THE EFFECT OF AN EIGHT-WEEK BICYCLE ERGOMETER TRAINING PROGRAM ON HIGH DENSITY LIPOPROTEIN-CHOLESTEROL (HDL-C), HDL2-C, HDL3-C AND TOTAL CHOLESTEROL (TC) CONCENTRATIONS

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To my parents, for their continuous encouragement and understanding, I dedicate this thesis.

ABSTRACT

Recent studies have suggested that HDL may regulate the tissue cholesterol from the arterial wall. Furthermore, low blood levels of HDL have been identified as a risk factor for coronary heart diseases. The role of the HDL subfractions, HDL2 and HDL3, remains controversial but it is believed that HDL2 assumes a protective role by transporting the cholesterol acquired from the cells to the liver for degradation and elimination. Physical activity, among other factors, is known to beneficially influence HDL and its subfractions.

Sixteen young women (19-30 years) participated in a bicycle ergometer training program, while nine women acted as control sujects and remained sedentary, to study the effect of exercise on HDL-c, HDL2-c, HDL3-c and total cholesterol concentrations. All subjects were untrained, non-smokers and non-users of oral contraceptives. The training group exercised three times a week for eight weeks, 35 minutes per session (5 minute work bout, 1 minute rest period) with heart rates corresponding to 75% of their VO2 max.

Weight, height, sum of four circumferences, percentage of body fat and VO2 max were determined prior to and at the completion of the eight- week study. Blood samples were drawn after an overnight fast at week zero (pre), four,

five, six, seven, eight and nine (post) and analyzed for the cholesterol content in the lipid and lipoproteins. The results indicated that the body weight, the BMI (w/h2) and the sum of circumferences did not change in either group, while with time, the percentage of body fat decreased significantly and VO2 max increased significantly in the combined group. No interconversion of the HDL subfractions was recorded as a function of the training program. Furthermore and contrary to what the literature has been suggesting, the changes observed in the HDL subfractions were primarily a function of modifications occurring in HDL3, and not in HDL2, as expected. The changes observed in the lipid and lipoproteins cannot be attributed to training due to an absence of significant between groups (training and control) differences on any variable measured.

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THE PROBLEM

1.1 INTRODUCTION AND RATIONALE

Ischemic Heart Disease (IHD) is at present classified as the major cause of death in men and women in Canada, notwithstanding the fact that its incidence is declining (Statistics Canada, 1984). Therefore, it is not suprising that a vast amount of research has been published in past decades pertaining to the different risk factors associated with the prevalence of IHD and related coronary heart diseases (CHD). Elevated blood pressure, obesity, smoking, diet, inactivity and blood lipids have been identified as possible contributors. The latter one, blood lipids, and more specifically, the high density lipoprotein (HDL) component, has been a most recent factor of interest in a number of studies, particularly since Miller and Miller's (1975) classic paper. These authors were the first to propose that HDL may regulate the tissue cholesterol pools and act in the clearance of cholesterol from the arterial wall. Miller et al. (1981) confirmed epidemiological data, establishing a positive correlation between atherosclerosis and low densi-

ty lipoprotein (LDL) and a negative correlation with HDL-cholesterol (HDL-c), in patients undergoing coronary angiography. The results also confirmed that high angiography scores (greater number of stenoses, greater narrowing of the artery and greater length of the stenoses) were associated with lower HDL-c (mainly the HDL2 subfraction) concentrations.

The available data has enabled researchers to better understand the role of HDL as a protective agent against the development of atherosclerosis: HDL's metabolic function is primarily one of transporting the cholesterol from the peripheral tissues to the liver for excretion or for further synthesis into bile acids (Glomset, 1968). Gotto et al. (1982) summarized concisely the different theories that have been advanced to explain HDL's protective role: HDL may prevent the uptake of LDL by the cells (LDL being the main carrier of the total cholesterol mass); HDL may act as a scavenger to remove cholesterol from nonhepatic tissues, including the arterial walls; and HDL may accelerate the clearance of triglyceride-rich lipoproteins.

The concentration of circulating HDL is influenced by many factors: gender, age, smoking, adiposity, diet, oestrogen use, heredity and inactivity. Women have higher HDL-c concentrations than men at any age, (Abbot et al., 1983; Albers et al., 1978; Shepherd et al., 1978), and in great

part, this is accounted for by the HDL2 subfraction, while HDL3 shows only a slight variation with gender (Anderson 1978). Smokers have lower mean HDL-c than non-smokers (Brunzell, 1984; Cowan, 1983; Enger et al., 1977; Heyden et al., 1979; Rabkin et al., 1981; Stubbe et al., 1982). Furthermore, a dose response with the number of cigarettes smoked per day has been reported (Wilson et al., 1983).. Cigarette smoking affects the HDL3 subfraction more than the HDL2 subfraction (Haffner et al., 1985). Also, smokers exercising three to four times a week, running two to four miles per session, demonstrated lipid profiles similar to those of sedentary non-smokers (48 mg/dl for smokers, 47 mg/dl for non-smokers) suggesting that smoking may negate the beneficial effect of physical activity (Stamford et al., 1984). Alcohol consumption has been shown to positively affect HDL, but more so the HDL3 subfraction (Jacqueson et al., 1983; Philipps et al., 1983), by increasing the synthesis of nascent HDL by the liver, and/or by interfering with the degradation of HDL micelles (Fraser et al., 1983) and/or by its influence on hepatic lipase (Hartung et al., 1983). The use of contraceptives and its effect on lipid metabolism has received much attention since its link to an increased risk of myocardial infarct. The research data provides conflicting results due to various combinations of progesterone and oestrogen concentrations. Conclusively, oestrogen use favors higher HDL concentrations (Cauley et al., 1982; Schaefer et

al., 1983; Wahl et al., 1983), be it either in an exogenous or endogenous form, and may indeed reflect the higher HDL-c concentrations found in women; progesterone dosage is negatively correlated with HDL-c concentrations (Bradley et al., 1978).

HDL-c concentrations have been shown to increase with aerobic physical activity with or without weight loss (Brunzell, 1984; Hartung et al., 1983; Huguchi et al., 1984; Philipps et al., 1982; Williams et al., 1983; Zimmerman et al., 1984). Most studies which have observed aerobic training benefits, have also demonstrated favorable lipid profiles (lower total cholesterol (TC), lower LDL-c, lower VLDL and lower triglycerides (TG) concentrations), but more importantly, an increase in HDL-c concentrations for the active subjects versus sedentary controls (Berg et al., 1983; Cowan, 1983; Durstine et al., 1983; Enger et al., 1977; Farrell et al., 1980; Hartung et al., 1984; Huguchi et al., 1984; Huttunen et al., 1979; Kiens et al., 1984; Lehtonen et al., 1978;1979; Lopez et al., 1974; Miller et al., 1979; Moore et al., 1983; Schriewer et al., 1983; Sutherland et al., 1984; Wood et al., 1976; 1983). A few studies, however, have failed to show any change in total HDL-c concentrations (Frey et al., 1982; 1983; Nye et al. 1980; Rainville et al., 1984; Williams et al., 1983; Wynne et al., 1980) and one group reported a decrease in HDL-c concentrations (Myhre et al., 1981). These conflicting results may in fact be a

function of experimental variables such as training mode, duration of training, blood sampling techniques, weight changes, caloric intake, hormonal intake and/or production and genetic susceptibility. Recently the need for quantifying the changes occurring in the two predominant HDL fractions, HDL2 and HDL3, has emerged, in view of the the discovery that HDL2 may actually be the antiatherogenic fraction due to its central role in determining plasma levels of HDL-cholesterol (Sphepherd et al., 1980). A number of studies have recently investigated the cholesterol content of the HDL subfractions (Anderson et al., 1978; Neel et al., 1984; Nicoll et al., 1981; Patsch et al., 1978; Ron et al., 1983; Schaefer et al., 1979). Studies researching the effects of aerobic training have shown that HDL2 is the least stable and is the fraction most influenced by training while HDL3 remains more stable and unlikely to be affected by training (Krauss et al., 1977b; Kuusi et al., 1982). However, most of the above studies involved male subjects. Few have investigated HDL subfractions in women (Krauss et al., 1977b; Lipson et al., 1979). There is therefore, a dearth of information with respect to the effects of physical activity on HDL-c subfractions in women. A number of reasons have contributed to this anomaly: the widespread use of different combinations of oral contraceptives has rendered data interpretation difficult; the fluctuations in HDL-c concentrations during the menstrual cycle have often been neglected

in analyses; the relatively high initial HDL-c levels found in women. Moreover, the effects of an aerobic training program on HDL2 and HDL3 in young women would provide us with a better understanding of the sequential pattern of alterations occurring in the HDL subfractions.

1.2 STATEMENT OF THE PROBLEM

The primary objective of this study was to observe any change occurring in HDL-cholesterol concentrations, and in particular, in its main subfractions, HDL2 and HDL3, in sedentary women subjected to an eight-week aerobic bicycle ergometer training program. More specifically, the study was designed to determine if three weekly exercise sessions of thirty-five minutes each, at heart rates in the range of 60% to 70% of maximal oxygen consumption (VO2 max) would alter the concentrations of HDL2-c and HDL3-c, favoring the formation of HDL2.

A secondary objective of the study was to examine total cholesterol concentrations in the same subjects, within the same time frame, to document changes occurring in cholesterol transport as a result of an aerobic training program.

1.3 EXPERIMENTAL HYPOTHESIS

On the basis of the information provided in the introduction, the following was hypothesized. Young women, submitted to an aerobic training program would demonstrate lower total cholesterol (TC) concentrations and higher HDL-c concentrations. Furthermore, concomitant changes would occur in the HDL2 and HDL3 subfractions, thus providing the trained subjects with higher HDL2-c/TC and HDL2-c/HDL-c ratios than those found in the control subjects.

1.4 DELIMITATIONS

The subjects were healthy sedentary young women (primarily students) who were all volunteers. Furthermore, risk factors associated with CHD such as obesity, smoking, high blood pressure and diet, were not studied. The results of the study are therefore only applicable to the experimental population and to the described exercising protocol.

1.5 LIMITATIONS

The lipids analyzed in the study were limited to HDL-c, HDL2-c, HDL3-c and total cholesterol concentrations. One major limitation of this study was the method of lipid analysis. The method of choice is ultracentrifugation. The method used in this experiment was a double precipitation method as described by Gidez et al. (1982). Furthermore, the control over changes in the diet and alcohol consumption (which may affect lipids and lipoproteins) was done solely by questionnaire.

1.6 STATISTICAL ANALYSIS

An analysis of variance with repeated measures was used to establish if significant differences existed between the training and control groups for the following variables: TC, HDL-c, HDL2-c, HDL3-c, HDL2-c/TC, HDL2-c/HDL-c, body weight, body mass index, sum of circumferences, percentage of body fat and VO2 max. A 0.05 level of probability was employed in all tests of statistical significance, and when significant differences were observed, Tukey's test was used to locate these differences.

1.7 DEFINITIONS

The following terms are used in this study:

1. Coronary Heart Disease (CHD):

Atherosclerosis, the major cause of CHD is characterized histologically by the accumulation of lipids, predominantly cholesterol derived from LDL, in the arterial wall together with a local connective tissue reaction (smooth muscle cell proliferation) (Miller et al., 1975), forming bulky plaques that inhibit the flow of blood until a clot eventually forms, obstructing an artery and causing a heart attack or a stroke (Brown et al., 1984).

2. Lipoproteins:

The lipoproteins are transport vehicles in the circulation for endogenously synthesized and exogenous (dietary) lipids needed for cellular metabolism. They are discrete water-soluble macromolecular complexes with finite dimensions and composition, all containing cholesterol (free and esterified), triglycerides, phospholipids and proteins (apolipoproteins). Four classes have been identified by ultracentrifugation according to their gravitional density, and are known as chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) (Haskell, 1984). (See Table 1 for lipid and protein contents of the human plasma lipoproteins).

3. Chylomicrons (CM) (d<0.95 g/ml):

Chylomicrons, the largest lipoproteins (containing 85% triglycerides (TG)), are synthesized by the intestine to transport dietary TG (which are hydrolyzed within the plasma by the enzyme lipoprotein lipase (LPL)) and cholesterol from the site of absorption in the intestinal epithelium to various cells of the body (Mahley et al., 1984).

4. Very Low Density Lipoproteins (VLDL) (d=0.95-1.006 g/ml):

VLDL are the major transport vehicles of endogenous TG from the liver to various tissues, including those TG newly synthesized and those derived from storage reservoirs (Haskell, 1984; Mahley et al., 1984).

5. Low Density Lipoproteins (LDL) (d=1.019-1.063 g/ml):

LDL, constituting the largest lipoprotein mass, are responsible for the delivery of the majority of the cholesterol (50% to 66% of total mass) to the various cells and tissues of the body to be used as a structural component. The effective delivery of cholesterol is possible by specific cell surface receptors (LDL or apo-B,E receptors) which bind and internalize the lipoproteins. The hydrolysis of the LDL within the lysosomes produces free cholesterol which is available for membrane biosynthesis or, in specific cells, as a precursor for steroid hormones (Innerarity et al., 1984).

6. High Density Lipoproteins (HDL):

HDL are mainly involved in the reverse cholesterol transport pathway, transporting cholesterol from the peripheral tissues to the liver for excretion or synthesis into bile acids, via interaction with the enzyme lecithin:cholesterol acyl transferase (LCAT) (Albers et al., 1976; Haskell, 1984). The HDL particle has been separated by rate zonal ultracentrifugation into two main subfractions: HDL2 (density=1.063-1.125 g/ml) and HDL3 (density=1.125-1.210 g/ml) (Shepherd et al., 1980). The subfractions are heterogenous (Barter et al., 1984b; Neel et al., 1984); HDL2 is lipid rich and protein poor (43% protein, 28% phospholipids, 23% cholesterol and 6% TG), while the opposite is true of HDL3 (58% protein, 22% phospholipids, 14% cholesterol and 5% TG) (Patsch et al., 1978; Schaefer et al., 1979). HDL3 is the more stable subfraction, while HDL2 may be influenced by physical activity, sex, hormones, menstrual cycle and pharmacological agents (Taskinen et al., 1981). HDL2 is the subfraction mainly responsible for the variation in the total HDL concentrations, and contributes to the inverse relationship associated with the prevalence of CHD (Anderson et al., 1978; Gotto et al., 1982). Women have greater HDL2 concentrations than their male counterparts, and this holds true for all age groups (Anderson et al., 1978; Haffner et al., 1985; Ottosson et al., 1985; Ron et al., 1983; Shepherd

et al., 1978; 1980). This may indeed be why women have higher total HDL-c concentrations than men, which renders them less susceptible to coronary diseases.

7. Apolipoproteins (Apo):

Apolipoproteins are constituents of the major lipoproteins (see Table 2 for distribution) and act as stabilizers for the lipoprotein, constitute recognition sites for cell receptors and act as cofactors for enzymes involved in the lipoprotein metabolism (Dufaux et al., 1982).

8. Lecithin:Cholesterol Acyl Transferase (LCAT):

LCAT's primary enzymatic role is its involvement in the reverse cholesterol transport function, facilitating the transfer of cholesterol from the peripheral cells into the HDL particles (Dufaux et al., 1982).

9. Lipoprotein Lipase (LPL):

LPL is the key enzyme for the catabolism of TG-rich lipoproteins (VLDL and CM), for circulating TG cannot be taken up by tissues until they are hydrolyzed by LPL (Haskell, 1984; Kinnunen et al., 1983; Lithell et al., 1981). The enzyme is located at the endothelial surface of various extrahepatic tissues, but its major effect is its activity at the level of the skeletal and adipose tissues (Dufaux et al., 1982; Haskell, 1984).

10. Hepatic Lipase (HL):

HL is an enzyme located exclusively on the sinusoidal surfaces of the liver's endothelial cells (Kinnunen et al., 1983). HL has a high phospholipase activity and can degrade the surface phospholipids of HDL, which is followed by a release of cholesteryl esters to the liver. From their results, Kuusi et al. (1980) suggest that HL is highly selective and hydrolyzes only the HDL2 subfraction.

11. Maximal Oxygen Consumption (VO2 max):

VO2 max is a quantitative statement of an individual's capacity for aerobic energy transfer, and has been identified as the point when oxygen consumption ceases to rise with further increases in workload (McArdle et al., 1981).

TABLE 1

LIPID AND PROTEIN CONTENTS OF PLASMA LIPOPROTEINS

	APPROXIMATE COMPOSITION (PERCENT)				
			Cholesterol		
Lipoprotein	Protein	TG	Free	Esters	Phospholipids
Chylomicron	2	85	2	5	7
VLDL	9	50	8	15	18
LDL	25	10	9	36	20
HDL	40	5	5	22	28

(Orten and Newhaus (1982), p. 284)

TABLE 2

PRINCIPAL APOPROTEINS OF PLASMA LIPOPROTEINS

	LIPOPROTEIN CLASS (MOL %)						
Apoproteins	CM	VLDL	IDL	LDL	HDL	HDL2*	HDL3*
A-I	3	-	-	-	46	65	62
A-II	3	-	-	-	23	10	23
A-IV	2	-	-	-	-	?	+
B-48	0.8	-	-	-	-	3 (B-48,	
B-100	+	1	13	74	-	B-100)	
C-I	20	8	26	-	18	13	5
C-II	22	20	9	-	2	(C-I, C-II,	
C-III	49	60	41	17	3	C-III)	
D	+	-	-	-	5	2	4
E	+	9	13	9	1	3	1
Others	-	-	-	-	-	4	5

+ represents trace
(*Dufaux et al. (1982); Gotto (1983))

II

REVIEW OF LITERATURE

The object of this chapter is to review the pertinent literature with a special emphasis on HDL-c and the HDL subfractions. Also of main concern for this study, is the effect of oestrogen on HDL-c, HDL2-c and HDL3-c. The first section provides a discussion of the regulatory role of exercise with respect to lipid metabolism; the origin, the role, the pathway and the interaction with the various enzymes for each of the lipoproteins are elaborated. The following two sections deal with longitudinal and comparative training studies, respectively. Both are further divided into long-term and short-term training (longitudinal studies only), studies involving women and studies pertaining to HDL subfractions. The last section summarizes the effect of oestrogen on HDL and its subfractions.

2.1 LIPID METABOLISM AND ITS REGULATION WITH AEROBIC EXERCISE

Lipids in the body are in a dynamic state: they may be oxidized for energy purposes, converted to essential constituents or stored in the form of TG as reserve fat (Orten

et al., 1982). The major families of lipids transported in the blood are cholesterol, cholesteryl ester, triglycerides, phospholipids and fatty acids. They appear in three forms: chylomicrons (CM), lipids associated with lipoproteins and unesterified fatty acids loosely bound to serum albumin (Gotto, 1984). Since lipids are insoluble in an aqueous medium, their transportation in blood is made possible by the association of more hydrophobic lipids to more hydrophilic ones, such as phospholipids, and combining the mixture with cholesterol and protein to form a globular hydrophilic lipoprotein particle, characterised by a density of less than 1.21 g/ml (Orten et al., 1982). Reference values (plasma) for the lipids and lipoproteins appear in Table 3 (Boehringer Mannheim, 1982, 1983; Gidez et al., 1982).

TABLE 3

REFERENCE VALUES FOR LIPIDS AND LIPOPROTEINS IN PLASMA

Lipids/Lipoproteins	CONCENTRATIONS mg/dl	mmol/l
TC	140-260	3.6-6.7
TG	30-135	0.8-3.5
VLDL-c	0-40	0-1.0
LDL-c	150-190	3.9-4.9
HDL-c	40-70	1.0-1.8
HDL2-c	10-30	0.3-0.9
HDL3-c	27-43	0.7-1.1

Lipoproteins are secreted by the liver and the intestine in a nascent form. During their circulation in the plasma, they undergo constant modification to a product that allows them to be recognized by cellular receptors in the various tissues and to be removed from the circulation. The intravascular modification of the lipoproteins is a function of a) enzymatic activity (LCAT, LPL and HL), and b) of the transfer of lipids and protein components between the particles themselves and with the cellular membranes (Patsch et al., 1984).

