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**EFFECTS OF EXERCISE TRAINING ON LIPID METABOLISM IN ELDERLY
MEN**

University of Arizona

M.S. 1983

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EFFECTS OF EXERCISE TRAINING
ON LIPID METABOLISM
IN ELDERLY MEN

by

Tamara Teague Baker

A Thesis Submitted to the Faculty of the
DEPARTMENT OF NUTRITION AND FOOD SCIENCE
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE

In the Graduate College
THE UNIVERSITY OF ARIZONA

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ABSTRACT

Thirty five sedentary men 50-73 years old participated in a 20 week randomized study. The study was designed to determine effects of aerobic exercise on plasma lipoprotein profile. Twenty were assigned to a running program and 15 served as controls. Fasting plasma samples were collected before and after a 20 week exercise program. Plasma lipoproteins were separated by ultracentrifugation and agarose-column chromatography into very low (VLDL), intermediate (IDL), low (LDL) and high (HDL) density lipoprotein fractions. A definite training effect was evident from significant increases in $\dot{V}O_2$ max and treadmill time observed in exercised subjects. Lipoprotein alterations in the runners indicated a significant increase in HDL-cholesterol and significant decreases in LDL-cholesterol, total triglyceride, IDL-protein and LDL-protein. The present study demonstrates that moderate physical training can increase HDL-cholesterol and alter other lipoprotein components and may subsequently reduce the risk of coronary artery disease (CAD).

INTRODUCTION

Research has identified lipoprotein abnormalities as a major risk factor in several human diseases. Lipoprotein disorders rarely cause serious illness in infancy or childhood, but predominantly surface later in life in connection with illness and death among the middle aged and elderly populations (Gordon et al., 1977; Havel et al., 1980). Currently, lipoprotein disorders are most frequently associated with coronary artery disease (CAD). CAD disease is the most common type of heart disease and the leading cause of death in the United States as well as many other countries (Albers et al., 1978; Rapaport, 1980; Miller and Miller, 1975).

Lipoproteins are divided into five major classes or fractions: chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Until recently, only the LDL fraction was linked with CAD (Ernest and Levy, 1980). LDL are the major carriers of cholesterol through the bloodstream. They deposit this cholesterol in the tissues and blood vessels of the body. Elevated levels of LDL gradually weaken the

strength of the arterial vascular system through the accumulation of lipids (predominantly cholesterol) on the arterial wall. This, in combination with a localized connective tissue and smooth muscle cell migration reaction, results in a partial to complete blockage of blood flow through the vessels. The reduction in blood flow signals an increase in cardiac work load eventually leading to a heart attack or myocardial infarction (MI) if not relieved (Rappaport, 1980).

In the past, manipulation of the LDL level was the focus of lipoprotein research and CAD prevention. Recently, another lipoprotein fraction, the HDL were found also to influence CAD (Albers et al., 1978). HDL are thought to remove cholesterol from the tissues and transport it through the body to the liver where it follows an excretory pathway (Mahley, 1981; Wood and Haskell, 1979; Krauss et al., 1977; Kiens et al., 1980). It is hypothesized that elevated levels of HDL-cholesterol (HDL-c) in the bloodstream may decrease the risk of CAD, while depressed HDL-c values may significantly increase the risk of CAD. The risk is highest in men 50 years and older with low HDL-c values (Wood et al., 1976; Shephard and Kavanah, 1978; Aniansson et al., 1980). As a result, researchers have concentrated their efforts in identifying ways to increase HDL-c. Moderate levels of aerobic exercise to increase HDL-c have been suggested as a possible strategy. Although several

cause-effect relationships have been shown, the correlation between HDL-c, aerobic exercise, and CAD is still under dispute.

The objective of this study was to gain insight on the relationship between aerobic exercise, lipoproteins, and CAD. The research presented here was designed to determine the effects of aerobic exercise on lipoprotein metabolism in elderly men.

LITERATURE REVIEW

Lipoproteins

Lipoproteins are spherical particles consisting of simple proteins combined with lipid components. The components are made up of cholesterol, phospholipids and triglycerides. All lipoproteins appear to possess a similar fundamental structure. That structure includes a non-polar, hydrophobic lipid core with a more polar surface coat of phospholipids and a globular apoprotein region embodied in the lipid core (Alaupovic, 1981).

Research on the protein components of lipoproteins was sparse until the late 1960's. At this point advanced procedures of solubilizing, separating, and characterizing proteins were perfected. As a result of this development, the role of proteins in lipid transport systems was more easily studied. Eight different apoproteins are now known to exist in the lipoprotein fractions (Havel et al., 1980). In addition to regulating specific metabolic functions of the lipoprotein, the apoproteins may direct the interaction of lipoproteins with cell surface receptors of specific cells (Mahley, 1981b).

Inside the surface coat of proteins and phospholipids is the nonpolar lipid core. This core consists primarily of triglycerides (TG) and cholesteryl esters. As the density of the lipoproteins increase, the cholesteryl ester content is also increased (Ernest and Levy, 1980; Mahley, 1981b; Levy, 1978).

The TG in the lipid core are produced in the endoplasmic reticulum of cells in the liver and small intestine. The rate of secretion of TG is dependent on the supply of fatty acids from intestinal fat absorption. TG are the principle form in which long-chain fatty acids are stored in the body. TG are also the principle form for the transportation of these fatty acids from the liver and intestine to the blood circulation (Alaupovic, 1981).

The other major component of the lipid core is the cholesterol or the cholesteryl ester component. Cholesterol serves three major functions in humans: 1) it is required in structural components of the plasma membrane of all cells; 2) it is a precursor for steroid hormones; and 3) it is a precursor for bile acids. Cholesterol exists in both the unesterified form (free cholesterol) or as an ester of a long-chain fatty acid. The cholesteryl

ester appears only to be synthesized for purposes of cholesterol transport through plasma and storage within cells. Plasma lipoproteins function to regulate cholesterol equilibrium on a cellular level (Havel et al., 1980).

Homeostasis of total body cholesterol is regulated by the liver. When dietary intake of cholesterol is low, the liver and intestine account for as much as 95% of the total endogenous cholesterol production (Dietschy and Wilson, 1970). When present in adequate amounts, dietary cholesterol depresses endogenous cholesterol synthesis. The secretion of bile acids by the liver provides the major route for excretion of excess cholesterol (Glomset, 1980).

The amount of cholesterol or TG within a lipoprotein varies from fraction to fraction. Lipoprotein fractions can be separated according to their size (by gel filtration), density (by ultracentrifugation), net surface charge (by electrophoresis), or other surface properties (precipitation and absorption techniques) (Ernest and Levy, 1980). Lipoprotein complexes can be divided into five major classes or fractions: chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). Approximate composition of these lipoproteins is shown in Figure 1.

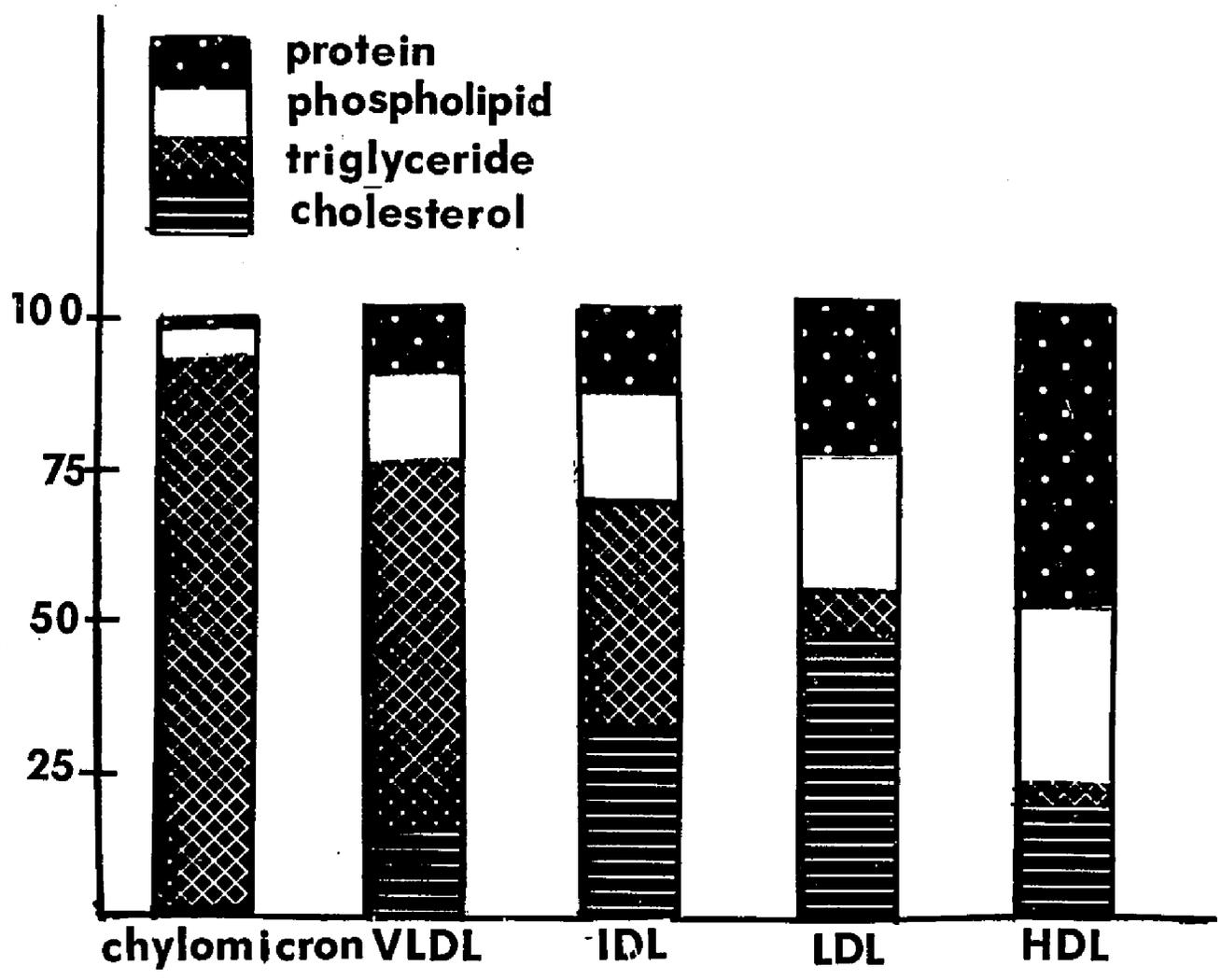


FIGURE 1
APPROXIMATE % COMPOSITION
OF LIPOPROTEINS

Chylomicrons are the largest and lightest of the lipoproteins. The bulk of the chylomicron is composed of triglycerides (80-95% by weight), originating entirely from exogenous (dietary) fat. The remaining 15-20% of the chylomicron is composed of cholesterol (2-7%), phospholipids (3-6%), and protein (1-2%) (Ernest and Levy, 1980). Chylomicrons are synthesized in the intestine and serve as a carrier for the transportation of dietary glycerides from the intestinal mucosa through the thoracic duct to the tissues (Havel, 1980).

Fat is ingested and emulsified, hydrolyzed into smaller fragments by the pancreatic lipase in the intestine, and these are solubilized in micelles by conjugated bile salts. Resynthesized fat is then associated with protein, cholesterol, and phospholipids in the cells of the intestine to form chylomicrons which enter the bloodstream via the lymphatics (Havel et al., 1980). Chylomicrons are normally the most abundant in the bloodstream after a meal, giving the plasma a milky appearance. The lipoproteins and TG are united and secreted by intestinal or liver cells into the bloodstream. After serving their transport function these lipoproteins are degraded and cleared from the body. The presence of chylomicrons in the fasting state (12-16 hours after a meal) indicates a defective handling of dietary fat and should be considered abnormal (Havel et al., 1980).

The VLDL are the second lightest and second largest of the lipoproteins. They are composed of approximately 55-65% TG (endogenous), 10-15% cholesterol, 15-20% phospholipids and 5-10% protein. A small amount of VLDL is present in the plasma during normal fasting. The presence of VLDL in the bloodstream will give a cloudy appearance to the blood. The triglyceride portion of VLDL is derived from the liver. The liver synthesizes glycerides de novo primarily from absorbed carbohydrates and fatty acids mobilized from adipose tissue (Alaupovic, 1981). Although the precise mechanism of degradation for VLDL is not yet established, it is generally thought that the VLDL are eventually degraded into IDL and then LDL through a complex metabolic process. High levels of VLDL are associated with obesity, glucose intolerance, hyperuricemia, and premature atherosclerosis (Havel et al., 1980). Both exogenous and endogenous triglycerides must be cleared from the plasma. This occurs predominantly in the liver, adipose tissue, and muscle where the glycerides are stored or metabolized.

