialysis." New. Eng. J. Med. 299: 1326-1329, 1978.

Ratcliff, R., K. Elliott, and C. Rubenstein. "Plasma lipid and lipoprotein changes with chronic training." Med. Sci. Sports. 10: 55, 1978.

Rennie, M.J., R. Edwards, C. Davies, S. Krywawych, D. Halliday, J.C. Waterlow, and D.J. Millward. "Protein and amino acid turnover during and after exercise." Biochem. Soc. Trans. 8: 499-501, 1980.

Rifkind, B.M. "The plasma lipoproteins." Angiology 33: 555-561, 1982.

Sauer, J., S. Skrede, J. Erikssen, and J.P. Blomhoff. "The relation between the levels of HDL cholesterol and the capacity for removal of triglycerides." Acta Med. Scand. 208: 199-203, 1980.

Schonfeld, G., J.S. Chen, and R.G. Roy. "Use of antibody specificity to study the surface disposition of apoprotein AI on human HDL's." J. Biol. Chem. 252: 6655-6659, 1977.

Shapiro, W. E.H. Estes, and H.L. Hilderman. "Diurnal variability of serum cholesterol at normal and reduced levels." J. Lab. Clin. Med. 54: 213-215, 1959.

Shepherd, J., C.J. Packard, J.M. Stavart, B.D. Vallance, T.D. Lawrie, and H.G. Morgan. "The relationship between the cholesterol content and subfraction distribution of plasma high density lipoproteins." Clin. Chim. Acta. 101: 57-62, 1980.

Siegle, A.J., L.M. Silverman, and R.E. Lopez. "Creatine kinase elevations in marathon runners: relationship to training and competition." Yale J. Biol. Med. 53: 275-279, 1980.

Steinberg, D. in The Control of Lipid Metabolism J.K. Grant (ed) New York: The Academic Press, 1963, p.111-143.

Sternberg, J.C. "A rate nephelometer for measuring specific proteins by immunoprecipitin reactions." Clin. Chem. 23: 1456-1464, 1977.

Streja, D and D. Mymin. "Moderate exercise and HDL cholesterol." JAMA 242: 2190-2192, 1979.

Tall, A.R. and D.M. Small. "Body cholesterol removal: role of plasma high-density lipoproteins." Adv. Lipid. Res. 17: 1-51, 1980.

Tall, A.R. and D.M. Small. "Plasma high-density lipoproteins." New Eng. J. Med. 299: 1232-1236, 1978.

Tan, M.H. and M.A. Dickenson. "High cholesterol diet raises HDL cholesterol in men." Clin. Res. 25: 703A, 1977.

Taskinen, M.R., E.A. Nikkila, S. Rehunen, and A. Gordin. "Effect of acute vigorous exercise on lipoprotein lipase activity of adipose tissue and skeletal muscle in physically active men." Artery 6: 471-483, 1980.

Thoden, J.S., B.A. Wilson and J.D. MacDougall. "Testing Aerobic Power". In Physiological Testing of the Elite Athlete. J.D. MacDougall, H.A. Wenger and J.J. Green (eds). Ottawa: Canadian Association of Sports Sciences, 1983.

Thompson, P.D., E. Cullinane, L.O. Henderson, and P.N. Herbert. "Acute effects of prolonged exercise on serum lipids." Metabolism 29: 662-665, 1980.

Wallace, R.B., D.B. Hunninghake, S. Reiland. "Alterations of plasma high-density lipoprotein cholesterol levels associated with consumption of selected medications." Circulation 62(Suppl. 4): IV77-IV82, 1980.

Widholm, K., E. Maxa, and G. Zyman. "Effect of diet and exercise upon the cholesterol and triglyceride content of plasma lipoproteins in overweight children." Eur. J. Ped. 127: 121-126, 1978.

Wilson, D.E. and R.S. Lees. "Metabolic relationships among the plasma lipoproteins. Reciprocal changes in the concentrations of very low and low density lipoproteins in man." J. Clin. Invest. 51: 1051-1057, 1972.

Wood, P.D. and W.L. Haskell. "The effect of exercise on plasma high density lipoproteins." Lipids 14: 417-427, 1979.

Wood, P.D., W.L. Haskell, H. Klein, S. Lewis, M.P. Stern, and J.W. Farquhar. "The distribution of plasma lipoproteins in middle-aged male runners." Metabolism 25: 1249-1257, 1976.