As dietary fats (triglycerides and cholesterol) are ingested, they appear as CM (the largest lipoproteins, containing 85% TG) in the thoracic duct during active intestinal absorbtion (Lakshmanan et al., 1983; Mahley et al., 1984) and contain mainly TG resynthesized from diglycerides in the wall of the gut. They are then transported through the lymph and emptied into the systemic circulation at the level of the jugular and subclavian veins (Guyton, 1976), after which the TG content is catabolized by the enzyme LPL at extrahepatic tissue sites, liberating glycerol and fatty acids. The fatty acids can then readily be used as an energy source at the level of the skeletal muscle or taken up by the adipocytes and stored as TG for future use (Lakshmanan et al., 1983; Mahley et al., 1984). The CM remnants (TG deprived and cholesterol enriched) in the circulation are rapidly taken up (absent after a 12-hour fast) and further me-

tabolized by the liver (Mahley et al., 1984), due to the appropriate exposure of the apolipoprotein E which serves as a ligand for high-affinity receptors located on hepatocytes (Patsch et al., 1984). The cholesterol and cholesteryl esters of the CM reaching the liver may be incorporated into lipoproteins and released back into the plasma, or converted to bile acids and secreted in the bile, or secreted in the bile as neutral sterols (Haskell, 1984; Gottto, 1984).

Most of the endogenous plasmatic TG are transported by the VLDL; the synthesis of VLDL particles, occurring mostly in the liver and in a small quantity in the intestine, is significantly influenced by the availability of carbohydrates, excess calories and fatty acids. Their removal from the circulation is similar to those of the CM; the TG content is hydrolyzed to free fatty acids (FFA) at extrahepatic sites by LPL, generating cholesterol enriched lipoproteins, including the intermediate density lipoprotein (IDL), the transient form which is further degraded to LDL in the bloodstream, followed by the subsequent hepatic uptake of the remnants (Dufaux et al., 1982; Levy et al., 1980).

Cholesterol is produced by most mammalian cells, is an essential structural component of all cell membranes, and is used as a precursor of bile acids, the steroid hormones and of vitamin D; it is not dietarily essential since it is readily synthesized by the cell (Patsch et al., 1984). The

exogenous cholesterol is derived from animal products such as meats, liver, eggs, milk lipids..., and directly affects, by feedback inhibition, the amount synthesized by tissue cells (Orten et al., 1982). LDL are the primary transporters of cholesterol in the circulation, and are derived mostly from VLDL in the plasma. However, some LDL formation may be from direct secretion of intermediate density lipoprotein (IDL) and LDL particles from the liver or from the catabolism of CM. The effective delivery of cholesterol to the cell, via LDL, is made possible by specific cell surface receptors referred to as LDL or apo B,E, receptors which bind and internalize the lipoproteins (Innerarity et al., 1984). Two pathways are accessible for cholesterol delivery: the LDL receptor pathway serves a physiologic function, and is used when cells express a need for cholesterol by increasing the availability of their surface receptors. This pathway is saturable and approximately 50% to 60% of the LDL are removed from the plasma following this route. Any excess LDL are removed from circulation through the nonreceptor (or scavenger) pathway which is not saturable, thereby allowing cells that possess the acetyl LDL receptor (i.e. macrophages, cells of the reticuloendothelial system (RES)) to accumulate uncontrolled amounts of cholesteryl esters. Thus, macrophages can incorporate modified LDL until they are massively enriched in cholesteryl esters and turn into foam cells, characteristic of early atherosclerotic lesions. It

is possible that CM remnants escaping the hepatic uptake may be incorporated by RES cells, leading also to foam cell formation (Patsch et al., 1984; Steinberg, 1978).

Most tissues are unable to catabolize or excrete the cholesterol from the body. The transport of cholesterol from such tissues to sites of catabolism (liver, adrenal cortex, gonads) and excretion (liver, skin, intestine) may be a function of HDL. HDL acts as an acceptor, present in the interstitial fluid adjacent to the cell surfaces; HDL is capable of removing cholesterol from the extravascular cells when not needed, by increasing its load of cholesterol (Nicoll et al., 1981; Reichl et al., 1982). In contrast to the CM, the remnants and the LDL, HDL does not contain the apo B which serve as ligands (with apo E) for the various receptors for adsorptive endocytosis of the lipoprotein. This suggests that HDL have a completely different function in cholesterol transport, one which has been termed reverse cholesterol transport. Two pathways are known to exist for the cholesterol acquired by HDL: a) the cholesterol can be returned to the liver for excretion when no need is expressed by the cells, or b) can by cholesteryl ester exchange to the apo B containing lipoproteins be delivered to cells in need (Patsch et al., 1984). The HDL particle has apoproteins which are located on or near its surface. Furthermore, the polar head of the phospholipids seem to be oriented near the surface, while the more hydrophobic compo-

nents (TG, cholestery esters) appear to constitute the central core of the particle (Yachida et al., 1983). The high density of the particle is a function of the high protein content (50% by weight) and the low lipid content (Levy et al., 1980). The HDL particles originate from the liver and the intestinal epithelial cells. The mature HDL are products of precursor particles, from TG-rich lipoprotein hydrolysis, which have been acted upon by the enzyme LCAT (Koga et al., 1982). During the catabolism of the CM and VLDL by the enzyme LPL, there is a release of surface components (unesterified cholesterol, phospholipids, apo-C...) which enter the HDL density range and by association with HDL3 or with HDL precursors secreted by the liver and/or by the intestine, form HDL2 particles. Turner et al.'s (1979) results provided some direct evidence that discoidal, presumably nascent HDL, are secreted by the splanchnic bed. This latter process is modulated by the enzyme LCAT and is activated by the apo A-1 (Dufaux et al., 1982; Gotto, 1983; Tall et al., 1978).

The HDL precursors referred to above originate as disc-shaped particles, consisting of phospholipids and free cholesterol micelles solubilized through the detergent action of the apoproteins (which are secreted from the liver). To these, as confirmed by Nestel et al. (1981), material from the CM and VLDL are added within the plasma, and within the interstitial space, cholesterol is extracted from the cellu-

lar membranes. Hopkins et al.'s (1984) data with the experimental drug Intralipid (phospholipid-TG emulsifier) in vitro, provided some evidence that the HDL are secreted into the plasma as nascent disk-like particles, composed mainly of proteins and phospholipids with virtually no cholesteryl esters. Patsch et al., (1978) were the first to propose from their in vitro experiment that the conversion of HDL3 (synthesized by the vascular endothelium at the cellular site of the LPL reaction) to HDL2 occurred through the assimilation of constituents freed from VLDL-TG, during a four-hour incubation with LPL. Schmitz et al., (1981) challenged the above results; their data suggested that the conversion from HDL3 to HDL2 was solely achieved by LCAT. After a 24 hour incubation with LCAT, a near complete interconversion of HDL3 to HDL2 occurred. The incubation resulted in increased levels of lipids and apoproteins (apo C, apo A-I and A-II) in HDL2, which was accompanied by a concomitant decrease of these components in the HDL3 subfraction. They concluded that HDL3 served as a primary substrate in the LCAT reaction and that the cholesteryl ester enrichment (from the previously free cholesterol) then entered the core of the HDL particle, resulting in the lower density particle with the ultimate conversion to HDL2. Glomset in 1968 was the first to hypothesize that LCAT may be the basis of HDL's assumed protective role. Through LCAT activity, the free cholesterol contained in HDL is transformed to cholesteryl ester which allows ad-

ditional free cholesterol to be taken up by HDL from specifically located cells as those of the arterial wall.

Taskinen and coworkers (1981) confirmed the relationship between the HDL subfractions and the LPL activity in the adipose tissue and skeletal muscle of 14 men and 8 women, providing direct evidence for a metabolic precursor-product relationship between the subfractions; the HDL2 particles are generated during the action of LPL on TG-rich lipoproteins, and the rate of this reaction is an important determinant of HLD2. The non-relationship between HDL3 and LPL demonstrates the presence of different metabolic pathways for the subfractions. Kinnunen (1979) supports the notion that a high LPL activity favors high HDL2 levels. In addition, the author suggests that the protective role of HDL2 could indeed reflect the antiatherogenic property of a high LPL activity. Koga et al. (1983) investigated changes in the HDL levels in patients with liver disease (hepatitis) and compared these to healthy individuals to elucidate the role of the liver in the metabolism of the subfractions. The above authors observed an inverse relationship between HDL-c and VLDL, suggesting that HDL is produced from precursor particles (TG-rich lipoproteins and nascent HDL). Furthermore, most changes arising in HDL-c occurred in HDL2, suggesting that HDL2 is produced during VLDL catabolism. The preferential decrease of HDL3 in patients with liver disease suggests a hepatic origin of this class of lipoproteins.

Furthermore, the HDL2 subfraction is larger and contains more cholesterol, carrying twice as many cholesterol molecules per unit apoprotein and being more efficient for the reverse cholesterol transport; HDL2 is viewed as the end product of the utilization of TG-rich lipoproteins (Patsch et al., 1984).

2.1.1 The role of LPL

LPL is the rate-limiting step in the hydrolysis process, and is activated by the apoprotein C-II, a surface component of VLDL and CM, the natural substrates of LPL; the apo-C is then transfered back to HDL3 to regenerate HDL2 particles (Kinnunen et al., 1983; Lakshmanan et al., 1983). Nikkila et al. (1978) measured the LPL activity in the adipose tissue and skeletal muscle of competitive runners and compared these results to those of less active subjects. The significantly greater LPLA (LPL activity) in the skeletal muscle and adipose tissue found in the long distance runners is aimed at increasing the capacity of the body to mobilize and utilize fat as a fuel. With prolonged acute exercise, the muscle derives its energy mainly from circulating free fatty acids (FFA) and from its own TG stores, whereas plasma triglyceride fatty acids contribute little. Therefore, the TG content of the muscle decreases and if an exercise session

is repeated at frequent intervals, as with endurance training, the muscle TG remain low. The exercise-induced catabolism of TG and the improved VLDL flux, associated with an increased exchange of surface lipids, may induce a compensatory elevated HDL pool in order to guarantee optimal muscle metabolism (Berg et al., 1983). The body must then restore the TG rapidly and does so by increasing its LPLA, enabling the tissue to take up circulating TG (from CM and VLDL) more efficiently than in the untrained state. The increased LPLA enhances the availability of fatty acids for the muscle (Lithell et al., 1979). Nikkila suggested that the different LPLA measured in the active and sedentary subjects may be a function of the training regime and/or the fiber composition. Most probably, as Nikkila's group suggests, the difference is accounted for by the training, because aerobic training does not in man, as it does in the rat, predominantly increase LPLA in the slow twich fibers. Lithell et al. (1981) also studied the possible relationship between fiber composition and the LPLA of the gastrocnemius in seventeen 48 year old males. Their results confirmed that the above relationship did not exist, but that LPLA depends on the number of capillaries in the muscle (enhanced with aerobic training) and not on fiber composition; these findings are not suprising, due to the fact that the enzyme LPL is located on the endothelial cells of the capillaries. Peltonen et al.'s (1981) longitudinal training study (15 weeks of

aerobic exercise in 29 males) demonstrated that the increased LPLA occurred within a short period of time; after one week, the mean recorded LPLA was 45% greater than its initial value. These authors concluded that the observed significant rise in HDL (1.20 mmo/l vs 1.28 mmol/l, $p<0.01$) was a function of the increased LPLA. Taskinen et al. (1980) measured the LPLA of the adipose tissue and of the skeletal muscle in 10 well trained men, before and after a 20 kilometre race. When comparing the two values, LPLA had increased more than two times in the skeletal muscle ($p<0.01$) and by 20% in the adipose tissue ($p<0.05$). They concluded that the skeletal muscle reacts this way to place itself in a better position for the uptake of circulating TG, which are either used immediately or used to replenish the muscle lipid stores. Nakamura et al. (1983) confirmed that LPLA is greater in women's adipose tissue than in men's, so much so that inactive women have greater LPLA than male long distance runners. From their findings, Sady et al. (1984) substantiated that LPLA of the muscle and of the adipose tissue of endurance runners were greater than those of sedentary subjects.

It is not known how LPLA increases with training, but Nikkila et al. (1978) proposed that the two factors may be linked to the reduced insulin secretion brought about with training, or to the enhanced intake and turnover of calories, or to an endothelial proliferation, as stated above.

2.1.2 The HDL Subfractions: HDL2 and HDL3

Evidence that the HDL subfractions are heterogenous has repeatedly been confirmed (Barter et al., 1984b; Levy et al., 1980; Neel et al., 1984; Nestel et al., 1981; Nye et al., 1981). With the notion that the major proportion of esterified cholesterol in the human plasma is derived from an interaction between LCAT and HDL (Durstine et al., 1983), Barter et al. (1984b) proposed an experiment to determine if any difference existed between the subfractions two and three when these were incubated with LCAT. It followed that both subfractions actually competed for any interaction with LCAT, and given a quantity of LCAT, cholesterol esterification was greater in HDL3. Furthermore, at physiological concentrations of lipoproteins, HDL2 will function as a competitive inhibitor of the cholesterol esterification reaction by displacing the enzyme LCAT from a more effective substrate, HDL3, to a less effective one, HDL2. Hopkins et al. (1984) confirmed that HDL3 appears to be the preferred substrate for LCAT, in view of the decreased cholesteryl ester content of the lipoprotein within the HDL3 density range. Barter et al. (1984a) provided some evidence that LCAT exists and functions physiologically as a component of an enzyme-apoprotein complex, and may interact with different lipoproteins (i.e. LDL and HDL3 but not HDL2). Moreover, the contribution to the formation of esterified cholesterol is a

function of concentration. The major proportion of esterified cholesterol formed by LCAT in the human plasma is incorporated initially into HDL; a subsequent transfer of this esterified cholesterol out of HDL accounts for most of the esterified cholesterol found in VLDL and LDL (Barter et al., 1984a; Mahley et al., 1984). Furthermore, in vitro, Grow et al. (1978) observed an exchange of apolipoproteins between the subfractions. Schaefer et al.'s (1979) findings support Grow's results.

2.1.3 The Role of HL

Hepatic Lipase (HL) is known to play a role in the catabolism of HDL2; as its primary physiological function, HL could remove from HDL2 the post-prandially (7 hours after ingestion of a meal) derived phospholipids so that HDL2 can continue to serve as a phopspholipid acceptor during renewed CM catabolism (Patsch et al., 1984). Bamberger et al. (1983) examined the proposal that the delivery of HDL-c to the liver is mediated by HL using human HDL and rat cells (chosen due to their large capacity for storage of cholesterol as cholesteryl ester and having highly regulated free cholesterol pools). The incubation of HDL with HL indicated a greater uptake of HDL-c by the rat hepatic cells, demonstrated by the accumulation of free cholesterol (radiola-

beled) and the actual cellular cholesterol mass. Sutherland et al. (1984) measured HL activity and HDL-c concentrations in 12 men participating in an 18 week running program. It has often been postulated that an inverse relationship exists between HL and HDL-c. Their data suggested that this relationship existed for pretraining values ($r=0.733$, $p<0.05$, $n=8$); with training, HDL increased significantly by 27% ($p<0.05$) and HL by 29% ($p<0.05$) after 18 weeks. Sutherland et al.'s results are not in accord with the accepted inverse relationship between plasma HDL-c levels and HL activity. Therefore, they do not support the concept that reduced HL activity is mainly responsible for the elevated levels of HDL-c with training. Kuusi et al.'s (1980) results support the hypothesis that HL is involved in the metabolism of HDL2; the inactivation of HL by a specific rat antiserum is followed by an increase in HDL-c. A significant negative correlation between HDL-c, HDL2 and HL were found, while no such correlation existed with HDL3. HL, therefore, serves in the removal of HDL particles, transfering the cholesterol from the blood to the liver and thereafter to the bile.

Most studies therefore suggest that the liver is the major site for HDL removal, but evidence exists that HDL can be removed by a variety of cells. Cultured human fibroblasts take up and metabolize HDL by adsorptive endocytosis but binding to cells is not mediated through high affinity re-

ceptors as for LDL. HDL do bind to fibroblasts at sites which appear to be highly specific, and separate from LDL; furthermore, the binding of HDL need not be followed by internalization and degradation of the particle but may rather serve to facilitate the transport of cholesterol between cells and lipoproteins (Nestel et al., 1981).

2.1.4 Aerobic Training and HDL

The effects of an aerobic training on lipoprotein metabolism have been elucidated. More often, lower LDL-c concentrations and higher HDL-c concentrations have been reported as a function of a training program. Haskell (1984) summarized from previous findings the following theories. Lower LDL concentrations may be a function of: a) a decreased synthesis by the liver of VLDL, precursors of LDL, or a normal hepatic VLDL production but an impaired conversion of the VLDL remnants into LDL particles; or, b) an increased fractional uptake of LDL by peripheral cells especially if LPL receptor activity is enhanced; or, c) an increased hepatic removal of LDL could occur. Moreover, no direct evidence exists for any of the above theories. Patsch et al. (1984) provided a schematic model for the transport of lipids by the plasma lipoproteins, as shown in figure 1.

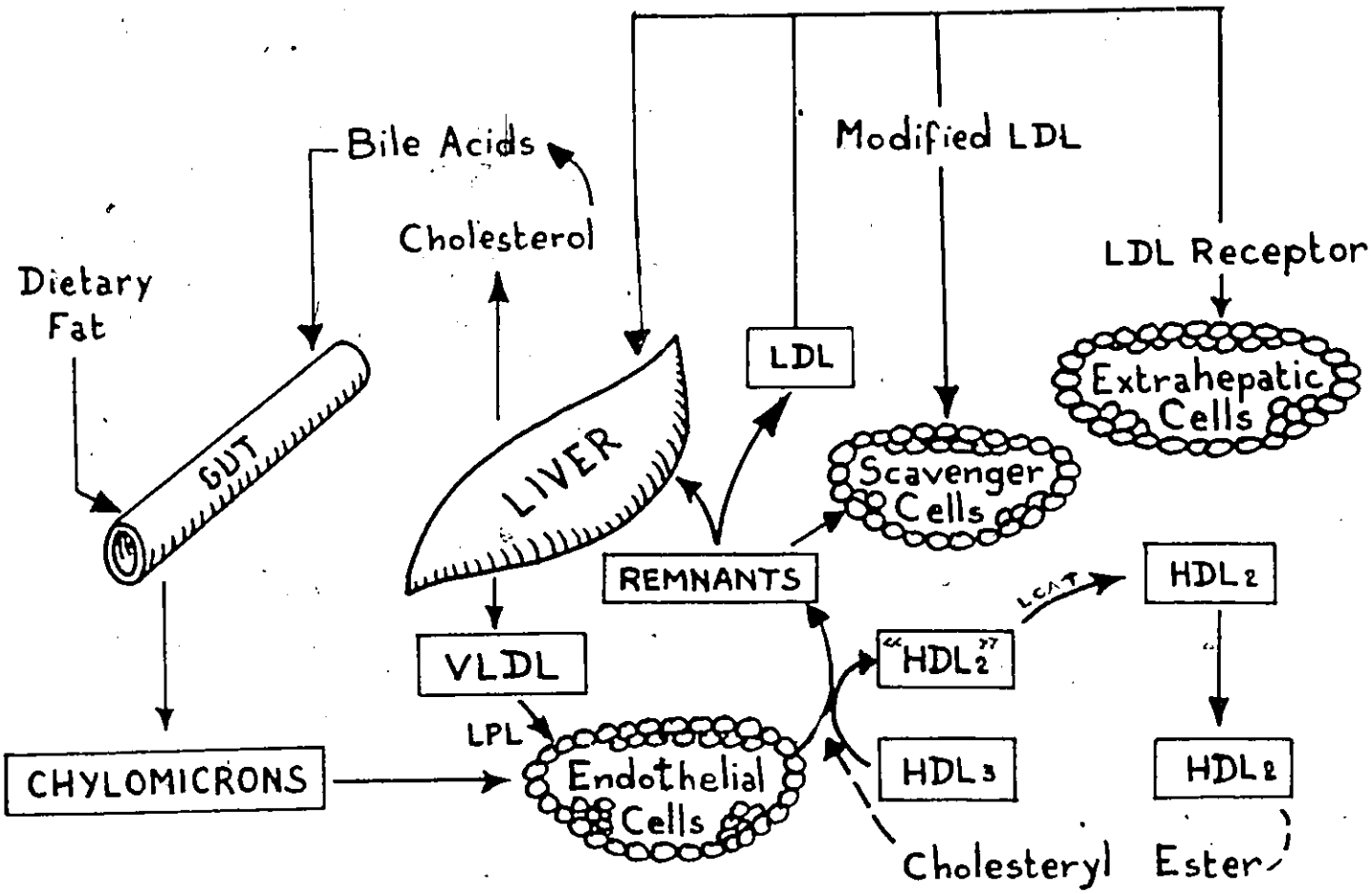

Figure 1: Model for Lipid Transport by Plasma Lipoproteins

The relatively higher HDL concentrations found in aerobically exercising subjects may be related to a greater LPLA which increases the catabolism of TG-rich lipoprotein, promoting the transfer of surface components and therefore, increasing the HDL mass. Also responsible for these changes are the lower HL activity and/or the greater LCAT activity that have been observed. No definite mechanism has been identified. Lowered TG and VLDL levels are repeatedly found in the active subject; TC concentrations produce conflicting results (Wood et al., 1979).