Lipoprotein lipase (LPL) is probably active in the breakdown of the large glyceride into smaller more soluble lipoproteins and fatty acids. Lipoprotein lipases catalyze the hydrolysis of TG in TG-rich lipoproteins. The broken down monoglycerides are either taken up into parenchymal

cells of the tissues close to the site of hydrolysis or are bound to plasma albumin and transported to other tissues (Ernest and Levy, 1980).

Removal of the bulk of TG from the core of TG rich lipoproteins, produces remnant particles with an excess of surface material. The excess of surface components are rapidly removed, and the remnant particle remains spherical. Some of the removed surface material, particularly the apoproteins C and E, are transferred to HDL during the hydrolytic process. Some of the surface phospholipids are also transferred to HDL, possibly in the same complex. As the size of the lipoprotein remnant diminishes due to LPL activity, its density increases and the particle is converted to IDL. The HDL particles interact with the plasma enzyme lecithin cholesterol- acyltransferase (LCAT) which esterifies excess cholesterol and transfers it to the IDL particle. The net result of this entire process is the replacement of TG with cholesteryl esters (Krauss, 1982).

The IDL fall in between the VLDL and LDL in size and density. They are normally not considered a separate lipoprotein class, but rather as an intermediate between VLDL and LDL. IDL are composed of approximately 40% TG, 30% cholesterol, 20% phospholipids, and 10% protein. IDL are considered an intermediate step rather than a separate lipoprotein class because they are rarely

present in the body for more than 2-6 hours (Ernest and Levy, 1980). Elevated plasma IDL levels are considered abnormal, and are associated with premature atherosclerosis, glucose intolerance, and hyperuricemia (Havel, 1980).

Once the conversion of VLDL to IDL is complete, the IDL particles are released from the capillary wall into circulation. They undergo further conversion in which most of the remaining TG are removed, and all apo-proteins are lost except B. The completion of this process results in the formation of LDL.

LDL are slightly heavier and smaller than IDL and are composed by weight of approximately 45% cholesterol, 22% phospholipids, 10% TG, and 25% protein. Clearance of LDL from the body primarily takes place through the liver. LDL are the principal carriers of cholesterol through the bloodstream (approximately 80%) and function in transporting and depositing cholesterol to peripheral tissues. Elevated levels of LDL are associated with premature atherosclerosis, and corneal arcus (Albers et al., 1978; Goldstein and Brown, 1977). LDL delivers cholesterol to extrahepatic cells and the liver by binding to high affinity receptors located in regions of the plasma membrane. At the binding site, cholesteryl esters are hydrolyzed by an acid lipase and apoprotein B is degraded

to amino acids. The liberated cholesterol from this reaction leaves LDL for use in cellular reactions. This pathway is known as the high affinity pathway (Brown et al., 1981).

When plasma LDL levels are high, another pathway, the scavenger pathway is utilized. In this pathway scavenger cells are used to degrade the excess LDL. When scavenger cells are overloaded with cholesteryl esters they are converted to "foam cells", the classic components of atherosclerotic plaques. In man, between 33-66% of the LDL is degraded by the high affinity pathway, the remaining LDL is degraded by the scavenger cell system (Brown et al., 1981).

In the steady state, the tissues excrete cholesterol into the plasma in amounts equal to the quantity taken up from LDL. The cholesterol leaving the tissue is believed to be absorbed onto HDL. The bulk of the HDL mass appears to be from the interaction of precursor particles (nascent HDL) secreted by the liver and intestine with lipids and proteins released during the catabolism of TG-rich lipoproteins. HDL carries roughly 20% of the cholesterol in the bloodstream. HDL is composed of approximately 45-50% protein, 30% phospholipids, and 20% cholesterol. HDL functions mainly in cholesterol and phospholipid exchange and esterification reaction within the plasma,

with the help of LCAT. HDL is also thought to serve as a transport mechanism for cholesterol from the peripheral cells to the liver, the major site of cholesterol excretion (Krauss, 1982). If HDL were allowed to react with LCAT in the presence of red cell membranes, a net transfer of unesterified cholesterol occurs from the membranes to the depleted HDL.

The fate of HDL is unknown. Human skin fibroblasts absorbing HDL by endocytosis appear to account for some of the degradation. The liver may also be partially responsible for degradation (Glomset, 1980; Mahley, 1981b).

Present research indicates that lipoproteins are one of the means by which glyceride and cholesterol balance is achieved. An imbalance in this glyceride and cholesterol metabolism may increase the risk of CAD (Goldstein and Brown, 1977; Mahley, 1981; and Miller, 1980).

Lipoproteins and Coronary Artery Disease

The atherosclerotic lesion consists of a plaque-like thickening of the intima, the inner most layer of the artery wall. The thickening is composed of proliferating smooth muscle cells surrounded by large amounts of interstitial substance that include collagen, elastin, and fibrin. Surrounding and within the smooth muscle

cells, massive amounts of lipids (primarily cholesteryl ester) are deposited. This lipid substance gives the atheroma its menacing character. The cholesterol in the atheroma has been suggested as the component primarily responsible for the damage generated from the lesion (Havel et al., 1980).

In human populations in which the mean plasma cholesterol is lower than about 160 mg/dl, symptomatic coronary atherosclerosis does not frequently develop even when other risk factors are present. Epstein et al. (1971) indicated that the probability of an individual developing a MI increases in proportion to his plasma cholesterol level.

Current evidence indicates that the cholesterol components of these atherogenic lesions originates from plasma lipoproteins, especially LDL. The most prevalent theory states that the primary lesion in atherosclerosis involves an injury to the endothelial cells that line the arterial intima. The endothelium's barrier system begins to break down, and the underlying subendothelial connective tissue becomes exposed to platelets and other blood elements (French, 1966). The platelets adhere to subendothelial collagen, aggregate, and release the contents of their granules (Ross and Glomset, 1976).

As the breakdown in the endothelium continues, large amounts of plasma constituents, including lipoproteins, penetrate the artery wall to the subendothelial layer. The aggregated platelets are thought to release factors that stimulate smooth muscle cells in the arterial media to divide. The proliferating smooth muscle cells migrate into the inner layer (the intima) where they appear to remove the foreign plasma constituents by endocytosis. The cholesterol released from the hydrolysis of LDL cannot leave the smooth muscle cells unless it is carried by a molecule that can solubilize it for transport into the plasma. Because of this, transport of cholesterol out of the smooth muscle cell is relatively slow. Faced with a load of cholesterol that it cannot excrete, the smooth muscle cell esterifies some of it for storage as cholesteryl esters (Goldstein and Brown, 1977).

The role of VLDL and TG in atherosclerosis and CAD is unclear. Evidence has been found to both support and disclaim the theory that VLDL-TG levels positively correlate with CAD (Alaupovic, 1981; Glomset, 1980; Brown et al., 1981).

It is suggested that the HDL-c may play a protective role in the atherosclerotic process through transport of cholesterol out of the cell. Metabolic studies in humans have suggested an inverse correlation between

the size of total body cholesterol pools and plasma HDL-c levels (Miller and Miller, 1975). Under certain conditions HDL-c can enhance the removal of cholesterol from cell membranes leading to the hypothesis that HDL may protect against atherosclerosis by facilitating the removal of cholesterol from smooth muscle cells in the arterial wall (Havel et al., 1980).

Throughout the life cycle, men and women who performed vigorous aerobic exercise and possessed higher HDL-c levels may have a much lower frequency of developing CAD compared to those who did not (Gordon et al., 1977 and Gofman et al., 1966).

Acute MI is associated with several hormonal and metabolic disturbances which are reflected as changes in the concentration of many serum components, particularly the lipoproteins. A significant decrease in HDL-c levels immediately following an MI, followed by a return to the initial level after three months, have been shown in a number of studies (Ronnemaa et al., 1980; Albers et al., 1978; Miller and Miller, 1975; Hulley et al., 1978; Castelli et al., 1977). A correlation was sometimes found between the magnitude of the MI and the decrease of HDL-c (Ronnemaa et al., 1980; Brunner et al., 1980). In those free of heart disease, elevated HDL-c levels were commonly seen and appeared to serve in a protective role. Individuals who

regularly exercised had high HDL-c. Even in those with heart disease, large amounts of exercise significantly increased HDL-c levels at a rate faster than non-exercisers with heart disease (Castelli et al., 1977). These observations lead to the hypothesis that exercise may protect against MI and other clinical attacks of CAD throughout the life cycle (Morris et al., 1973). Even in men 80 years and older who exercised, the prevalence of degenerative peripheral vascular disease was significantly less and higher HDL-c levels were observed in cross sectional studies.

Increasing evidence supports the hypothesis that HDL may promote the removal of cholesterol from cells into the blood and thereby promote excretion of cholesterol from the body via the liver. Methods to increase the transport of cholesterol through the catabolic pathway may have a potential therapeutic value. In controlling lipoprotein, cholesterol, and TG equilibrium, however, a number of other conditions, both exogenous and endogenous come into play.

Influencing Factors on Lipoprotein Metabolism

In looking at lipoprotein differences and abnormalities a number of conditions are recognized as possible influences on lipoprotein levels. Smoking, race, sex, hormones, drugs, diabetes, body weight, diet, age

and exercise are all identified as factors that may alter lipoprotein values.

Smoking has been shown in a number of studies to lower the level of HDL-c in the bloodstream (Hulley et al., 1978; Garrison et al., 1978; Rabkin et al., 1981; Enger et al., 1977). The evidence is not concrete however, due to studies showing no alterations in HDL-c with smoking (Gordon et al., 1977). In general, the effect of smoking on lipoproteins can only be an estimate because the effect of smoking cannot be isolated from the factors previously mentioned. The mechanisms by which smoking alters lipoprotein metabolism are unknown.

Racial variations also revealed lipoprotein diversity. In looking at Black, Japanese and White populations, those with CAD had lower HDL-c, and higher LDL-c, total cholesterol and TG when compared with baseline levels within their respective populations (Tyroler, 1975; Castelli et al., 1977). Comparisons between the three races showed Blacks to have higher HDL-c values and lower incidence of heart disease (Tyroler, 1975).

Within the same race, women showed a higher HDL-c and lower incidence of heart disease than men. Those women regularly participating in aerobic exercise showed the highest HDL-c as well as the lowest LDL-cholesterol (LDL-c) and total cholesterol levels (Wood et al., 1977).

When compared with their male counterparts in a short term aerobic exercise program, the plasma lipoprotein values of women showed little increase if any with aerobic exercise, while a definite elevation in HDL-c was observed in most men undergoing similar training (Brownell et al., 1982; Frey et al., 1982). Although the mechanism is not known, hormonal differences (primarily estrogen) are suggested as a possible explanation for the diversity. Treatment with gonadal hormones or hormonal derivatives may exert marked effects on serum HDL-c. Studies on estrogen and progesterone therapy in women have shown an increase in HDL-c associated with estrogen usage and a reduction in HDL-c and TG with the combined estrogen and progesterone therapy. In addition, oral contraceptive therapy generally elevates HDL-c in women. However, oral contraceptives exert no effect on serum lipoproteins in diabetic women (Gordon et al., 1977; Krauss et al., 1978).

A number of other drugs are associated with low levels of HDL-c. These drugs are commonly used in the treatment of hyperlipidemia, hypertriglyceridemia, osteoporosis and heart disease. An excellent review on the influence of pharmaceuticals on HDL-c was recently published (Krauss, 1982). Hormones such as insulin,

thyroxin, glucagon, epinephrine and adrenal and pituitary factors may also interact with the lipoproteins and subsequently alter their levels by increasing or decreasing lipogenesis and or lipolysis in liver or adipose tissue (Ernest and Levy, 1980).

It is well established that insulin dependent diabetes is associated with increased incidence of atherosclerosis and excessive mortality from CAD (Nikkila, 1981). The degree to which HDL-c and other lipoproteins are involved in the etiology of this disease is not yet known. Untreated insulin-dependent diabetics show a strong negative correlation to HDL-c in most cases (Gordon, 1977). These values return to normal range however, after insulin therapy (Nikkila, 1981). HDL-c concentrations are regulated by two endothelial lipolytic enzymes, LPL and hepatic lipase (HL), both of which are insulin sensitive. Insulin treatment results in the elevation of tissue LPL, and plasma HDL-c levels (Nikkila, 1981).