Wood, P.D., W.L. Haskell, M.P. Stern, S. Lewis, and C. Perry. "Plasma lipoprotein distributions in male and female runners." Ann. NY. Acad. Sci. 301: 748-763, 1977.

Yano, K., B.B. Rhoads, and A. Kagan. "Coffee, alcohol and risk of coronary heart disease among Japanese men living in Hawaii." New Eng. J. Med. 297: 405-409, 1977.

APPENDIX 1

TOTAL AMOUNT OF EXERCISE PERFORMED BY EACH SUBJECT

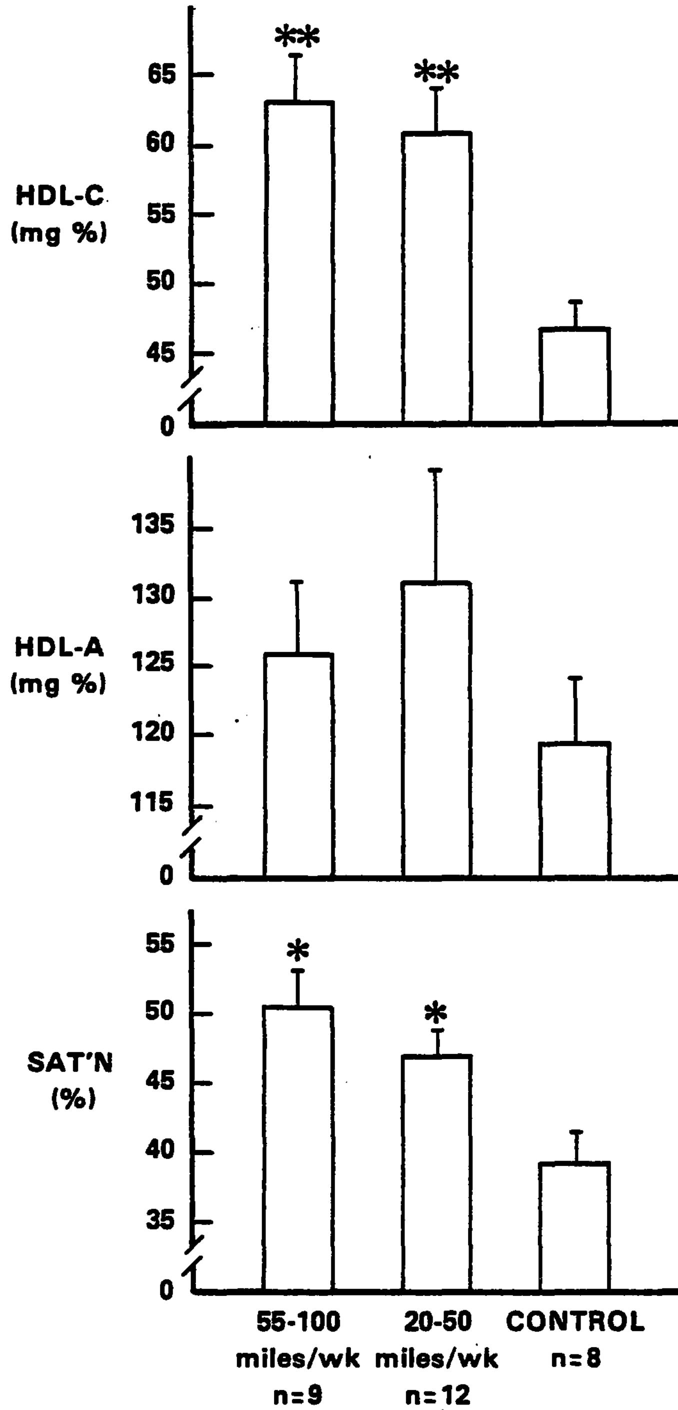
Subject	Total Exercise Time (min)		Equivalent Distance (km)
	60% VO_2 max	90% VO_2 max	
A.S.	56	36	9.6
T.R.	57.5	37	11.2
J.K.	57	37	8.8
C.H.	58	37.5	9.6
S.L.	58.3	38	11.2
G.J.	54.5	36	9.6
J.C.	55	36	9.6
C.W.	58	38	12
M.O.	56	36.5	11.2
D.E.	57.5	37.5	12
N.O.	58	38	9.6
D.M.	59	37.5	8.8