The following series of studies have measured lipoprotein levels, more specifically HDL-c, to substantiate the evidence that the antiatherogenic lipoprotein HDL-c (HDL2 in particular) may be altered favorably with aerobic training. Data from longitudinal training studies will be considered first, to be followed by the data derived from comparative training studies.

2.2 LONGITUDINAL AEROBIC TRAINING STUDIES

First choice resides with longitudinal training studies when the goal is to identify the effects of aerobic training on lipoprotein metabolism. The training studies reported here vary in length, ranging from four weeks to eight months. The training programs are diverse: cross-country

skiing, jogging/running, calisthenics... In most studies, little has been done to control weight aside from verbal indications that no drastic change in the diet should be undertaken during the course of the study; dietary recalls were used periodically to assess if the subjects acted accordingly. When significant changes in body weight occurred (which may affect lipoprotein levels, as stated in the introduction), these were reported. Blood samples, unless otherwise stated, were taken after a 12 to 16 hour fast, ascertaining that the data reported were fasting values. The longitudinal studies have further been divided into short-term programs, consisting of training for less than 10 weeks, and into long-term programs, for those exceeding the 10 week mark.

2.2.1 Short-term Training Programs

Huguchi et al. (1984) trained five men (midly active) for four weeks aged 28 to 31 years, running on a treadmill for 50 minutes, five times a week, so that energy expenditure equaled nine kcal per kilogram (kg) of body weight per session. No significant changes occurred in body weight, percentage (%) of body fat and VO2 max as determined by a bicycle ergometer test (pre 48.1 ml/kg/min vs post 48.3 ml/kg/min), while HDL-c levels increased significantly by 22% after two weeks (54 milligrams per decilitres (mg/dl) vs

66 mg/dl, $p<0.01$) and by 35% (54 mg/dl vs 73 mg/dl, $p<0.05$) at four weeks. They concluded that increases in HDL-c can occur without weight changes or increases in VO2 max; this latter factor may be a function of the short training time.

Lopez et al. (1974) studied the effects of 30 minute sessions of aerobic exercise (jogging, bicycling and calisthenics) with an average energy expenditure of seven mets, four times a week for seven weeks, in 13 medical students. An increase in total HDL was observed from 286 mg/100ml to 332 mg/100ml, as a function of the training regime.

Frey et al. (1983) exercised 12 young sedentary men on a bicycle ergometer for 30 minute bouts at 70% of max heart rate (HR) reserve, three times a week, for ten weeks. A significant improvement in VO2 max and a decline in TC levels (171.5 vmg/dl vs 168 mg/dl, $p<0.02$) were noted. An initial fall in HDL-c was measured at week five; overall, no significant change occurred in HDL-c. Schriewer et al. (1983) also trained nine young men (of which 2 dropped out) for ten weeks. The training consisted of a 30 minute run (5 to 7 kilometres (km)), three times a week, with heart rates ranging between 120 and 160 beats per minute (bpm). Blood samples were taken weekly following a 12-hour fast. Overall, TC did not change, whereas HDL-c levels increased by 0.08 millimoles per litre (mmol/l) to 0.31 mmol/l above initial values; no change in weight was observed.

Short-term training programs have produced conflicting results. Huguchi et al. (1984) suggested that changes in HDL-c occurred as early as the second week of training (with no change in VO2 max), while Frey et al. (1983) failed to show any change in HDL-c after ten weeks of training despite an increase in VO2 max. The differences observed in the various studies may well be a function of initial levels, intensity and frequency of training, etc..., as summarized in Table 4.

TABLE 4

LONGITUDINAL TRAINING STUDIES (SHORT-TERM)

AUTHORS	DETAILS ON STUDY	EXERCISE PROTOCOL	KEY RESULTS
Frey et al. (1983)	n=12 young sedentary men	-bicycle ergometer, 70% max HR reserve, 3X/wk for 10 wks	-TC decr. sign.(S) -HDL-c decr. at 5 wks; overall, no diff.
Huguchi et al. (1984)	n=5 men age:28-31 yrs	-running on tread. 50 min., 5X/wk, 4 wks, 9 kcal/kg/ session	-HDL-c inc. by 35%
Lopez et al. (1974)	n=13 medical students	-30 min. aerobic exercise, 4X/wk, 7 wks, 7 Mets/sess.	-HDL inc. S.
Schriewer et al. (1983)	n=9 men 2 dropped out	-30 min. run (5-7km) 3X/wk, HR=120-160 bpm	-HDL-c inc.

2.2.2 Long-term Training Studies

Huttunen et al.'s (1979) findings in a hundred sedentary men aged 40 to 45 years, equally divided into a control and exercising groups, training three to four times a week for

four months, demonstrated a significant ($p<0.01$) rise in HDL-c concentrations (1.27 mmol/l to 1.41 mmol/l (n=44)). Peltonen et al. (1981) had 29 sedentary men, aged 31 to 49, participating in various aerobic activities for 15 weeks, while nine men of the same age range acted as the control group. A 14% increase ($p<0.001$) in physical performance was observed, with no change in body weight. TC levels decreased slightly while HDL-c showed a significant ($p<0.01$) rise (1.20 mmol/l vs 1.28 mmol/l), occurring primarily in the first three weeks after which the concentrations reached a plateau throughout the remaining period. Postheparin plasma and adipose tissue LPLA were higher in the trained, the former, as early as the first week. Also, a trend towards lowered HL activities in the exercise group were observed after three weeks, but only significant ($p<0.05$) after ten weeks.

Twelve men with an average age of 27 years, participated in a 18 week self-regulated marathon running training program; blood samples were taken at week one, six and 18. HDL-c levels as reported by Sutherland et al. (1984), were not significantly different from baseline values at week six, but at week 18, HDL-c concentrations were 27% above initial levels. Kiens et al.'s (1984) objective was to verify if further increases in HDL-c could be observed in already trained men, after an enhanced training period. Eight men, aged 30 to 40 years, with VO2 max values ranging from 47 ml/kg/min to 67 ml/kg/min, followed a running program and fur-

ther increased the frequency and distance of their training sessions. VO2 max increased by 11% ($p<0.01$) after 12 weeks with no further change thereafter; at the 6 week mark, HDL-c levels were lower (1.57 mmol/l) but not significantly different than initial levels (1.66 mmol/l); at week 24 (n=6), a significant rise to a mean value of 1.92 mmol/l ($p<0.05$) was observed. TC demonstrated a similar decline at 6 weeks (5.22 mmol/l vs 5.33 mmol/l), with a final mean concentration of 5.10 mmol/l at 24 weeks. The conclusion drawn from the study was that regardless of the initial HDL-c concentration, HDL-c levels can be influenced with further training even though VO2 max levels off, and presumably no training effect per se is taking place. Myhre et al. (1981) worked with cross-country skiers over an eight month period during which the intensity and duration of training varied according to the seasonal training schedule. Six male skiers with a mean VO2 max of 75.2 ml/kg/min were compared to five control subjects, with a mean VO2 max of 39.6 ml/kg/min. Their results indicated that increases in HDL-c (non-fasting samples) were a function of physical endurance training of a lower intensity (70-80% of VO2 max vs 80-95% of VO2 max) and of longer duration (60 hours/week vs 35 hours/week). With fewer hours of training but of greater intensity, HDL-c levels were lower (1.7 mmol/l vs 2.1 mmol/l) but still significantly higher than those of the sedentary individuals (1.5 mmol/l).

From the literature, it is therefore suggested that training programs lasting more than ten weeks can favorably increase the HDL-c concentrations and may even decrease TC levels (Huttunen et al., 1979). Table 5 provides a summary of the long-term training studies.

TABLE 5

LONGITUDINAL TRAINING STUDIES (LONG-TERM)

AUTHORS	DETAILS ON STUDY	EXERCISE PROTOCOL	KEY RESULTS
Huttunen et al. (1979)	n=100 men (sedentary) age:40-45 yrs	-aerobic program 3-4X/wk, 4 months	-HDL-c inc. S.
Kiens et al. (1984)	n=8 men age:30-40 yrs	-enhanced running (frequency & int.) program for 24 wks	-HDL-c decr. at 6 wks (NS); -HDL-c inc. S. 24 wks; -TC decr. 6 wks; -VO2 max inc.
Myhre et al. (1981)	n=6 male X-country skiers n=5 control	-X-country skiing program, 8 months	-HDL-c inc. a function of low int. & long duration
Peltonen et al. (1981)	n=29 sed. men n=9 control men age:31-49 yrs	-aerobic activities for 15 weeks	-TC decr. -HDL-c S. inc. -LPLA S. inc.
Sutherland et al. (1984)	n=12 men age:27 yrs	-18 wk self-regulated marathon running program	-HDL-c inc. by 27% at 18 wks

2.2.3 Training Studies Involving Women

Wynne et al. (1980) and Frey et al. (1982) studied two groups of women to determine if a rigorous bicycle ergometer exercise program would elicit changes in HDL-c concentrations. The ten-week program consisted of three weekly sessions of 30 minutes each, inducing 70% of the max HR reserve. In both studies, training consisted of five minute work bouts interrupted by two minute rest periods; in Frey's study, an additional six women participated in a continuous 30 minute work session. Wynne's group consisted of 19 women (of which 13 were training), aged 19 to 30 years; all of the subjects were on a specific oral contraceptive (OC) (50 ug mestranol, 1 mg norethisterone). Over the ten week period, VO2 max increased from 29.21 ml/kg/min to 33.9 ml/kg/min ($p<0,001$), with a concomitant decline in the percentage of body fat (28.3% vs 26.3%, $p<0.01$) in the exercising group; no changes were observed in HDL-c (56 mg/dl (pre) vs 57 mg/dl (post)), TC and LDL-c levels while TG concentrations decreased by 15%.

In Frey's study, 12 young women (no OC) participated in the interval training, while six participated in the continuous training described above. Blood samples were taken at week zero, two, five and ten. Overall, VO2 max increased (28.5 ml/kg/min vs 33 ml/kg/min, $p<0.0001$), percentage of

body fat declined (28.5% vs 26%, $p<0.0018$), while total body weight remained unchanged. TC increased (166.9 mg/dl vs 179.1 mg/dl, $p<0.05$), while no change was observed in HDL-c when comparing week zero's mean value (61.6 mg/dl) to week ten's (mean of 62 mg/dl); an initial decline occurred at week two (56.3 mg/dl), followed by a rise at week 5 (58.1 mg/dl). Further, the above authors have proposed that blood samples be taken at the same time within the menstrual cycle to avoid possible cyclic changes in certain lipids. Also, the suggestion was made that the training period may not have been long enough to elicit lipoprotein changes in view of the high initial HDL values.

Farrell et al. (1980) determined the sequential pattern of changes in HDL-c and TC in seven sedentary men and nine sedentary women. The training constituted of running at 70% of VO2 max for 30 minutes, three to four times a week for eight weeks. The group improved its fitness level as indicated by the results of the VO2 max test (9.9% above initial values, $p<0.05$); improvements were noted as early as the fourth week of training. Overall, TC increased (162.8 mg/dl vs 174.5 mg/dl) with a high at two weeks (184 mg/dl); HDL-c concentrations were significantly higher at eight weeks versus the two week value only (57.4 mg/dl vs 51.1 mg/dl ; 53.8 mg/dl at week zero). No explanation was offered for the decline observed at week two. They concluded that alterations in lipoproteins suggest a lag time behind im-

provements in cardiovascular fitness, whereas the initial four weeks may act as a stabilization period.

Twenty-two women (mean age of 28.4 years), running five to six days a week and averaging 44.9 miles a week, participated in a four to seven month study. Significant changes occurred in total body weight (decrease), percentage of body fat (decrease), lean body weight (increase) and HDL-c concentrations (increase by 5 mg/dl, $p<0.01$); no significant change in the TC to HDL-c ratio was observed (Rotkis et al., 1981).

Lipson et al. (1979) trained five men and six women aged 19 to 22 years for six weeks by having them jog on a treadmill at 50% to 75% of their VO2 max, for 30 minutes a day; diet was adjusted so that the body weight was kept constant. The authors failed to report the number of days per week for which the subjects were training. A training effect was documented by a rise in VO2 max values (n=8, pre 44 ml/kg/min to post 49 ml/kg/min, $p<0.001$). TC concentrations decreased from 156 mg/dl to 137 mg/dl ($p<0.05$) and so did HDL-c concentrations (47 mg/dl to 42 mg/dl); when the six women only were considered, the decrease (50 mg/dl to 41 mg/dl) was significant. Further, HDL2, HDL3, LDL, VLDL and TG showed no significant change. The authors concluded that when diet and weight are controlled, no increase is seen in HDL-c levels, which may even decrease with a concomitant de-

crease in TC concentrations. Lipson's group is one of a few that have quantified HDL subfractions in women.

Training studies involving women have produced a great number of discrepancies with respect to HDL and TC values (Table 6). Wynne et al. (1980) and Frey et al. (1982) have found no change in HDL-c, while Farrell et al. (1980) and Rotkis et al. (1981) reported increases in HDL-c; Lipson et al. (1979) saw a decline in HDL-c levels with training. TC increased in some studies (Frey's and Farrell's), did not change in others (Wynne's and Rotkis') and declined in Lipson's study.

TABLE 6

TRAINING STUDIES INVOLVING WOMEN

AUTHORS	DETAILS ON STUDY	EXERCISE PROTOCOL	KEY RESULTS
Farrell et al. (1980)	n=7 sed. men n=9 sed. women	-running at 70% VO2 max, 30 min., 3-4X/ wk, 8 wks	-TC inc. S. -HDL-c decr. at 2 wks; overall, inc. S.
Frey et al. (1982)	n=12 women interval T. n=6 women cont. T. no OC	-10 wk bicycle erg., 3X/wk, 30 min./sess. 70% max HR reserve (cont.) -interval:work 5 min. rest 2 min.	-VO2 max inc. S. -no diff. in HDL-c -TC inc S.
Lipson et al. (1979)	n=5 men, 6 women age:19-22 yrs	-jog on treadmill at 50-75% VO2 max, 30 min./day, 6 wks	-TC, HDL decr. S. -no diff. HDL2 & HDL3
Rotkis et al. (1981)	n=22 women age:28.4 yrs	-running 5-6X/wk, average 44.9 miles/ wk, 4-7 months	-TC unch. -HDL-c S. inc. by 5 mg/dl
Wynne et al. (1980)	n=13 active n=6 control All on specific OC age:19-30 yrs	-10 wks bicycle erg. 3X/wk, 30 min./sess. 70% max HR reserve	-VO2 max inc. -no diff. in TC & HDL-c

2.2.4 HDL Subfractions in Training Studies

The following researchers have gone beyond the analysis of HDL-c as a whole component. By analytical ultracentrifugation, HDL has been further divided into the HDL2 and HDL3 subfractions. The results become more suggestive if one accepts the premise that HDL2 is the protective "agent".

Nye et al. (1981) measured the cholesterol content in HDL, HDL2 and HDL3 at different intervals (pre, 2, 6 and 10 weeks) in subjects participating in a calisthenics program. The 17 men, aged 30 to 45 years were asked to participate in two weekly sessions of 30 to 45 minutes each, plus any other activity they wished to take part in; only seven acted accordingly, while the ten other exercised less than twice weekly. Overall, TC levels decreased from 6.38 mmol/l to 6.08 mmo/l; LDL concentrations were lower at two weeks (versus pre, $p<0.001$) and remained below baseline values at ten weeks ($p<0.005$); VLDL did not change significantly. Total HDL-c concentrations had decreased significantly at two weeks (1.55 mmol/l vs 1.45 mmol/l, (approximations only), $p<0.005$), after which they increased slightly (non significantly (NS)). HDL2-c followed the same pattern as HDL-c; a decline at two weeks (0.5 mmol/l vs 0.35 mmol/l) was followed by a rise at six weeks (0.55 mmol/l vs 0.35 mmol/l, $p<0.025$). HDL3 demonstrated the exact opposite pattern; at

two weeks, a significant rise was recorded (1.05 mmol/l vs 1.1 mmol/l, $p<0.025$), followed by a decline between weeks two and six (1.1 mmo/l vs 0.95 mmol/l, $p<0.025$) and a further decline from week six to week ten (0.95 mmo/l vs 0.9 mmol/l, $p<0.01$). Overall, when computing pre and post values, HDL2 showed a rise of 0.19 mmol/l ($p<0.001$) while HDL3 fell significantly ($p<0.001$) by 0.20 mmol/l. These results provide further substantiation to the finding that changes in HDL subfractions may occur even though no changes are observed in total HDL-c levels. The authors proposed that a selective redistribution of apo-C peptides occurred, favoring the formation of HDL2; as a consequence, a change in the HDL2/HDL3 ratio was initiated.

Cowan (1983) investigated lipoprotein changes in 40 men, aged 29 to 56 years, engaged in a cardiac rehabilitation program. The training consisted of three daily periods (20 to 40 minutes each) of jogging, calisthenics, swimming, cycling and brisk walks, five days a week for three weeks, with work intensities progressing from 70% (week 1) to 75% (week 2) to 80% (week 3) of the max HR. Fasting blood samples were taken on days two and seventeen. The results showed that HDL concentrations increased during this period by 0.87 mmol/l to 0.95 mmol/l and that HDL2 concentrations increased by 0.14 mmol/l to 0.19 mmol/l; no results were offered for HDL3. The level of probability was said to be highly significant but was not reported.

Since weight loss is often associated with endurance training, it became relevant for Williams et al. (1983) to investigate to what extent the higher HDL-c and HDL2 and lower HDL3 values consistently found in higher mileage runners were a function of their body composition. Eighty-one sedentary middle-aged men participated in the study; 48 of them were assigned to a jogging-running program, three to five days a week, with exercise heart rates ranging from 70% to 85% of maximal HR, five times a week for a one year period; 33 men acted as control subjects and led a sedentary lifestyle. Body density was evaluated by hydrostatic weighing (Siri's equation) and by the skinfold method. After the one year period, the mean body weight and percentage of body fat had decreased in the exercising group (n=36) (-1.4 kg and -1.3% respectively), while the same components had increased in the non-exercising group (n=28) (1.1 kg and 2.8%); the changes were significant for both groups ($p<0.001$). HDL-c concentrations did not increase significantly more in the runners versus the control subjects (1.3 mg/100ml vs 0.1 mg/100ml); percentage of body fat was found to have a significant negative correlation ($r=-0.44$, $p<0.01$) with HDL-c concentrations for the first six months, while the relationship was weaker ($r=0.21$) for the remaining period. HDL2 changes mirrored those of the HDL-c correlation ($r=0.45$, $p<0.01$) even though HDL2 constitutes only one third of the total HDL mass; HDL3 was shown to be independant of

body composition and negatively correlated ($r=-0.42$, $p<0.01$) to the miles run over the one year period. The above results suggested that a large proportion of the observed differences in HDL-c and HDL2 mass during the one year exercising period were directly related to concurrent weight changes. Further, evidence that LPLA was increased with fat loss and that LPLA was positively correlated with HDL-c and HDL2 substantiated the notion that weight loss associated with aerobic training favors increases in HDL-c and HDL2-c. On the other hand, controlled weight loss may not necessarily increase HDL-c concentrations. Therefore, the physiological processes accompanying weight loss through exercise versus dietary weight loss alone may be very different. No explanation was offered for the 17 subjects who did not complete the study.

The same group of workers directed by Wood et al. (1983), studied the lipoprotein profile of 81 men (48 exercising and 33 controls), aged 30 to 55 years, to assess if a dose-response existed with the distance run. Even though no statistical significance was found, lipoprotein profiles were more favorable for the active subjects (n=46); at the same time, the exercising group was deemed fitter and leaner. Those men (n=24) who ran in excess of eight miles per week for a period of one year demonstrated increases of 4.4 mg/dl ($p=0.045$) in their plasma HDL-c levels and of 33 mg/dl in their total HDL2 mass ($p=0.059$). From these findings,

an eight mile per week running program of one year appeared to be the threshold at which significant changes in HDL2 and HDL3 levels were observed.