In diabetics who are not dependent on insulin low HDL-c values are also common, but are associated more with obesity and hypertriglyceridemia than with the diabetic state itself. Obesity is a parameter closely linked to energy balance and HDL levels. Lower plasma levels of HDL-c have been observed in obese individuals

in comparison to non-obese individuals. In addition a number of studies have shown no change in HDL-c or been negatively correlated to weight loss in obese subjects (Keins et al., 1981; Rabkin, 1981; Krauss, 1982). The metabolic basis for the reduction in HDL-c in obesity is still under investigation.

The importance of specific dietary components in the regulation of serum lipid levels is well established. Glueck and Morrison (1981) suggested that the development of atherosclerosis may relate back to nutritional patterns of early childhood. The development of atherosclerosis was primarily mediated by the dietary influence on lipid and lipoprotein metabolism. Furthermore, animal studies restricting dietary cholesterol early in life significantly decreased the incidence of atherosclerosis later in life. There is little information regarding the effects of these components on HDL. Dietary modifications have primarily dealt with fat intake. Studies have shown a positive correlation between high saturated fat diets, plasma total cholesterol, and LDL-c. Results for HDL-c however, have varied (Dwyer et al., 1981; Kritchevsky, 1976; Shephard et al., 1978, Kiens et al., 1981; Johnson et al., 1982; Kramsch et al., 1981). High polyunsaturated fat diets were negatively correlated to plasma total cholesterol, LDL-c, and HDL-c (Vessby et al., 1980; Shepherd et al., 1978).

Research on protein sources of diets were also investigated. Higher total cholesterol and HDL-c levels were observed in rats fed casein (animal protein) as compared to those fed soy (plant protein). However, the HDL-c/total cholesterol ratio was not significantly altered in either diet (Park and Liepa, 1982). In addition, studies in humans comparing vegetarian and non-vegetarian populations provided similar findings (Sacks et al., 1975).

High complex carbohydrate diets were negatively correlated to HDL-c, some studies also noted a marked increase in TG as well (Dwyer et al., 1981; Amerychx et al., 1981; Schonfeld et al., 1976; Kritchevsky, 1976).

Coffee consumption provided mixed results in regard to HDL-c levels, while alcohol consumption and HDL-c showed a significant positive correlation (Yano et al., 1977; Krauss et al., 1977). Alcohol consumption was also positively correlated with TG levels (Krauss et al., 1977; Johansson and Medhus, 1974). In summary, diet modifications appear incapable of modifying the HDL-c/total cholesterol ratio, although they can lower the LDL-c level.

Identifying methods of increasing the level of HDL-c in the body becomes even more significant as individuals grow older. A steady decrease in HDL-c has been observed with age. In the older male population, low HDL-c levels are the major risk factor for coronary disease (Gordon et al., 1977). Although research is limited in this area,

the most consistent exception to low HDL-c levels is found in older individuals partaking in regular aerobic exercise. These individuals showed significantly higher HDL-c and significantly lower level of degenerative peripheral vascular disease (Enger et al., 1977; Schneider et al., 1980).

Lipoproteins and Exercise

Improved psychological disposition, increased muscle strength and a greater aerobic capacity have been demonstrated in numerous aerobic exercise training studies (Froelicher et al., 1980; Shephard, 1978; and Spirduso, 1980). Moderate aerobic exercise training is thought to reduce the deterioration of physiological capacity by an average of eight to nine years (Shephard, 1978). Fewer electrocardiogram (ECG) abnormalities (Epstein et al., 1976), enhanced cardiac performance (Stein et al., 1978) and increased work capacity and maximum O₂ consumption were all seen after aerobic exercise training (Frick et al., 1970).

In animals, intense exercise training has resulted in an increase in blood flow and cardiac and arterial collagen metabolism in rats and mice, respectively (Heikkinen and Vvori, 1972; Spear et al., 1978). In addition, aerobic exercise resulted in an increase in the diameter of the coronary arteries and heart size, as well as improved hemodynamics in monkeys (Kramsch et al., 1981).

Exercise may serve only to improve the muscular and vascular activity of the heart, or it may contribute to decreasing the risk of CAD through positive influences on serum lipids (Froelicher et al., 1980).

Exercise and HDL-c first received emphasis after the classic paper of Miller and Miller (1975) compiling evidence indicating that low levels of HDL-c are strongly associated with increased risk of CAD in man. Based on the assumption that exercise could be effective in the prevention of CAD through increasing HDL-c, a series of aerobic exercise studies were conducted. These experiments were of two natures; cross sectional and longitudinal.

Many of the cross sectional studies comparing populations such as distance runners (Wood et al., 1977; Lehtonen and Vikkeri, 1978a; Lehtonen and Viikari, 1978b), cross country skiers (Lehtonen and Viikari, 1978b; Enger et al., 1977) and lumberjacks (Lehtonen and Viikari, 1978a) to their sedentary peers showed the exercising individuals to have significant compositional differences in their plasma lipoprotein fractions and subfractions. It was hypothesized that the intensity and duration of aerobic exercise may be positively correlated with the amount of increase in HDL-c (Myhre et al., 1981). Cross sectional studies, however, cannot eliminate the influences of variables such as diet, relative body weight, other serum lipids and genetic endowment. These variables may lead

to both a high capacity for physical exercise and high HDL-c levels (Krauss, 1982).

One method of minimizing variables is by conducting longitudinal training studies. These studies, conducted on healthy sedentary individuals, measure the effect of increased physical activity on plasma lipoprotein concentrations. Although longitudinal studies may not control all variables, they are able to provide some degree of uniformity in training levels, initial level of adiposity, diet and lifestyle; factors that would influence lipoprotein levels. Longitudinal studies that have a control group in addition to the training group further minimize variables by providing a means of measuring external changes such as seasonal variations. Several longitudinal aerobic exercise studies on males demonstrated increases in HDL-c and alterations in the other serum lipid fractions (Huttunen et al., 1979; Kiens et al., 1980; Wood and Haskell, 1979; Brownell et al., 1982). These results were similar to those seen in many of the cross sectional studies. There have been longitudinal studies however, which found no changes or decreases on HDL-c with aerobic exercise (Lipson et al., 1980; Nye et al., 1981; Hørby-Petersen et al., 1982; Frey et al., 1982).

Although longitudinal studies control for many of the obvious variables, they may not adequately regulate technical variables. Poor experimental design, inadequate

intensity and duration of training, and laboratory procedures lacking the desired level of sensitivity could all drastically affect lipoprotein fractions and components.

As a result of the the forementioned variables, the outcome of exercise on lipoprotein metabolism is unresolved. The possible relationship between elevated HDL-c levels and a low risk of CAD makes additional knowledge in the exercise lipoprotein area extremely valuable. This is particularly true in the male population 50 years and older. The major risk factor for CAD in this population is low HDL-c. After careful assessment of past investigations, this research was designed to minimize previously mentioned variables.

MATERIALS AND METHODS

Subjects

Over one hundred and fifty male applicants 50 years and older from the Tucson community responded to newspaper advertisements soliciting sedentary volunteers for a controlled exercise training program. Telephone interviews of these subjects produced a group of nearly 90 males possibly eligible for the study. Those eligible attended one pre-screening orientation meeting and two baseline visits to the Exercise Physiology Laboratory at the University of Arizona. Informed consent, medical clearance forms, photographic releases, pre-exercise health forms and activity questionnaires were all completed by each subject prior to testing to screen out subjects contraindicated by health problems.

Eligibility requirements for participation in the study were listed in the informed consent forms (see Appendix B).

Forty sedentary men age 50-73 years old were eligible according to these criteria. Fifteen were randomly assigned to the control group and twenty five to the training group. Due to apathy, conflicting work schedules and illness, five members of the training group

Table 1. Baseline Characteristics of Exercise and Control Groups¹

Baseline	Treatment Group	Value
Height (cm)	Ex	178.04 \pm 1.64
	C	174.85 \pm 1.61
Weight (kg)	Ex	82.36 \pm 2.67
	C	77.71 \pm 2.34
Age (years)	Ex	57.53 \pm 1.26
	C	58.98 \pm 1.40

¹Mean \pm Standard Error of the Mean (SEM).

failed to complete the exercise program. Fifteen control and 20 exercise subjects completed the entire study. The baseline characteristics of these subjects are given in Table 1.

This study was approved by the Human Subjects Committee of the University of Arizona.

Experimental Design

Following baseline evaluation and assignment to groups, the exercise participants joined a 20 week walk/run aerobic fitness program for approximately one hour/session, three days/week at 6:00 am or 4:00 pm. These sessions consisted of a 15 minute muscle stretching warm up, and 5 minute cool down, and a 45 minute run/walk phase. The training program was progressive in nature so it was not expected for all subjects to run the entire 45 minutes until approximately the sixth week of the study. Subjects maintained individual training heart rates between the range of 70 to 85% of their maximal heart rate throughout their training. Heart rates were calculated using the formula suggested by Karvonen et al. (1957). The peak rate recorded from the two baseline treadmill tests prior to training was assumed to be the maximal value. Exercise heart rates were monitored by the subjects at approximately the 15th, 30th, and 45th minutes of exercise during each training session. Subjects were periodically checked by the supervisor to ensure accuracy

in heart rate determination to maintain the training heart rate. All training sessions were supervised.

Training sessions took place on both grassy and paved terrain. Each subject was asked to report his three forementioned heart rates to the session supervisor immediately following each exercise session. A record was kept on each subject's attendance and heart rate data. Subjects attended an average of 93% of scheduled sessions. All sessions missed were made up by subjects attending one extra supervised exercise session for every day absent, which resulted in 100% attendance. Approximately one half of the way through the training program, two of the exercise subjects sustained injuries. They completed the remaining training sessions on a bicycle ergometer (Model Schwinn Biodyne) working at 90-100 revolutions/minute using a tension appropriate to uphold their training heart rates. The tension was periodically adjusted to maintain the prescribed heart rates.

Subjects reported to the University of Arizona's Exercise and Sport Sciences Laboratory for post-testing approximately 48 hours after the completion of the final training session.

Biochemical Procedures

All subjects reported to the Exercise and Sports Sciences Laboratory both before and after the twenty week

period. Subjects abstained from eating for 12-16 hours before each visit. At each visit 20 ml of venous blood was drawn in vacutainer tubes providing 1.5 mg/ml disodium EDTA, while the subject remained in a supine position. Blood was immediately refrigerated at 4°C.

Lipoprotein Separation

The isolation of plasma began within one hour after blood collection by centrifugation at 2000 g at 4°C for 30 minutes. Ten ml of plasma were drawn off and placed in polycarbonate ultracentrifuge bottles. The solvent density of plasma was then raised to d 1.225 by adding solid KBr (0.3517 g of KBr/ml of plasma). Ten ml of plasma were overlaid with 100 ml of d 1.225 buffered solution containing Na azide (Scanu and Granda, 1966). Tubes were centrifuged in a type 30 rotor for 24 hours at 4°C and 28,000 rpm's in a Beckman model L ultracentrifuge. Tubes were carefully removed and the top 1-2 ml containing the lipoprotein concentrate were pipeted off, placed in a separate tube and refrigerated at 4°C until injected into an agarose column for separation. All samples were injected within 3 days after the plasma-lipoprotein separation.

Columns were prepared by using sepharose Cl-4B (Pharmacia Fine Chemicals) in 2.5 cm internal diameter and 90 cm bed height columns. Less than 75 mg of

lipoprotein cholesterol in a 2-4 ml volume were applied to each column and eluted at 21 ml/hr with 0.1M-NaCl-0.01% EDTA, pH 7.4 (Margolis, 1967). Lipoproteins were separated by size. Fractions of the eluate were collected at 24 minutes per fraction by an LKB fraction collector (Model 2111 LKB, Rockville, MD). The fractions of the column eluate containing individual lipoprotein peaks were collected as indicated in Figure 2A and 2B. The tubes for each lipoprotein fraction were combined and made up to 50, 50, 60 and 80 ml with a saline solution for VLDL, IDL, LDL, and HDL, respectively.