APPENDIX 2 - DESCRIPTION OF SUBJECTS IN PILOT STUDY

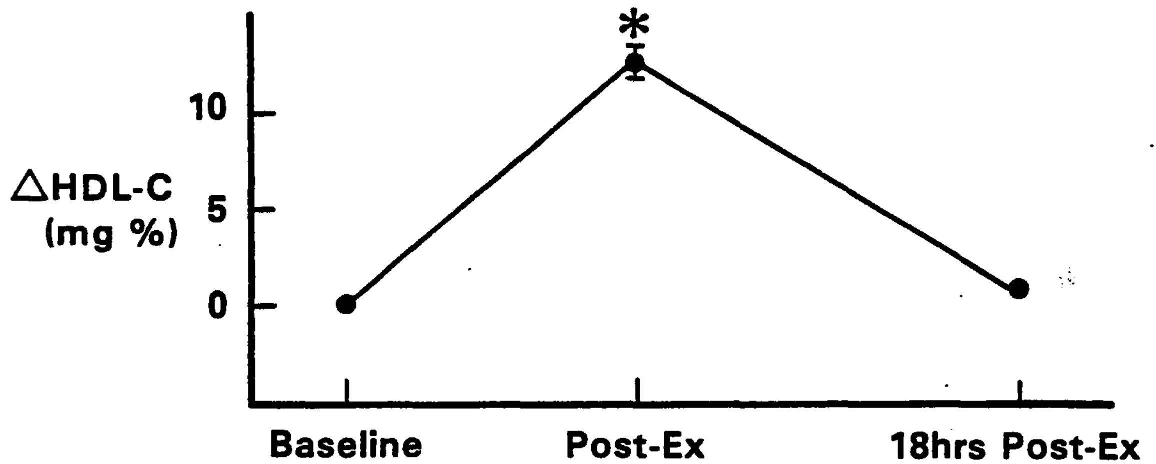
DESCRIPTION OF GROUPS

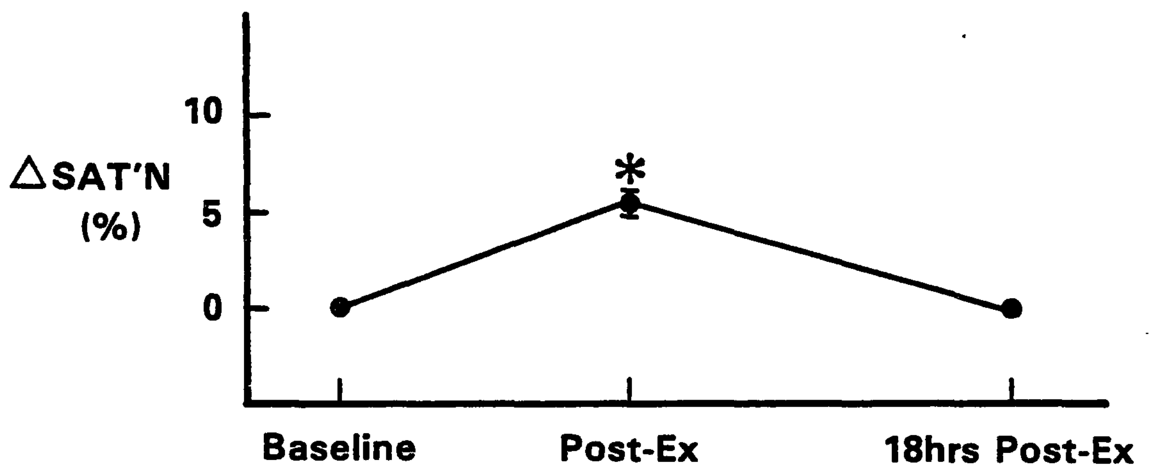
GROUP I (n=9) 55-100 miles/wk		GROUP II (n=12) 20-50 miles/wk		GROUP III (n=8) CONTROL	
SUBJECT	AGE (yrs)	SUBJECT	AGE (yrs)	SUBJECT	AGE (yrs)
C.W.	34	K.W.	28	C.H.	25
R.Z.	37	G.J.	24	T.F.	34
P.B.	20	J.K.	29	D.P.	27
M.O.	19	R.E.	39	B.N.	24
P.Br.	32	K.F.	45	J.M.	31
P.F.	37	D.H.	51	B.D.	52
G.M.	26	R.L.	38	A.S.	23
J.C.	31	T.E.	25	P.O.	39
B.R.	18	B.H.	26		
		N.O.	41		
		D.M.	39		
		T.J.	19		
X ± S.E.	28.2 ± 2.5		33.7 ± 2.8		31.9 ± 3.5