Most researchers that have subfractionned HDL have found increased HDL2 values in active subjects, with a concomitant rise in HDL-c. Nye et al. (1981) observed no change in HDL-c and a decline in HDL3. Most increases in HDL-c occur primarily in HDL2. Furthermore, it has been suggested that HDL2 is a primary contributor of the observed inverse relationship between HDL and the prevalence of CHD (Anderson et al., 1978). Table 7 provides a summary of the above studies.

TABLE 7

TRAINING STUDIES AND HDL SUBFRACTIONS

AUTHORS	DETAILS ON STUDY	EXERCISE PROTOCOL	KEY RESULTS
Cowan et al. (1983)	n=40 men age:29-56 yrs	-cardiac rehab., 3X/ day, 20-40 min./ sess., 5X/wk, 3 wks	-HDL-c, HDL2 inc. S.
Nye et al. (1981)	n=17 men age:30-45 yrs	-aerobic program 30-45 min./sess., 2X/wk, 10 wks	-n=7; TC decr. NS -HDL-c S. decr. at 2 wks, & followed by inc.; -HDL2 same as HDL-c; -HDL3 opp. HDL2
Williams et al. (1983)	n=48 men exercise n=33 control middle-aged	-jog-run program 5X/wk, 70-85% max HR, 1 year	-n=36 ex. -n=28 c. -HDL-c inc. NS
Wood et al. (1983)	n=48 active n=33 control age:30-55 yrs	-one year running program	-thresh. 8 miles/ wk for S. inc. HDL-c, HDL2

2.3 COMPARATIVE TRAINING STUDIES (AEROBIC)

Comparative studies have often been used as an alternative for longitudinal studies. Even though they bring forward valuable information concerning the effects of training, they are nevertheless less conclusive than longitudinal findings; the latter permit the inference that training did or did not induce certain lipolytic changes. Comparative studies on the other hand, fail to ascertain to what extent constitutional characteristics (weight, genetics, diet...) may render someone suitable to one form of exercise or another, and hence potentialy influence the results. Nevertheless, comparative studies are used since they are less time consuming. The following is an array of such studies; some researchers have focused on HDL-c concentrations only, while others have quantified the subfractions in various populations, from electricians to runners.

In 1976, Wood and coworkers determined fasting lipoprotein levels in 41 men, running more than 15 miles a week; their results were compared to those of control subjects. Runners had significantly ($p<0.05$) lower TG (70 mg/dl vs 146 mg/dl), TC (200 mg/dl vs 210 mg/dl), LDL-c (125 mg/dl vs 139 mg/dl) and higher HDL-c (64 mg/dl vs 43 mg/dl) levels (control group: n=743 for TC and TG, n= 147 for HDL and LDL). The differences between the two groups were only partially accounted for by the degree of adiposity.

Enger et al. (1977) compared 220 male cross-country skiers (mean age of 41 years) to four control groups: group 1, included 269 men aged 40 to 59 years, of which 247 were CHD-free; group 2, (a subgroup of 1), included 67 men who were within 10% of their ideal body weight; the last two groups were blood donors (non-fasting blood samples): group 3 was composed of 87 men, aged 19 to 59 years, and group 4 included 24 women aged 19 to 62 years, not on any OC. The skiers (non-fasting) were found to have significantly ($p<0.001$) higher HDL-c values and HDL-c/TC ratios than all of the male control groups; these same active men had values equivalent to those of the female control group. In the trained men, 28% of the TC mass was found to be transported via HDL, 22% in the male control groups and 27% in the women control group.

Lehtonen et al. (1978) compared 12 lumberjacks (mean working time of 13.9 years) to 15 electricians who were not regularly participating in any physical activity. TC levels were highest in the lumberjacks (5.79 mmol/l vs 5.20 mmol/l, ns), and so were HDL-c concentrations (1.93 mmol/l vs 1.42 mmol/l, $p<0.001$). Those lumberjacks that were overweight ($n=3$) had the lowest HDL-c values.

Nikkila et al. (1978) compared eight male sprinters and 12 male and six female long distance runners to a control group of 16 women and ten men who were considerably less ac-

tive. The sprinters were averaging 20-35 km/week, the long distance runners 100-130 km/week, while the control group were not exceeding 15 km/week. Higher HDL-c levels were evident in the men (66 mg/dl) and women (74 mg/dl) long distance runners while the sprinters' HDL-c values (50 mg/dl) were no different than those of the control subjects (47 mg/dl for the men and 61 mg/dl for the women). LPLA in the adipose tissue of the long distance runners (gluteal region) was 2.7 times greater ($p<0.05$) than that of the controls, while LPLA of the skeletal muscle (vastus lateralis) was 1.7 times greater ($p<0.01$); sprinters' LPLA was no different than that of the controls.

Lehtonen et al. (1979) compared 23 active males (25 km/week) to 15 age-matched controls; they measured HDL-c, Apo A-I and A-II levels. HDL-c and Apo A-I were significantly higher in the active subjects (1.77 mmol/l vs 1.42 mmol/l, $p<0.01$; 2.16 g/l vs 1.65 g/l, $p<0.001$, respectively). Apo A-II was no different in the two groups. Further, the highest HDL-c levels were found in those subjects logging more than 70 km/week. Also, the higher Apo A-I concentration was said to reflect the LCAT mediated reaction favoring the formation of HDL-c (HDL2 more specifically).

Masarei et al. (1982) also found a significant ($p<0.01$) correlation between HDL-c and the results of the W170 endurance fitness test in 43 males, senior administrators of major companies.

Comparative studies exposed above (see Table 8) have all demonstrated higher HDL-c concentrations for the more active subjects. A few suggestions have been offered as possible contributors for these increased levels. Nikkila et al. (1978) suggested that the higher LPLA was responsible; Wood et al. (1976) believed that the degree of adiposity may have contributed to a certain extent; Lehtonen et al. (1979) believed that higher Apo A-I levels may have accentuated the LCAT reaction and favored HDL formation.

TABLE 8

COMPARATIVE STUDIES

AUTHORS	DETAILS ON STUDY	EXERCISE PROTOCOL	KEY RESULTS
Enger et al. (1977)	n=220 male skiers n=356 men, 24 women (no OC) control age:19-62 yrs	-X-country skiing	-skiers: S. high. HDL-c, HDL-c/ TC
Lehtonen et al. (1978)	n=12 lumber-jacks n=15 elect.		-lumber.: higher TC (NS), HDL-c S.
Lehtonen et al. (1979)	n=23 active men n=15 sed.men	-running 25 km/wk	-active: inc. Apo A-I, HDL
Masarei et al. (1982)	n=43 men	-W170 endurance fitness test	-HDL-c S. corr. to W170
Nikkila et al. (1978)	n=8 sprint., 12 l-d men 6 l-d women n=10 men, 16 women control	-sprinters: 20 km/wk -long-dist.:130 km/wk -control: <15 km/wk	-HDL-c & LPLA > in l-d; -sprint. same as control
Wood et al. (1976)	n=41 active n=147 (HDL, LDL); 743 (TC, TG) controls All men	-running >15 miles/wk	-runners: S. lower TC, TG, LDL; -HDL-c S. higher

2.3.1 Comparative Studies and HDL Subfractions

Miller et al. (1979) verified the relationship between HDL values and physical fitness in 11 young men participating in a spectrum of physical activity, with the most active ones being cross-country skiers. From the blood samples, the authors analyzed the HDL-c and the Apo A-I components, which they believed reflected the HDL2 and the HDL3 subfractions respectively. The results proved that a strong relationship existed between aerobic capacity (determined by a bicycle ergometer VO2 max test) and HDL-c levels ($r=0.81$, $p<0.01$), and a trend only with Apo A-1; the ratio HDL-c/Apo A-I produced the strongest correlation with VO2 max ($r=0.88$, $p<0.001$). The authors concluded that physical fitness itself raised HDL-c via the HDL2 subfraction and assumed when drawing these conclusions that HDL-c and Apo A-I were appropriate indicators of HDL2 and HDL3, respectively.

Kuusi et al.'s (1982) study examined the relationship between physical fitness (determined by a bicycle ergometer test) and HDL2, HDL3, HL and LPL in 27 young men enroled in the army, with similar dieting and activity patterns. HDL2-c was found to correlate significantly ($r=0.52$, $p<0.01$) to physical fitness; no correlation was found with HDL3-c, while HL was negatively correlated to fitness ($r=-0.57$, $p<0.01$). No relationship was found with TC, TG, VLDL-TG and

LDL-c concentrations. HDL-c and HDL2-c but ~~not~~ HDL3-c, were inversely related to HL activity. No relationship was found with LPLA. The authors suggested that the increased HDL2 observed with training may be a function of dual mechanisms: 1) an increased rate of formation during an accelerated hydrolysis of TG-rich lipoprotein by LPL, and 2) a diminished removal by the liver via HL activity.

Laporte et al. (1983) measured HDL-c levels in males aged 18 to 60 years across a spectrum of physical activity. The group of subjects included 56 spinal injuries, 11 disabled with chronic pain, 197 normal controls, 16 joggers (2-40 miles/week) and seven marathon runners (more than 80 miles per week). HDL-c values showed a gradient, relating to the level of physical activity, from a mean of 27 mg/dl for the spinal cord injured subjects, to a mean of 61 mg /dl for the marathon runners. In the disabled, the reduced level of activity was primarily associated with HDL3, while physical activity above sedentariness was associated with increases in both HDL2 and HDL3 levels.

As Table 9 indicates, comparative studies like longitudinal training studies have reported that HDL and HDL2 are related to the level of aerobic activity. Furthermore, HDL3 which showed no association with physical activity remained practically unchanged.

TABLE 9

COMPARATIVE STUDIES AND HDL SUBFRACTIONS

AUTHORS	DETAILS ON STUDY	EXERCISE PROTOCOL	KEY RESULTS
Kuusi et al. (1982)	n=27 young men		-HDL2 S. corr. w. physical fitness -HDL and HDL2 inv. corr. w. HL
Laporte et al. (1983)	n=287 men	-spectrum of physical activity (P.A.)	-HDL-c shows a gradient with P.A.
Miller et al. (1981)	n=11 young men	-spectrum of P.A.; most active: X-country skiers	-strong rel. between VO2 max & HDL-c

2.3.2 Comparative Studies Involving Women

Wood et al. (1977) studied lipid profiles in male and female long distance runners and compared this group to an age-matched control group. The runners were averaging a minimum of 15 miles per week for the past year and reported no

significant weight loss; 41 men (35-59 years, average of 37 miles/week) and 43 women (30-59 years, average of 31 miles/week) were included in the study group, while 145 men and 101 women acted as controls. When comparing the two groups, runners were found to be significantly ($p<0,05$) different than the control (n=743 males and 934 females for TG and TC). Table 10 summarizes the findings; all differences were significant at the 0.05 level.

TABLE 10

LIPID CONCENTRATIONS IN RUNNERS AND CONTROLS

	CONCENTRATIONS IN MG/DL			
	MEN		WOMEN	
Lipoproteins	Runners	Controls	Runners	Controls
TG	70	146	56	123
TC	200	212	193	209
LDL-c	125	139	113	124
HDL-c	64	43	75	56

These results indicate that runners carry more cholesterol in the HDL component and less in the LDL component. Also, the higher concentrations of cholesterol found in HDL for the runners did not necessarily indicate that higher plasma concentrations of the total HDL macromolecules were present, but was probably indicative. Furthermore, in a number of women and in a few male runners, HDL was the princi-

pal carrier of cholesterol, such that the ratio of HDL-c to LDL-c exceeded unity. This is rarely seen except in very young children and in populations where atherosclerosis is pratically non existent.

Hartung et al. (1984) computed HDL-c values in pre- (n=136) and post- (n=37) menopausal women, aged 24 to 58 years, not using any OC, with a subject population being comprised of long distance runners, joggers and inactive subjects. HDL-c was invariably higher in the exercising groups (independent of the menopausal status), with the highest HDL-c values in the more active subjects ($p<0.001$). The HDL-c/TC ratio yielded a significant difference ($p<0.001$) between the long distance runners and the inactive women. Body fat was different between the groups and was related to the physical activity level. Rainville et al. (1984) confirmed that premenopausal trained women (range: 25-75 miles/week) (n=10) had higher HDL-c (NS), HDL-c/LDL-c ratio ($p<0.05$) and HDL-c/TC ratio ($p<0.05$) than premenopausal untrained women (n=10). Associated with the above, Rainville and coworkers suggested that trained women are less prone to CHD than untrained women.

Diet is often said to influence HDL-c concentrations. A study undertaken by Moore et al. (1983) verified the effect of the exercise level and the diet on HDL-c in 49 long distance runners (mean of 41.8 km/week), 49 joggers (mean of

9.7 km/week) and 47 control subjects (less than 1.6 km/ week); none of these women were using oral contraceptives. The groups were different one from the other when weight ($p<0.02$), percentage body fat ($p<0.001$), TG concentrations ($p<0.02$), HDL-c concentrations ($p<0.01$) and the HDL-c/TC ratio ($p<0.001$) were compared. Also, HDL-c levels in women running more than 49.9 km/week were the highest (13% above those of the control group); no significant difference was found when comparing the diet composition of the groups, except that fiber consumption was greater in the more active groups ($p<0.01$). The authors suggested a threshold of 19.6 km/week before any substantial rise (i.e. 8 mg/dl) occurred in HDL-c levels. The mechanism responsible for this increase is still in question but may be due to a reduced synthesis or an enhanced catabolism of lipids or apolipoproteins, an increased uptake of VLDL via LPLA or greater LCAT activity and Apo A-I concentrations. Much remains to be confirmed.

Krauss et al.'s (1977b) findings are valuable for they are probably among the few HDL subfraction studies including women. Six women runners, aged 34 to 46 years, not taking any hormones, and seven male runners aged 42 to 58 years were compared to control subjects. The results from the blood samples demonstrated that the runners, men and women, had higher HDL2 values (119 mg/dl, $p<0.005$ and 218 mg/dl, $p<0.05$, respectively) and HDL levels than their control counterparts for HDL2 (53 mg/dl and 122 mg/ dl); HDL-c val-

ues were not reported. All the results were significant at the 0.05 level. (Note that HDL values reported here were for the total HDL subfractions and not solely for the cholesterol content, as most of the above studies). HDL3 was different only in the males: the runners had the highest concentrations (259 mg/dl vs 227 mg/dl, $p<0.05$). Apos A-I and A-II were also measured; Apo A-I levels were higher in the men (163 mg/dl vs 120 mg/dl, $p<0.001$) and in the women (176 mg/dl vs 130 mg/dl, $p<0.001$) runners; Apo A-II levels were almost identical in both groups. The conclusion drawn from these findings was that the higher HDL values found in the runners were almost all totally reflected in the HDL2 subfraction and associated with a rise in Apo A-I but not Apo A-II.

The literature presented above demonstrated that highly active women (i.e. long distance runners) have higher HDL-c levels (and HDL2) than their sedentary counterparts. Moreover, a threshold of 19.6 km/week has been suggested by Moore et al. (1983) to produce substantial changes in HDL. It is considerably higher than the proposed men's threshold of 8 miles per week (Wood et al., 1983). Table 11 reviews the different studies.

TABLE 11

COMPARATIVE STUDIES INVOLVING WOMEN

AUTHORS	DETAILS ON STUDY	EXERCISE PROTOCOL	KEY RESULTS
Hartung et al. (1984)	n=136 premeno. n=37 post. (active)	-long dist. runners ,joggers, inactive	-HDL-c > in most active
Krauss et al. (1977b)	n=7 runners men; 6 women runners (no OC)		-runners: S. > HDL2
Moore et al. (1983)	n=49 l-d n=49 joggers n=47 controls	-l-d: 41.8 km/wk -joggers: 9.7 km/wk -controls:<1.6 km/wk	-HDL-c 16 mg/dl > in l-d vs controls
Rainville et al. (1984)	n=18 trained n=10 untrained premenopausal	-25 miles/wk	-S. > HDL-c, HDL-c/ TC in T.
Wood et al. (1977)	n=41 men, 43 women:active n=145 men, 101 women:sed. age:30-59 yrs	-runners: minimum 15 miles/wk	-runners: < TG, TC & LDL-c; > HDL-c; all S.

2.4 THE EFFECT OF OESTROGEN ON HDL REGULATION

The cholesterol content in HDL and HDL2 is greater in women than in men (HDL-c: 1.32 mmol/l vs 1.14 mmol/l, $p<0.01$) (Shepherd et al., 1980). The following groups of researchers have built upon this observation to elucidate the link between oestrogen levels and HDL-c concentrations.

Two groups of researchers studied women to determine if lipoprotein concentration changes occurred in the course of the menstrual cycle. Mendoza et al. (1979) measured LPLA and lipoproteins in 24 women, aged 20 to 35 years, with menstrual cycles ranging from 28 to 30 days and not taking any oral contraceptives; blood was taken after a 12 to 14 hour fast on days seven and 21 of the cycle, to represent the follicular and luteal phases , respectively. LPLA was significantly ($p<0.001$) higher (78 U/ml vs 50.9 U/ml) in the luteal phase; this activity was significantly associated with lower TG concentrations (96.7 mg/dl vs 86.7 mg/dl, $p<0.05$). Furthermore, TC, LDL and HDL (45.6 mg/dl vs 42.8 mg/dl) levels did not differ significantly from one phase to the other. Shepherd et al. (1980) confirmed from their findings in 12 women (mean age of 30 years) that hormonal changes during the menstrual cycle appeared not to affect HDL-c and apo A-I levels. Moreover, no cyclic differences were observed in the HDL subfractions. Therefore, the researchers concluded that no special care was necessary for blood sampling.

Similarly, Kim et al. (1979) studied 14 women with regular menstrual cycles of various lengths (21 to 37 days), having not received any medication for three months prior to the beginning of the study. Estimations were made for the menstrual, follicular, ovulatory and luteal phases for each of these women, who were then followed for three consecutive cycles; blood samples were taken every three to five days and the results were assigned to one of the above phases after which means were compiled. The women's results were then compared to ten age-matched men who were arbitrarily assigned to a 28 day cycle and from whom blood was also taken every three to five days. The results confirmed that women have significantly ($p<0.01$) lower TC concentrations in the late luteal phase (188 mg/dl) vs the menstrual and early follicular phases (208 mg/dl); furthermore, the women's lowest luteal values were significantly ($p<0.05$) lower than those of the men during the whole cycle (210 mg/dl); LDL-c followed the same pattern. Overall, HDL-c concentrations were higher ($p<0.05$-0.01) in the women than in the men (65 mg/dl vs 57 mg/dl); also, there was an observed trend in the women for higher (but ns) HDL-c concentrations in the luteal phase versus the follicular phase (66 mg/dl vs 64 mg/dl). From these results, the authors deduced that oestrogen may influence the cholesterol removal process by increasing the HDL mass.

Krauss et al. (1979) studied the fluctuations in the HDL subfractions (HDL3, HDL2a and HDL2b), as influenced by endogenous hormones in the normal menstrual cycle, in four subjects. HDL3 and HDL2a levels remained the same throughout the cycle, whereas HDL2b increased at or just after ovulation after which it declined rapidly, suggesting that factors other than oestrogen, such as the luteinizing hormone, are likely to be involved; blood samples were taken within eight hours of a light fat-free breakfast and/or lunch. Nakamura et al. (1983) supported that HDL2 increased around ovulation time in healthy premenopausal women; it is believed that oestrogen suppressed hepatic TG lipase and favored higher HDL2-c levels, while androgenic steroids had the opposite effect.

Demacher et al. (1982) observed that HDL-c values fell during the contraceptive cycle from 57 mg/100ml to 50 mg/100ml ($p<0.05$) (n=8) and rose again to starting values during the pill-free days; also, TC levels declined significantly during the cycle (maximimum of 0.40 mmol/l) as a result of the lower HDL-c. In non users (n=10), no significant changes were seen in TC, TG, LDL-c and HDL-c concentrations during one cycle.