Procedure for Plasma Protein Determination

The protein content of the lipoprotein fractions were measured colorimetrically using the methods of Lowry et al. (1951). Graded levels of protein standards, 0, 25, 50, 100, 150 and 200 mg were used to construct a standard curve. Bovine albumin (A4378, crystallized and lyophilized, Sigma Chemical Company, St. Louis, MO) was used as the protein standard. Plasma protein in solution reacted with copper in alkaline solution. The products formed were used to quantitatively reduce a phosphomolybdic-phosphotungstic reagent. The purple color complex was quantitated on a Coleman Model 6120 Junior II spectrophotometer.

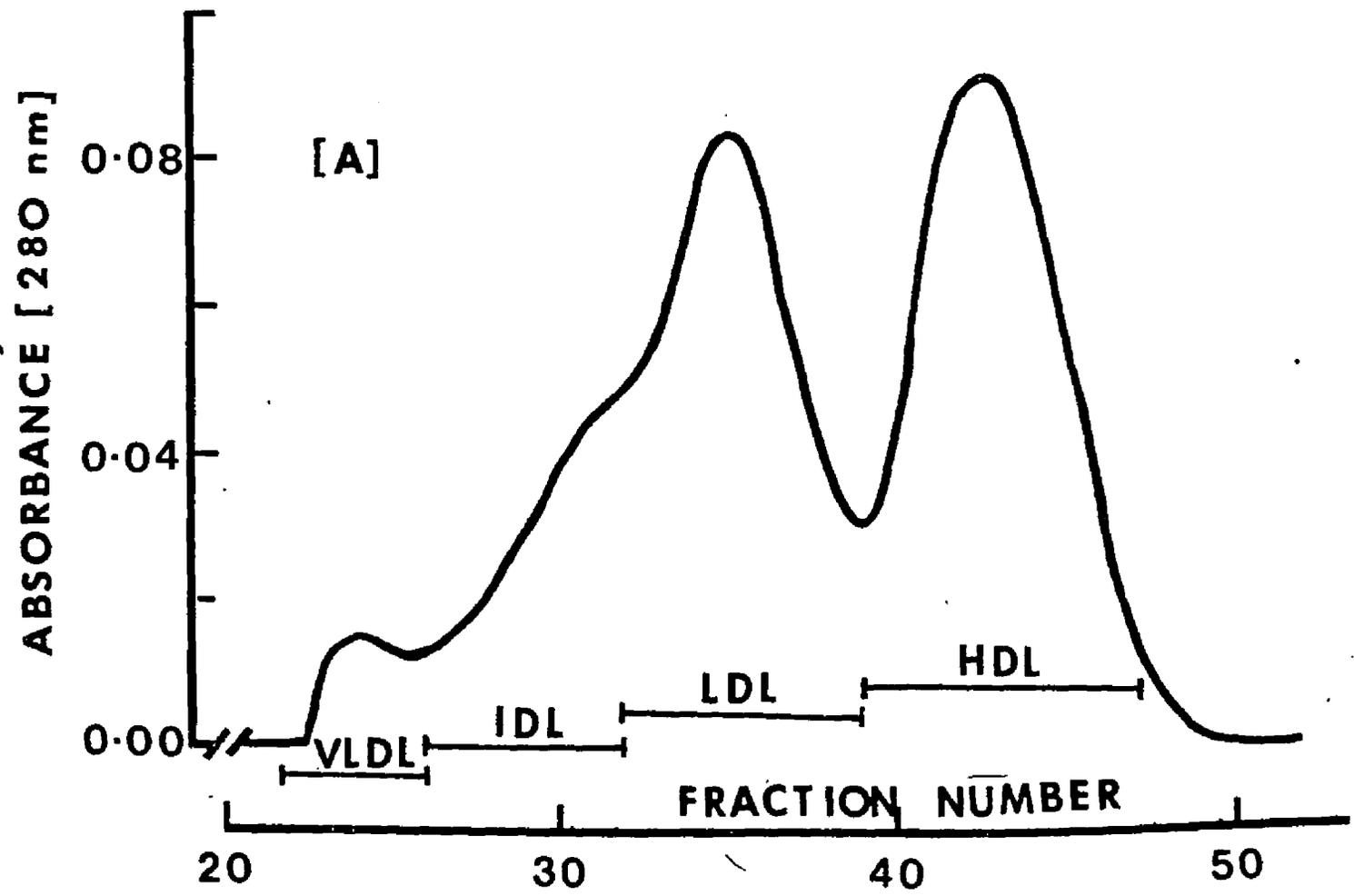


Figure 2A. Characteristic Lipoprotein Separation Prior to Aerobic Exercise Program.

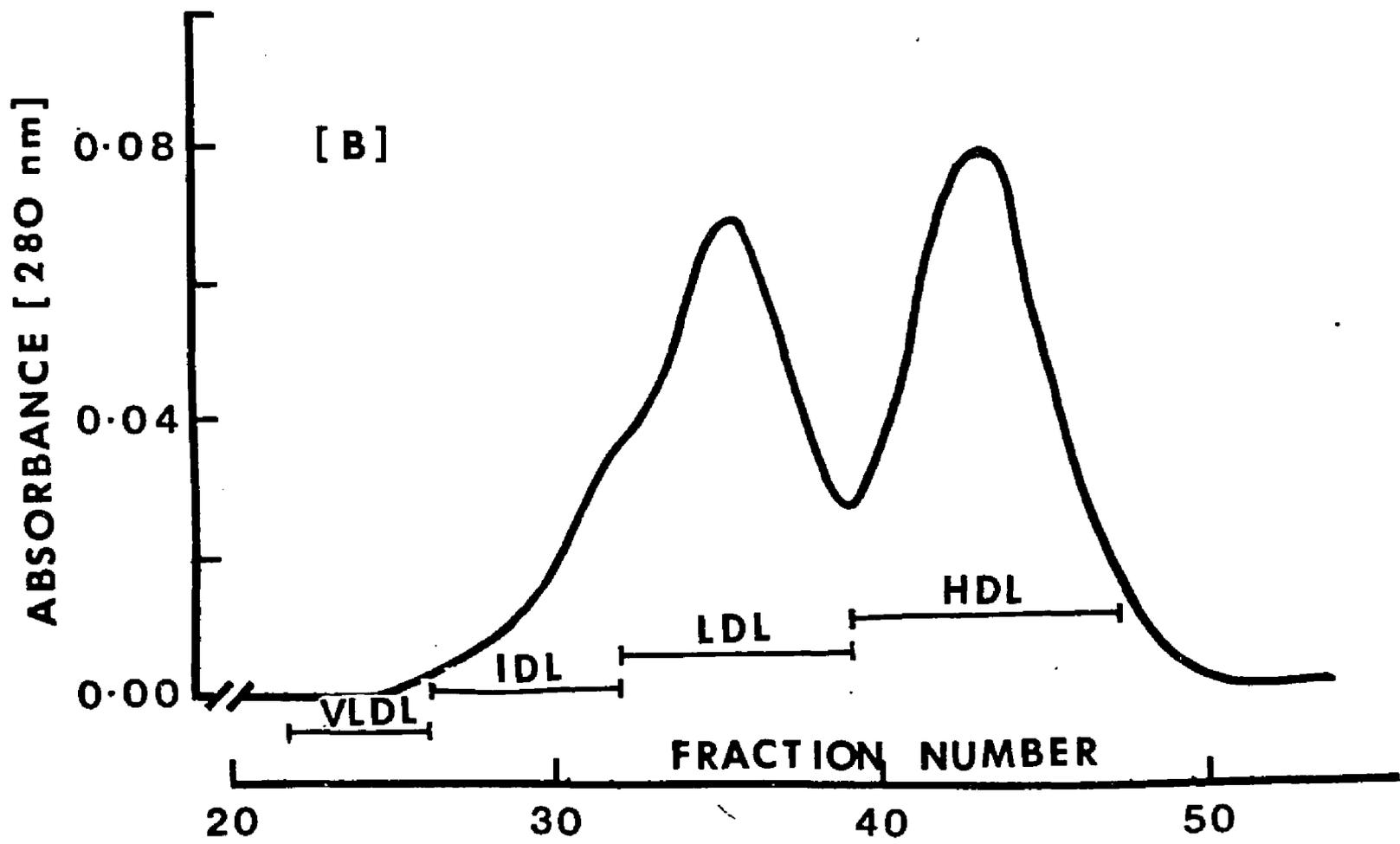


Figure 2B. Characteristic Lipoprotein Separation Following a 20 Week Aerobic Exercise Program.

Concentration of Lipoprotein Fractions

In order to analyze the cholesterol and triglyceride content of the lipoprotein fractions, 1/2 of the VLDL, IDL and LDL solution were concentrated down to 1 ml, 1 ml, and 2 ml, respectively. Fifty-five ml of the HDL solution were concentrated to 1 ml. Centriflo filter cone (CF 25, Amicon Corp., Danvers, MA) was used to concentrate the fractions. An aliquot of 3.3 ml from each sample was pipeted into labeled cones and centrifuged at 3000 rpm for 20 minutes. This centrifugation step was repeated until the filtration was completed. Cones were washed with Na azide saline solution and final concentrate values were made up to 1 ml for VLDL, and IDL, and 2 ml for LDL and HDL.

Procedure for Plasma Cholesterol Determination

Plasma cholesterol levels were quantitatively determined by using an enzymatic kit (Boehringer Mannheim, Indianapolis, IN). Certified standards of cholesterol, 50, 100, 125, 150 and 200 mg/dl (Boehringer Mannheim) were used to construct the standard curve. Cholesteryl esters in the samples were converted to free cholesterol by the cholesterol esterase. In the presence of oxygen, free cholesterol was oxidized by cholesterol oxidase to cholest-4-en-3-one and equivalent amounts of hydrogen

peroxide. The hydrogen peroxide formed was used to transform methanol to formaldehyde in the presence of a catalase. Formaldehyde then reacted with ammonium ions and acetylacetone in the Hantzsch reaction to produce a yellow product, 3,5-diacetyl-1,4 dihydrolutidine which was directly proportional to the concentration of cholesterol. The yellow color was read at 410 nm against a blank using a Beckman model 25 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA).

Procedure for Plasma Triglyceride Determination

Plasma triglyceride values for all experiments were measured colorimetrically using an enzymatic kit from Boehringer Mannheim (Indianapolis, IN). A certified standard (Boehringer Mannheim) was used. Plasma triglycerides were hydrolyzed to yield fatty acids and free glycerol using the lipase/esterase solution. Liberated glycerol was then phosphorylated. The glycerol-3-phosphate formed was reacted with NAD^+ to form dihydroxyacetone phosphate, NADH and H^+ . The NADH and H^+ then reduced the 3-(4,5-Dimethyl Thiazolyl-2)-2,5 Diphenyl Tetrazolium Bromide (MTT) yielding the reduced form (MTT.H). The amount of MTT reduced was proportional to the glycerol in the specimen. The reduced form of MTT gave the purple chromatic dispersion and was measured at 560 nm against a blank using a Bausch and Lomb model 710 spectrophotometer.

Physiological Procedures

Body composition and cardiorespiratory measurements were conducted on 40 subjects prior to and 35 subjects immediately following the 20 week training program.

Height and weight measurements were taken with a GPM anthropometer and a Homs Beam scale, respectively. Body density was determined by hydrostatic weighing using ten trials (Behnke and Wilmore, 1974). Residual lung volume, used in the calculation of body density, was assessed by a modification of the oxygen dilution technique (Behnke and Wilmore, 1974) with the subject out of the water in a sitting position similar to the position used when weighed underwater. Relative body fat was estimated from body density according to the equation of Siri (1956).

Maximal oxygen consumption and maximal heart rate were assessed during a continuous walk-run test until exhaustion on a Quinton motorized treadmill (model #24-72). Subjects were familiarized with the equipment and testing procedures prior to data collection. Subjects were told to go to the point of exhaustion (sign symptom limited, volitional fatigue) and were verbally encouraged throughout the test to continue working as long as possible. Electrocardiogram (ECG) and heart rate were monitored throughout the test on a Hewlett-Packard ECG/Phono System

(model #1514A) from a twelve-lead electrocardiogram. A 10 second recording strip was obtained at the 45 second mark of each minute during the testing. Metabolic and respiratory function were monitored continuously at 60 second intervals throughout the testing period using a Beckman Metabolic Cart (MMC). Rating of Perceived Exertion was monitored using the Borg Scale (Borg, 1974) at the 50 second mark into each minute in the protocol. Equipment calibration was conducted at regular intervals throughout the testing process.