APPENDIX 3 - HDL CHARACTERISTICS OF PILOT STUDY SUBJECTS



APPENDIX 4 - ACUTE HDL CHANGES AFTER A 9-12 KM RUN



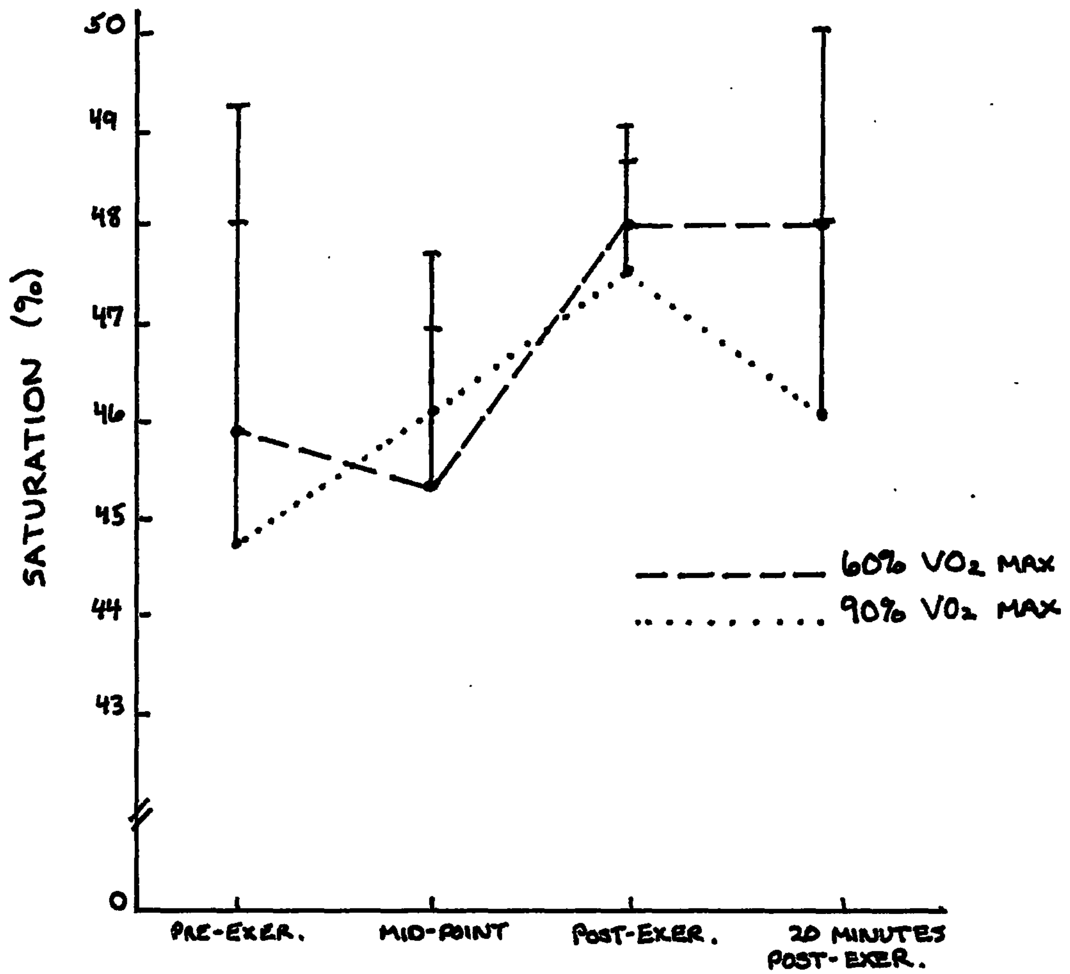


* Difference in variable is significant at $p < .01$

APPENDIX 5

CHANGE IN SATURATION WITH ACUTE EXERCISE

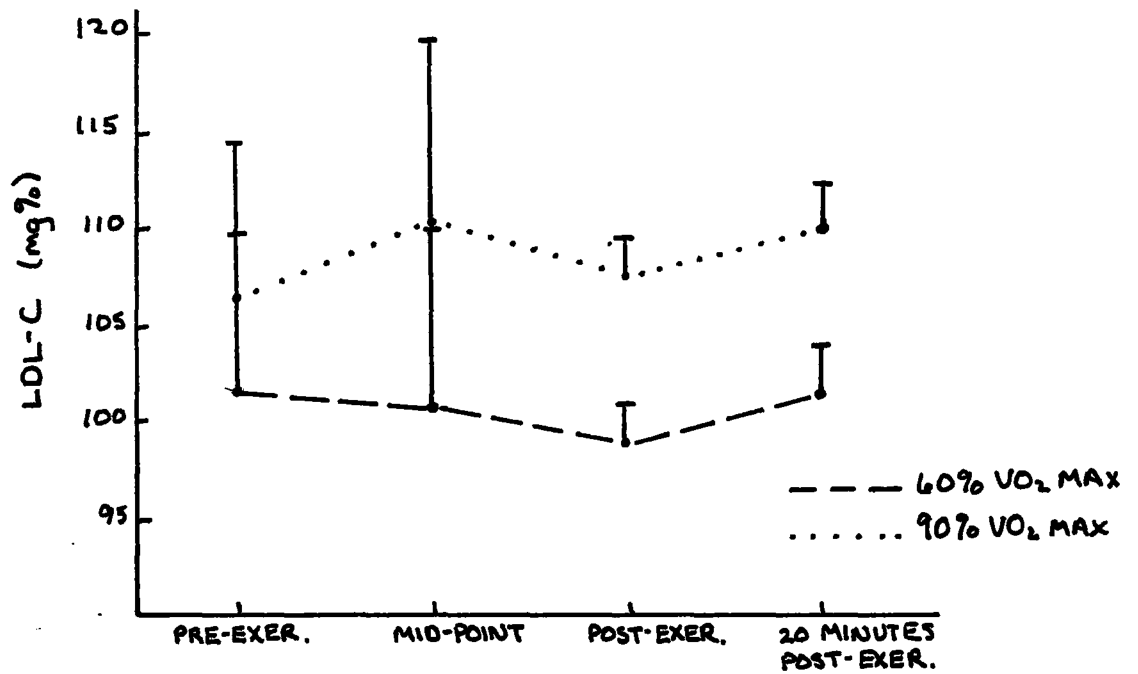
($\bar{X}$ ± SEM)



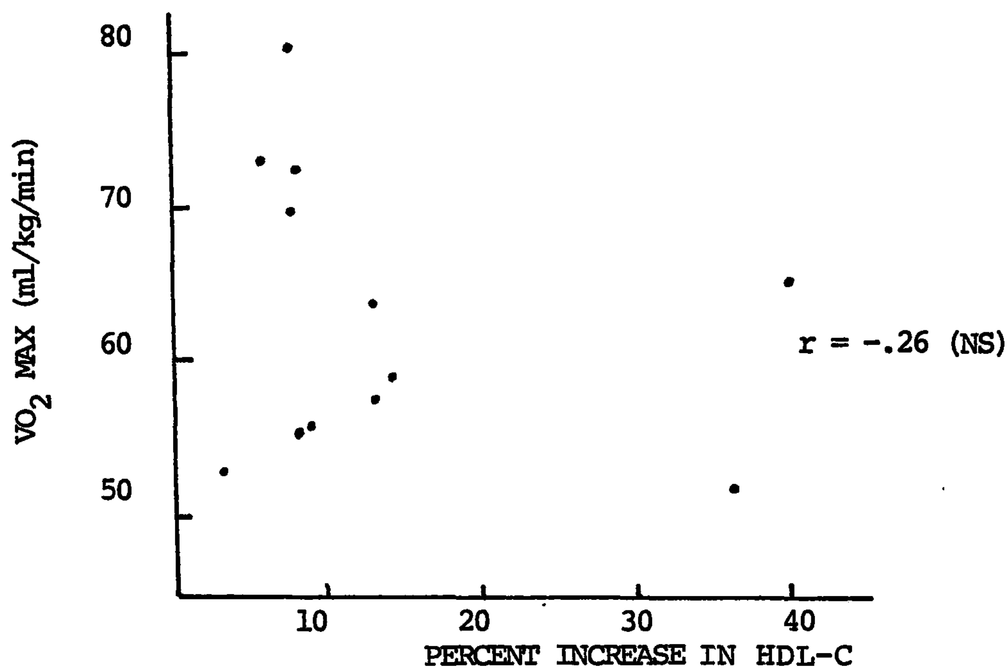
APPENDIX 6

CHANGE IN LDL-C WITH ACUTE EXERCISE

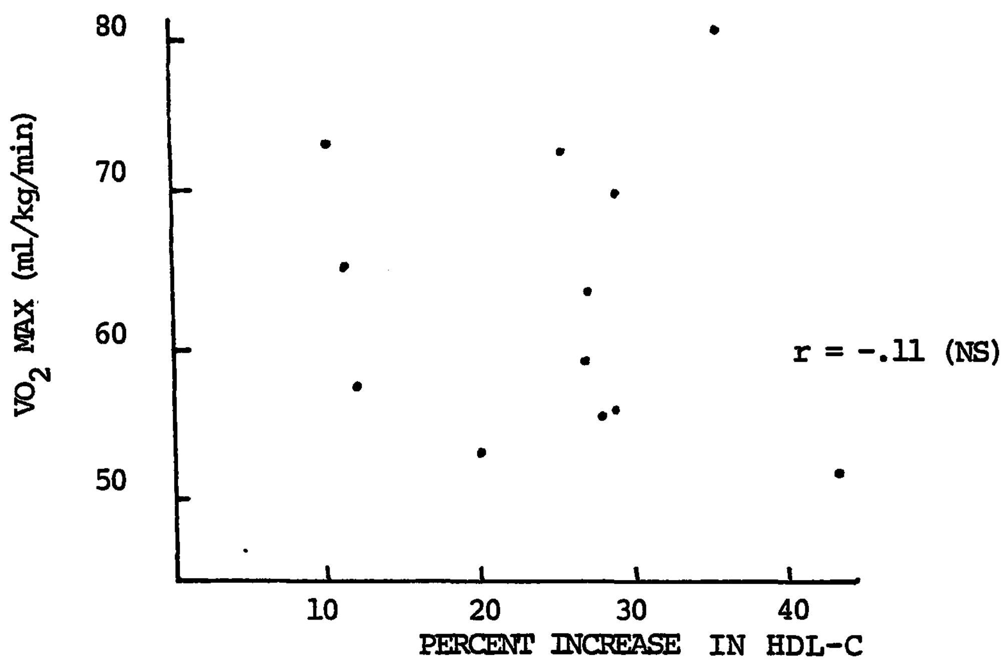
($\bar{X}$ ± SEMΔ)



APPENDIX 7 – RELATIONSHIP BETWEEN VO_2 MAX (ml/kg/min) AND PERCENT INCREASE IN HDL-C AFTER CONTINUOUS EXERCISE AT 60% VO_2 MAX.



RELATIONSHIP BETWEEN VO_2 MAX (ml/kg/min) AND PERCENT INCREASE IN HDL-C AFTER INTERVAL EXERCISE AT 90% VO_2 MAX.



APPENDIX 8 - ANOVA TABLES AND F-RATIOS

PAGE 3 ANOVA HDLC

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

	SOURCE	ERROR TERM	SUM OF SQUARES	D.F.	MEAN SQUARE	F	PROB.
1	MEAN	S	270550.888	1	270550.8880	193.42	.0000
2	S		15386.708	11	1398.7916		
3	C	SC	51.100	1	51.1000	.98	.3443
4	T	ST	988.480	3	329.4934	32.27	.0000
5	SC		575.613	11	52.3285		
6	ST		336.904	33	10.2092		
7	CT	SCT	124.719	3	41.5731	6.29	.0017
8	SCT		219.157	33	6.5108		

PAGE 3 ANOVA HDLA

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

	SOURCE	ERROR TERM	SUM OF SQUARES	D.F.	MEAN SQUARE	F	PROB.
1	MEAN	S	1227035.66	1	1227035.659	500.47	.0000
2	S		26969.20	11	2451.745		
3	C	SC	514.76	1	514.763	1.93	.1924
4	T	ST	2476.03	3	825.343	25.24	.0000
5	SC		2925.20	11	265.927		
6	ST		1079.01	33	32.697		
7	CT	SCT	275.71	3	91.904	3.01	.0439
8	SCT		1006.75	33	30.508		

PAGE 3 ANOVA SATN

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

	SOURCE	ERROR TERM	SUM OF SQUARES	D.F.	MEAN SQUARE	F	PROB.
1	MEAN	S	207930.481	1	207930.4812	775.82	.0000
2	S		2926.141	11	266.0125		
3	C	SC	12.391	1	12.3913	.89	.3666
4	T	ST	96.567	3	32.1889	4.89	.0064
5	SC		153.739	11	13.9762		
6	ST		217.233	33	6.5828		
7	CT	SCT	26.384	3	8.7946	1.40	.2599
8	SCT		207.179	33	6.2782		

PAGE 3 ANOVA TC

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

	SOURCE	ERROR TERM	SUM OF SQUARES	D.F.	MEAN SQUARE	F	PROB.
1	MEAN	S	3023652.22	1	3023652.224	393.00	.0000
2	S		84631.09	11	7693.735		
3	C	SC	1248.56	1	1248.556	1.47	.2514
4	T	ST	1031.46	3	343.819	8.65	.0002
5	SC		9369.31	11	851.755		
6	ST		1312.13	33	39.762		
7	CT	SCT	337.96	3	112.652	3.03	.0429
8	SCT		1225.32	33	37.131		

PAGE 3 ANOVA TRI

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

	SOURCE	ERROR TERM	SUM OF SQUARES	D.F.	MEAN SQUARE	F	PROB.
1	MEAN	S	938814.19	1	938814.193	58.59	.0000
2	S		176244.97	11	16022.270		
3	C	SC	2468.68	1	2468.685	1.65	.2259
4	T	ST	4809.13	3	1603.043	3.07	.0413
5	SC		16499.85	11	1499.986		
6	ST		17240.53	33	522.440		
7	CT	SCT	109.66	3	36.555	.18	.9117
8	SCT		6845.67	33	207.444		

APPENDIX 8 - (cont.)

PAGE 3 ANOVA FFA

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

	SOURCE	ERROR TERM	SUM OF SQUARES	D.F.	MEAN SQUARE	F	PROB.
1	MEAN	S	53.509121	1	53.509121	207.23	.0000
2	S		2.840360	11	.258215		
3	C	SC	.057428	1	.057428	.45	.5176
4	T	ST	2.668164	3	.889388	11.62	.0000
5	SC		1.413880	11	.128535		
6	ST		2.525860	33	.076541		
7	CT	SCT	.038358	3	.012786	.20	.8971
8	SCT		2.132744	33	.064629		

PAGE 3 ANOVA LACTATE

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

	SOURCE	ERROR TERM	SUM OF SQUARES	D.F.	MEAN SQUARE	F	PROB.
1	MEAN	S	24409.88	1	24409.882	111.77	.0000
2	S		1747.17	8	218.396		
3	C	SC	4949.41	1	4949.413	63.07	.0000
4	T	ST	3491.70	2	1745.851	42.58	.0000
5	SC		627.80	8	78.475		
6	ST		656.07	16	41.004		
7	CT	SCT	2474.98	2	1237.489	42.51	.0000
8	SCT		465.77	16	29.111		

PAGE 3 ANOVA LDLC

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

	SOURCE	ERROR TERM	SUM OF SQUARES	D.F.	MEAN SQUARE	F	PROB.
1	MEAN	S	1050096.152	1	1050096.152	137.34	.0000
2	S		84104.771	11	7645.888		
3	C	SC	1468.127	1	1468.127	1.48	.2486
4	T	ST	112.404	3	37.468	.44	.7229
5	SC		10890.737	11	990.067		
6	ST		2782.633	33	84.322		
7	CT	SCT	84.271	3	28.090	.47	.7042
8	SCT		1965.748	33	59.568		

PAGE 3 ANOVA HCT

ANALYSIS OF VARIANCE FOR DEPENDENT VARIABLE 1

	SOURCE	ERROR TERM	SUM OF SQUARES	D.F.	MEAN SQUARE	F	PROB.
1	MEAN	S	190126.2507	1	.19013E+06	5053.87	0.0000
2	S		413.8197	11	37.620		
3	C	SC	9.8496	1	9.8496	2.37	.1517
4	T	ST	29.3197	3	9.7732	12.86	.0000
5	SC		45.6582	11	4.1507		
6	ST		25.0788	33	.75996		
7	CT	SCT	17.9499	3	5.9833	9.13	.0002
8	SCT		21.6361	33	.65564		