Wallace et al. (1977) studied the TG and TC concentrations in 18,461 women, aged 15 to 74 years. Women younger than 49 years, and users of contraceptive pills, had

slightly greater TC levels (5%), and greater TG levels (48%); no control was performed to assess the type of pill used. Similarly, Wahl et al. (1983) described the effects of ten hormone preparations on lipoprotein concentrations; 375 women were using OC, 284 women were on oestrogen therapy, while 1,086 women acted as the control group. The highest TG levels were found in those women using OC with high oestrogen dosage; HDL-c followed the same pattern (73 mg/dl vs 54 mg/dl for the non users, $p<0.05$). Oestrogen therapy in 58 postmenopausal women invariably increased (significantly $p<0.001$) HDL (1.20 mmol/l to 1.31 mmol/l) and HDL2 (0.63 mmol/l to 0.73 mmol/l), and decreased TC (6.67 mmol/l to 6.30 mmol/l); HDL3 remained unchanged (0.56 mmol/l to 0.58 mmol/l) (Ottosson et al., 1985).

Bradley et al. (1978) concluded from their findings in 4,978 women aged 21 to 62 years (592 OC users, 964 on hormone therapy), that HDL was positively associated with oestrogen dosage, while progesterone dosage was negatively associated. Albers et al. (1976) demonstrated that women on combined OC (n=80) had HDL values somewhat similar to those on no medication (n=99), with a mean value of 56 mg/dl. Those women (n=29) using an oestrogen pill had the highest HDL-c values (62 mg/dl). Albers et al. (1978) confirmed these findings.

Hennekens et al. (1979) examined the relationship between the use of OC in 190 women aged 21 to 39 years, and TG, TC and HDL-c concentrations. In users, TG levels were higher (95 mg/100ml vs 73 mg/100ml, p=0.002), and so were TC levels (198 mg/100ml vs 189 mg/100ml); HDL-c levels were slightly lower (47 mg/100ml vs 50 mg/100ml). Wallace et al. (1979) studied lipoprotein concentrations in 424 OC users, 162 oestrogen users and 2,020 non users of hormones, aged 15 to 74 years; most OC users (41.9%) were between 20 and 24 years. OC users had significantly greater TC (by 2-16 mg/dl), TG (by 30-60 mg/dl), LDL-c and VLDL-c (by 2-8 mg/dl) values; HDL-c levels were not significantly different in the groups, whereas oestrogen use in the older women (55-59 years) increased HDL-c by 15 to 20% ($p<0.0002$). Also, those women taking OC with an oestrogen concentration greater than 50 ug had significantly ($p<0.01$) higher HDL-c, TC, TG and VLDL-c levels than other OC users (n=129).

Other researchers have recognized the need of studying the effect of OC on the HDL subfractions. Krauss et al. (1977a) found non significant increases in TC and TG concentrations in 18 combined OC users (20 to 39 years) compared to 19 age-matched controls, while HDL-c levels were significantly greater (386.4 mg/dl vs 328.3 mg/dl, $p<0.05$), and almost totally accounted for by HDL3 (275 mg/100ml vs 223 mg/100ml, $p<0.005$), while HDL2 remained similar in both groups (111.2 mg/dl vs 105.0 mg/dl). Oestrogen use in women

(aged 44 to 66 years) appeared to be associated with higher HDL2 (a,b) levels, but not HDL3 (Krauss et al., 1979). Cauley et al. (1982) studied the effects of exogenous oestrogen intake in postmenopausal women. HDL-c levels were higher (76.3 mg/dl vs 65.1 mg/dl) and so were HDL2-c levels (39.1 mg/dl vs 24.2 mg/dl), while HDL3-c levels were lower (37.4 mg/dl vs 40.8 mg/dl) in the users (n=37), versus the non users (n=40) ($p<0.05$ for all differences).

Schaefer et al. (1983) administered synthetic oestrogen (0.1 mg ethinyl estradiol) to five young women for 28 days. HDL-c concentration increased significantly by 43% ($p<0.05$) from its baseline value of 47 mg/dl; HDL2a, HDL2b and HDL3 also increased significantly ($p<0,01$) by 150%, 27% and 26.9% , respectively. Also, TC, TG and VLDL-c levels were significantly higher (19.5%, 87% and 123.1%, $p<0,05$), while LDL-c remained unchanged. Furthermore, HL activity decreased by 43.8% while no significant change was observed in LPLA.

From the above studies it can be observed that oestrogen use has been associated with higher HDL-c values (dose-related) than those of non users as shown in Table 12. It would then seem that OC use would provide some benefit. However, concomitant changes have also occurred in the other lipids and lipoproteins (TC, TG and LDL), promoting unfavorable profiles and therefore increasing the risk of CHD. A limited number of studies have reported the potency of the

OC used by the subjects which renders the comparison of different studies difficult if not impossible due to the wide range of OC on the market.

The present chapter has enumerated the discrepancies existing in the literature. The majority of longitudinal and comparative have shown that HDL correlated highly with the level of physical activity, and more importantly, that HDL2 reflected HDL-c levels; HDL3 levels did not change with aerobic activity and may have decreased slightly. Oestrogen use favored higher HDL-c levels, this being associated with modifications in various lipoproteins and lipids.

It is difficult to provide a cause and effect relationship between HDL and aerobic activity and HDL and oestrogen levels. The variations that arised in the results were a function of numerous factors which have been enumerated earlier. More rigorous control of such variables must therefore be exerted before any conclusive results can be reported.

TABLE 12

THE EFFECT OF OESTROGEN ON HDL REGULATION

AUTHORS	DETAILS ON STUDY	KEY RESULTS
Albers et al. (1976)	n=80 women on combined OC n=99 women on no OC n=29 women on oestrogen OC	-Combined OC users have HDL similar to no OC; Oestrogen OC users: highest HDL-c
Bradley et al. (1978)	n=592 OC users n=964 on hormone therapy n=3,422 controls age:21-64 yrs	-HDL-c ass. pos. with oestrogen dosage; HDL-c - ass. with prog. dosage.
Cauley et al. (1982)	n=37 oestrogen users n=40 non users postmenopausal	-HDL-c, HDL2 S. higher in users
Demacher et al. (1982)	n=8 OC users n=10 non users	-users:HDL-c, TC decr. S. during contra. cycle; -non users: no diff. in HDL-c & TC in MC
Hennekens et al. (1979)	n=190 users age:21-39 yrs no report on OC dosage.	-users: > TG, TC & < HDL-c
Kim et al. (1979)	n=14 women, no OC n=10 age-matched men MC:21-37 days Blood samples:every 3-5 dys	-TC decr. S. in luteal phase -HDL-c inc. NS in luteal phase
Krauss et al. (1977a)	n=18 OC users n=19 controls age:20-39 yrs	-HDL-c > in users (mostly HDL3) -HDL2 similar in both groups

Krauss et al. (1979)	n=4 women	-HDL2, HDL3: no diff. throughout MC; HDL2b inc. at ovulation
Mendoza et al. (1979)	n=24 women age:20-35 yrs MC:28-30 days Blood sample on days 7 and 21 of MC	-LPLA inc. in luteal phase -no diff. betw. phases re TC, LDL-c and HDL-c
Nakamura et al. (1983)		-HDL2 inc. at ovulation time
Ottosson et al. (1985)	n=58 postmenopausal women with oestrogen therapy	-HDL, HDL2 inc. S; HDL3 unch.; -TC decr.
Schaefer et al. (1982)	n=5 young women Administration of synthetic oestr. for 28 days	-HDL-c inc. 43% -HDL2, HDL3 inc. -TC, TG inc.
Shepherd et al. (1980)	n=12 women age:30 yrs	-no change: HDL-c HDL2, HDL3, Apo A-I within MC
Wahl et al. (1983)	n=658 OC users n=1086 controls	-HDL-c in OC users with high oestrogen dosage
Wallace et al. (1977)	n=18,461 women	-OC users: inc. TC, TG
Wallace et al. (1979)	n=2,606 OC users and oest. users and non users age:15-74 yrs	-HDL-c NS diff. between groups

III

METHODOLOGY

3.1 INTRODUCTION

The purpose of this study was twofold. The initial objective was to determine if a bicycle ergometer training program with three weekly sessions of 35 minutes each, would favorably decrease TC concentrations and increase HDL-c concentrations in the exercising group. Furthermore, and more importantly, the HDL subfractions were to be analyzed to verify if the ratio between HDL2 and HDL3 had been altered in favor of greater HDL2 production, and therefore reducing the concentration of HDL3-c. A control group was used to establish whether the changes observed in the lipid concentrations were a consequence of the training program.

3.2 SUBJECTS

All subjects were untrained women (primarily students), aged 18 to 30 years and not taking oral contraceptives; the term untrained in this context refers to the abstinence of participation in any regular training program. Sixteen women were assigned to the training group while nine women remained sedentary, acting as control subjects (i.e. not taking part in any regular physical activity). Most subjects had less than 35% of body fat as determined by four skinfold measurements, and a BMI index of less than 25. None of the subjects had a resting blood pressure exceeding 95 mmHg for the diastolic and 140 mmHg for the systolic.

The subjects were informed of the objectives of the study and fully understood the implications involved before agreeing to participate. A questionnaire (#1, Appendix E) was administered to all interested subjects and utilized as a screening tool. The questionnaire pertained mostly to personal lifestyle habits with items on general health, diet, smoking, alcohol consumption, physical activity pattern, hormonal intake... Subjects were asked not to modify their food and alcohol consumptions nor their exercise pattern over the course of the study (with the exception of the experimental protocol). Drastic changes which may have occurred and possibly have affected the results were evaluated

by a second questionnaire (Appendix E), completed at the end of the study. Once the questionnaire had been completed, each subject's personal information was assessed to warrant accessibility to the study. The subjects had to meet the following selection criteria to be included:

1) a woman, aged between 18 and 30 years;
2) not using any oral contraceptive or any medication which may alter blood lipids;
3) menstruating regularly;
4) have a "normal" blood pressure (as stated above);
5) not participating in a regular training program (i.e. 3-4 times/week);
6) non-smoker;
7) not practising any extreme lifestyle habits (i.e. diet, alcohol...);
8) agree to participate in the assigned control or exercising group and follow the testing and experimental protocols.

Admissible subjects were then assigned to the control or exercising groups. Furthermore, each woman's menstrual cycle (MC) was assessed (by the use of the questionnaire) to determine an approximation of the ovulation day, according to the average length of the MC (last 3 MC or more).

3.3 TESTING PROCEDURE

The study consisted of three phases: the preparation period (week 0 and preceeding weeks), the training program (weeks 1 to 8), and the post-training period (week 9).

Phase 1 (Pre 4 Weeks):;

One month prior to the beginning of the study, advertisements were posted on university grounds. Interested subjects were then asked to complete questionnaire 1 (Appendix E).

Pre 2 weeks: All screened out participants (i.e. those that did not meet the selection criteria described above) were notified. All screened in subjects were briefed individually. The information session consisted of exposing the subject to the purpose of the study and the commitments involved (training sessions, blood sampling...). If the subject agreed to participate, a consent form was signed (Appendix E) before any testing was undertaken. Furthermore, arrangements were made for a) a blood sampling day, b) a pre-testing session, and c) weekly training times for the subjects assigned to the exercising group.

Pre 1 week: A blood sample was taken (see blood sampling section for more details). The scheduled pre-testing session consisted of determining the following parameters for all subjects.

1) BMI: weight in kilograms, height in centimetres

2) 4 skinfold measurements (subscapular, supra-iliac, biceps and triceps) from which the body density was calculated as specified by Durnin and Womersley's (1974) linear regression equations. Furthermore, the density was used to calculate the percentage of body fat according to Siri's equation : 495 divided by density, minus 450 (Katch et al., 1983).
3) 4 circumference measurements (chest, waist, hip and gluteal).
4) resting blood pressure;
5) VO2 max on the bicycle ergometer (see VO2 max section).

Phase 2:

The training regime included three weekly sessions (Mondays, Wednesdays and Fridays), leaving Tuesdays and Thursdays available for make up sessions. The exercise sessions lasted 35 minutes each, consisting of having the subject pedalling at 50 revolutions per minute (rpm), following the rhythm of a metronome (set at 100 beats per minute). The training consisted of five minute work bouts separated by one minute rest periods, repeated for six sets. The intensity of work induced heart rates that corresponded to 60% to 70% of the maximal oxygen consumption as determined by the VO2 max test (pre); 60- 65% of VO2 max was chosen as the training intensity for week 1, while 70% of VO2 max was chosen for the following weeks of training. The training con-

sisted of a gradual increase in workload for the safety of the subject and to ascertain maximal participation on behalf of the subjects. A warm up period (achieved by pedalling with a low resistance) was allotted for a few minutes at the beginning of each session. The heart rates were monitored every five minutes to ascertain that the training intensity was maintained. Each training session was terminated by a progressive decrease in the workload.

Phase 3

On week 9, all subjects were reevaluated for the following:

1) blood sample;
2) body weight, skinfold and circumference measurements;
3) VO2 max;
4) questionnaire 2 (Appendix E).

Table 13 shows the flow chart of events with respect to the testing procedures.

TABLE 13

SEQUENCE OF EVENTS FOR TESTING

Prior to week 0:
Recruitment of subjects via questionnaire 1 (Appendix E).

Week 0:
Subjects assigned to exercising or control groups.
Blood sample taken (pre).
VO2 max test, height, weight, blood pressure, circumference and skinfold measurements.

Weeks 1 to 8:
Blood samples-(week 4, 5, 6, 7 and 8).
Training: 35 min./3 times a week/60-70% of VO2 max (exercising group only).

Week 9:
Questionnaire 2 (Appendix E).
VO2 max, weight, skinfold and circumference measurements
Blood sample (post).

3.4 DETERMINATION OF VO2 MAX

The following procedure was followed to obtain a maximal oxygen consumtpion value for all subjects (American College of Sports Medicine, 1983/84; Thoden et al ., 1982). All bicycles were calibrated before each test.

1. The metronome tempo was set at 100 single beats per minute so that the subject was pedalling at 50 revolutions per minute (rpm).

2. The height of the saddle was adjusted to suit the subject.
3. With the subject seated on the bicycle ergometer but without touching the pedals, the mark on the pendulum was set at "0" on the scale.
4. At the beginning of the test, the brake belt was slack; the desired workload was then set and timing started.
5. Two minute work stages were used, with an initial workload of 1.0 kilopond (kp); increments were of 0.5 kp per stage.
6. The HR was recorded every minute by auscultation.
7. Gas samples were collected throughout the entire test.
8. Attainment of VO2 max was assured when a plateau (as indicated by a change < 100 ml/min.) or a slight drop in oxygen consumption occurred as the workload was increased beyond the intensity that first resulted in a maximal value. If any symptoms appeared, as described by the American Heart Association (1975), the test was terminated at once.
9. Following a 15 minute rest, the subject completed a ride to exhaustion at the load equal to the last load completed in the progressive phase. The gas collection protocol remained the same.
10. VO2 max was reported in ml/min/kg as the highest value attained in either the progressive or exhaustive test.

3.5 BLOOD SAMPLING.

The blood samples were drawn from an antecubital vein into Vacutainer tubes containing 1.0-1.4 mg of dry EDTA per ml of blood, between 7:30 and 9:30 a.m., after a 12 to 16 hour fast. The subjects were asked if they had complied with the request; if not, an alternate day was scheduled. The plasma was separated from the whole blood after being centrifuged for ten minutes. Thereafter, the plasma was pipetted off and frozen in glass tubes until analysis. In all, seven blood samples were drawn from each subject at weekly intervals:

a) sample 1, consisting of the pre-training sample (week 0);
b) samples 2 to 6, consisting of the training or control samples (weeks 4, 5, 6, 7 and 8) taken in the morning, after a training day;
c) sample 7, consisting of the post sample (last training sample taken on Monday or Tuesday of week 9).

Krauss et al. (1979), Mendoza et al. (1979) and Shepherd et al. (1980) have determined that no significant difference exists between the follicular and luteal phases of the menstrual cycle with respect to HDL-c and TC concentrations. Kim et al's (1979) results suggested that TC levels were significantly lower in the luteal phase and that HDL-c levels were higher (but ns) in that same phase. Also, a signif-

icant rise observed on the day of ovulation (Krauss et al., 1979) warrants special care for blood sampling. Ovulation is said to occur 12 to 16 days before the onset of the next period (Hafez, 1980; Shangold, 1984). Therefore, to avoid misleading results, days 12 to 16 prior to the expected commencement of the menstrual flow (averaged from the 3 previous cycles) were avoided for blood withdrawal.

Each subject (control and exercising) was assigned a blood sampling day (Monday to Friday). Thereafter, that day was respected unless predicted calculations coincided with the designated "ovulation period" (days 12 to 16 prior to onset of menstrual flow). In any event, an alternate day was arranged to reflect as accurately as possible a week's interval between the preceeding and succeeding blood samples. Each subject was also asked to report the day of onset of the menstruation for each cycle to further control the possible effect of ovulation on HDL-c and TC concentrations. Sample 7 was taken on the Monday or Tuesday immediately following the last week of training (before the post-test) so that each subjects' results were comparable with respect to the delay after the last training session.

3.6 BLOOD ANALYSIS.

The blood sampling protocol was identical for the control and training subjects. Fasting plasma (12 to 16 hours) was used for all determinations of TC, HDL and HDL subfractions.

3.6.1 TC Analysis

The TC concentrations were analyzed enzymatically, using the Cholesterol C-system, CHOD-PAP method. The first step of the procedure consisted in quantitatively splitting all the cholesterol esters present in the plasma into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, free cholesterol was then oxidized by cholesterol oxidase to cholest-4-en-3-one. Following, the hydrogen peroxide reacted in the presence of peroxidase with phenol and 4-aminophenazone to form a p-quinone imine dye. The intensity of the colour formed was proportional to the cholesterol concentration and could be measured photometrically at 500 nm (Siedel et al. 1981). The Preciset Cholesterol Kit which contains six standard TC concentrations, was used to prepare calibration curves; the standard concentrations are 50, 100, 150, 200, 300 and 400 mg/dl. Furthermore, two standards were chosen for all assays performed and were

used to calculate the TC concentrations. Quality control was assessed by analyzing a pooled human plasma sample for all assays (Appendix A for assay).

3.6.2 Analysis of HDL-c, HDL2-c and HDL3-c Concentrations

In the precipitation method described by Gidez et al. (1982), the apo B containing lipoproteins (VLDL and LDL) were removed by precipitaion while HDL remained in the supernatant and its cholesterol content was measured. The precipitation reagents were the polyanionic heparin and the divalent cation manganese. The mechanisms of the precipitation reaction are not clear but it is believed that the interaction between the negatively charged groups of heparin and the protein moieties of the lipoproteins is probably important. Divalent metal ions interacted with negatively charged groups of phospholipids which facilitated the formation of insoluble complexes. The larger lipid rich proteins, VLDL and LDL, formed insoluble complexes more readily than did the smaller protein-rich HDL. The insoluble complexes were then sedimented by low speed centrifugation. The cholesterol remaining in the supernatant solution represented HDL-c, and measured as described for the TC analysis. Following the precipitaion of apo B containing lipoproteins with heparin and manganese, HDL2 was precipitated by the addition of dextran sulfate (mol. wt. 15,000). An aliquot of the superna-

tant HDL3-c (dextran sulfate supernatant) was removed after centrifugation and analyzed for its cholesterol content as described for TC analysis. HDL2-c was calculated as the difference between total HDL-c and HDL3-c. HDL2 determined by this method correlates well with results obtained by preparative ultracentrifugation ($r=0.91$, $n=295$) and analytical ultracentrifugation ($r=0.92$, $n=17$), as reported by Gidez et al. (1982). The Special Control Serum Kit, a lyophilized control serum based on human serum was used for the control of accuracy. The control serum was used for all assays performed and used to calculate the HDL-c concentrations (Appendix B for Assay).

To assess the reproducibility of the above techniques, ten aliquots of pooled plasma were analyzed for TC, HDL-c, HDL2-c and HDL3-c on the same day. Thereafter, an aliquot of this pooled plasma was used for all assays. The coefficients of variation for these samples (day to day and within day) are reported in Appendix C.

3.7 STATISTICAL ANALYSIS

An analysis of variance (ANOVA), with repeated measures was used for the analysis of all variables measured in the exercising and control groups using the Bio-Medical Data

Processing (BMDP) Statistical package. The data for the dependent variables TC, HDL-c, HDL2-c, HDL3-c, HDL2-c/TC and HDL2-c/HDL-c included seven repeated measures (week zero (pre), four, five, six, seven, eight and nine (post)), while the analysis of the remaining dependent variables included two (pre and post) repeated measures: weight, BMI, sum of four circumferences, percentage of body fat, and VO2 max. The independent variable was the training program. The two-way analysis of variance produced three F ratios with a corresponding level of probability. The "groups" F ratio expressed the degree of difference between the groups' (training and control) total means, with respect to the effect of the treatment: exercise or no exercise (i.e. the means of the "weekly" measures combined). The "weeks" F ratio expressed the degree of difference between the total group's weekly means (i.e. the weekly mean for the training and control groups combined was compared to other combined weekly means). The interaction ("weeks" and "groups"; W * G) F ratio expressed the effect of having both main effects combined. Significant F ratios were revealed when differences between the means were greater than those due to chance alone.