All participants underwent a minimum of two maximal treadmill tests prior to training and one maximal treadmill test after training. Two maximal treadmill tests were conducted prior to training to establish reliability, the criterion being that the two determinations of maximal oxygen consumption ($\dot{V}O_2$ max ml/kg/min) differ by less than 2 ml as outlined by Taylor et al. (1955). In the event that this criterion was not met, a third treadmill test was given. The treadmill protocol, controlled by a Quinton Programmer (model #644) was developed for subjects of a lower fitness level and is listed on the following page:

<u>TIME</u>	<u>SPEED</u>	<u>GRADE</u>
1 min	1.7 mph	6%
2 min	2.2 mph	6%
3 min	2.2 mph	8%
4 min	2.6 mph	8%
5 min	2.6 mph	10%
6 min	3.0 mph	10%
7 min	3.0 mph	12%
8 min	3.4 mph	12%
9 min	3.4 mph	14%
10 min	3.8 mph	14%
11 min	4.2 mph	14%
12 min	4.6 mph	14%
13 min	5.0 mph	14%
14 min	5.8 mph	14%
15 min	6.2 mph	14%
16 min	6.6 mph	14%

The treadmill protocol remained the same for all subjects pre and post training and was designed to allow maximal cardiorespiratory values to be obtained between 7-14 minutes. A minimum of 48 hours was allowed for recovery between maximal treadmill tests prior to training. The post-training maximal treadmill test was conducted approximately 48 hours after the final training session.

Nutritional Analysis

Three day food records were completed on consecutive days both pre and post training by the exercise and

control group. Subjects were asked to record their dietary intake on Sunday, Monday and Tuesday so weekend and weekday dietary habits could be assessed. The dietary records were reviewed with each subject to clarify any questionable areas and further guarantee accuracy in amounts and types of foods. Subjects were requested to keep content of diet as similar as possible throughout the study, with exercise subjects increasing only the size of portions to meet extra caloric needs.

Dietary analysis of daily calorie, protein, fat, saturated fat, carbohydrates, and alcohol intake were computed for pre and post test periods using the Diet Analysis Computer Program developed by the University of Arizona Health Science Center. The program was derived from the Agricultural Handbook No. 8 data (U. S. Department of Agriculture, Washington, D.C.).

Statistical Analysis

Differences between pre and post values for all data were calculated. These values were separated into exercise and control categories. The population variances of each parameter measured were compared. A regular Student's t-test was used when the variances were homogenous. The t-test was based on separate variance estimates (Minium, 1978) when the variances were heterogenous.

RESULTS

Exercise

Following the 20 week exercise program, mean relative fat ($p < 0.001$) fat weight ($p < 0.001$) and total body weight ($p < 0.01$) were significantly reduced in exercise subjects (Table 2). Significant decreases in resting systolic blood pressure (BP) ($p < 0.05$), resting heart rate (HR) ($p < 0.001$) and maximum HR ($p < 0.05$) were also established in these subjects (Table 3). The exercise group demonstrated significant increases in total treadmill time ($p < 0.001$), relative $\dot{V}O_2$ max ($p < 0.001$), respiratory exchange ratio (R) ($p < 0.05$), absolute $\dot{V}O_2$ max ($p < 0.001$) and ventilation equilibrium ($\dot{V}E$) ($p < 0.001$) as well (Table 4).

Biochemical

The mean values of pre and post testing and their differences (Δ) for cholesterol, TG and protein of the lipoprotein fractions are given in Tables 5, 6 and 7, respectively. Reductions in LDL cholesterol ($p < 0.005$) LDL TG ($p < 0.001$), total TG ($p < 0.05$), IDL protein ($p < 0.005$) and LDL protein ($p < 0.001$) are observed. Exercise subjects also showed significant elevation in HDL-c values ($p < 0.01$).

Table 2. Effect of Exercise on Body Composition of Exercise and Control Groups¹

Body Composition	Treatments	Time		Differences (Δ)	P value ²
		Pre	Post		
Residual					
Volume (L)	Ex	2.00 ± 0.11	1.89 ± 0.09	-.10 ± 0.05	NS ³
	C	1.97 ± 0.13	1.93 ± 0.12	-.05 ± 0.04	
Relative Fat (%)	Ex	27.34 ± 0.88	25.19 ± 0.83	-2.16 ± 0.39	.001
	C	26.87 ± 1.46	27.26 ± 1.30	.39 ± 0.35	
Fat Wt (kg)	Ex	22.72 ± 1.22	20.43 ± 1.10	-2.28 ± 0.43	.001
	C	21.18 ± 1.49	21.55 ± 1.38	.37 ± 0.35	
Lean Wt (kg)	Ex	59.65 ± 1.74	60.09 ± 1.73	.45 ± 0.28	NS
	C	56.53 ± 1.32	56.52 ± 1.16	-.01 ± 0.42	
Total Wt (kg)	Ex	82.36 ± 2.67	80.52 ± 2.55	-1.84 ± 0.52	.010
	C	77.71 ± 2.34	78.07 ± 2.17	.36 ± 0.55	

¹Mean ± SEM

²P values were derived from t test. Significant changes were obtained by comparing the Δ between the exercise and control groups.

³NS=non significant

Table 3. Cardiovascular-Respiratory Measurements of Exercise and Control Groups¹

Fitness Measurements	Treatments	Time		Differences (Δ)	P value ²
		Pre	Post		
Treadmill Time (seconds)	Ex	604.61 ± 15.01	711.24 ± 15.09	106.64 ± 7.17	.001
	C	575.54 ± 19.15	573.56 ± 22.76	1.98 ± 7.24	
$\dot{V}O_2$ max (ml/kg/min)	Ex	31.47 ± 0.97	36.79 ± 0.96	5.32 ± 0.63	.001
	C	29.10 ± 1.16	28.72 ± 1.34	-.28 ± 0.43	
$\dot{V}O_2$ (L/min)	Ex	2.57 ± 0.11	2.96 ± 0.11	.38 ± 0.05	.001
	C	2.24 ± 0.10	2.24 ± 0.11	.00 ± 0.03	
R ($\dot{V}CO_2/\dot{V}O_2$)	Ex	1.14 ± 0.02	1.28 ± 0.01	.14 ± 0.02	.050
	C	1.23 ± 0.03	1.30 ± 0.02	.06 ± 0.03	
VE (L/min)	Ex	116.35 ± 4.39	128.97 ± 5.02	12.62 ± 2.38	.001
	C	101.80 ± 4.17	98.71 ± 4.23	-3.84 ± 1.65	

¹Mean ± SEM

²P values were derived from t test. Significant changes were obtained by comparing the Δ between the exercise and control groups.

Table 4. Cardiovascular-Respiratory Measurements of Exercise and Control Groups¹

Physiological Measurements	Treatments	Time		Differences (Δ)	P value ²
		Pre	Post		
Resting HR (beats/min)	Ex	67.40 ± 2.42	60.64 ± 1.99	-6.76 ± 1.5	.001
	C	68.41 ± 2.40	72.01 ± 2.90	3.59 ± 2.1	
Maximum HR (beats/min)	Ex	176.45 ± 2.18	172.05 ± 2.74	-4.40 ± 1.67	.050
	C	170.89 ± 3.08	169.86 ± 3.35	- .24 ± 0.88	
Systolic BP	Ex	140.05 ± 3.80	125.80 ± 2.42	-14.25 ± 2.76	.050
	C	134.67 ± 3.10	130.00 ± 2.58	- 4.67 ± 2.05	
Diastolic BP	Ex	91.68 ± 2.09	82.90 ± 2.85	- 8.78 ± 2.93	NS ³
	C	92.40 ± 1.68	87.13 ± 1.47	- 5.27 ± 1.54	

¹Mean ± SEM

²P values were derived from t test. Significant changes were obtained by comparing the Δ between the exercise and control groups.

³NS=non significant

Table 5. Effect of Exercise on¹ the Cholesterol Content of Plasma Lipoproteins in Elderly Male Subjects

Lipoprotein Fractions mg/dl	Treatments	Time		Differences		P value ²
		Pre	Post	(Δ)		
VLDL	Ex	0.60 ± 0.16	0.28 ± 0.07	-0.324 ± 0.177		NS ³
	C	3.13 ± 2.12	1.24 ± 0.53	-1.891 ± 2.11		
IDL	Ex	6.02 ± 1.18	1.57 ± 0.46	-4.453 ± 1.185		NS
	C	17.08 ± 4.39	16.43 ± 4.55	-0.658 ± 1.920		
LDL	Ex	196.83 ± 10.46	186.94 ± 9.59	-9.887 ± 2.680		.005
	C	165.20 ± 12.39	173.97 ± 9.90	8.768 ± 5.401		
HDL	Ex	37.27 ± 2.58	43.45 ± 2.88	6.188 ± 2.909		.010
	C	34.99 ± 2.89	31.40 ± 2.13	-3.586 ± 1.866		
Total Cholesterol	Ex	240.72 ± 12.29	232.24 ± 10.89	-8.475 ± 4.806		NS
	C	220.40 ± 12.66	223.04 ± 10.54	2.636 ± 7.443		

¹Mean ± SEM

²P values were derived from t test. Significant changes were obtained by comparing the Δ between the exercise and control groups.

³NS=non significant.

Table 6. Effect of Exercise on the Triglyceride Content on Plasma Lipoproteins in Elderly Male Subjects¹

Lipoprotein Fractions mg/dl	Treatments	Time		Differences (Δ)	P value ²
		Pre	Post		
VLDL	Ex	6.38 ± 1.92	5.25 ± 3.49	-1.127 ± 3.339	NS ³
	C	10.81 ± 3.63	8.98 ± 2.98	-1.836 ± 1.342	
IDL	Ex	19.92 ± 3.59	12.22 ± 2.41	-7.689 ± 2.114	NS
	C	40.19 ± 10.43	28.62 ± 7.35	-11.572 ± 6.476	
LDL	Ex	51.69 ± 3.73	38.00 ± 2.44	-13.678 ± 2.444	.001
	C	44.99 ± 7.17	50.11 ± 5.33	5.123 ± 4.300	
HDL	Ex	10.26 ± 2.34	11.96 ± 1.44	1.699 ± 2.322	NS
	C	5.58 ± 1.37	12.26 ± 2.01	6.683 ± 1.842	
Total	Ex	88.24 ± 7.49	67.45 ± 5.57	-20.795 ± 5.650	.050
	C	101.57 ± 16.13	99.97 ± 13.29	-1.602 ± 7.897	

¹Mean ± SEM

²P values were derived from t test. Significant changes were obtained by comparing the Δ between the exercise and control groups.

³NS=non significant

Table 7. Effect of Exercise on the Protein Concentration of Plasma Lipoproteins in Elderly Male Subjects¹

Lipoproteins Fractions mg/dl	Treatments	Time				Difference (Δ)	P value ²
		Pre		Post			
VLDL	Ex	1.05 ± 0.30	0.82 ± 0.23	-0.235 ± 0.390	NS ³		
	C	5.51 ± 2.76	6.45 ± 3.56	-0.933 ± 0.965			
IDL	Ex	14.74 ± 2.03	6.55 ± 1.22	-8.190 ± 1.860	.005		
	C	23.63 ± 5.79	28.77 ± 6.80	5.140 ± 1.968			
LDL	Ex	106.88 ± 4.66	86.91 ± 5.54	-19.975 ± 3.931	.001		
	C	84.97 ± 8.35	92.69 ± 6.23	7.726 ± 5.823			
HDL	Ex	184.65 ± 18.89	196.10 ± 14.08	11.445 ± 23.094	NS		
	C	190.69 ± 19.82	187.77 ± 13.22	-2.927 ± 14.980			
Total Protein	Ex	307.32 ± 19.02	290.37 ± 18.09	-16.955 ± 24.003	NS		
	C	304.79 ± 25.92	315.67 ± 24.27	10.887 ± 17.453			

¹Mean ± SEM

²P values were derived from t test. Significant changes were obtained by comparing the Δ between the exercise and control groups.

³NS=non significant

Table 8. Daily Nutrient Intakes from Three Day Recalls for Exercise and Control Groups¹.

Nutritional Data	Treatment	Time		Differences (Δ)	P value ²
		Pre	Post		
Kilo Calories (per day)	Ex	2616 ± 126	2672 ± 148	56 ± 22	NS ³
	C	2630 ± 392	2179 ± 226	-451 ± 166	
Protein (gm/day)	Ex	109 ± 6	103 ± 6	-6 ± 0	NS
	C	110 ± 19	84 ± 7	-26 ± 12	NS
Fat (gm/day)	Ex	107 ± 7	109 ± 8	2 ± 1	NS
	C	118 ± 24	90 ± 10	-28 ± 14	
Carbohydrates (gm/day)	Ex	295 ± 24	319 ± 27	24 ± 3	NS
	C	267 ± 26	260 ± 34	-7 ± 8	
Saturated Fat (gm/day)	Ex	19 ± 2	20 ± 2	1 ± 0	NS
	C	18 ± 3	15 ± 2	-3 ± 1	
Alcohol (% total calories/day)	Ex	3 ± 1	2 ± 1	-1 ± 0	NS
	C	2 ± 1	1 ± 1	-1 ± 0	

¹Mean + SEM

²P values were derived from t-test. Significant changes were obtained by comparing the Δ between the exercise and control groups.

³NS=non significant.

Nutrition

Results of dietary information requested from subjects are presented in Table 8. The values indicate no significant changes in composition of diets for both control and exercise subjects during the experimental period. Since only one of the 3 days during dietary recalls involved exercise training, the usual increase in caloric intake associated with the exercise was not observed.

DISCUSSION

Physiological Results

The pre and post training assessments of body composition showed a significant reduction in the exercise group's level of adiposity and total body weight following 20 weeks of aerobic exercise (Table 2). This is a typical finding in previously sedentary men placed on an exercise program (Williams et al., 1982; Shephard and Kavanah, 1978). Cross sectional research comparing serious runners to sedentary individuals in the same age group, have also shown runners to have a significantly lower percent fat and body weight (Lehtonen and Viikari, 1978; Shephard and Kavanah, 1978). No significant change was seen in the runners' lean weight over the 20 week period. This finding was not unusual, particularly in the case of elderly men. A number of other studies have also found lean weight to remain the same after only a short duration of training (Johnson et al., 1982; Wood et al., 1983; Farrell and Barboriak, 1980). In addition, lean muscle mass steadily decreases after approximately thirty years of age, making it particularly difficult for the elderly to increase muscle mass. In studies where highly significant

improvement in strength has been noted, the efficiency of electrical activity related to muscle hypertrophy did not change and muscle mass remained constant (deVries, 1970). From these findings, short durations of aerobic exercise training do not appear effective in increasing lean weight.

Fitness measurements (work time on the treadmill) $\dot{V}O_2$ ml/kg/min, absolute $\dot{V}O_2$ L/min, R, and $\dot{V}E$ improved for the exercisers when compared to their sedentary peers (Table 3). A training effect in the exercise group was evident by the highly significant increase in performance time on the standardized multistage treadmill test. A mean increase of 106.64 seconds was shown for the runners. This increase in endurance could be due to a number of factors such as a greater activation of the central nervous system (CNS) and improved respiratory functions (deVries, 1970).

The significant increase in absolute $\dot{V}O_2$ max (.38 L/min) and in relative $\dot{V}O_2$ max (5.32 ml/kg/min) is also indicative of a training effect in the exercise group. The increase in absolute $\dot{V}O_2$ max (L/min) is a measure showing the absolute O_2 consumption. The relative $\dot{V}O_2$ max (ml/kg/min) measures the efficiency of O_2 consumption by adjusting the absolute value for the weight variations. An increase in $\dot{V}O_2$ max is a function, in part of maximal cardiac output (the ability of the heart to deliver blood

to the working muscles). Increased cardiac output and the ability of the circulation to shift blood from non-working to working muscles as well as the ability of those muscles to extract O_2 from the blood are associated with increased $\dot{V}O_2$ max levels. In prolonged exercise, there is a high correlation between O_2 consumption and energy expenditure (Lamb, 1978).

The two individuals injured one half of the way through the study were post tested on both a bicycle ergometer and the Quinton motorized treadmill. Post testing on the bicycle and treadmill resulted in similar max HR values on both of the injured exercise subjects. These values were comparable to the rest of the exercise group and fell within the mean values. $\dot{V}O_2$ max treadmill data for the cyclists was also within the means of the exercise group, however, the $\dot{V}O_2$ max data on the bicycle test was slightly lower. The lower $\dot{V}O_2$ max may be due to the reduced amount of muscle mass engaged in exercise with cycling, which would result in lower oxygen needs (Astrand and Radahl, 1977).

The significant increase in the R of the exercise group (.14 L) demonstrates an increase in the amount of CO_2 given off in relationship to the amount of O_2 consumed.

The $\dot{V}E$ indicates the rate of pulmonary ventilation of O_2 and CO_2 . The increase in the exercise groups' $\dot{V}E$

indicates an increase in the number of open capillaries in the lungs responding to a greater flow of blood around the lungs (Astrand and Rodahl, 1977). In aerobic exercise, the lung function is commonly the limiting factor in the elderly (Astrand and Rodahl, 1977).

The significant decreases in the remaining physiological measurements of the exercise group, resting HR and resting systolic BP also signify a training effect (Table 4). The reduction in maximal HR observed in this study signifies a training response but it is not a typical training effect.

The decrease in resting and maximal HR of the exercise participants indicates the effect of exercise and coincides with previous studies (Wood et al., 1983; Huttunen et al., 1979). This bradycardia is thought to be the result of an increase in maximal cardiac output caused by greater stroke volume. The overall effect of these changes gives a more efficient cardiovascular system and a lower maximal HR.

Changes were also noted in the resting systolic BP. A decrease in resting systolic BP was seen in the exercise group after the 20 week period. Decreases in resting systolic BP have also been seen in the results of other exercise studies (Froelicher et al., 1980; deVries, 1970). Under normal circumstances, an increase in cardiac output and greater blood volume would increase blood pressure.

In those who exercise, however, a general peripheral vasodilation occurs simultaneously and is responsible for the decrease in blood pressure (Lamb, 1978).

The physiological alterations observed in exercise subjects when compared to the sedentary controls, show significant changes in fitness parameters. Significant improvements in respiratory and cardiovascular functions as well as significant reductions in adiposity and total body weight demonstrate a training effect in the elderly population of this study.

Although the majority of aerobic exercise studies show a training effect, there is a variety of protocol differences in the studies: Exercise and HDL-c discrepancies could be due to protocol design differences. Both the amount and intensity of training are important and are positively associated with elevated HDL-c concentrations (Myhre et al., 1981). Fairly consistent changes have been noted in aerobic exercise programs ranging for 4 months or more (Leon et al., 1979; Huttunen et al., 1979) with mild to moderate training. Lehtonen et al. (1979) and Huttunen et al., (1979) observed a positive correlation between weekly running distance and plasma HDL-c concentration. Likewise, other studies have shown the fastest runners, skiers and individuals with the most

aerobic endurance to have the highest HDL-c concentrations (Myhre et al., 1981; Enger et al., 1977; Williams et al., 1982).

Pre and post testing schedules are also an important consideration. Our subjects were tested approximately 48 hours after their last aerobic exercise session to avoid a diminishing effect. The possibility that the effect of aerobic exercise may diminish with time is suggested by the reduction in HDL-c observed within a two week period when runners were reduced from 10 miles/day to five miles/day under controlled conditions (Krauss, 1982).

Similarly, a 12% increase of HDL-c was observed in 20 men immediately following a 70 km race, with a gradual fall off over the following three days (Enger et al., 1980). At the same time, individuals temporarily immobilized by spinal fractures showed a reduction in HDL-c when compared with normally active control subjects (Nikkila et al., 1980).

Discrepancies among past lipoprotein and exercise studies may in part be linked to a relatively new finding related to the physiological adaptations of exercise training. Chronic exercise apparently triggers the elevation of plasma renin activity and vasopressin (Convertino et al., 1980a; Convertino et al., 1980b). This results in the retention of water and sodium in the body causing an increase

in plasma volume. Research indicated that an exercise intensity greater than 50% of the maximal oxygen uptake (VO_2 max) appeared necessary to generate this reaction, but the exact quantities of increase are not known (Convertino et al., 1980a; Convertino et al., 1980b). Alterations in plasma volume may not occur in studies with low levels of intensity. Studies performed at high intensities however, may actually have alterations in lipoprotein fractions, but would be unable to properly identify them without plasma volume measurements and calculations.

The quantitative changes in plasma volume of the elderly are not yet known. The individuals exercising in this study were training above 50% of their maximal O_2 uptake, but lipoprotein changes were still observed. One possible explanation for this is that the increase in plasma volume of the elderly may not be significant enough to alter their lipoprotein values.

Nutritional Evaluation

Subjects were screened initially to eliminate those with dietary habits contraindicated. Total caloric intake (kcal/day), protein (gm/day), fat (gm/day), carbohydrates (gm/day), saturated fat (gm/day), and alcohol (percent of total kcal/day) showed no significant

changes throughout the study (Table 8). Many existing publications did not evaluate diet for compositional or caloric alterations (LaRosa et al., 1982; Nye et al., 1981). Dietary changes could, in part, account for the conflicting results seen regarding exercise and its effects on lipoprotein fractions. This is true particularly when evaluating the alcohol, carbohydrate and saturated fat consumption of the diet (Kritchevsky, 1976). In this study, results show dietary intake of exercise and control subjects to be constant. This indicates that diet was not a major influence on lipoprotein alterations.

Lipoprotein Data

A number of studies have related 3 to 4 hours/week of aerobic exercise to alterations in composition and amount of lipoproteins. Methodology is another possible explanation for the variation in lipoprotein research. The reliability and consistency of lipoprotein separation, concentration and analyzation has varied from study to study. Sequential ultracentrifugation, for example, may cause structural alterations of the proteins due to long periods of exposure to high salt concentrations and the high g forces involved (Rudel et al., 1974). Samples for this study were separated within a couple of hours from the time of the blood draw. The methodology used in this study (separation by ultracentrifugation and

agarose column chromatography) was a mild method and the lipoproteins were not subjected to extreme gravitational pull. In addition, the agarose column chromatography method is highly reproducible (Weisgraber and Mahley, 1980). Using this methodology, a significant decline in LDL-c and increase in HDL-c in the exercise subjects were observed (Table 5). In contrast, the control group showed no significant changes, indicating that seasonal variations or other non-specific factors were not responsible. Past studies have reported conflicting results in regard to LDL-c and HDL-c shifts (Kiens, et al., 1981; Huttunen et al., 1979; Lipson et al.; Nye et al., 1982). This study demonstrated that significant alterations in lipoprotein cholesterol could be seen in exercise levels of 3 hours or 9-12 miles of running/week.

The present finding of marked differences between elderly runners and controls in respect to distribution of plasma cholesterol between LDL and HDL is of considerable interest. Low HDL-c is the primary risk factor for CAD in the elderly. An increase in HDL-c therefore may reduce the risk of CAD (Wood and Haskell, 1976). Changes in the quantity of cholesterol carried by the lipoproteins may be due to an actual alteration of the lipoproteins or to an influence from other factors such as LCAT. LDL delivers cholesterol to extrahepatic cells and the liver

by binding to high affinity receptors on plasma membranes. In the steady state, tissues excrete cholesterol into plasma in quantities equal to amounts taken up from LDL (Brown et al., 1981). Excreted cholesterol that binds to HDL is esterified by LCAT. High levels of LCAT have been reported in runners (Krauss, 1982). LCAT appears to promote shifts in the distribution of lipids and apoproteins between HDL and other lipoproteins. HDL particles appear to be the best substrate for LCAT, which catalyzes the esterification of free cholesterol located on the outer surface of these lipoprotein particles. The esterified cholesterol then moves from the surface into the nonpolar core of the HDL or is transferred to VLDL or LDL. The modified HDL is then ready to accept additional free cholesterol from various peripheral cells. Eventually, the HDL particles with cholesterol are removed by the liver (Alaupovic, 1981). If aerobic exercise is capable of elevating LCAT then an increase in cholesterol transfer to HDL (via LCAT) may result. The transfer to HDL may reduce the binding, uptake and degradation of LDL by peripheral cells, which has been suggested as a possible explanation for the inverse relationship between HDL-c and LDL-c (Glomset, 1980).

In some studies, alterations in body weight have influenced lipoprotein levels. Conflicting results in

regard to HDL-c levels have been reported in weight reduction studies. In obese individuals, high adiposity is often correlated with relatively low levels of HDL-c when compared with individuals of normal weight (Krauss, 1981). In the past, weight loss by calorie restriction has resulted in further reduction of HDL-c in some studies. However, no change in HDL-c was seen in other studies (Rabkin et al., 1981; Keins et al., 1981). Studies on the combination of exercise and calorie restriction to reduce adiposity have often shown increased HDL-c. When the calorie restriction is continued in the absence of exercise, however, HDL-c dropped (Krauss, 1982). This suggests that changes in body composition and levels of energy expenditure could work together to increase HDL-c, but energy expenditure appears to have a dominant influence.