If a significant interaction effect (W * G) was revealed, simple main effects were then calculated. The level of probability $p<0.05$ was accepted as significance. Tukey's technique (pairwise comparisons) was used for post-hoc anal-

ysis to determine where the differences between the means were located.

IV

RESULTS AND DISCUSSION

4.1 INTRODUCTION

The objective of the study was to verify the effect of an eight-week bicycle ergometer training program on cholesterol transport. The following lipids, lipoproteins and ratios were studied: total cholesterol, HDL-c, HDL2-c, HDL3-c, HDL2-c/TC and HDL2-c/HDL-c. In addition, body composition and maximal oxygen consumption were measured. The following chapter is divided into two parts: a presentation of the results, followed by a discussion.

4.2 RESULTS

The results have been subdivided into two sections. The first section summarizes the findings on the following dependent variables: body weight, body mass index (BMI), sum of circumferences, percentage of body fat and maximal oxygen consumption (VO2 max); all were analyzed by means of a two-way ANOVA with two repeated measures. The variables analyzed

in the second section (two-way ANOVA with seven repeated measures) were those of total cholesterol, HDL-c, HDL2-c, HDL3-c, HDL2-c/TC and HDL2-c/HDL-c. Prior to the analysis of the results, independent t-tests and tests of the homogeneity of variances were conducted on the pre measurements of the dependent variables. For all variables, the t-tests demonstrated that the groups (training and control) were not initially different one from the other and that their variances were homogeneous, therefore fulfilling the basic requirements of the analysis of variance (ANOVA). Stastical analysis of all the variables proceeded as elaborated in chapter III. All raw scores appear in Appendix D. On the basis of the questionnaire (Appendix E) responses, it appeared that eating habits and alcohol consumptions were not modified during the course of the study. Furthermore, none of the subjects smoked or used oral contraceptives. The control group did not participate in any regular program of physical activity, while the training group participated solely in the prescribed training program.

4.2.1 Dependent Variables with Two Repeated Measures

Body weight, body mass index (BMI), sum of circumferences, percentage of body fat and maximal oxygen consumption measurements were measured prior to and at the completion of the study.

Body Weight

The pre and post body weight means and standard deviations (kilograms) for the training and control groups are presented in table 14.

TABLE 14

MEANS AND STANDARD DEVIATIONS FOR BODY WEIGHT IN kg

GROUPS / WEEKS		PRE	POST
Training (n=16)	mean:	61.9	62.0
	S.D.:	8.4	9.0
Control (n=9)	mean:	59.9	59.7
	S.D.:	10.0	10.2

The results of the two-way ANOVA (table 15) demonstrated that the two main effects, groups and weeks and the interaction between the effects groups and weeks (G * W) were all non significant. Therefore, both groups were said to have similar body weights throughout the study period.

TABLE 15

RESULTS OF THE ANOVA FOR BODY WEIGHT

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	52.69	1	52.69	0.31	0.58
Error within	3857.91	23	167.74		
Weeks	0.03	1	0.03	0.03	0.87
G * W	0.33	1	0.33	0.31	0.58
Error between	24.50	23	1.07		

Body Mass Index (BMI)

In table 16, the means and standard deviations are given for the body mass index of the training and control groups, measured at week 0 and 9.

TABLE 16

MEANS AND STANDARD DEVIATIONS FOR BMI IN kg/cm2

GROUPS / WEEKS		PRE	POST
Training (n=16)	mean:	23.1	23.1
	S.D.:	3.9	3.9
Control (n=9)	mean:	22.3	22.2
	S.D.:	2.6	2.6

As shown in table 17, the two-way analysis of variance did not produce any significant F ratios. Once again, the training and control groups were said to have comparable pre and post body mass indices.

TABLE 17

RESULTS OF THE ANOVA FOR THE BODY MASS INDEX

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	7.87	1	7.87	0.32	0.58
Error within	566.52	23	24.63		
Weeks	0.05	1	0.05	0.36	0.56
W * G	0.01	1	0.01	0.07	0.79
Error between	3.50	23	0.15		

Sum of Circumferences

The means and standard deviations for the sum of circumferences for the two experimental groups are presented in table 18.

TABLE 18 .

MEANS AND STANDARD DEVIATIONS FOR THE SUM OF CIRCUMFERENCES IN cm

GROUPS / WEEKS		PRE	POST
Training (n=16)	mean:	292.2	293.8
	S.D.:	22.4	26.4
Control (n=9)	mean:	286.6	285.9
	S.D.:	19.8	19.6

Results of the ANOVA are presented in table 19. Even though slight changes in the sum of circumferences are observed in the mean measurements of each group, both main effects and the interaction between them produced non significant F ratios.

TABLE 19

RESULTS OF THE ANOVA FOR THE SUM OF CIRCUMFERENCES

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	530.7	1	530.7	0.52	0.48
Error between	23626.9	23	1027.3		
Weeks	2.8	1	2.8	0.11	0.74
G * W	16.0	1	16.0	0.64	0.43
Error within	577.8	23	25.1		

Percentage of Body Fat

The means and standard deviations for the percentage of body fat for both groups are given in table 20.

TABLE 20

MEANS AND STANDARD DEVIATIONS FOR THE PERCENTAGE OF BODY FAT

GROUPS / WEEKS		PRE	POST
Training (n=16)	mean:	33.5	31.6
	S.D.:	6.2	6.9
Control (n=9)	mean:	30.4	28.8
	S.D.:	5.5	4.8

The results of the ANOVA presented in table 21 revealed non significant F ratios for the main effect groups and the interaction between the groups and weeks. However, the effect of the repeated measures (weeks) was found to be significant with a level of probability of 0.001. The combined group's (control and training) mean percentage of body fat decreased significantly over the ten week period from 32% (pre) to 30.2% (post), as observed in table 20.

TABLE 21

RESULTS OF THE ANOVA FOR THE PERCENTAGE OF BODY FAT

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	103.61	1	103.61	1.44	0.24
Error within	1650.55	23	71.76		
Weeks	34.21	1	34.21	13.31*	0.001
W * G	0.24	1	0.24	0.09	0.76
Error between	59.10	23	2.57		

* significant at the respective level of probability

Maximal Oxygen Consumption

VO2 max means and standard deviations measured in the training and control groups are shown in table 22.

TABLE 22

MEANS AND STANDARD DEVIATIONS FOR MAXIMAL OXYGEN CONSUMPTIONS IN ml/min/kg

GROUPS / WEEKS		PRE	POST
Training (n=16)	mean:	27.5	32.7
	S.D.:	5.2	6.1
Control (n=9)	mean:	28.9	31.4
	S.D.:	3.2	3.7

Presented in table 23 are the results of the ANOVA which demonstrated that the groups effect and the interaction effect were not significant, but that the effect of the repeated measures (weeks) provided a significant F ratio with a level of probability of 0.0001. From the combined group's means, derived from the training and control groups (table 22), it can be observed that the mean maximal oxygen consumptions increased significantly with time irrespective of the treatment group (exercise or no exercise) from 28.2 ml/min/kg to 32.1 ml/min/kg. Therefore, a factor other than the exercise program must have contributed to the higher post VO2 max values.

TABLE 23

RESULTS OF THE ANOVA FOR THE MAXIMAL OXYGEN CONSUMPTIONS

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	0.05	1	0.05	0.0	0.97
Error within	975.0	23	42.39		
Weeks	172.21	1	172.21	22.89*	0.0001
W * G	21.86	1	21.86	2.91	0.10
Error between	173.01	23	7.52		

* significant at the respective level of probability

From the analysis of the above dependent variables, the following can be observed: the exercise program did not modify the mean body weight, the mean BMI nor the mean sum of circumferences. Nevertheless, the combined group (control and training) manifested the same pattern of change for the percentage of body fat and the maximal oxygen consumption: the percentage of body fat decreased significantly while the VO2 max increased significantly.

4.2.2 Dependent Variables with Seven Repeated Measures

The following dependent variables total cholesterol, HDL-c, HDL2-c, HDL3-c and the ratios of HDL2-c to Total cholesterol and HDL2-c to HDL-c were analyzed by a two-way ANOVA including seven repeated measures (pre, weeks 4, 5, 6, 7, 8, and post). The following results were observed.

Total Cholesterol

The means and standard deviations for the total cholesterol concentrations (mg/dl) are presented in table 24.

TABLE 24

MEANS AND STANDARD DEVIATIONS FOR TOTAL CHOLESTEROL CONCENTRATIONS IN mg/dl

GROUPS / WEEKS	PRE	4	5	6	7	8	POST
Training (n=16)							
mean:	160.0	154.6	164.7	165.9	163.5	164.2	166.9
S.D.:	26.2	21.5	29.9	23.9	25.1	21.3	24.4
Control (n=9)							
mean:	176.4	176.2	175.2	166.5	171.6	175.9	176.4
S.D.:	19.0	25.0	27.3	30.0	27.2	23.9	25.3

Results of the two-way ANOVA on the total cholesterol concentrations are presented below in table 25. Both main effects, groups and weeks, were found to be non significant. The interaction between the main effects produced a significant F ratio with a level of probability of 0.03. When simple main effects were calculated, the following observations were revealed. The difference between groups was significant at week four, i.e. the mean of the training group (154.6 mg/dl) differed significantly from the mean of the control group (176.2 mg/dl) at week four with a confidence interval of 95%. Furthermore, significant differences were found in the training group only, at the 0.05 level of probability. Tukey's post-hoc technique (table 26) revealed that the mean total cholesterol concentration at week four (154.6 mg/dl) was significantly different than the concentration at week six (165.9 mg/dl) and the post concentration (166.9 mg/dl). No significant change was observed with respect to the pre total cholesterol measurement.

TABLE 25

RESULTS OF THE ANOVA FOR TOTAL CHOLESTEROL CONCENTRATIONS

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	5074.33	1	5074.33	1.35	0.26
Error between	86664.10	23	3768.00		
Weeks	700.20	6	116.70	1.12	0.35
G * W	1512.96	6	252.16	2.42*	0.03
Error within	14388.79	138	104.27		

* significant at the respective level

TABLE 26

RESULTS OF TUKEY'S TECHNIQUE ON THE TRAINING GROUP'S TOTAL CHOLESTEROL MEANS

WEEKS	PRE	4	5	6	7	8	POST
MEANS	160.0	154.6	164.7	165.9	163.5	164.2	166.9
PRE	160.0	---	5.4	4.7	5.9	3.5	4.2	6.9
4	154.6		----	10.1	11.3*	8.9	9.6	12.3*
5	164.7			----	1.2	1.2	0.5	2.2
6	165.9				----	2.4	1.7	1.0
7	163.5					----	0.7	3.4
8	164.2						----	2.7
POST	166.9							----

* significant at the 0.05 level

High Density Lipoprotein-Cholesterol (HDL-c)

Presented in table 27 are the means and standard deviations for the HDL-c concentrations measured at the different intervals, for the training and control groups.

TABLE 27

MEANS AND STANDARD DEVIATIONS FOR HDL-CHOLESTEROL CONCENTRATIONS IN mg/dl

GROUPS / WEEKS	PRE	4	5	6	7	8	POST
Training (n=16)							
mean:	56.8	61.7	60.9	62.3	61.4	64.6	67.2
S.D.:	10.8	10.9	11.0	10.3	10.9	9.5	11.2
Control (n=9)							
mean:	60.0	62.8	61.8	59.6	60.2	61.2	58.4
S.D.:	14.0	16.9	17.6	14.4	15.4	14.7	13.5

The results of the ANOVA on the HDL-c values (table 28) demonstrated that both main effects, groups and weeks, were not significant but that the interaction effect produced a significant F ratio at a level of probability of 0.003. Furthermore, calculations of the simple main effects demonstrated that significant differences existed within the training group's mean, at the 0.05 level of probability. Tu-

key's technique applied to the training group's means (table 29) revealed the following significant differences: the pre HDL-c mean (56.8 mg/dl) was significantly different from the means at week six (62.3 mg/dl), at week eight (64.6 mg/dl) and the post (67.2 mg/dl) mean. The means at week four, five and seven differed significantly from the post mean. Therefore, as of the sixth week of training (with the exception of week 7), significant increases in HDL-c concentrations were observed when these means were compared to the pre mean of 56.8 mg/dl.

TABLE 28

RESULTS OF THE ANOVA FOR HDL-CHOLESTEROL CONCENTRATIONS

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	98.94	1	98.94	0.11	0.75
Error within	21500.94	23	934.82		
Weeks	324.19	6	54.03	2.12	0.06
W * G	537.19	6	89.53	3.51*	0.003
Error between	3524.89	138	25.54		

* significant at the respective level

TABLE 29

RESULTS OF TUKEY'S TECHNIQUE ON THE TRAINING GROUP'S HDL-CHOLESTEROL MEANS

WEEKS		PRE	4	5	6	7	8	POST
MEANS		56.8	61.7	60.9	62.3	61.4	64.6	67.2
PRE	56.8	----	4.9	4.1	5.5*	4.6	7.8*	10.4*
4	61.7		----	0.8	0.6	0.3	2.9	5.5*
5	60.9			----	1.4	0.5	3.7	6.3*
6	62.3				----	0.9	2.3	4.9
7	61.4					----	3.2	5.8*
8	64.6						----	2.6
POST	67.2							----

* significant at the 0.05 level

High Density Lipoprotein 2-Cholesterol (HDL2-c)

The means and standard deviations for the HDL2-c concentrations of the training and control groups are given in table 30.

TABLE 30

MEANS AND STANDARD DEVIATIONS FOR HDL2-CHOLESTEROL CONCENTRATIONS IN mg/dl

GROUPS WEEKS	PRE	4	5	6	7	8	POST
Training (n=16)							
mean:	26.4	28.1	27.5	28.1	28.0	26.8	30.7
S.D.:	7.7	7.9	9.1	9.2	7.6	8.1	9.4
Control (n=9)							
mean:	31.2	30.1	28.7	26.4	26.0	26.9	23.4
S.D.:	15.2	14.9	14.6	11.8	11.4	14.9	9.7

The results of the ANOVA (table 31) on the groups' HDL2-c mean concentrations mirrored those of the HDL-c concentrations. Both main effects provided F ratios that were not significant, while the F ratio for the interaction between the factors groups and weeks was found to be significant at the 0.009 level of probability. Because a significant interaction effect was detected, simple main effects were calculated. With a confidence interval of 95%, the effect of the repeated measures produced a significant F ratio for the control group's HDL2-c mean. Tukey's post-hoc analysis (table 32) on these means revealed that the pre mean (31.2 mg/dl) was significantly different (p=0.05) from the post mean

(23.4 mg/dl), due to a gradual decrease in HDL2-c concentrations throughout the course of the study.

TABLE 31

RESULTS OF THE ANOVA FOR HDL2-CHOLESTEROL CONCENTRATIONS

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	7.46	1	7.46	0.01	0.91
Error within	13656.14	23	593.75		
Weeks	118.27	6	19.71	0.71	0.65
W * G	503.21	6	83.87	3.00*	0.009
Error between	3854.76	138	27.93		

* significant at the respective level

TABLE 32

RESULTS OF TUKEY'S TECHNIQUE ON THE CONTROL GROUP'S HDL2-CHOLESTEROL MEANS

WEEKS		PRE	4	5	6	7	8	POST
MEANS		31.2	30.1	28.7	26.4	26.0	26.9	23.4
PRE	31.2	----	1.1	2.5	4.8	5.2	4.3	7.8*
4	30.1		----	1.4	3.7	4.1	3.2	6.7
5	28.7			----	2.3	2.7	1.8	5.3
6	26.4				----	0.4	0.5	3.0
7	26.0					----	0.9	2.6
8	26.9						----	3.5
POST	23.4							----

* significant at the 0.05 level

High Density Lipoprotein 3-Cholesterol (HDL3-c)

The means and standard deviations for the HDL3-c concentrations for the two groups are reported in table 33.

TABLE 33

MEANS AND STANDARD DEVIATIONS FOR HDL3-CHOLESTEROL CONCENTRATIONS IN mg/dl

GROUPS WEEKS	PRE	4	5	6	7	8	POST
Training (n=16)							
mean:	30.5	33.5	33.3	34.3	33.4	37.7	36.4
S.D.:	4.8	6.7	3.3	4.3	5.0	7.0	4.2
Control (n=9)							
mean:	28.8	32.7	33.1	33.1	34.3	34.3	34.9
S.D.:	7.9	7.5	6.5	4.9	5.7	7.4	5.5

Presented in table 34 are the results of the two-way ANOVA on the HDL3-cholesterol concentrations. The weeks effect provided a significant F ratio with a level of probability of 0.0001, while the interaction and groups effects were both found to be non significant. Following the ANOVA, Tukey's technique was used to determine at what week the combined group's means (mean derived from the training and control groups) differed. The results revealed that the pre combined group mean (29.9 mg/dl) was significantly different ($p=0.05$) than all succeeding combined group means (table 35). This indicated that both groups exhibited similar pattern of change throughout the weeks.

TABLE 34

RESULTS OF THE ANOVA FOR HDL3-CHOLESTEROL CONCENTRATIONS

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	52.06	1	52.06	0.39	0.54
Error within	3093.95	23	134.52		
Weeks	604.06	6	100.68	6.44*	0.0001
W * G	60.62	6	10.10	0.65	0.6928
Error between	2156.36	138	15.63		

* significant at the respective level

TABLE 35

RESULTS OF TUKEY'S TECHNIQUE ON THE COMBINED GROUPS HDL3-CHOLESTEROL MEANS

WEEKS	PRE	4	5	6	7	8	POST
MEANS	29.7	33.1	33.2	33.7	33.9	36.0	35.7
PRE	29.7	----	3.4*	3.5*	4.0*	4.2*	6.3*	6.0*
4	33.1		----	0.1	0.6	0.8	2.9	2.6
5	33.2			----	0.5	0.7	2.8	2.5
6	33.7				----	0.2	2.3	2.0
7	33.9					----	2.1	1.8
8	36.0						----	0.3
POST	35.7							----

* significant at the 0.05 level

High Density Lipoprotein 2-Cholesterol to Total Cholesterol Ratio

Presented in table 36 are the means and standard deviations for the HDL2-c to total cholesterol ratio, as measured in the training and control groups.

TABLE 36

MEANS AND STANDARD DEVIATIONS FOR THE RATIO OF HDL2-CHOLESTEROL TO TOTAL CHOLESTEROL CONCENTRATIONS

GROUPS WEEKS	PRE	4	5	6	7	8	POST
Training (n=16)							
mean:	0.166	0.182	0.166	0.170	0.172	0.166	0.184
S.D.:	0.05	0.05	0.04	0.05	0.04	0.05	0.06
Control (n=9)							
mean:	0.177	0.172	0.162	0.161	0.152	0.154	0.133
S.D.:	0.07	0.08	0.07	0.07	0.06	0.08	0.06

The ratio of HDL2-cholesterol to total cholesterol were analyzed by a two-way ANOVA (table 37); the results demonstrated that the two main effects and the interaction between the two of them produced non significant F ratios.

TABLE 37

RESULTS OF THE ANOVA FOR THE RATIO OF HDL2-CHOLESTEROL TO TOTAL CHOLESTEROL CONCENTRATIONS

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	0.007	1	0.007	0.41	0.53
Error within	0.399	23	0.017		
Weeks	0.006	6	0.001	1.00	0.43
W * G	0.012	6	0.002	2.10	0.06
Error between	0.136	138	0.001		

High Density Lipoprotein 2-Cholesterol to High Density Lipoprotein Cholesterol Ratio

Presented in table 38 are the means and standard deviations for the ratio of HDL2-c to HDL-c, as observed in the two experimental groups.