Other alterations in lipoproteins such as TG and protein changes could also account for cholesterol changes by triggering a series of reactions within the lipoproteins or acting as a carrier in their metabolism (Brown et al., 1981). A high level of HDL-c has been previously noted to be associated with a low level of total plasma TG (Wood et al., 1976). Fasting TG have been found to be lower in physically well trained men than in their sedentary counterparts (Wood et al., 1976; Bjorntorp et al.,

1972). In this research study, a significant decrease in TG was also observed with exercise (Table 6).

One factor that influences TG catabolism is LPL activity. A training study in rats demonstrated increased skeletal muscle LPL activity after exercise (Borensztajn et al., 1975). In lipid transport, the TG rich lipoproteins react with LPL and release most of their TG and excess surface material (phospholipids and cholesterol). This surface material is thought to be transferred to the HDL. An increase in LPL is commonly found in well trained men. Lipoprotein lipases are synthesized within cells of various tissues, but their active forms are situated extracellularly at the surface of capillary endothelia. The enzymes are most active in the capillaries of adipose tissue, cardiac and red skeletal muscle, and the lactating mammary gland. Lipoprotein lipases are readily displaced from the endothelial surface by negatively charged polyelectrolytes, including heparin (Havel et al., 1980). The degree of capillarization is a particularly strong determinant of heparin elutable LPL activity in the skeletal muscle. The capillarization of muscles in those who exercise is higher than sedentary individuals due to the increased blood flow requirement of working muscles (Kiens et al., 1980). LPL appears to be sensitive to external influences such as diet and physical exercise. Hepatic

lipase (HL) however, does not have the same sensitivity. HL appears to concentrate on the removal of HDL particles from the circulation via the liver. HL shows little change with exercise (Kiens et al., 1980). Aerobic exercise, therefore, may cause a decrease in TG activity through influencing LPL, without stimulating HL activity to breakdown HDL (Kuusi et al., 1980; Kiens et al., 1980).

Changes in cholesterol and TG components of the lipoproteins may also be linked to their protein distribution. In this study, the exercise group significantly decreased the IDL and LDL protein concentrations, indicating that a redistribution is possibly taking place. The HDL protein appeared to be increased but the results were not significant. Several past studies have looked at the role of apo-proteins in relationship to lipoprotein distributions and found significant correlations with a number of factors. Apo-protein AI is thought to initiate the LCAT reaction and apo-protein E is thought to act in locating receptor sites in the liver (Alaupovic, 1981; Kiens et al., 1980; Glomset, 1980; Brown, et al., 1981). In the two major cholesterol carrying lipoprotein fractions (LDL and HDL) the shifts in protein coincide with the cholesterol shifts. This may indicate that the protein components in some way act to promote the redistribution of cholesterol. Although subfractioning and electrophoresis were not

performed in this study, the changes in protein composition of the different lipoprotein fractions indicate that some kind of relationship may exist.

The results of the present study indicate that moderate aerobic training in elderly men may promote plasma lipoprotein pattern changes in the direction which may reduce the risk of CAD. These patterns are particularly significant when realizing that the greatest risk factor for the prevalence of CAD among the elderly is low HDL-c. Although it is impossible to control for all variables in longitudinal studies, the magnitude and specificity of changes seen in this study suggest a mechanism by which physical activity might play a role as an independent behavioral risk factor for CAD.

SUMMARY

This study investigated the influence of exercise on lipoprotein fractions and their components in the elderly population (50-73 years old). Thirty five sedentary men were recruited and completed the study. Twenty participated in a 20 week walk-run aerobic fitness program, and 15 served as controls, maintaining their previous level of sedentary activity. Body composition and cardio-respiratory measurements were assessed pre and post and indicated a training effect on those who exercised. Biochemical analyses of the plasma lipoproteins were also assessed pre and post on all 35 subjects. A significant increase in HDL-c, and significant decreases in LDL-c, total TG, IDL-protein, and LDL-protein were observed in exercised subjects. The control group showed no significant changes during the study. These results are consistent with the concept that aerobic exercise conditioning may decrease the risk of CAD.

APPENDIX A

LIST OF ABBREVIATIONS

BP	blood pressure
C	control
CAD	coronary artery disease
cm	centimeters
CO ₂	carbon dioxide
dl	deciliters
ECG	electrocardiogram
EDTA	ethylenediamine-tetracetic acid
EX	exercise
g	gravitational force
gm	grams
HDL	high density lipoproteins
HDL-c	high density lipoprotein cholesterol
HL	hepatic lipase
HR	heart rate
IDL	intermediate density lipoproteins
KBr	potassium bromide
Kcal	kilo calories
Kg	kilogram
LCAT	lecithin-cholesterol acyltransferase

LDL	low density lipoproteins
LDL-c	low density lipoprotein cholesterol
MI	myocardial infarction
mg	milligrams
ml	milliliter
MTT	diphenyl tetrazolium bromide
Na azide	sodium azide
NS	non significant
O ₂	oxygen
R	respiratory exchange
RV	residual volume
rpm	revolutions per minute
SEM	standard error of the mean
TG	triglyceride
$\dot{V}_{CO_2} / \dot{V}_{O_2}$	ventilation of carbon dioxide production/ oxygen consumption
\dot{V}_E	ventilation equilibrium
$\dot{V}LDL$	very low density lipoproteins
$\dot{V}O_2$	ventilation of oxygen

APPENDIX B
MISCELLANEOUS

University of Arizona

A Research Study of the Effects of Exercise Training on Lipid Metabolism in Elderly Men

MEDICAL CLEARANCE FORM

Patient's Name _____ Physician's Name _____

Date of Last Examination _____ Physician's Address _____

City _____ State _____ Zip _____ Phone _____

Medical Examination:

History (pertinent comments):

Present Medication:

Allergies:

Electrocardiogram Interpretation (if available):

Blood Pressure:

12 Hour Fasting Blood Chemistries (if available): Cholesterol _____

HDLC _____ Triglycerides _____ Uric Acid _____ Other _____

I have examined the above patient and have found him medically qualified to participate in the research study, including the training and the exercise stress tests.

Date Physician's Signature

Please return this form and a copy of the 12-lead resting electrocardiogram, if available, to Dr. Thomas Rotkis as soon as conveniently possible.

Exercise and Sport Sciences Laboratory
c/o Thomas C. Rotkis, M.D., PhD.
Lipid Metabolism Study
McKale Center - 228
University of Arizona
Tucson, Arizona 85721

EFFECTS OF EXERCISE TRAINING
ON LIPID METABOLISM IN ELDERLY MEN

Subject's Consent for Training Study Participation

A University of Arizona Research Project

I have received an oral explanation of the training study. I understand the following:

Purpose: The study is designed to analyze biochemical and physiological effects of exercise on healthy sedentary men 50 years of age and older.

Objectives: The biochemical effects of exercise that will be monitored on each subject include: the amount of cholesterol found in the blood and the fractions that comprise it (protein, fat, etc.); the amount of lipoprotein lipase (substance which aids in breaking down fat) in the blood; and the number of platelets (substance in the blood which aids in blood clotting) in the blood.

The physiological effects of exercise that will be monitored on each subject include: the level of cardiovascular fitness; how well the lungs are functioning; maximum heart rate; how much of the subject's weight is fat and how much is muscle; the level of flexibility; how efficiently the heart is functioning; blood pressure.

All biochemical and physiological testing will take place in the Exercise and Sport Sciences Laboratory, Room 228 McKale Student Center, located on the campus of the University of Arizona.

Exercise: Subjects chosen for the training portion of the study will participate in a 20 week walk/run aerobic fitness program. Subjects will train 45 minutes per day, 3 days a week, at the same time each day. (Since the training program is progressive in nature, it is not expected that all subjects will be capable of exercising for the full 45 minutes a day from the outset of the study. Subject's work time per day will be gradually increased as their fitness level indicates.) Subjects will maintain individual training heart rates within a range obtained by adding 60% of the difference between heart rate (HR) max and HR rest to HR rest for the lower limit of the range and by adding 85% of the difference between HR max and HR rest

to HR rest for the upper limit of the range. The peak heart rate recorded in the treadmill tests conducted prior to training will be assumed to be the maximal value.

Exercise heart rates will be monitored by the subject at approximately the 15th, 30th, and 45th minutes of exercise during each training session. Subjects involved in the training program are to record the length of each training session, the date and time when each session occurs, the approximate distance covered each session, and the heart rate attained at each of the three designated times during the session.

Training sessions will be conducted on the campus of the University of Arizona from August, 1982 to January, 1983.

Subjects chosen as controls will not participate in the exercise portion of this study and will not engage in any training programs during the course of the study.

Respiratory Calorimetry (Maximal Treadmill Tests): Each subject will complete a minimum of 3 and a maximum of 4 treadmill tests to exhaustion. The exercise test will be performed on a motor-driven treadmill with the amount of effort required increasing gradually each minute until the point of volitional fatigue (participant's decision to stop the test due to physical tiring) is reached. Symptoms such as dizziness, chest pain, or unusual fatigue would be reasons for terminating the treadmill test.

The maximal treadmill tests require all subjects to wear electrodes in order to record heart rate and to be connected to a Beckman Metabolic Cart via a tube connected to a mouthpiece and supported by a head brace. The nose will be closed to breathing by means of a nose clip while the mouthpiece is in place to force breathing to take place through the mouth.

During the tests a physician will be in the lab, and an exercise physiologist will monitor the subject's heart rate, blood pressure, and electrocardiogram. There is a very remote possibility that certain abnormal changes may occur during testing. This could include abnormal blood pressure or abnormal electrocardiogram responses. Every effort will be made to minimize the possibility of these occurrences by close observation of the subject's performance during the exercise test. Further, a variety of emergency equipment is located in the lab and will be available for use by the supervising physician if necessary.

Prior to the twenty week training program, each subject will undergo a minimum of 2 and a maximum of 3 maximal treadmill tests. The third test will be necessary in the event that the maximal oxygen consumption values obtained in the first two tests are not within five percent of one another.

After the twenty week training program is completed, each subject will undergo 1 maximal treadmill test no sooner than 48 hours and no later than 120 hours (5 days) after the final training session.

Body Composition Assessment: The body composition tests include a series of measurements of various bone diameters, muscle girths, and fat skinfold thickness. In addition, the subject will be submerged in a tank of water and will be asked to hold his head underwater for several seconds (the tank is only $3\frac{1}{2}$ feet deep so the ability to swim is not required). The underwater weighing is used to determine the density of the subject's body.

Lung Function Assessment: Lung function will be determined by a procedure which involves taking a maximum inspiration (taking the deepest breath possible) and then exhaling the greatest volume of air possible as quickly as the subject can.

Flexibility Assessment: This test is designed to measure the subject's flexibility in the lower back area. The subject will be asked to sit on a pad with his back straight and flush against the wall. The subject will then be asked to lean forward stretching out his arms as far as possible in front of him.

Dietary Recall: Subjects will be asked to list all food and fluids ingested for a period of 3 consecutive days (72 hours) once prior to the training portion of the study and once after training is completed.

Blood Sampling: Subjects will have blood drawn a total of 6 times during the study. Prior to training, subjects will have a total of 45cc (approximately 5 tablespoons) of blood drawn. Two of the blood draws will involve the removal of 20cc (less than $2\frac{1}{2}$ tablespoons) of blood and a third blood draw of 5cc (approximately $\frac{1}{2}$ tablespoon) will be conducted ten minutes after the injection of heparin (see section titled Injections for explanation). After training, the same procedure (as described in the previous two sentences) will be conducted. Therefore,

a total of 6 veni punctures in an anticubidal vein (a vein just below the elbow on the forearm) will be performed for a total withdrawal of 90cc of blood (less than 10 table-spoons). There will be a slight discomfort associated with the blood draws and there is a possibility of a hematoma occurring (local swelling with blood that should disappear after a day or two).

Injections: Heparin will be injected into the subject's bloodstream once prior to training and once after training is completed. Heparin is used clinically as an anticoagulant (stops the formation of blood clots) and the dosage to be injected in this study (60 International Units/kilogram of body weight) is much less than the clinical dosage. There will be a slight discomfort associated with the injections and there is a possibility of a hematoma occurring.

Miscellaneous:

1. Participation in this research is entirely voluntary.
2. Individuals with past histories of any of the following should not participate in this study:
 - Heart Disease (including open heart surgery, heart attack, and stroke).
 - Diabetes within the past 3 years
 - Hemophilia (abnormal tendency to bleeding) and/or a history of bruising easily.
 - Cholesterol levels in excess of 300 mg/dl within the past 3 years.
3. Subjects may not take any prescribed or over-the-counter drugs, or any other drugs, on days when they are to have blood drawn and on days when they are to perform maximal treadmill tests. No drugs may be taken on days preceeding the blood draws and treadmill tests if such drugs have effects that last long enough to affect blood values or treadmill performance.
4. Subjects are not to take aspirin 48 hours before and within 48 hours after receiving heparin.
5. It is estimated that subjects will spend a total of 68 hours on activities related to the study (this includes all testing and training).

6. No medical coverage will be provided by the investigators during training except for first aid for minor injuries.
7. Subjects are to provide their own clothing and shoes for all training and testing sessions.
8. The investigators will at any time answer any inquiries concerning procedures to be used.
9. Any subject is free to withdraw from the study at any time and for any reason without prejudice.
10. The subjects will be anonymous in any publication(s) of the results of this study.
11. Subjects are not to ingest any solid food for five hours prior to maximal treadmill tests and not for twelve hours prior to blood draws.
12. Each 45 minute training session will be preceded by 15 minutes of stretching and flexibility exercises and will be followed by 5-10 minutes of light warm-down drills. Subjects involved in the training portion of the study are expected to participate in the warm-up and warm-down exercises.

Physical Examinations: Each subject is required to obtain a clearance from their personal physician in order to be permitted to participate in the study. Physical exams will not be given to the subjects (but a physician will be present during testing and will analyze all heart data obtained in order to determine whether any abnormalities exist). If at any time a participant or the investigators believe that the health of the subject may be impaired, the subject may drop, or be asked to drop, from the study.

Risks: Potential risks are considered to be minimal. Subjects may experience minor inconvenience and discomfort due to wearing the headgear during testing in the lab. Muscle soreness may occur in some subjects as a result of training and/or testing. While soreness may be uncomfortable to some subjects, it is not expected to present undue difficulty. (Warm-up exercises will be performed prior to training sessions in order to loosen-up muscles to be exercised; this should reduce the possibility of both muscle soreness and injury.) Venous blood samples and heparin injection have

been described on pages three and four, respectively of this consent form and the risks of each are described therein. In individuals with a high prevalence of coronary artery disease vigorous exercise could precipitate chest pain, a variety of arrhythmias and, in extreme cases, death (the testing prior to training will give the investigators a good indication of each subject's level of coronary artery disease and subjects contraindicated for training will not be permitted to participate in the training study).

Benefits: Subjects will gain special understanding of the scientific methods as applied to Exercise Physiology research. Subjects involved in the training program will receive instruction in the areas of flexibility drills, effective training methods as applied to running, proper running form, and how to use target heart rates for training. Subjects involved in the training program will also benefit from an increased level of fitness and may increase their level of high density lipoprotein cholesterol, which is associated with a decreased risk of cardiovascular disease. All subjects will receive biochemical and physiological testing valued well in excess of \$500.00. Subjects will also receive a thorough explanation of all testing results.

Compensation for Injuries: I understand that in the event of physical injury resulting from the research procedures, financial compensation for wages or time lost is not available, and the costs of medical care and hospitalization is not available and must be borne by the subject. I understand that Tammy S. Teague (790-0250 h or 626-1670 w) and/or R. Douglas Allen (326-1009 h or 626-3290) will provide more information upon my request.

I have read the above subject's consent. The nature, demands, risks, and benefits of the study have been explained to me. I understand that I may ask questions and that I am free to withdraw from the study at any time without incurring ill will. I also understand that this consent form will be filed in an area designated by the Human Subjects Committee and that my data will be kept in strict confidence with access restricted to the principle investigators or authorized representatives of the particular department.

Subject's Signature

Date

Witness' Signature

Date

I have carefully explained to the subject the nature of the above study. I hereby certify that, to the best of my knowledge, the subject signing this consent form understands clearly the nature, demands, benefits, and risks involved in participating in this study. A medical problem or language or educational barrier has not precluded a clear understanding of his involvement in this project.

Investigator's Signature

Date

PHOTOGRAPHIC RELEASE

I hereby grant to Tammy S. Teague and R. Douglas Allen absolute permission and all rights to copyright, publish, display, and use for any legal purpose, any or all photographs (together with descriptive text or statements) in which I or my property appear.

Dated: _____

_____ Signature

_____ Street

_____ City State Zip

_____ Print Name

THREE DAY DIETARY RECALL

FOR: _____ DATE: _____

BREAKFAST		MIDMORNING SNACK		LUNCH	
TIME:		TIME:		TIME:	
ITEM	AMOUNT	ITEM	AMOUNT	ITEM	AMOUNT
AFTERNOON SNACK		DINNER		EVENING SNACK	
TIME:		TIME:		TIME:	
ITEM	AMOUNT	ITEM	AMOUNT	ITEM	AMOUNT



THE UNIVERSITY OF ARIZONA

TUCSON, ARIZONA 85724

HUMAN SUBJECTS COMMITTEE
ARIZONA HEALTH SCIENCES CENTER 2305

78

TELEPHONE: 626-6721 OR 626-7575

20 July 1982

Mr. R. Douglas Allen
Department of Physical Education
McKale Center, Room 228
MAIN CAMPUS

Dear Mr. Allen:

We are in receipt of your project, "Effects of Exercise Training on Lipid Metabolism in Elderly Men", which was submitted to the Human Subjects Committee for review. The procedures to be used in this project pose no more than minimal risk to the subjects participating and the drug to be used is FDA-approved and requires no Investigational New Drug exemption. Regulations issued by the U.S. Department of Health and Human Services (45 CFR Part 46.110) authorize approval of this type project through the expedited review procedure, with the condition that the subjects' anonymity is maintained. Although full Committee review is not required, a brief summary is submitted for their information and comment, if any, after administrative approval is granted. This project is approved effective 20 July 1982.

Approval is granted with the understanding that no changes will be made in either the procedures followed or in the consent form to be used (copies of which we have on file) without the knowledge and approval of the Human Subjects Committee and the Departmental Review Committee. Any physical or psychological harm to any subject must also be reported to each committee.

A university policy requires that all signed subject consent forms be kept in a permanent file in an area designated for that purpose by the Department Head or comparable authority. This will assure their accessibility in the event that university officials require the information and the principal investigator is unavailable for some reason.

Sincerely yours,

Milan Novak

Milan Novak, M.D., Ph.D.
Chairman

MN/jm

cc: Patricia C. Fairchild, Ph.D.



THE UNIVERSITY OF ARIZONA

TUCSON, ARIZONA 85721

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DEPARTMENT OF PHYSICAL EDUCATION
EXERCISE & SPORT SCIENCES LABORATORY
McKALE MEMORIAL CENTER

July 14, 1982

Dear Physician,

Your patient has expressed an interest in participating in a research study conducted out of the Exercise And Sport Sciences Laboratory at the University of Arizona. Before allowing your patient to participate, we ask that the attached medical clearance form be filled out and signed by you. Please forward a copy of a recent resting 12-lead electrocardiogram, if available.

The study involves a battery of physiological tests which include a minimum of 3 maximal exercise stress tests conducted on a treadmill (a 12-lead electrocardiogram, blood pressure and maximal oxygen consumption will be assessed throughout each test), hydrostatic weighing, lung function, and flexibility. Biochemical testing will also be conducted which involves 6 blood draws on separate occasions amounting to a total withdrawal of 90cc of blood throughout the course of the study. On two occasions heparin will be injected (60 I.U./kg body weight) prior to drawing blood.

Subjects participating in the exercise portion of the study will participate in a 20 week walk/run aerobic fitness program. Subjects will train 3 days/week for 45 min/day within a target heart rate range. Each exercise session will be preceded by 15 minutes of stretching and will be followed by 5 minutes of warm-down exercises. (Control subjects will not participate in the exercise portion of the study.)

A detailed description of the forementioned program has been provided to your patient. We would encourage you to talk over the ramifications of this program with your patient so that his decision to participate will be made on a sound and rational medical basis. Your cooperation will be greatly appreciated. We will send you, on the approval of your patient, a complete set of the data collected in our laboratory including an interpretation.

The attached form is for your use in providing us with a minimum medical history and status of your patient, and there is a place for your signature indicating the patient is medically qualified to participate in this research study.

Please feel free to call our lab (626-5590) should questions arise. We would like to thank you for your cooperation and would also like to extend an invitation to you to visit our testing facility in the near future.

Sincerely,

R. Douglas Allen
Principle Investigator

PRE-EXERCISE PERSONAL INVENTORY

NAME _____ DATE _____ ID# _____

Please answer the following questions by responding as follows:

- () YES
 () NO
 (?) UNKNOWN

Fill in the numerical value
 where appropriate

HAVE YOU EVER HAD....

- () Rheumatic Fever
 () Heart Murmur
 () High Blood Pressure
 () Any Heart Trouble
 () Disease of the Arteries
 () Varicose Veins
 () Lung Disease
 () Epilepsy
 () Arthritis
 () Rheumatism
 () Migraine Headaches
 () Operations (Bone, Joint, Other, if yes describe): _____

- () Injuries (Fracture, Torn Cartilage, Etc. describe): _____

HAVE YOU RECENTLY HAD....

- () Chest Pain
 () Shortness of Breath
 () Heart Palpitations
 () Cough on Exertion
 () Coughing of Blood
 () Back Pain
 () Swollen, Stiff, or Painful Joints (explain): _____

- () Foot Problems
 () Muscle Cramps

HAVE ANY OF YOUR RELATIVES HAD....

- () Heart Attacks (Under age 55)
 () High Blood Pressure
 () Cholesterol (260 or higher)
 () Diabetes
 () Congenital Heart Disease
 () Heart Operations

DO YOU NOW....

- () Drink Coffee
 cups/day
 () Drink Tea
 cups/day
 () Drink Cola, Tabs, Etc.
 cans/day
 () Drink Beer
 cans/week (12 oz.)
 () Drink Wine
 oz/week
 () Drink Alcohol
 oz/week (1 shot = 1 oz.)
 () Smoke Cigarettes
 cigarettes/day
 () Smoke a Pipe
 Bowls Tobacco/day
 () Smoke Cigars
 cigars/day
 () Take prescription medication, if yes, list drug and dosage: _____

HAVE YOU EVER...

- () Smoked Cigarettes
 () Smoked a Pipe
 () Smoked Cigars

If yes, how long ago did you quit?

- Months
 Years

DIRECTIONS: Please answer the following questions as accurately as possible. Place a circle around the appropriate letter or number for each question.

1. Which of these exercises are you doing on a regular basis?

a. None	f. Jogging
b. Walk for exercise	g. Lift weights
c. Ride a bicycle	h. Taekwondo, karate, or judo
d. Swim	i. Competitive sports (List _____)
e. Do Calisthenics	j. Other (List _____)

2. How many days per week do you exercise?

a. None	e. Four
b. One	f. Five
c. Two	g. Six
d. Three	h. Seven

3. How much time do you spend on exercise each day?

a. None	e. 45 to 60 minutes
b. Less than 15 minutes	f. 60 to 75 minutes
c. 15 to 30 minutes	g. 75 to 90 minutes
d. 30 to 45 minutes	h. 90 minutes or more

4. If you exercise select the odd or even number which best describes the intensity (how hard) of your work outs.

6	14
7 Very, very light	15 Hard
8	16
9 Very light	17 Very hard
10	18
11 Fairly light	19 Very, very hard
12	20
13 Somewhat hard	

5. Indicate the MAJOR or MAIN reason why you exercise (Select one answer).

a. I do not exercise	
b. It makes me feel good	
c. I am trying to lose weight	
d. It is good for your health	
e. I am required to exercise	
f. My doctor told me to exercise	
g. Other (Explain _____)	

6. Have you ever had a physical injury as a result of participating in sports or an exercise program?

a. Yes (Explain _____)	
b. No	

7. Have you ever had any back, hip, knee, ankle, or foot problems while participating in an exercise program?

a. Yes (Explain _____)	
b. No	

8. Have you ever been advised by a physician not to exercise because of a medical problem?

a. Yes (Explain _____)	
b. No	

NAME _____

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