TABLE 38

MEANS AND STANDARD DEVIATIONS FOR THE HDL2-CHOLESTEROL TO HDL-CHOLESTEROL RATIO

GROUPS / WEEKS	PRE	4	5	6	7	8	POST
Training (n=16)							
mean:	0.456	0.451	0.443	0.441	0.448	0.411	0.447
S.D.:	0.07	0.08	0.07	0.09	0.07	0.09	0.09
Control (n=9)							
mean:	0.501	0.462	0.446	0.431	0.416	0.421	0.387
S.D.:	0.14	0.12	0.11	0.08	0.09	0.13	0.09

From the results of the two-way ANOVA presented in table 39, the following is observed. The groups effect and the interaction effect were both non significant while the weeks effect was significant with a level of probability of 0.01. Computations of the simple main effects detected the following differences between the means. Tukey's technique revealed that the pre mean ratio of 0.479 was significantly different from the mean ratio at week 8 (0.416) and from the post mean ratio (0.417) (table 40).

TABLE 39

RESULTS OF THE ANOVA FOR THE HDL2-CHOLESTEROL TO HDL-CHOLESTEROL RATIO

SOURCE	SUM OF SQUARES	DEGREES FREEDOM	MEAN SQUARE	F RATIO	LEVEL OF PROBABILITY
Groups	0.001	1	0.001	0.03	0.87
Error within	0.810	23	0.035		
Weeks	0.069	6	0.012	3.02*	0.01
W * G	0.040	6	0.007	1.73	0.12
Error between	0.525	138	0.004		

* significant at the respective level

TABLE 40

RESULTS OF TUKEY'S TECHNIQUE ON THE COMBINED GROUPS HDL2-CHOLESTEROL TO HDL-CHOLESTEROL RATIO MEANS

WEEKS		PRE	4	5	6	7	8	POST
MEANS		0.479	0.457	0.445	0.436	0.432	0.416	0.417
PRE	0.479	----	0.022	0.034	0.043	0.047	0.063*	0.062*
4	0.457		-----	0.012	0.013	0.020	0.041	0.040
5	0.445			-----	0.009	0.008	0.029	0.028
6	0.436				-----	0.004	0.020	0.010
7	0.432					-----	0.020	0.010
8	0.422						-----	0.001
POST	0.387							-----

4.2.3 Summary

Table 41 provides a summary table of the statistical analysis of results on the variables studied.

TABLE 41

SUMMARY TABLE: STATISTICAL ANALYSIS

VARIABLE	ANOVA Groups	ANOVA Weeks	ANOVA W*G	SIMPLE MAIN EFFECTS Groups	SIMPLE MAIN EFFECTS Weeks	TUKEY'S TECHNIQUE Sign. Dif.
BODY WEIGHT	NS	NS	NS	-	-	-
BMI	NS	NS	NS	-	-	-
SUM OF CIRC.	NS	NS	NS	-	-	-
% BODY FAT	NS	0.001	NS	-	-	-
VO2 MAX	NS	0.0001	NS	-	-	-
TOTAL CHOL.	NS	NS	0.03	wk 4	T.	T: 4 vs 6 4 vs 9
HDL-C	NS	NS	0.003	-	T.	T: 0 vs 6 0 vs 8 0 vs 9 4 vs 9 5 vs 9 7 vs 9
HDL2-C	NS	NS	0.009	-	C.	C: 0 vs 9
HDL3-C	NS	0.0001	NS	-	-	0 vs 4 0 vs 5 0 vs 6 0 vs 7 0 vs 8 0 vs 9
HDL2-C/ TC	NS	NS	NS	-	-	-
HDL2-C/ HDL-C	NS	0.01	NS	-	-	0 vs 8 0 vs 9

Note: significance levels are reported;
week 0 = pre
week 9 = post

4.3 DISCUSSION

The effects of an eight week bicycle ergometer aerobic training program were studied in sixteen young women. A non exercising group of nine women was used as a control group to establish the effect of exercise on cholesterol transport. Prior to the analysis of the data, both experimental groups (training and control) were found to be equal; pre means and variances for all variables measured were not significantly different between the two groups.

The training program did not produce any significant changes in body composition. Body weight, body mass index and the sum of circumferences remained unchanged. However, it was not expected that a program of this intensity would alter body compostion. Nevertheless, the percentage of body fat decreased significantly in the combined group from a combined pre mean (32.1%) to a combined post mean (30.2%). A decline in the percentage of body fat could have resulted from a) experimental measurement errors; b) an increase in fat free mass with no change in total body weight; or c) a combination of the above two factors. With the absence of

a significant interaction effect and groups effect as observed in table 21, the significant change observed with time has little significance and could not have been a function of the training program.

Significant increases in VO2 max were observed in the combined group. The mean increase reported for the training group was of a greater order (27.5 ml/min/kg to 32.7 ml/min/kg) than that of the control group's mean increase (28.9 ml/min/kg to 31.4 ml/min/kg). Increases of the same magnitude have previously been reported by Wynne et al. (1980)(29.2 to 33.9 ml/min/kg) and Frey et al. (1982) (28.5 to 33 ml/min/kg). Both groups conducted bicycle ergometer training programs in young women, three times a week, 30 minutes per session at 70% of maximal heart rate reserve, for ten weeks. Contrary to most studies, maximal oxygen consumptions should not increase in a group said to remain "inactive" (control group). Keeping in perspective the population being tested and the conditions of the maximal bicycle test, the following explanation is presented. None of the subjects (with the exception of one or two) had ever performed a test to exhaustion. Also, having to wear a mouthpiece throughout the test was very difficult for some and may have contributed, in certain instances, to test termination. Furthermore, no verbal encouragement was given and as soon as the subject indicated her end point the test was terminated within 15 seconds, even though the subjects often

appeared only somewhat exhausted. Taking the above factors into consideration, an exhaustive end point was often not reached. More so, it is believed that the results of a symptom limited exercise test conducted in a population such as utilized in this study are very much subjective and may not be indicative of a true VO2 max. Furthermore, with the verification of the individual results, it was observed that only 13 subjects attained a true VO2 max (as described in chapter III) for the pre-test (8 from the exercising group, 5 from the control group) and only 11 subjects for the post-test (6 and 5 respectively). Due to the fact that the groups were not significantly different from each other and that both groups exhibited parallel changes with time, the changes observed in VO2 max were not a function of the training program and no conclusive training effect can be reported.

The main objective of the present study was to quantify "weekly" concentrations of total cholesterol, HDL-cholesterol, HDL2-cholesterol and HDL3-cholesterol in order to verify the effect of exercise on cholesterol transport. Frey et al. (1982) and Wynne et al. (1980) are the few authors who have studied exercise induced lipid modifications in women. As described above, both groups conducted bicycle ergometer training programs for ten weeks. Wynne's group took pre and post measurements, while Frey's study consisted of blood samples at week zero, two, five and ten. Both groups of au-

thors measured total cholesterol, HDL-c, LDL-c and triglycerides. Therefore, no elaborate picture exists on "weekly" measurements of HDL and HDL subfractions.

Increases in total cholesterol concentrations as observed in the exercising group have also been reported by Farrell et al. (1980) (running program) and Frey et al. (1982) (bicycle program). In the present study, significant differences in mean concentrations of total cholesterol were observed only as a function of a decreased total cholesterol concentration at week four (154.6 mg/dl) which then became significantly different from weeks six's concentration of 165.9 mg/dl and the post value of 166.9 mg/dl. The observed increase in total cholesterol concentrations in the training group may have been a function of the observed increase in HDL-c, but one may only speculate because other cholesterol transporters (i.e. VLDL, LDL) have not been measured. Nothing can be concluded on a training effect per se with respect to total cholesterol concentrations. It has repeatedly been reported that total cholesterol concentrations decreased or remained unchanged as a function of exercise. Lipson et al. (1979) (running program for six weeks) and Peltonen et al. (1981) (aerobic activities for 15 weeks) reported decreases in total cholesterol concentrations. Furthermore, HDL-c decreased in Lipson's study group and increased in Peltonen' group. In Rotkis et al.'s (1981) study (four to seven month running program), total cholesterol

concentrations remained unchanged while HDL-c concentrations increased significantly. Wynne's (1980) results demonstrated no change in total cholesterol and HDL-c concentrations, within a ten week bicycle ergometer training program. Therefore, much remains to be established on the effects of exercise on total cholesterol concentrations.

In view of non conclusive VO2 max results, it cannot be concluded that the changes observed in HDL and its subfractions are due to the training program. Therefore, the increased levels of HDL-c (training group) are presumably a function of various factors known to influence HDL-c and to which exercise may have played an accessory role. With respect to HDL subfractions, the results obtained in the present study are contrary to what the literature has suggested. Nye et al.'s (1981) results are a prime example of the changes expected to occur with training. This group of researchers demonstrated an increase in HDL-c concentrations (as a function of a calisthenics program), which was mirrored by an increase in HDL2-c concentrations, while HDL3-c concentrations decreased. Cowan's (1983) results supported the above. In the present study, the changes in HDL2-c were observed in the control group only, with a significant decline between the pre and the post means. This decline in HDL2-c was counterbalanced by an increase in HDL3-c. No change was expected in the control group's values. Those observed are possibly a function of a) decreased enzymatic ac-

tivity; b) experimental errors; c) decreased formation of HDL2 particles, or d) a combination of these factors. With respect to HDL3-c concentrations, both groups reacted in the same manner; the increases with time were parallel and because the groups were not different from each other, the training program may not have contributed to this change. In the control group, the increase in HDL3-c was accompanied by a decrease in HDL2-c, while in the training group the increase in HDL3-c was not accompanied by a significant change in HDL2-c. Contrary to what t the literature has suggested, the changes observed in this study are primarily a function of modifications occurring in the HDL3 subfraction and not in the HDL2 subfraction. It is not known what contributed in modifying HDL and its subfractions in the studies referred to above. Nye et al. (1981) proposed that the changes were a function of a selective redistribution of apo-C peptides, while Williams et al. (1983) suggested that the changes were a function of fat loss (induced by exercise). From the results obtained in this study, it cannot be concluded that a conversion of the HDL subfractions occurred, favoring HDL2 formation and decreasing the formation of the HDL3 particles, as has been suggested by Patsch et al. (1978), Schmitz et al. (1981), Taskinen et al. (1981), (1983), and Wood et al. (1983). Also, even though the groups were statistically similar, individual concentrations of lipoproteins demonstrated a wide range of values. These

individual variations may have been due to the degree of adiposity, diet, metabolism and most likely to genetics. Furthermore, initial and weekly differences observed may have been a function of hormonal variations arising throughout the menstrual cycle. Even though careful estimation of ovulation was practised, much remains questionable on the effect of female hormones on lipoproteins and lipids metabolism. Also, because the changes expected in HDL and the subfractions are relatively small, the higher the initial values are, less changes are to be expected.

Triglycerides, total cholesterol, HDL and LDL concentrations have been used as possible predictors of CHD. Miller et al.'s major finding in 1981 was the association between the severity of CAD and the cholesterol content found in HDL2, and more so because HDL2 has a greater ratio of cholesterol to protein content than does HDL3. Haffner et al. (1985) determined that atherosclerosis (as measured by coronary angiography) was associated with a decline in HDL2 rather than the HDL3 subfraction. Dufaux et al. (1982) suggested that HDL2 was a stronger negative risk factor than HDL3. Koga et al.'s (1983) findings suggested that most changes in HDL occur within the HDL2 subfraction. Therefore, the use of ratios becomes important if one believes that HDL2-c is the protective subfraction (i.e. the one responsible for transporting the cholesterol to the liver). Furthermore, the HDL2-c/TC and HDL2-c/HDL-c ratios provide

indications on the efficiency of this transport mechanism. The observed HDL2/TC ratio changes throughout the weeks were not significant but the overall increase observed in the training group and the decrease observed in the control group should leave the training group in a more favorable position, i.e. at the end of the training program, more of the total cholesterol mass was being transported by the HDL2 subfraction, most probably a function of the reciprocal HDL2-c trends seen in the control and training groups. With respect to the HDL2-c/HDL-c ratio both groups exhibited similar changes (decrease), but as a function of different variables. In the training group, the decreased ratio was a function of a significant rise in HDL-c, while in the control group the decreased ratio was a function of a decline in HDL2-c levels. Initially (pre measurements), both groups' HDL2-c/HDL-c ratios were extremely high (0.456 for the training group and 0.501 for the control group) which conferred on these subjects a low risk of developing coronary heart diseases on the basis of these two lipoproteins, and more so, less chance of observing significant modifications.

Initially, the total group had high HDL-c values (mean of 58.4 mg/dl) and HDL2-c values (mean of 28.8 mg/dl), and low HDL3-c values (29.7 mg/dl); furthermore, the group was deemed moderately heavy (mean weight of 60.9 kg) with a higher than average percentage of body fat (32.1 %) compared

to women of the same age group; the group was also unfit (mean VO2 max of 28.2 ml/min/kg). Furthermore, no significant weeks effects were observed prior to week six of the study (when compared to the pre mean values); most significant changes were solely a function of differences between the pre and post means.

Most studies have, in conjunction with determining lipid and lipoprotein concentrations, measured various enzymes such as LPL and HL, and apoproteins (A-I, A-II) to further explain the changes observed in cholesterol transport and establish the possible mechanisms involved. This was not feasible within the limitations of this study.

V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 SUMMARY

This study was designed to quantify the changes occurring in HDL-c, HDL2-c, HDL3-c and total cholesterol concentrations in women following a bicycle ergometer training program. The subjects in the study were young women between 18 and 30 years (primarily students) who were non smokers and sedentary. Sixteen women were exercised three times a week, 35 minutes per session (five minute work bouts, one minute rest periods) with heart rates corresponding to 75% of VO2 max, for eight weeks. Nine women participated as controls. Weight, height, sum of circumferences, percentage of body fat and VO2 max were determined prior to and at the completion of the eight-week study. Blood samples were drawn after an overnight fast at week zero (pre), four, five, six, seven, eight and nine (post) for analysis. The day of ovulation was determined for each woman so that it could be avoided for blood withdrawal due to its possible influence on blood variables. The results indicated that the body weight , the BMI and sum of circumferences did not change in either

group, while the percentage of body fat decreased significantly in the combined group; VO2 max increased significantly in the combined group with time. No interconversion of the HDL subfractions was recorded as a result of the training program. Furthermore, the changes observed in HDL-c levels were primarily a function of changes taking place in the HDL3 subfraction. The changes observed cannot be attributed to a training effect due to the absence of significant between groups differences on any variables, including VO2 max.

5.2 CONCLUSIONS

Within the scope of this study, the following conclusions may be drawn:

1. The exercise stimulus may not have been sufficient to produce significant training effects per se, since VO2 max was not significantly improved relative to control subjects (as measured by VO2 max values). Therefore, no conclusive remarks can be drawn on the effect of the training program. By the same token, body compostion did not change as a function of the exercise program.

2. The training group manifested significant increases in HDL-c across time though at no point were these values sig-

nificantly different from those of the control group. Furthermore, most changes observed in HDL were a function of changes in HDL3 rather than the expected changes in the HDL2 subfraction. It cannot be concluded that an interconversion of the HDL subfractions occurred in the training group or as an effect of the training. When the training group was examined in itself, the earliest significant modifications in any of the HDL values (compared to pre-training) were observed at week six.

3. In this study there were no significant effects or trends that could be attributed to the training program, since no significant between groups effects were obtained. The non significant changes observed in the training group alone did follow "beneficial" trends (i.e. increases in HDL-c, HDL2-c, HDL3-c and HDL2-c/HDL-c) but unfortunately, the control group also showed considerable and sometimes significant, though unexplainable changes. These latter, combined with sizeable, though not significant, pre-test differences between groups, appear to have effectively annuled the significance of the training group trends that appeared in the data.

5.3 RECOMMENDATIONS

The present structure of the study has permitted the quantification of changes occurring in cholesterol transport. However, after the completion of such an experiment, a few changes are recommended if such a study is to be conducted again.

Firstly, it is recommended that the intensity and frequency of the exercise program be increased to 75-80% of VO2 max and to four sessions per week, respectively. Also, VO2 max determination should be conducted (as described in chapter III), two days in a row. Thereafter, the highest value would be used as the true VO2 max. With respect to VO2 max determination in a population such as the one studied, the elaboration of a VO2 max test is questionable in view that very few subjects are able to attain a true end point. In such a situation, very little if anything can be concluded with respect to a training effect.

Secondly, it is believed that the control of ovulation day be monitored by daily body temperature charts, which would eliminate fluctuating values observed in certain subjects. This in itself entails a minimum of three months for baseline values. Also, a fast of 14 hours would be preferred to one of 12 hours for all blood samples. Finally, with respect to cholesterol determination, the gold standard

method "ultracentrifugation" should definitely yield more accurate results.

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Appendix A

TOTAL CHOLESTEROL ASSAY: BOEHRINGER MANNHEIM

Reagents	Concentrations
Tris buffer	100 mmol/l ph 7.7
Magnesium aspartate	50 mmol/l
4-aminophenazone	1 mmol/l
Sodium cholate	10 mmol/l
Phenol	6 mmol/l
3,4-dichlorophenol	4 mmol/l
Hydroxyoplyethoxy-n-alkanes	0.3 %
Cholesterol esterase	0,4 U/ml
Cholesterol oxidase	0.25 U/ml
Peroxidase	0.2 U/ml

Preparation of Solution:

Content of one bottle is dissolved in 100 milimiters of redistilled water. Reagent solution is ready to use after ten minutes.

Procedure:

1. Tubes are labelled.
2. To 2 ml of the reagent solution is added 20 ul of EDTA plama. For each assay, two standard concentrations are used and treated as plasmic samples.
3. The sample is mixed thoroughly with the vortex and left standing for ten minutes.
4. The sample is transferred to a glass cuvette.

5. The spectrophotometer is set at 500 nm and the absorbance of the sample is read against a reagent blank (2 ml of reagent solution).
6. The equation used for the calculation of the total cholesterol concentration is:

   Concentration of the standard divided by the absorbance of the standard, which is then multiplied by the absorbance of the sample = mg/dl of cholesterol.

* Two standards are used; the mean of the two (concentration divided by the absorbance) is used as the multiplying factor for all samples in the assay.

Appendix B

ASSAY FOR HDL-CHOLESTEROL, HDL2-CHOLESTEROL AND HDL3-CHOLESTEROL CONCENTRATIONS

Reagents and Preparation of Solutions:

1. Heparin 5000 units/ml:

   10000 USP units/ml of heparin are diluted in 1:1 ratio of saline (0.9 % NaCl).

2. 2 M MnCl2 4H2O MW: 197.9 Dalton:

   39.58 grams of MnCl2 4H2O are dissolved in distilled water; flask is filled up to 100 ml.

3. EDTA solution 8 mmol/l MW: 372.24 Dalton:

   2.977 grams are dissolved with distilled water up to 1 liter.

4. Boehringer Cholesterol Reagent (Cat. # 237574):

   Content of one bottle is dissolved with 100 ml of EDTA solution.

5. Dextransulphate MW: 15000 Dalton:

   1.43 g/dl of Dextransulphate are dissolved in 0.15 M NaCl.

Note: Solution 1, 2 and 3 can be prepared in advance; solutions 4 and 5 should be prepared for each assay.

Procedure:

1. Tubes are labelled.
2. To 2 ml of EDTA plasma is added:
   a) 80 ul of the heparin solution;
   b) 100 ul of the MnCl2 solution;

   samples are mixed with vortex after each addition.
3. Samples are left to stand on ice for 30 minutes and then

centrifuged at 3000 r.p.m. for 30 minutes at 4 degrees Celcius.

4. Aliquots of the clear heparin-Mn^{2+} supernatant are withdrawn immediately for HDL2 precipitation and analysis of total HDL-cholesterol.
5. To 1 ml of the heparin-Mn^{2+} supernatant is added 0.1 ml of the Dextransulphate solution; after mixing thoroughly with the vortex, the samples are left to stand at room temperature for 20 minutes then centrifuged at at 3000 r.p.m. for 30 minutes at 4 degrees Celcius.
6. An aliquot of the clear supernatant is removed immediately for HDL3-cholesterol determination.
7. Cholesterol determination for HDL and HDL3:
   To 2 ml of cholesterol reagent is added 0.1 ml of the supernatant. Samples are mixed thoroughly and left to stand for 10 minutes at room temperature. The absorbance is read at 500 nm in a glass cuvette against blank (2ml of reagent solution) within one hour.
8. Cholesterol concentrations of the samples are calculated as follow:
   A standard (Special Control Serum for HDL-c) is used for all assays.

   Standard Concentration for Special Control Serum divided by its absorbance = multiplying factor

   NOTE: HDL2-c = HDL3-c - HDL-c.
9. With each run, a blank with 2 ml of saline instead of serum is prepared and treated as a sample.

Appendix C

COEFFICIENT OF VARIATION FOR POOLED PLASMA (10 SAMPLES)

Total Cholesterol:

within day	186.4 mg/dl (5.8)	c.v.: 3.1%
day to day	168.2 mg/dl (18.3)	c.v.: 10.9%

HDL-Cholesterol:

within day	65.0 mg/dl (4.1)	c.v.: 6.3%
day to day	63.3 mg/dl (3.5)	c.v.: 5.5%

HDL2-Cholesterol:

within day	27.1 mg/dl (4.1)	c.v.: 15.1%
day to day	26.7 mg/dl (2.0)	c.v.: 7.6%

HDL3-Cholesterol:

within day	37.9 mg/dl (2.0)	c.v.: 5.3%
day to day	36.6 mg/dl (3.1)	c.v.: 3.1%

Note: ( ) denotes standard deviations.

Appendix D

RAW SCORES

GROUPS		WEIGHT (kg)		BMI (kg/cm2)		SUM CIRC. (cm)	
	WEEKS	Pre	Post	Pre	Post	Pre	Post
Training	1	70.8	67.2	32.0	30.4	330.6	328.6
	2	64.5	64.2	24.5	24.4	294.3	295.6
	3	58.0	57.3	20.6	20.4	283.2	280.4
	4	58.6	58.4	20.5	20.4	288.0	280.8
	5	76.5	77.5	31.2	31.6	338.9	344.0
	6	66.9	68.8	25.2	25.9	297.4	303.5
	7	57.8	58.3	20.9	21.1	273.7	280.9
	8	54.0	53.5	19.0	18.8	270.1	261.5
	9	56.2	55.5	21.9	21.6	275.4	281.4
	10	54.9	56.2	21.7	22.2	275.7	278.2
	11	56.0	57.8	19.1	19.1	278.8	277.0
	12	69.2	71.2	24.5	25.2	316.5	325.9
	13	64.4	65.0	23.1	23.0	294.5	316.8
	14	50.5	48.3	19.2	18.4	266.7	258.5
	15	54.3	53.4	21.0	20.7	274.2	266.0
	16	78.0	79.9	25.3	25.9	316.8	322.4
Control	1	55.8	55.0	23.1	22.7	281.8	278.8
	2	64.3	65.8	23.1	23.7	300.1	303.3
	3	73.2	73.5	23.8	23.9	301.5	296.9
	4	72.5	73.4	25.2	25.5	308.3	313.2
	5	49.0	49.0	19.6	19.6	264.4	265.6
	6	64.6	62.5	24.5	23.7	300.5	294.4

	7	59.2	57.5	20.3	19.6	290.2	283.9
	8	57.7	57.0	23.7	23.5	285.1	287.7
	9	43.2	43.8	17.5	17.7	247.2	249.1

GROUPS		% BODY FAT (%)		VO2 MAX (ml/min/kg)	
	WEEKS	Pre	Post	Pre	Post
Training	1	42.5	41.5	19.9	29.7
	2	41.1	36.7	24.9	29.2
	3	21.4	20.5	29.9	37.4
	4	35.3	27.8	25.3	28.8
	5	42.5	42.5	17.8	22.8
	6	36.7	38.2	36.5	38.8
	7	24.6	22.3	31.9	45.0
	8	31.0	29.2	29.9	32.6
	9	33.9	33.9	33.0	35.3
	10	32.9	34.3	26.5	30.0
	11	33.9	33.9	27.7	30.8
	12	36.7	36.7	25.5	28.2
	13	35.3	30.1	24.0	26.3
	14	26.9	24.6	26.7	38.8
	15	26.0	21.9	35.9	41.5
	16	35.3	32.0	24.6	28.7
Control	1	33.8	32.9	31.6	36.5
	2	31.0	31.5	31.4	30.3
	3	33.1	30.1	27.7	29.0

4	33.8	32.0	30.2	29.3
5	30.4	29.7	32.6	27.2
6	35.3	32.5	25.1	27.7
7	30.5	26.0	24.0	31.2
8	28.3	26.4	26.5	34.9
9	17.0	17.9	31.4	36.8

TOTAL CHOLESTEROL (MG/DL)

GROUPS

WEEKS		PRE	4	5	6	7	8	POST
Training	1	148.7	141.7	147.5	153.3	142.9	136.8	145.6
	2	126.8	128.7	127.7	124.4	131.7	132.3	138.1
	3	179.9	172.1	187.7	202.8	183.0	185.9	169.6
	4	131.8	153.1	149.8	164.5	157.5	157.7	155.4
	5	169.9	172.1	152.1	169.7	166.5	160.0	162.3
	6	147.2	142.5	153.2	158.2	139.8	152.7	147.2
	7	158.1	160.5	154.4	165.8	181.7	161.8	171.1
	8	144.9	133.3	123.5	155.8	146.7	155.8	143.0
	9	188.2	194.3	213.7	206.7	209.3	200.7	210.4
	10	225.3	195.7	225.3	211.2	207.6	207.3	225.5
	11	179.9	159.7	198.9	155.1	156.3	182.2	168.9
	12	155.7	142.5	144.1	157.0	144.4	152.8	163.8
	13	136.9	157.4	176.5	167.0	172.5	175.9	179.2
	14	178.8	130.6	157.9	148.1	162.9	164.8	173.6
	15	160.5	161.7	184.2	177.5	185.9	158.9	178.6

	16	128.2	127.1	138.5	137.8	126.8	141.8	137.8
Control	1	179.9	194.8	182.8	166.1	181.1	178.1	188.1
	2	177.6	167.0	167.4	147.3	172.9	194.1	177.7
	3	169.9	185.6	163.6	160.9	167.8	172.9	165.0
	4	154.1	133.3	148.4	135.6	139.7	153.3	141.4
	5	188.2	184.2	181.6	177.2	185.8	200.8	192.2
	6	177.7	183.0	188.4	177.7	163.8	162.7	171.0
	7	207.6	201.9	226.8	189.7	187.3	191.0	207.4
	8	189.0	197.8	187.0	221.9	219.2	200.4	207.0
	9	143.9	138.5	131.0	122.0	126.7	130.0	138.1

HIGH DENSITY LIPOPROTEIN-CHOLESTEROL (MG/DL)

GROUPS

WEEKS		PRE	4	5	6	7	8	POST
Training	1	58.9	60.5	56.2	69.8	72.5	71.0	67.1
	2	40.8	44.5	50.2	44.1	44.2	48.7	52.1
	3	50.6	57.7	65.7	63.2	67.1	61.9	61.4
	4	69.7	76.9	73.2	78.7	67.1	78.9	79.5
	5	58.7	58.9	60.5	68.5	76.8	67.2	71.5
	6	60.2	60.1	59.3	54.4	59.4	59.3	60.2
	7	63.6	69.2	60.5	56.6	59.4	68.1	72.9
	8	33.7	40.8	40.7	49.9	38.4	42.4	40.2
	9	64.5	72.9	68.0	62.2	64.9	66.8	66.9
	10	76.0	78.6	80.7	67.1	72.5	76.6	82.6
	11	64.9	65.9	73.8	64.9	61.0	73.5	74.2

	12	49.8	49.8	46.7	53.7	46.7	62.0	68.5
	13	48.3	70.7	71.9	79.7	69.9	69.1	75.1
	14	55.1	59.7	52.7	56.3	60.2	61.1	62.4
	15	62.8	66.8	62.7	73.8	69.0	67.0	81.1
	16	51.8	53.8	50.9	54.7	53.9	59.3	59.1
Control	1	73.6	76.9	76.8	70.1	70.4	68.0	78.4
	2	70.5	71.3	69.0	73.1	69.5	67.7	68.2
	3	61.5	69.0	58.9	61.6	62.9	63.4	59.4
	4	64.3	48.0	51.8	52.5	57.8	59.0	54.7
	5	43.6	44.3	45.2	44.6	49.9	43.0	39.2
	6	42.7	54.0	55.8	50.0	46.7	47.0	45.2
	7	81.8	95.0	99.9	87.1	91.9	92.4	72.2
	8	46.0	45.5	46.3	48.6	43.3	60.3	44.9
	9	56.1	61.0	52.5	48.4	49.7	49.8	63.1

HIGH DENSITY LIPOPROTEIN2-CHOLESTEROL (MG/DL)

GROUPS

WEEKS		PRE	4	5	6	7	8	POST
Training	1	30.4	19.8	23.6	36.0	31.1	37.9	33.1
	2	14.4	19.2	18.7	15.6	12.1	15.0	11.9
	3	21.6	24.0	31.7	25.8	33.0	27.0	27.4
	4	39.1	44.2	38.1	44.1	34.1	41.0	46.2
	5	24.0	30.6	23.7	34.7	39.5	30.2	34.9
	6	25.8	26.0	26.4	17.6	28.4	23.1	23.7
	7	30.4	25.8	32.1	18.4	28.4	29.7	34.4

	8	11.5	14.4	14.5	21.6	14.7	10.8	11.4
	9	30.2	33.8	31.2	33.8	33.4	32.2	30.2
	10	33.7	30.3	46.6	27.5	27.3	15.6	35.7
	11	32.2	30.5	35.8	33.7	30.8	28.3	32.7
	12	21.3	19.2	13.0	16.9	17.2	24.4	32.2
	13	17.8	40.6	35.7	44.6	34.5	34.0	38.9
	14	25.6	30.5	20.9	24.7	27.4	27.9	30.1
	15	35.8	32.2	27.3	30.7	32.5	28.5	42.8
	16	27.9	29.3	21.5	23.3	23.5	23.9	26.3
Control	1	31.6	40.9	35.3	35.0	27.5	25.9	36.3
	2	33.6	27.9	36.2	31.9	34.1	22.8	28.3
	3	28.8	25.6	27.9	22.0	25.8	20.6	17.1
	4	35.6	21.4	23.5	20.9	25.3	23.5	23.2
	5	15.2	14.7	18.0	14.8	23.2	12.0	12.2
	6	19.8	18.9	12.1	18.8	11.7	15.6	12.6
	7	66.9	62.7	61.4	52.8	50.4	63.4	35.4
	8	18.5	21.0	18.7	21.0	13.6	32.1	14.9
	9	30.9	37.7	24.9	20.6	22.1	26.1	31.0

HIGH DENSITY LIPOPROTEIN3-CHOLESTEROL (MG/DL)

GROUPS

WEEKS		PRE	4	5	6	7	8	POST
Training	1	28.5	40.7	32.6	33.8	41.4	33.1	34.0
	2	26.4	25.3	31.5	28.5	32.1	33.7	40.2
	3	29.0	33.7	34.0	37.4	34.1	34.9	34.0

	4	30.6	32.7	35.1	34.6	33.0	37.9	33.3
	5	34.7	28.3	36.8	33.8	37.3	37.0	36.6
	6	34.4	34.1	32.9	36.8	31.0	36.2	36.5
	7	33.2	43.4	28.4	38.2	31.0	38.4	38.5
	8	22.2	26.4	26.2	28.3	23.7	31.6	28.8
	9	34.3	39.1	36.8	28.4	31.5	34.6	36.7
	10	42.3	48.3	34.1	39.6	45.2	61.0	46.9
	11	32.7	35.4	38.0	31.2	30.2	45.2	41.5
	12	28.5	30.6	33.7	36.8	29.5	37.6	36.3
	13	30.5	30.1	36.2	35.1	35.4	35.1	36.2
	14	29.5	29.2	31.8	31.6	32.8	33.2	32.3
	15	27.0	34.6	35.4	43.1	36.5	38.5	38.3
	16	23.9	24.5	29.4	31.4	30.4	35.4	32.8
Control	1	42.0	36.0	41.5	35.1	42.9	42.1	42.1
	2	36.9	43.4	32.8	41.2	35.4	44.9	39.9
	3	32.7	43.4	31.0	39.6	37.1	42.8	42.3
	4	28.7	26.6	28.3	31.6	32.5	35.5	31.5
	5	28.4	29.6	27.2	29.8	26.7	31.0	27.0
	6	22.9	35.1	43.7	31.2	35.0	31.4	32.6
	7	14.9	32.3	38.5	34.3	41.5	29.0	36.8
	8	27.5	24.5	27.6	27.6	29.7	28.2	30.0
	9	25.2	23.3	27.6	27.8	27.6	23.7	32.1

HIGH DENSITY LIPOPROTEIN2-CHOLESTEROL TO TOTAL CHOLESTEROL RATIO

GROUPS

WEEKS		PRE	4	5	6	7	8	POST
Training	1	0.20	0.14	0.16	0.23	0.22	0.28	0.23
	2	0.11	0.15	0.15	0.13	0.09	0.11	0.09
	3	0.12	0.14	0.17	0.13	0.18	0.15	0.16
	4	0.30	0.29	0.25	0.27	0.22	0.26	0.30
	5	0.14	0.18	0.16	0.20	0.24	0.19	0.22
	6	0.18	0.18	0.17	0.11	0.20	0.15	0.16
	7	0.19	0.16	0.21	0.11	0.16	0.18	0.20
	8	0.08	0.11	0.11	0.14	0.10	0.07	0.08
	9	0.16	0.17	0.15	0.16	0.16	0.16	0.14
	10	0.15	0.15	0.21	0.13	0.13	0.08	0.16
	11	0.18	0.19	0.18	0.22	0.20	0.15	0.19
	12	0.14	0.13	0.09	0.11	0.12	0.16	0.20
	13	0.13	0.26	0.20	0.27	0.20	0.19	0.22
	14	0.14	0.23	0.13	0.17	0.17	0.17	0.17
	15	0.22	0.20	0.15	0.17	0.17	0.18	0.24
	16	0.22	0.23	0.16	0.17	0.19	0.17	0.19
Control	1	0.18	0.21	0.19	0.21	0.15	0.15	0.19
	2	0.19	0.17	0.22	0.22	0.20	0.12	0.16
	3	0.17	0.14	0.17	0.14	0.15	0.12	0.10
	4	0.23	0.16	0.16	0.15	0.18	0.15	0.16
	5	0.08	0.08	0.10	0.08	0.12	0.06	0.06
	6	0.11	0.10	0.06	0.11	0.07	0.10	0.07
	7	0.32	0.31	0.27	0.28	0.27	0.33	0.17
	8	0.10	0.11	0.10	0.09	0.06	0.16	0.07
	9	0.21	0.27	0.19	0.17	0.17	0.20	0.22

HIGH DENSITY LIPOPROTEIN2-CHOLESTEROL TO HIGH DENSITY LIPOPROTEIN-CHOLESTEROL

GROUPS	WEEKS	PRE	4	5	6	7	8	POST
Training	1	0.52	0.33	0.42	0.52	0.43	0.53	0.49
	2	0.35	0.43	0.37	0.35	0.27	0.31	0.23
	3	0.43	0.42	0.48	0.41	0.49	0.44	0.45
	4	0.56	0.57	0.52	0.56	0.51	0.52	0.58
	5	0.41	0.52	0.39	0.51	0.51	0.45	0.49
	6	0.43	0.43	0.45	0.32	0.48	0.39	0.39
	7	0.48	0.37	0.53	0.33	0.48	0.44	0.47
	8	0.34	0.35	0.36	0.43	0.38	0.25	0.28
	9	0.47	0.46	0.46	0.54	0.51	0.48	0.45
	10	0.44	0.39	0.58	0.41	0.38	0.20	0.43
	11	0.50	0.46	0.49	0.52	0.50	0.39	0.44
	12	0.43	0.39	0.28	0.31	0.37	0.39	0.47
	13	0.37	0.57	0.50	0.56	0.49	0.49	0.52
	14	0.46	0.51	0.40	0.44	0.46	0.46	0.48
	15	0.57	0.48	0.44	0.42	0.47	0.43	0.53
	16	0.54	0.54	0.42	0.43	0.44	0.40	0.45
Control	1	0.43	0.53	0.46	0.50	0.39	0.38	0.46
	2	0.48	0.39	0.52	0.44	0.49	0.34	0.41
	3	0.47	0.37	0.47	0.36	0.41	0.32	0.29
	4	0.55	0.45	0.45	0.40	0.44	0.40	0.42
	5	0.35	0.33	0.40	0.33	0.46	0.28	0.31

6	0.46	0.35	0.22	0.38	0.25	0.33	0.28
7	0.82	0.66	0.62	0.61	0.55	0.69	0.49
8	0.40	0.46	0.40	0.43	0.31	0.53	0.33
9	0.55	0.62	0.47	0.43	0.44	0.52	0.49

Appendix E

QUESTIONNAIRES AND CONSENT FORM

Questionnaire 1

General Information:

1. Do you smoke?

____ yes

____ no

If yes, how many cigarettes per day? _____

2. Do you consume any alcohol?

____ yes

____ no

If yes, what is your average consumption per day or per week (please specify)?

3. Are you presently following a diet (i.e. weight reducing, vegetarian, lacto-ovo (dairy products but no meat)?

____ yes

____ no

If yes, please specify the type of diet.

4. Are you presently taking any medication (including oral contraceptives)?

____ yes

____ no

If yes, please specify the type of medication, length and frequency of intake.

If no, have you ever used any oral contraceptives? Specify when.

Menstrual Pattern:

5. Do you menstruate regularly?

____ yes

____ no

6. Can you recall the dates of onset of your last 3 cycles (i.e. first day of bleeding)?

   If yes, please indicate the actual dates.

   If no, can you provide an approximation of the length of your menstrual cycle.

7. Do you or have you ever experienced episodes of:

   ____ amenorrhea (no menstruation for at lest 3 consecutive cycles)

   ____ oligomenorrhea (skipped one cycle)

   ____ other related menstrual disorders

   If yes for any of the above, please elaborate.

Physical Activity:

8. Are you presently participating in a regular training program (i.e. 3-4 times/week, 30 minutes/session)?

   ____ yes

   ____ no

   If yes, specify the type of activity, number of days per week, duration of sessions.

   If no, when was the last time (if ever) you participated in such a program?

9. Do you practise any type of physical activity?

   ____ yes

   ____ no

   If yes, give details on the type, frequency and duration of the activity.

General Health:

10. Have you ever been told by your doctor that you have cardiac problems?

11. Have you ever experienced any chest pain?

12. Do you ever feel dizzy or faint?

13. Have you ever been told by your doctor that you have any bone or joint problems (i.e. arthritis)which may be aggravated by exercise?

14. Do you know of any physical reason not mentionned above which forbids you to participate in a physical training program?

15. When was your last medical examination?

Questionnaire 2

SINCE ENTERING THE STUDY:

1. Have you started to smoke?

   ---- yes

   ---- no

2. Do you consume any alcohol?

   ____ yes

   ____ no

   If yes, specify your average consumption per day or per week.

3. Has your daily food intake changed (i.e. types of foods and quantities)?

   ____ yes

   ____ no

   If yes, please elaborate.

4. Have you, throughout the course of the study, been following a diet (i.e. deliberately reducing or increasing your food intake)?

   ____ yes

   ____ no

5. Are you taking any oral contraceptives, or other medication?

   ____ yes

   ____ no

   If yes, specify the type of medication and when started.

6. Have you observed any change in your menstrual cycle

(which may have been caused by the training program) (i.e. length of menstrual cycle, number of days of bleeding, cramps...)?

____ yes

____ no

If yes, please elaborate.

7. Have you undertaken any type of regular training program apart from your participation in the study?

____ yes

____ no

If yes, please give details.

Consent Form

I, , understand by signing this consent form, that I will be subject to the following exercise and experimental protocols.

I understand that my weight, height, four skinfold and four circumference measurements will be taken at two intervals throughout the study, and that I will perform two maximal oxygen consumption tests on a bicycle ergometer. The tests consist of pedalling to "exhaustion" at a set speed during which the workloads will be increased at predetermined intervals. Following this test (after a 15 minute rest), I will complete a ride to exhaustion at the last workload completed in the progressive phase. This is to ascertain that a VO2 max value is obtained. Heart rates will be monitored throughout.

I understand that seven blood samples will be drawn by a qualified technician.

I understand that I may be assigned to the control or exercising group. If assigned to the control group, I will not take part in the exercise program. If assigned to the exercising group, I will be training on a bicycle ergometer 3 times a week, 35 minutes per session for eight weeks, with heart rates ranging from 60% to 70% of VO2 max.

I understand that just as with other types of exercise and fitness tests, there are potential risks such as lightheadedness, fainting, chest discomfort, fatigue... In agreeing to participate in such an experiment, I waive any legal recourse against the members of the Department of Kinanthropology (staff and/or students) from any and all claims resulting from personal injuries sustained or death resulting from the exercise or experimental protocols. This waiver shall be binding upon my heirs and my personal representatives.

Signature:

Date: