THE EFFECT OF A SINGLE BOUT OF DIFFERENT EXERCISE INTENSITIES ON LIPID AND LIPOPROTEIN CHOLESTEROL SUBFRACTIONS

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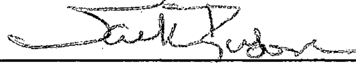

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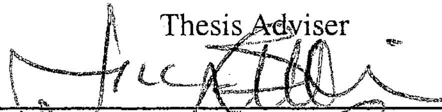

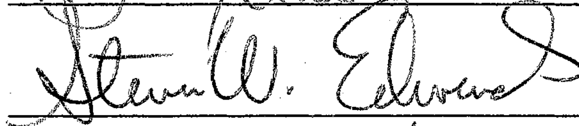

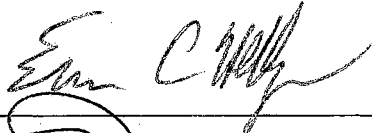

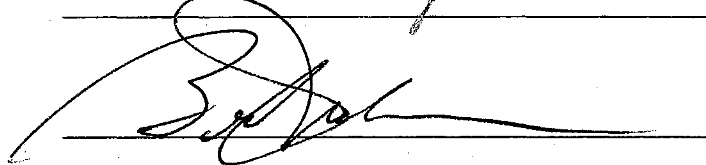

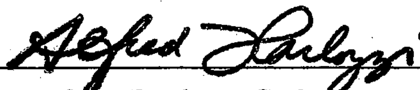

Dean of the Graduate College

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NOMENCLATURE

ACTH	adrenocorticotropic hormone
AnT	anaerobic threshold
Apo	apolipoprotein
BE	before exercise
bpm	beats per minute
cAMP	3'5'-cyclic adenosine monophosphate
CE	cholesterol ester
CETP	cholesteroly ester transfer protein
ECG	electrocardiogram
ETC	eletron transport chain
FAD	flavin adenine dinucleotide
FC	free cholesterol
FFA	free fatty acids
GH	growth hormone
h	hour (hours)
Hct	hematocrit
HDL-c	high-density lipoprotein cholesterol
HR	heart rate
HSL	hormone sensitive lipase

HTGL (or HL)	hepatic triglyceride lipase
IPE	immediately post-exercise
LCAT	lecithin cholesterol acetyltrasferase
LDL-c	low-density lipoprotein cholesterol
LDH	lactate dehyrogenase
LPL	lipoprotein lipase
LT	lactate threshold
NAD	nicotinamide adenine dinucleotide
OBLA	onset of blood lactate accumulation
PVC	plasma volume change
RER	respiratory exchange ratio
RPE	rate of perceived exertion
TC	total cholesterol
TG	triglycerides
TSH	thyroid-stimulating hormone
VE	ventilatory equivalent
VLDL-c	very low-density lipoprotein cholesterol
VO_2	oxygen uptake
$VO_{2\,max}$	maximal oxygen uptake
24 h	24 hours

CHAPTER I

INTRODUCTION

Cardiovascular diseases (CVD), especially atherosclerotic heart disease and stroke, are the leading causes of death in the United States, affecting more than 69 million Americans (Barbara, Cooper, & Dorothy, 1976; Hsia, 1990). Clinical studies have suggested that positive relationships exist among coronary heart disease, total serum cholesterol, and low-density lipoprotein cholesterol (LDL-c) in humans (Anderson, Castelli, & Levy, 1987; Levi, 1981). In contrast, high-density lipoprotein cholesterol (HDL-c) has been shown to reduce the incidence of coronary heart disease (CHD). In other words, HDL-c has been highly correlated with a decreased risk of CHD (American Heart Association, 1982; Gordon et al., 1989).

Triglycerides and cholesterol form the major components of plasma lipids and circulate as complex lipoprotein particles (TABLE I). It has been considered that elevated LDL-c concentration is an independent risk factor for the development of atherosclerosis (Ginsberg, 1994). Cholesterol is the major component of the atherosclerotic plaque, can be synthesized in the liver, is a normal constituent of bile, and is served as a precursor of various steroid hormones (Thomas, 1993). LDL-c, which is the major cholesterol carrier in the body, is divided into at least three major LDL subfractions: LDL_1, LDL_2, and LDL_3 (TABLE II). LDL_3 is the smallest and most dense

LDL subfraction, containing higher percentages of cholesterol ester and apoB, and lower percentages of triglycerides in contrast to LDL_1. LDL_1 is not considered to be atherogenic in contrast to LDL_3 which is known as the most atherogenic LDL subfraction (Austin, 1992). VLDL appears to be the major source of LDL (Sigurdsson, Nicoll, & Lewis, 1975). However, the role of VLDL in the atherosclerotic process has not received much attention because VLDL particles undergo a series of metabolic changes to the intermediate-density lipoprotein cholesterol (IDL-c) and then LDL via enzyme action. For instance, the liver secretes VLDL particles, and a portion of this lipoprotein particle is converted to VLDL remnants (i.e., IDL or LDL) or used for the formation of nascent HDL-c through lipoprotein lipase activity. In addition, cholesterol ester from HDL_2-c is transferred into other lipoproteins such as VLDL, IDL, LDL, or chylomicrons by the action of cholesterol ester transfer protein (CETP) (Murray, Granner, Mayes, & Rodwell, 1996; Ziogas, 1997). All of the lipoprotein's classes actually vary in composition, size, density, and function. However, several major classes of lipoproteins are divided into five classes based on the their physicochemical characteristics (Gotto, Pownall, & Havel, 1986). These include chylomicrons, VLDL, IDL, LDL, and HDL (HDL_2 and HDL_3) (TABLE II).

TABLE I

AVERAGE LIPID CONCENTRATIONS IN POSTABSORPTIVE PLASMA

	mg/dl	*μm/L*
Keto acids	10	0.1
Free fatty acids	10	0.4
Triglycerides	100	1.2
Cholesterol (total)	185	4.8
Low density	120	
High density	50	
Very low density	15	

Source: Berne, R.M. and Levy, M.N. 1998.

TABLE II

MAJOR LIPOPROTEIN CLASSES

Lipo-protein	Source	Density (g/ml)	TG (%)	Cholesterol Free (%)	Esters	Phospho-lipid (%)	Protein (%)
Chylo	Intestine	<.96	88	1	3	8	1-2
VLDL	Liver/Intestine	.96-1.006	56	8	15	20	7-10
IDL	VLDL/Chylo	1.006-1.019	29	9	34	26	11
LDL	VLDL/Chylo	1.019-1.063	13	10	48	28	21
HDL_2	Intestine/Liver	1.063-1.125	16	10	31	43	33
HDL_3	Intestine/Liver	1.125-1.210	13	6	29	46	57

Source: Durstine and Haskell, 1994. * (Chylomicrons: Chylo)

It is generally accepted that endurance trained athletes have higher HDL-c concentrations than sedentary counterparts (Hartung, Foreyt, Mitchell, Vlasek, & Gotto, 1980; Huttunen et al., 1979). This pattern may be partially attributed to the therapeutic effect of regular aerobic exercise on the lipid profile. It is unknown, however, if these findings represent a true chronic adaptive change with training rather than an acute and transient response to the most recent exercise bout (Hicks, Macdougall, & Muckle, 1987).

The mechanisms by which exercise confers a beneficial effect on lipid concentrations have not been fully explained. After a single bout of exercise, acute increases in HDL-c have been observed in some studies (Crouse, et al., 1997; Gordon et al., 1998) but not in others (Angelopoulos & Robertson, 1993; Cullinane, Lazarous, Thompson, Saratelli, & Herbert, 1981; Nikkila, Taskinen, & Sane, 1987), whereas data for a more chronic effect of exercise have been provided in various longitudinal and cross-sectional investigations (Thune, Njolstad, Lochen, & Forde, 1998; Thompson, et al., 1997). Differences of exercise intensity, duration, and caloric expenditure may complicate the interpretation of these studies. Furthermore, subjects' initial state of training and baseline concentration of HDL-c further complicate research findings (Zmuda et al., 1998).

HDL-c can be separated into two major subclasses: HDL_2-c and smaller, denser HDL_3-c. People with low risk for CHD have low LDL-c and VLDL-c and higher HDL concentration of HDL_2-c and HDL_3-c (Krauss et al., 1987). As compared with sedentary men, active individuals such as long-distance runners have higher HDL_2-c concentrations and lower LDL-c and VLDL-c concentration (Gidez, Miller, Burstein, Slagle, & Eder, 1982; Williams et al., 1986). Gidez and colleagues (1982) obtained 368 blood samples from students, faculty, employees of medical school, and from patients with CHD, hyperlipidemia, and diabetes to measure HDL-c and HDL subfractions. They reported that although CHD patients have low both HDL_2 and HDL_3, the relative larger decrease in HDL_2 compared with HDL_3 was found. Moreover, hypertriglyceridemic patients had greatly reduced total HDL concentration due to the reduction of both HDL_2 and HDL_3 concentrations but the greatest change was in HDL_2. In contrast, hyperchloesterolemic patients had modest decreases in HDL and HDL_2, but not in HDL_3. Potentially, HDL_2

may be the more variable HDL subfraction and changes in total HDL are primarily a reflection of changes in HDL_2. Therefore, measurement of HDL_2 might be a more determining factor in prediction of CHD than total HDL (Gidez et al.).

Purpose of Study

Exercise training leads to decreased total cholesterol and increased HDL-c, potentially reducing the incidence of CHD as well as causing favorable changes in lipid and lipoprotein cholesterol (Crouse et al., 1997; Stein et al., 1990; Miller, Rao, Lewis, Myhre & Mjos, 1979; Ratliff, Elliott & Rubenstein, 1978). Many of the lipid changes after a single bout of exercise are similar to those reported after chronic exercise training. Therefore, if chronic aerobic exercise has a therapeutic effect on the lipid profile, it's worth to measure acute changes of blood lipid and lipoprotein profiles with response to a single bout of aerobic exercise. Because this measurement may also provide some pattern changes of lipid and lipoprotein profiles with response to acute aerobic exercise if there is an effect of acute aerobic exercise on lipid and lipoprotein profiles.

In addition, it appears that caloric expenditure, duration, and intensity of exercise are directly related to the degree of changes in lipid and lipoprotien profiles. In assessing exercise intensity, the lactate threshold (LT) provides the best differentiation between aerobic and anaerobic exercise (Tim, Holger, Gabriel, & Wilfried, 1999). Lactate threshold refers to the highest workload without a gradual increase in blood lactate that can be maintained over a long duration (Stegmann & Kindermann, 1982). However, many investigators use percentages of $VO_{2\ max}$ or of maximal heart rate in metabolic, hormonal, or immunological studies as a criterion for intensity although variability in

physiological responses to submaximal exercise may occur due to wide ranges of exercise intensities compared with the LT (Tim et al., 1999). For instance, heart rate is influenced by extraneous variables such as pathological illness. The $VO_{2\ max}$ can be used as an objective criterion as exercise intensity but not individualized for each participant as a same intensity to exercise, i.e., lactate threshold may occur between 50 and 85% of $VO_{2\ max}$ based on his or her training status (Dwyer & Bybee, 1983). This variability in physiological responses to the wide ranges of exercise intensities using percentages of $VO_{2\ max}$ or of maximal heart rates as a criterion for an exercise intensity to especially study lipid metabolism may cause different response to exercise. Consequently, the evaluation of lactate concentration may be the most reliable criterion for exercise intensity to study lipid metabolism.

Accordingly, the purpose of this study was to determine the effect of a single bout of aerobic exercise at different exercise intensities measured from the evaluation of lactate concentration on lipid and lipoprotein cholesterol subfractions in college men, assuming each subject equally expend 350 kcal of energy during different exercise intensities (70% of lactate threshold and lactate threshold (LT) intensities).

Hypotheses

The following null hypotheses were tested:

Ho_1: There were no significant differences between LT intensity and 70% LT intensity (intensity condition) in lipids and lipoprotein concentrations with response to a single session of aerobic exercise in men.

Ho_2: There were no significant differences over time in lipids and lipoprotein

concentrations with response to a single session of aerobic exercise immediately post-exercise (IPE) or 24 h post-exercise (24 h) in comparison to the baseline (before exercise; BE).

Ho_3: There were no significant interaction effects of the two independent conditions on the (intensity × time) lipid and lipoprotein concentrations with response to a single session of aerobic exercise in men.

Delimitations

Certain delimitations were set by the investigator of this study, which may have affected the results and conclusions drawn. The following delimitations were acknowledged:

1) Eighteen healthy college-age males served as subjects due to the availability of this population and the lack of available adolescent and elderly populations.
2) Each subject completed two counterbalanced running or walking treadmill tests at 70% of LT intensity and LT intensity, for the duration sufficient to expend 350 kcal of energy.
3) Heart rate (HR), electrocardiography (EKG), percentage of body fat (% BF), body mass index (BMI), and $VO_{2\ max}$ were only physiological markers tested.
4) Blood samples, taken at approximately the same time of day, were collected: before exercise (for baseline), immediately post-exercise (exercise response), and 24 hours post exercise (24 hours exercise response).
5) Primary chemical measures analyzed, adjusted for plasma volume changes (PVC), were: triglyceride (TG), total cholesterol (TC), free cholesterol (FC),

cholesterol ester (CE), HDL-c, HDL_2-c, HDL_3-c, LDL-c, and VLDL-c. But CE, HDL_2-c, LDL-c, and VLDL-c were calculated by using appropriate equations (TABLE IV) based on the TG, TC, FC, or HDL_3-c values. Blood lactate concentrations were analyzed to determine 70% of LT and LT, which were used for exercise intensities during exercise trials.

6) Normal daily variations in individual lipid and lipoprotein cholesterol profiles were not assessed.

Limitations

The limitations set in this study reflected the effect of the delimitations on the collection and interpretation of the data and the ability to expand the scope of inference beyond the sample population. Generalizations made from the results were compromised by the following limitations.

1) The small number of subjects (n = 18) constrained the power of testing for statistical significance.
2) Ability to inference beyond on exercising populations was not possible within investigation parameters.
3) No attempt was made to assess non-physical stimuli and their effects on lipid and blood variables.
4) Generalizations from the results should not be made beyond the male subject pool. The acquisition of subjects from a group of volunteers was not by random sampling that may prevent inference beyond the study sample.

Assumptions

For the purpose of this study, the researcher accepts the following assumptions.

1) Subjects abstained from exercise for 24 hours before testing session as specified.
2) Subjects were not currently taking any medications or substances known to alter lipid metabolism as indicated in completion of questionnaire and maintained normal diet as requested.
3) Subjects fasted for 12 hours preceding collection of blood samples as requested.
4) The level of work output by each subject was dependent on the degree of motivation, competitiveness and willingness to complete the exercise bout.

Definitions

1) Adventitia: the outermost covering of a structure or organ such as outmost layer of the three layers of an artery (Ross & Glomset, 1976).
2) Anaerobic threshold (AnT): the point at which the metabolic demands can no longer be met by available aerobic sources and at which an increase in anaerobic metabolism occurs, reflected by an increase in blood lactate accumulation (Wilmore & Costill, 1994).
3) Apolipoprotein: The apolipoproteins are the nonlipid, protein portions of a lipoprotein.
4) Arteriosclerosis: "the pathological conditions in which there are thickening, hardening, and loss of elasticity of the walls of arteries" (Thomas, 1993, p. 151).

5) Atheroma: "fatty degeneration or thickening of the walls of the larger arteries occurring in atherosclerosis" (Thomas, 1993, p. 168).

6) Atherosclerosis: a multi-factorial process that leads to the accumulation of cholesterol in the intima layer.

7) Chelating agent: an agent used to prevent coagulation in collected blood samples.

8) Chemotaxis: The movement of certain, additional white blood cells in response to products formed in an immunologic or inflammation reaction (Thomas, 1993).

9) Cholesterol ester transfer protein (CETP): a hydrophobic glycoprotein synthesized and secreted by hepatic, muscle, and adipose tissue. It is associated primarily with HDL particles and functions in the transfer of lipids between the HDL, LDL, and VLDL fractions.

10) Chylomicrons: a class of lipoproteins transport dietary cholesterol and tirglycerides after digestion and absorption of fat in food.

11) Collagen: the protein substance of the white fibers (collagenous fibers) of skin, tendon, bone, cartilage, and all other connective tissue.

12) Diabetes Mellitus: a chronic disorder of carbohydrate metabolism, characterized by hyperglycemia and glycosuria (sugar in urine) and resulting from inadequate production or utilization of insulin. Diabetes mellitus is classified according to two syndromes: Type I, or insulin-dependent diabetes mellitus (IDDM)- the patients secrete little or no insulin and Type II, or non-insulin-dependent diabetes mellitus (NIDDM)- insulin is produced, but insulin becomes less

effective due to the receptor defect (Thomas, 1993).

13) Distensibility: the ability of vessels to expand.

14) Ester: Fats are esters, formed by the combination of an organic acid with the alcohol glycerol (Thomas, 1993).

15) Ethylenediaminetetraacetic acid (EDTA): a chelating agent used to prevent coagulation.

16) Fibrin: "a whitish, filamentous protein formed by the action of thrombin on fibrinogen" (Thomas, 1993, p. 726).

17) Fibrinogen: "a plasma protein that is converted into fibrin by thrombin in the presence of calcium ions" (Thomas, 1993, p. 726).

18) Fibroblast: a connective tissue cell.

19) Hematocrit: the total volume of erythrocytes, expressed as a percentage (%) of blood.

20) Hemoglobin: the iron-containing pigment of the red blood cells. Its function is to carry oxygen from the lungs to the tissues. It is expressed as milligrams per deciliter (mg/dl) of blood (Thomas, 1993).

21) Hypercholesterolemia: a condition in which greater than normal amounts of cholesterol is present in the blood. High levels of cholesterol and other lipids may lead to the development of atherosclerosis.

22) Intima: "innermost coat of a structure such as a blood vessel" (Thomas, 1993, p. 1010).

23) Ketoacids: "any organic acid containing the ketone CO (carbonyl radical)" (Thomas, 1993, p. 1050).

24) Intermediate-density lipoprotein (IDL): a lipid-protein complex with a density between VLDL and LDL. The product has a relatively very short half-life and is normally in the blood in very low concentrations.

25) Lipoprotein (a): represents a class of plasma lipoproteins similar to LDL but containing a large glycoprotein molecule called apo-lipoprotein (a) or apo (a).

26) Lumen: "the space within an artery, vein, intestine, or tube" (Thomas, 1993, p. 1136).

27) Media: middle tissue layer of an artery.

28) Monocytes: a mononuclear, phagocytic, white blood cell derived from the myeloid stem cells. Monocytes are one of the first lines of defense in the inflammatory process.

29) Mucopolysaccharide (proteoglycans): it is polysaccharides that form chemical bonds with water, contain hexosamine and sometimes proteins, and is thick gelatinous material. This thick gelatinous material forms intercellular ground substance and basement membranes of cells; it is found in mucous secretions and synovial fluid (Thomas, 1993).

30) Phagocyte: a class of mobile white blood cells, which engulf foreign antigens and cell debris. An important component of the body's immune system.

31) Platelet: "a round or oval disk, 2 to 4 micrometers in diameter, found in the blood of vertebrates. Platelet plays an important role in blood coagulation, hemostasis, and blood thrombus formation" (Thomas, 1993, p. 1526).

32) Polysaccharide: a carbohydrate that contains three or more molecules of simple carbohydrates. Examples of polysaccharides are dextrins, starches, glycogens,

celluloses, gums, inulin, and pentose.

33) Respiratory exchange ratio (RER): The ratio of carbon dioxide expired to oxygen consumed at the level of the lungs (Kenney, 1995).

34) Rheology: "study of the deformation and flow of materials" (Thomas, 1993, p. 1718).

35) Smooth muscle: muscle tissue that lacks cross striations on its fibers; involuntary in action and found principally in visceral organs.

36) Thrombin: "an enzyme formed in shed blood from prothrombin that reacts with soluble fibrinogen, converting it to fibrin, which forms the basis of a blood clot" (Thomas, 1993, p. 1988).

37) Thrombus: "a blood clot that obstructs a blood vessel or a cavity of the heart. Anticoagulants are used in prevention and treatment of this condition" (Thomas, 1993, p. 1991).

38) Very Low-Density Lipoprotein (VLDL): these large, buoyant lipoproteins are secreted by the liver and contain a relatively large amount of triglyceride. Other components are cholesterol and phospholipid, the A and C apoliproteins, some apo E, and a copy of apo B-100.

CHAPTER II

REVIEW OF LITERATURE

Atherosclerotic Cardiovascular Disease

Multivariable factors of CVD

Cardiovascular disease (CVD) is a multifactorial phenomenon that requires more than one factor to manifest the disease. For this reason, a multivariate analysis helps us better understand the pathogenesis of the disease and provides better direction for preventing CVD (Michael & Donald, 1995).

The Framingham Study (Gordon, Castelli, Hjortland, Kannel, & Dawber, 1977) and others (Gordon et al., 1989) have identified a number of cardiovascular risk factors that contribute to the major atherosclerotic diseases. In the Framingham study, lipid and lipoprotein values were obtained on 2,815 men and women aged 49 to 82 years at Framingham, Massachusetts. At these age ranges, the major potent lipid risk factor was HDL cholesterol, which was inversely related to with the incidence of coronary heart disease ($p < 0.001$) in either men or women. In persons with HDL-c levels below 35 mg/dl, the incidence rate is more than eight times in persons with HDL-c levels 65 mg/dl or above. In addition, Gordon et al. reported that a 1 mg/dl increase in HDL-c

was associated with a 2% in men and 3% in women decrease in CHD. However, there are remarkable differences in their influence on the specific cardiovascular outcomes. In other words, the various cardiovascular risk factors affect disease rates in both sexes at all ages but with different magnitudes, i.e., diabetes and a low level of HDL cholesterol function more powerfully in women than in men (Kannel, 1987).

The cigarette smoking is a well-known risk factor for atherosclerosis that may be due to loss of distensibility and compliance of large arteries from smoking (Kool, Hoeks, Struijker Boudier, Reneman, & Van Bortel, 1993). Smoking is more influential in men than in women and loses its effect when the smoker quits smoking (Kannel, McGee, & Castelli, 1984).

Obesity and weight gain can cause all the major atherogenic risk factors (Higgins, Kannel, Garrison, Pinsky, & Stokes, 1987). Moreover, obesity and non-insulin-dependent diabetes mellitus (NIDDM; Type II) are closely linked with each other (Rios, 1998). Generally if people are over age 45 and significantly overweight, insulin resistance might develop with weight increase although the pancreas still produces insulin. In other words, the body can not use it effectively with weight increase and age.

Physical inactivity is a risk factor for cardiovascular disease and one mechanism may involve changes to lipoprotein metabolism. In other words, regular exercise may reduce the progression of atherosclerotic vascular disease in both obese and the general population (Hardman, 1999; Leaf, Parker, & Schaad, 1997).

The Framingham Study established hypertension as a major cardiovascular risk factor. Hypertension is a major risk factor for cardiovascular disease and death in both sexes (O'Donnell & Kannel, 1998), and the prevalence of hypertension in diabetics is

significantly higher than in the general population, being twice as prevalent (Navales et al., 1999).

Plasma fibrinogen is also a major independent risk factor for CVD, stroke, and peripheral arterial disease (Giannasi, Ferrari, & Galetta, 1995; Naito, Hayashi, & Iguchi, 1994) because it strongly affects blood coagulation, blood rheology and platelet aggregation (Ernst & Koenig, 1997). Dense LDL (LDL_{-3}) compared with buoyant LDL (LDL_{-1}) is associated with a more atherogenic type of lipoprotein profile, with increased triglyceride and apoprotein B and decreased HDL-c and apoprotein A-I (Slyper, 1994). Several prospective studies also report that lipoprotein (a) (Lp(a)) excess has been identified as a powerful predictor of premature atherosclerotic vascular disease (Stein & Rosenson, 1997).

Homocysteine, which is an intermediate formed during the metabolism of the essential sulfur-containing amino acid methionine, is an independent risk factor for CVD (Greenlund et al., 1999). The causes are still under study, but likely patients with elevated homocysteine include folate and B vitamin deficiencies and possibly renal disease. At least one study (Greenlund et al.) reported that homocysteine correlated positively with age, but another study did not (Yoo et al., 1999).

Valentine and colleagues (2000) reported early CVD is more common in first-degree relatives of individuals with premature peripheral vascular disease (PVD) than healthy individuals. This finding indicates that susceptibility to premature PVD has a familial basis.

The Pathogenesis of Atherosclerosis

Increased risk for atherosclerosis and its complications are traditionally associated with factors such as cigarette smoking, obesity, diabetes, physical inactivity, low HDL-c level (< 35 mg/dl), Lp(a), hemocysteine, hypertension, plasma fibrinogen, hypercholesterolemia, and familial predisposition as previously described.

Atherosclerosis is an intimal disease of arteries (Davies & Woolf, 1993). Normally, atherosclerosis is known to be an immune and inflammatory response of the intima to injury and the injury is initiated by lipid (Ross, 1986). Although native plasma lipids, especially LDL, freely enter the intima, they do not initiate an inflammatory response, are not ingested by monocytes, and do not damage tissue. However, oxidized lipid is chemotactic for monocytes, induces migration, and initiates inflammatory responses (Davies & Woolf). Atherosclerosis is a pathological phenomenon that begins with a fatty streak, containing and surrounded by deposits of lipid. Most of this lipid that is found within the fatty streak is in the form of cholesterol and cholesterol ester (Ross & Glomset, 1976).

The atherosclerotic lesion may be classified into three different types such as fatty streak, fibrous plaque, and complicated lesion. The fatty streak is characterized by a focal accumulation of relatively small number of intimal smooth muscle cells that contain and are surrounded by deposits of lipid, cholesterol and cholesterol ester. This fatty streak causes no obstruction (Ross & Glomset, 1976). The fibrous plaque is the most characteristic lesion of advancing atherosclerosis. It is grossly whitish and is elevated; therefore, it bulges out into the lumen of the artery that has developed atherosclerosis.

The complicated lesion may be a fibrous plaque. The notable characteristic of this lesion is the presence of calcium. This lesion is often associated with occlusive disease (Ross & Glomset). These focal atherosclerotic lesions are characterized by three fundamental phenomena: 1) proliferation of smooth muscle cells, 2) deposition of intracellular and extracellular lipid, and 3) accumulation of extracellular matrix compounds including collagen, elastic fibers and mucopolysaccharides (proteoglycans) (Ross & Glomset). Surprisingly, atherosclerosis is usually a slowly progressive disease causing no symptoms until a major artery is closed (National Institutes of Health, 1977).

The Structure of Arterial Wall - The normal muscular and elastic arteries are composed of three distinct layers: intima, media, and adventitia. The intima is the innermost layer and is composed of a narrow region bounded on the luminal side by a single continuous layer of endothelial cells and bounded peripherally by a fenestrated sheet of elastic fibers, called the internal elastic lamina. Normally the number of these cells increases with age (Ross & Glomset, 1976). The middle layer of the muscular artery is the media, which is composed of smooth muscle cells, surrounded by various amounts of collagen, small elastic fibers and mucopolysaccarides. The morphology of media, in contrast to the intima, does not change with age. The adventitia is the outermost layer of the artery. It consists of fibroblasts and smooth muscle cells that are loosely arranged between bundles of collagen and surrounded by mucopolysaccharides. It is typically separated from the media by a discontinuous elastic tissue, called external elastic lamina (Ross & Glomset). Normally, the intima is related to atherosclerosis.

As previously described, atherosclerosis is an intimal disease of arteries in which fatty lesions develop on the lumen of the arteries. These lesions, including fatty streak, fibrous plaque, and complicated lesion, are also called plaques. Atherosclerosis is a pathological phenomenon that begins by accumulation of fatty streak, containing and surrounded by deposits of lipid. With time, the fatty streak that develops and grows larger causes proliferation of surrounding fibrous and smooth muscle tissues to form larger plaques, eventually reducing the blood flow. The cholesterol accumulation on the surfaces of arterial walls and the cellular proliferation becomes large enough to bulge plaques into the lumen, sometimes causing complete occlusion. Even without occlusion, the fibroblasts of the plaque accumulate, grow larger, and then cause the arteries to harden so that distensibility is lost. This is called hardening of the artery or arteriosclerosis. Where the plaques bulge out into the flowing blood, the roughness of their surfaces increases the rise of blood clots, with thrombus or embolus formation, resulting in the sudden blocking of blood flow (Guyton & Hall, 1996).

Mechanism to Alter Lipids and Lipoproteins

The Enzymes associated with lipid and lipoprotein metabolism

Five major enzymes that significantly influence lipid metabolism are lipoprotein lipase (LPL), lecithin cholesterol acetyltrasferase (LCAT), hepatic triglyceride lipase (HTGL), cholesteroly ester transfer protein (CETP), and hormone-sensitive lipase (HSL).

Lipoprotein lipase (LPL) is a lipolytic enzyme that controls TG hydrolysis (lipolysis). This enzyme hydrolyzes the TG within the chylomicrons and VLDL into

FFAs and glycerol on the outer endothelial surface of the cell for storage or use of the adipose tissue or muscle (Kinnunen, Virtanen, and Vainio, 1983). In addition, HDL concentrations may be affected by activity of LPL. This may be due to release of phopholipid and apo A-I during hydrolysis of chylomicrons. This process contributes to the formation of nascent HDL (Murray et al., 1996).

Lecithin cholesterol acetyltrasferase (LCAT) reaction promotes cholesterol esterification that is the major source of HDL CE (Eisenberg, 1984). LCAT catalyzes plasma CE (a storage form of cholesterol) and this LCAT activity causes cholesterol in HDL to be esterified. Consequently, LCAT causes to move CE into the HDL-c core that creates a concentration gradient and draws in cholesterol from tissues and from other lipoproteins. Therefore, HDL_3 becomes less dense, forming HDL_2, which transports cholesterol to the liver (Murray et al., 1996).

Hepatic triglyceride lipase (HTGL) interacts with lipoproteins in the liver and may play a role in the reconversion of HDL_2 to HDL_3 in contrast to the activity of LCAT (Superko & Haskell, 1987). A HDL cycle is known as reverse cholesterol transport. The cycle includes uptake and esterification of cholesterol by HDL_3 that becomes less dense, forming HDL_2. In contrast to this cycle, HTGL hydrolyzes HDL phospholipid and TG, allowing the particle to release its CE to the liver, providing the particle becomes smaller and denser, and re-forming HDL_3, which reenters the cycle (Murray et al., 1996).

Cholesteroly ester transfer protein (CETP) has been also linked to HDL particles; that is, it facilitates transfer of CE from HDL_2 to other lipoproteins (VLDL, IDL, LDL, chylomicrons). Therefore, CETP is involved in the formation of HDL_3 (Tall, 1986).

Hormone-sensitive lipase (HSL) is the intracellular fraction of LPL (Oscai, 1983) that hydrolyzes TG in the adipose tissue to form FFAs and glycerol. Since glycerol cannot be utilized readily in this tissue, it diffuses out into the plasma and then is utilized by liver and kidney because these organs possess an active glycerol kinase that is responsible for the utilization of glycerol. However, the FFAs formed by hydrolysis can be reconverted in the tissue to acyl-CoA and reesterified with glycerol 3-phosphate to form TG. Thus, there is a continuous cycle of lipolysis (hydrolysis) and reesterification within the tissue. However, when the rate of lipolysis exceeds the rate of reesterification, FFAs accumulate and diffuse into the plasma, where they bind to albumin and raise the concentration of plasma FFAs (Murray et al., 1996).

The Enzyme Responses to Exercise

Considerable research has been conducted to understand the mechanisms related to how exercise affects enzymes that control plasma lipids and lipoproteins, but the understanding of mechanisms is still incomplete. The major enzymes are lipoprotein lipase (LPL), hepatic triglyceride lipase (HTGL), Cholesteroly ester transfer protein (CETP), hormone-sensitive lipase (HSL), and lecithin cholesterol acyltransferase (LCAT), which are responsible for the lipid and lipoprotein metabolism. These enzymes are also associated with exercise-induced changes in plasma lipid and lipoprotein.

LPL is an enzyme located in skeletal and cardiac muscle that is involved in the catabolism of free fatty acids and triglycerides, and is believed to play a major role in the formation of HDL as described previously. LPL activity is accelerated and the

catabolism of FFAs and TG is increased when energy demand is required. Increased LPL activity also contributes to increase in the conversion of VLDL to HDL (Stefanic, Terry, Haskell, & Wood, 1988). Following aerobic exercise training in sedentary individuals, there is an increase in capillary density (Kines & Lithell, 1989). This result indicated that the increase in capillary density results in elevation of LPL activity due to a larger number of binding sites for the enzyme on the capillary endothelial surface. This means that there is a positive correlation between LPL activity and capillary density (Kines & Lithell).

HTGL (or HL) is an enzyme found in the liver. It is considered to play a major role in the conversion of HDL_2 to HDL_3, and to participate in the conversion of VLDL to LDL as mentioned previously (Stefanic et al., 1988). The activity of HL is decreased by vigorous exercise (Haskell, 1986) and also by chronic exercise (Nikkila, Taskinen, Rehunen, & Harkonen, 1978). A reduction in HL activity is associated with a reduced conversion of HDL_2 to HDL_3 (Stefanic et al.). The conversion of VLDL to LDL is also slower when HL activity is reduced.

CETP facilitates transfer of CE from HDL_2 to other lipoproteins (VLDL, IDL, LDL, chylomicrons). Therefore, CETP is involved in the formation of HDL_3 (Tall, 1986). The short-term effects of prolonged intense exercise on plasma lipid and lipoproteins, and mass and activity of cholesterol ester transfer protein (CETP) were studied in eight male endurance-trained athletes over the first week after a bicycle marathon. Plasma concentrations of cholesterol, TGs, LDL-c, apo B, CETP, and CETP activity were significantly reduced in the recovery period compared with pre-exercise values. However, HDL cholesterol and HDL_2 cholesterol were significantly increased in

the post-exercise period, whereas HDL_3 cholesterol showed a tendency to decrease in the late recovery period (Foger et al., 1994). Other researchers also have reported that CETP concentration decreased significantly in response to training in both men and women (Seip et al., 1993; Serrat-Serrat et al., 1993).

In contrast, a significant increase in LCAT has been observed in trained versus sedentary subjects. This LCAT activity is correlated with HDL-c (Gupta, Ross, Myers, & Kashyap, 1993; Tsopanakis, Kotsarellis, & Tsopanakis, 1988). This enzyme causes CE to move into the HDL-c core, creating a concentration gradient and attracting cholesterol from tissues and other lipoproteins. Hence, HDL_3 becomes less dense, forming HDL_2.

Hormone-sensitive lipase (HSL), which is the intracellular fraction of LPL, hydrolyzes TG in the adipose tissue to form FFAs and glycerol. During exercise, catecholamines prevent release of insulin and causes secretion of other hormones such as cortisol, glucagon, and GH. These hormones increase the FFA mobilization as an energy source and in turn liver produces more VLDL as a result of increased FFAs in the blood. This response may increase the HDL-c (Oscai, 1983).

The Apolipoproteins (Apoproteins) associated with lipid and lipoprotein metabolism

To understand plasma lipid and lipoprotein metabolism it is very important to know the apolipoproteins. The protein moiety of a lipoprotein is known as an apolipoprotein (apoprotein) (Levi, 1981). Some apoproteins are essential and cannot be eliminated, whereas others are free to transport to other lipoproteins. One or more apoproteins are existing in each lipoprotein. Apoproteins perform several important roles as following

(Levi, 1981; Murray et. al., 1996): act as enzyme co-factors (i.e., Apo.C-II for LPL and Apo.A-I for LCAT), provide as ligand for interaction with lipoprotein receptors in tissue (i.e., Apo. B-100 and E for the LDL receptor, Apo. E for the remnant receptor, and Apo. A-I for the HDL receptor), provide structural stability to the lipoproteins, and play crucial roles in determining the metabolic fate of the particles on which they reside.

Most apoproteins are synthesized by the liver or small intestine. The major apoproteins and their primary functions are as following TABLE III.

TABLE III

APOLIPOPROTEINS OF HUMAN PLASMA LIPOPROTEINS

Apoproteins	Lipoproteins	Molecular Mass (Da)	Comments
Apo A-I	HDL, Chylo	28,000	LCAT activator. Ligand for HDL receptor.
Apo A-II	HDL, Chylo	17,000	Structure is two identical monomers joined by a disulfied bridge. Inhibitor of LCAT?
Apo A-IV	Secreted with Chylo but transfers to HDL	46,000	Associated with the formation of TG-rich lipoproteins. Function unknown. Synthesized by intestine.
Apo B-100	LDL, VLDL, IDL	550,000	Synthesized in liver. Ligand for LDL receptor.
Apo B-48	Chylo,Chylo remnants	260,000	Synthesized in intestine.
Apo C-I	VLDL,HDL, Chylo	7,600	Possible activator of LCAT.
Apo C-II	VLDL,HDL, Chylo	8,916	Activator of lipoprotein lipase.
Apo C-III	VLDL,HDL, Chylo	8,750	Inhibits LPL.
Apo D	Subfraction of HDL	19,300	May act as lipid transfer protein.
Apo E	VLDL,HDL, Chylo	34,000	Functions in the catabolism of chylo, VLDL, and HDL. Ligand for chylo remnant receptor in liver and LDL receptor.

Murray et al. (1996) * (Chylomicrons: Chylo)

Lipid and Lipoprotein Metabolism

Lipid (TG, cholesterol, CE, and FC) - Lipids are any one of a group of fats, characterized by their insolubility in water and solubility in fat solvents. The major lipids

found in human are triglycerides (TG), phospolipids, cholesterols, glycolipids, and fat-soluble vitamins. Most of lipids made by the liver are not used by liver. Therefore, they must be transported in the blood to other cells as chylomicrons, free fatty acids, and lipoproteins. Triglycerides, cholesterol (cholesterol ester and free cholesterol), phospholipid, and protein are the major components of plasma lipoproteins and circulate as complex lipoprotein particles (TABLE II). Triglycerides come from intestinal absorption of dietary lipids and synthesized in the liver (Murray et al., 1996).

Dietary (exogenous) TG is carried in plasma by chylomicrons while endogenous triglycerides are carried in plasma by VLDL. Hence, chylomicrons and VLDL are the vehicles of transport of TG from the liver to the extrahepatic tissues for use or storage. TG is the predominant lipid in chylomicrons and VLDL, whereas cholesterol and phospholipid are the predominant lipids in LDL and HDL (TABLE II).

TG stores in adipose tissue are continually undergoing lipolysis and reesterification that involve different reactants and enzymes. The resultant of these two processes determines the magnitude of the FFA in adipose tissue, which in turn is the source and determinant of the level of plasma FFA circulating in the plasma (Murray et al., 1996).

Cholesterol also comes from synthesis and diet. Actually, all tissues containing nucleated cells are able to synthesize cholesterol. Cholesterol is present in tissues and plasma lipoproteins either as FC (unesterified form of cholesterol) or CE (a storage form of cholesterol). It is synthesized in many tissues from acetyl-CoA and is ultimately eliminated from the body in the bile as cholesterol or bile salts (Eisenberg, 1984). CE is a storage form of cholesterol found in most tissues and is transported in the hydrophobic core of lipoproteins. CE in the diet is hydrolyzed to FC that mixes with dietary FC and

biliary cholesterol before absorption from the intestine in together with other lipids (Murray et al., 1996). FC is removed from tissues by HDL and transported to the liver for conversion to bile acids in the process known as reverse cholesterol transport.

Lipoproteins (Chylomicrons, VLDL, IDL, LDL, HDL-c, and HDL-c subfractions) - Hundreds of lipid and protein molecules build up the lipoproteins, which are spherical particles. Triglycerides, cholesterol, phospholipid, and protein are the major components of plasma lipoproteins and circulate as complex lipoprotein particles (TABLE II). TG and CE (esterified form of cholesterol) are insoluble in aqueous environments (hydrophobic) and constitute the core of the lipoproteins, whereas phospholipids and free cholesterol (unestered form of cholesterol) that are soluble in both lipid and aqueous environments (amphipathic) cover the surface of the particles. The last part of lipoprotein is apolipoprotein (or apoprotein), which also fills up the surface of the lipoproteins and plays important roles in the regulation of lipid transport and lipoprotein metabolism (Ginsberg, 1994) (Figure 1).

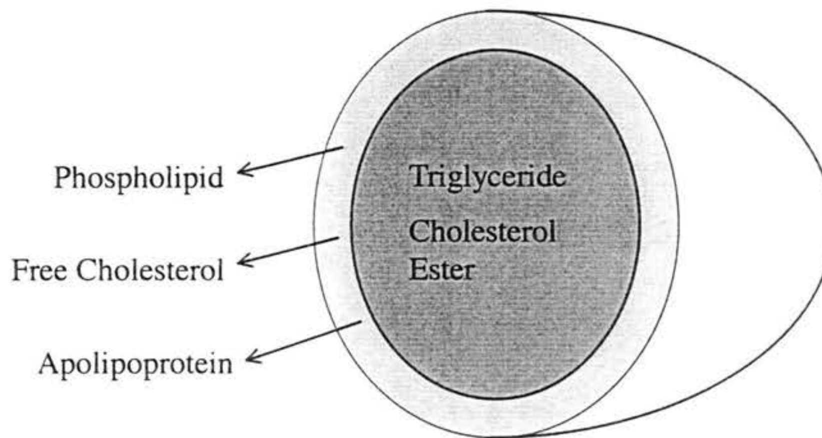


Figure 1. Schematic representation of a cross section through a lipoprotein (Ginsberg, 1994).

All of the lipoprotein's classes actually vary in composition, size, density, and function. However, several major classes of lipoproteins are divided into five classes based on the their physical-chemical characteristics (Gotto et al., 1986). These include chylomicrons, VLDL, IDL, LDL, and HDL (HDL_2 and HDL_3) as described previously (TABLE II).

First, nascent chylomicrons that contain Apo.A and B-48 enter the circulation from the small intestine. Nascent chylomicrons are converted to chylomicrons with the addition of Apo.C and E from HDL-c. When chylomicrons circulate in the capillaries of adipose tissue and skeletal muscles, LPL catabolizes the TG of chylomicrons into FFA and glycerides. That is the beginning of the metabolism of lipids and lipoproteins (Eisenberg and Levy, 1976). During this process, Apo.A and C from chylomicrons are released and stuck to existing HDL-c or used in the formation of nascent HDL-c. Hence, chylomicron remnants are formed, transported to the liver through an Apo. E receptor, and cleared by the liver while the FFA are re-esterified or oxidized within the mitochondria of adipose tissue or muscle cell (Eisenberg, 1979; Visich et al., 1996).

VLDL is formed in the liver, increased with excess carbohydrate-rich diets, and released into the plasma as nascent VLDL with Apo. B-100, C, and E as noted in the TABLE III. VLDL-c is formed with additional Apo.C and E added from HDL-c. Most of the cholesterol in VLDL is retained in the VLDL remnant (IDL) that is taken up by the liver or converted to LDL, which in turn is taken up by the LDL receptor in liver and extrahepatic tissues. When VLDL-c circulates in adipose tissue and skeletal muscle, LPL has the same action as mentioned with chylomicrons. After the action of LPL, Apo.C is released to bind with existing HDL-c or used for the formation of nascent HDL-c, and VLDL is hyrolyzed to IDL-c (VLDL-c remnant). The IDL-c can either be taken up by the liver via Apo.E receptor mediated activity or further hydrolyzed into LDL probably by LPL and HTGL (Visich et al., 1996). The residual IDL-c is converted into LDL-c with Apo.B-100 on its surface. Apo.B-100 is recognized by the hepatic and extrahepatic LDL receptors. The number of LDL-c receptor and each cell need for cholesterol influence the plasma LDL-c levels. In other words, when the need is low, cells make fewer receptors and LDL-c is eliminated at lower rates (Wolinsky, 1997; Shepard, 1992).

HDL is initially formed as a precursor lipoprotein. The initial particle, called nascent HDL, is a discoidal lipoprotein made of phopholipid, apoproteins, and small amounts of FC (Eisenberg, 1984). Both the small intestine and liver produce HDL and its precursors. HDL precursors come from two sources: from direct secretion of discoidal HDL (nascent HDL) from liver and small intestine into plasma or lymph; and from the surface components of TG-rich lipoproteins (chylomicrons and VLDL) during their catabolism in peripheral tissue via LPL (Tall & Small, 1978), i.e., as chylomicrons and VLDL are hydrolyzed, they lose not only their TG, but also surface remnants of

chylomicrons (Apo.A-I, A-II, C and phopholipid) and VLDL (Apo.E, C and phospholipid). Hence, during hydrolysis, protein and lipid constituents (such as some CE and phospholid) leave the chylomicron and VLDL and are converted into the nascent HDL through recombination of plasma Apo.A-I-phospholipid complexes with cholesterol in the blood stream (Nikkila et al., 1987). The nascent HDL (discoidal HDL) enter the blood stream and are transformed into the spherical particles (HDL) after interaction with LCAT. This change of the shape from discoidal (nascent HDL) to spherical (HDL) particles is conducted by migration of esterified cholesterol (from esterification of HDL FC) into the core of nascent HDL through interaction with LCAT because nascent HDL preferred substrate (Nikkila et al.). Therefore, the surface layer will then take more unesterified cholesterol from circulating lipoproteins and cell membrane, and then additional uptake of CE (Haskell, 1986; Miller, 1980), resulting in formation of the HDL-c (Figure 2).

Origin of High Density Lipoprotein

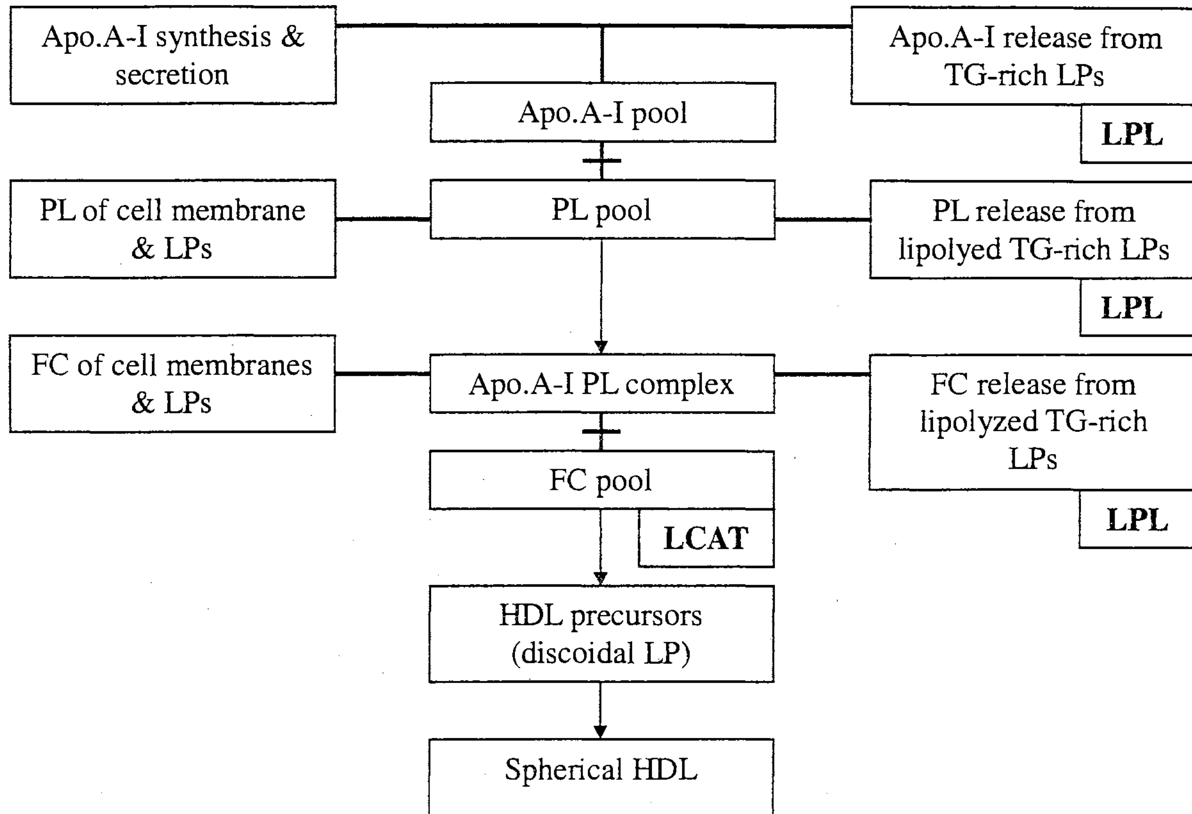


Figure 2. Schematic representation of HDL formation (Eisenberg, 1984).
* (Phospholipid: PL, Lipoproteins: LPs, Free cholesterol: FC, & Total Triglyceride:TG).

Total HDL is separated into HDL_2 and HDL_3 subfractions and contains at least eight recognized apoproteins (Apo.A-I, A-II, A-IV, C-I, C-II, C-III, D, and E) as described in TABLE III. These subclasses have been analyzed by a variety of ultracentrifugal techniques. These involve analytical ultracentrifugation of HDL in the density interval 1.063-1.21 g/ml: preparative ultracentrifugation at d 1.063-1.125 g/ml for HDL_2 and d 1.125-1.21 g/ml for HDL_3 (Gidez et al., 1982).

The HDL_2 subfraction has a larger (mean particle diameter 8 nm and lipid-to-protein ratio 1.48) and less dense than HDL_3 (mean particle diameter 5 nm and lipid-to-

protein ratio 0.8). The lipid composition of both subfractions is about 38% phosphatidylcholine, 27% CE and 6% TG by weight but HDL_2-c has a higher lipid and Apo.A-I concentration that make a larger and less dense particle than HDL_3-c (Miller, 1980).

The LPL activity appears to have a crucial role in the regulation of plasma HDL_2 and total HDL level, i.e., patients lacking LPL or apoprotein C-II do not have any HDL_2 in their plasma and the concentration of HDL_3 is very low. Furthermore, formation of true HDL_2 needs the presence of LCAT (Dieplinger, Zechner, & Kostner, 1985). That is, lipolysis of TG-rich lipoproteins (chylomicrons and VLDL) via LPL converts HDL_3-c to HDL_2-c. In other words, surface particles such as phospholipids, FC, and Apo.C are transferred from VLDL and chylomicrons to HDL (especially HDL_3-c) following breakdown of the TG-rich lipoproteins by LPL. Following esterification of FC in HDL_3-c, true HDL_2-c is formed by LCAT reaction (Nikkila et al., 1987). Dieplinger et al. suggested that the rate of HDL_2 formation depends on the rate of flux of the surface components from TG-rich lipoproteins that is in turn determined by the activities of LPL, LCAT, and the substrate availability (Figure 3).

LPL Activity

Low Medium High

Lipolysis of Chylomicrons and VLDL

↓

Phopholipid, FC, Apo.C

LCAT

Dense HDL_3 → Light HDL_3 → HDL_2a → HDL_2b

(1.140-1.210) (1.125-1.140) (1.080-1.125) (1.063-1.080)

Apo.A-I

↑

Free Apo.A-I pool from (probably present as VLDL A-I phospholipid complex)

↓

Catabolism

Figure 3. Scheme on the redistribution of HDL particles according to variation of LPL activity from low to high (Nikkila et al., 1987).

The main difference between HDL_2-c and HDL_3-c is their content of CE. HDL_2-c can be also converted back to HDL_3-c by two pathways: the role of the heparin-releasable hepatic lipase (HL or HTGL) and the role of the core lipid transfer reaction (CETP). For instance, HDL_2-c is converted back to HDL_3-c by transfer of CE from HDL_2-c to TG-rich lipoproteins and transfer of TG from TG-rich lipoproteins to HDL_2-c by lipid transfer reaction (CETP). After lipolysis of TG in HLD_2-c by LPL and primarily HL, HDL_2-c is converted back to HDL_3-c (Eisenberg, 1984) (Figure 4).

HDL_2-c ⟶ HDL_3-c Conversion

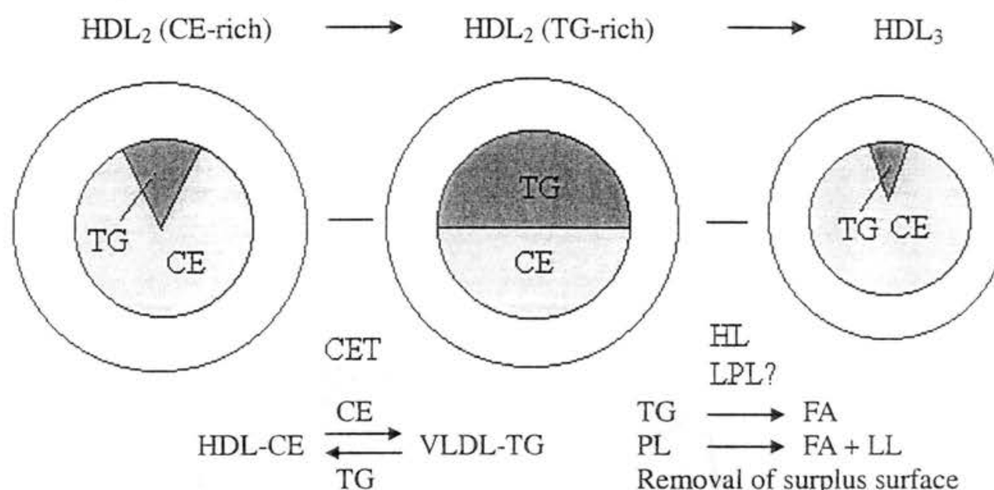


Figure 4. A schematic demonstrates the conversion of HDL_2 to HDL_3 through a two-step process. In the first step, CE from CE-rich HDL_2 is transferred into VLDL and chylomicrons that simultaneously act as acceptors for CE and as also donors of TG without change in size and density of HDL_2. In the next step, continuing replacement of CE by TGs and TG hydrolysis coupled with removal of surplus surface via HL and LPL results in complete conversion of HDL_2 to HDL_3 (Eisenberg, 1984).

Hormones

A major role of hormone action is to increase enzyme activity. Many hormones that influence either the rate of esterification or the rate of lipolysis affect the rate of release of FFAs from adipose tissue (Murray et al., 1996).

Insulin inhibits the release of FFAs from adipose tissue. It causes a decrease in circulating plasma FFAs due to enhancement of lipogenesis. An essential action of insulin in adipose tissue is to inhibit the activity of the HSL, reducing the release of FFAs and glycerol by inhibition of the synthesis of cAMP (Murray et al., 1996). This insulin activity plays a prominent role in the regulation of adipose tissue metabolism. The levels

of plasma insulin decrease as a function of exercise duration. This is most likely due to the inhibitory effect of an increased catecholamine release on the beta cell of the pancreas (Galbo, Christensen, & Holst, 1977). A lowering of blood glucose with prolonged exercise directly enhances hepatic glucose release and stimulates to produce the hormones, glucagon and epinephrine. This helps to maintain the level of blood glucose by increasing energy available from FFA mobilization in the adipocytes and also increasing glucose-sparing action (Mcardle, Katch, & Katch, 1996).

Several hormones promote the release of FFAs from adipose tissue and raise the plasma FFA concentration by increasing the rate of lipolysis of the TG stores. These include catecholamines (epinephrine & norepinephrine), glucagon, adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), growth hormone (GH), and cortisol. Many of these activate the hormone-sensitive lipase (HSL) (Murray et al., 1996).

Catecholamines act rapidly in promoting lipolysis. This process is conducted through stimulating the activity of adenyl cyclase that converts ATP to cAMP, converting inactive HSL into active HSL. Lipolysis is controlled largely by the amount of cAMP present in the tissue. This cAMP is further degraded to 5'-AMP by the enzyme (cyclic 3',5'-nucleotide phosphodiesterase) that is inhibited by methylaxanthines such as caffeine and theophylline. Therefore, drinking of caffeine causes elevation of plasma FFA in humans (Murray et al., 1996).

Hormonal Responses to Exercise - Hormonal regulation of energy metabolism seems to depend on both the intensity and duration of exercise. Various hormones work

to ensure glucose and FFA availability for muscle energy metabolism. Stimuli from either the working muscles or motor centers via the central nervous system modify the response of the endocrine system directly via pituitary hormones or indirectly via the sympathoadrenal system. The initial response to the onset of exercise is enhancement of sympathoadrenal activity (i.e., catecholamines) and secretion of pituitary hormones (i.e., GH, TSH, and ACTH), which cause a decrease in the plasma insulin concentration and increase in all other hormones' concentration. Because of this shift in hormone balance, a modification of the metabolism of intra- and extra-muscular triglycerides and glycogen as fuels for muscular exercise occurs. When exercise is prolonged, the hormonal response is influenced by additional factors such as temperature, glucose availability, oxygen tension, and changes in plasma volume (Thornton, 1985).

Prolonged exercise is accompanied by decreases in the muscle and liver glycogen stores, causing to secretion of GH, cortisol, and glucagon from the different glands for the compensation of decrease in blood glucose. The increased release of GH and cortisol occurs simultaneously with an increase in sympathetic activity. The increase in blood concentrations of FFAs and amino acids that accompanies increases in GH and cortisol provides substrates for gluconeogenesis and alternate fuels for skeletal muscle energy metabolism (Robergs & Roberts, 1997).

GH release from the anterior pituitary also causes a decrease in carbohydrate breakdown and a subsequent mobilization and use of lipids as an energy source. During exercise, GH stimulates lipid release from adipocytes while inhibiting glucose uptake by the cells. This glucose-sparing action helps maintain blood glucose at fairly high levels and contributes to sustain prolonged exercise (Mcardle et al, 1996).

Cortisol is the major glucocorticoid that is the stress hormone secreted by the adrenal cortex. Physical or emotional stress causes the hypothalamus to secrete corticotropin-releasing hormone, which stimulates the anterior pituitary to release ACTH. In turn, ACTH causes glucocorticoid release (i.e., cortisol) by the adrenal cortex. Cortisol secretion also accelerates lipid mobilization for energy. Most research indicates that cortisol output increases with exercise intensity but even during more moderate exercise, plasma cortisol rises if the exercise duration is sufficiently long (Brandenberger & Follenius, 1982).

The major function of glucagon in the body is to stimulate both glycogenolysis and gluconeogenesis by the liver. During prolonged, high-intensity exercise or food restriction, the pancreas' α-cells are stimulated to release glucagon that contributes to blood glucose regulation. This causes an almost immediate release of glucose from the liver (Felig, Wahren, Hendler, & Ahlborg, 1972).

The adrenal medulla secretes epinephrine and norepinephrine, collectively called catecholamines. These hormones prolong and augment sympathetic activity. A primary function of epinephrine in energy metabolism is to stimulate glycogenolysis in the liver and active muscle, and lipolysis in adipose tissue and active muscle; norepinephrine is also a powerful stimulator of lipolysis in adipose tissue (Wahrenberg, Engfeldt, Bolinder, & Arner, 1987). Epinephrine release from the adrenal medulla increases with exercise, and the magnitude of this increase is related to the intensity and duration of exercise (Mora & Coyle, 2000; Farrell, 1987).

ACTH (adrenocorticotropic hormone) acts also directly to enhance lipid mobilization from adipose tissue, increase the rate of gluconeogenesis, and stimulate

protein catabolism. ACTH concentrations are also increased during exercise if intensity is greater than 25% of $VO_{2\ max}$ (Galbo, 1983).

As mentioned previously, exercise is known to alter insulin concentration and also change the sensitivity of β-receptors via action of catecolamines. In other words, during the exercise the decrease of plasma insulin level that occurs and, therefore reduces fat storage. The increased sensitivity of β-receptors in the pancreas to catecholamines is associated with increased lipolysis (Askew, 1984).

Consequently, these changes of enzyme activity, sympathoadrenal activity, and hormonal response to aerobic exercise seem to be associated with an increase in HDL concentration and a decrease in triglycerides and VLDL-c concentrations in the blood. However, factors such as exercise intensity, exercise duration, body fat changes, and baseline plasma lipid levels may effect the response of plasma lipids and lipoprotein (Tran & Weltman, 1983).

The Effects of Chronic and Acute Aerobic Exercise on Lipids and Lipoproteins

Lipid Response to Chronic Aerobic Exercise

The effects of several modes of aerobic exercise on blood lipids have been investigated. These include walking, jogging, running, cycling, and other aerobic activities (Crouse et al., 1997; Cullinane et al., 1981; Gordon et al., 1998; Giada et al., 1991; Zmuda et al, 1997.). Great attention has been given to cross-sectional and longitudinal studies about acute and chronic effects, males and females, healthy and

unhealthy, and young and elderly people. Even though there are many contradictory reports, there is widespread agreement that chronic aerobic exercise has the ability to favourably change the blood lipid and lipoprotein of those who participate in aerobic exercise, i.e., increase HDL-c in combination with decrease TG concentration (Schwartz et al., 1992).

After aerobic exercise training, increases in HDL-c concentration were reported in a number of studies (Crouse et al., 1997; Gordon et al., 1998; Hartung et al., 1980; Huttunen et al., 1979; Pietila, 2000; Thompson, 1997). These responses were accomplished in general by increases in HDL_2-c (Crouse et al.; Aellen, Hollmann, & Boutellier, 1993) and was associated with a decrease in TG concentration (Cullinane et al., 1981).

Hicks and colleagues (1987) demonstrated increases in HDL-c after 9 to 12 Km on a treadmill run at intensities of 60% and 90% of $VO_{2\ max}$. However, greater increases in HDL-c were observed with the higher intensity exercise. Huttunen et al. (1980) reported that 4 months of mild-to-moderate physical activity consisting of 3 to 4 weekly sessions increased HDL-c and VO_2, and decreased TG in 50 inactive middle-aged men.

To examine acute and chronic effects of exercise at two different intensities (50% or 80% of $VO_{2\ max}$) on lipids and apolipoproteins, 26 hypercholesterolemic men trained and expended 350 kcal per session three times per week for 6 months. Serum lipid and apolipoprotein concentrations were measured before, immediately after, 24 h, and 48 h after exercise on four different occasions corresponding to 0, 8, 16, and 24 weeks of training. TG fell and total cholesterol, HDL-c, HDL_3-c, apo-AI, and apo-B rose 24 and 48-hrs after exercise regardless of training or intensity. Total cholesterol, HDL_3-c, apo

A-I, and apo B were lower and HDL_2-c was higher after training than before training (Crouse et al., 1997).

Zmuda et al. (1998) investigated the HDL response to 12 months of endurance exercise training without weight loss in 17 men aged 26 to 49 years with initially low (<40 mg/dl) or normal HDL-c level (>44 mg/dl). However, they reported that the ability to increase HDL-c levels through endurance exercise training is limited in subjects with low initial HDL-c due to the failure of change in TG metabolism.

Lipid Response to Acute Aerobic Exercise

Changes in lipid and lipoprotein concentrations have been observed immediately after a prolonged single bout of exercise (Angelopoulos & Robertson, 1993; Crouse et al., 1997). Many of the lipid changes after a single bout of exercise are similar to those reported after chronic exercise training.

Cullinane et al. (1981) demonstrated that acute decreases in TG and LDL-c with little change in HDL-c occurred in sedentary male who are aged 21-29 years after a single 30 min exercise session. Nine sedentary men (mean age, 23 years) were studied during and after treadmill exercise at 65% $VO_{2\ max}$ during 30 min. Blood samples were obtained prior to exercise and at 5 min, 24 and 48hours after exercise. There were no significant differences in TG and total cholesterol over time points. Total HDL-c remained higher at 5 min and 24 h post-exercise than at the pre-exercise level. Total HDL-c declined to pre-exercise values 48 h post-exercise. HDL_3-c levels were higher at

the 5 min and 48 h post-exercise time points than at the pre-exercise (Angelopoulos, Robertson, Goss, Metz, & LaPorte, 1993).

Short-term lipid responses to a single bout of exercise were examined immediately before and after an ultramarathon run (50-80 km) in 9 endurance athletes (Kaminsky, Kanter, Nequin, Lesmes, & Saeger, 1988). In this study, they reported decreases in plasma-volume-adjusted VLDL-c and TG, whereas there were increases in LDL-c and no changes in HDL-c and TC.

Gordon et al.(1996) reported plasma-volume-adjusted HDL-c increased above baseline values in moderately trained men after a prolonged treadmill run expending 800 kcals at 75% $VO_{2\ max}$. However, no changes in TC, TG, LDL-c were observed.

Crouse et al. (1995) also reported short-term changes in blood lipid and apo concentrations after a single bout of exercise in healthy hypercholesterolemic men at 80% of $VO_{2\ max}$ or 50% of $VO_{2\ max}$ cycle ergometer exercise balanced for caloric expenditure 350 kcal. HDL-c and apo-B increased at 24 h after exercise. However, LDL-c decreased immediately after exercise and then increased at 48 h later after exercise.

Lipid concentrations were measured in 11 trained and 10 untrained men before and after a single bout of cycle ergometry at 80 % of max heart rate (Kantor, Cullinane, Herbert, & Thompson., 1984). The trained and untrained males exercised for 2 and 1 h, respectively. LDL-c was lower in both groups at 24 h post-exercise and returned to baseline values thereafter. HDL-c concentrations were higher 48 h after the exercise in both groups. However, the HDL-c response pattern was different between two groups. In the trained subjects, the elevations in HDL-c were contributed by greater HDL_2-c, whereas by HDL_3-c in the untrained subjects.

The results from these studies vary slightly. Durstine and Haskell (1994) outline several potential factors involved in the inconsistencies of training (chronic) studies to elicit an increase in HDL including: the duration of the study, the volume of the exercise, alterations in body composition, dietary intake, weight change, and initial HDL-c concentration. However, several potential factors that include in the inconsistent results from acute exercise studies might be the volume of exercise (intensity, duration, and caloric expenditure), training status, gender, and initial HDL-c concentration.

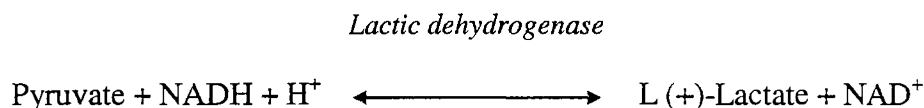
Lactate Threshold as A Criterion for Exercise Intensity

Lactate production and removal

Carbohydrates are the only significant foods that can be used to provide energy without use of oxygen (Robergs & Roberts, 1997). The lactate that is important as the end product of anaerobic glycolysis is formed from pyruvate, coming from mainly glucose.

The glucose is metabolized in the cytoplasm of skeletal muscle cells to produce pyruvate in the glycolytic pathway. Under anaerobic condition, pyruvate in the cytoplasmic pathway is more likely converted into lactate with the oxidation of NADH to NAD^+ through the lactate dehyrogenase (LDH) (Murray et al., 1996):

Lactic dehydrogenase



$$\text{Pyruvate} + \text{NADH} + H^+ \longleftrightarrow \text{L (+)-Lactate} + NAD^+$$

The enzymes of the glycolysis pathway are found in the extramitochondrial soluble fraction of the cell cytosol. When 1 mmol glucose is metabolized to lactate, it provides

either 2 mmol (blood glucose) or 3 mmol (muscle glycogen) ATP. Therefore, during intense exercise the glycolytic pathway converts glucose to pyruvate and then a large portion of the pyruvate is converted to lactate rather than transported into the mitochondria.

Conversely, under aerobic conditions, pyruvate is taken up into mitochondria through the mitochondria membrane, and after conversion to acetyl-CoA is oxidized to CO_2 and hydrogen by the citric acid cycle (Krebs cycle) (Robergs & Roberts, 1997). The hydrogen released during glycolysis and the Krebs cycle combines with two coenzymes known as NAD (nicotinamide adenine dinucleotide) and FAD (flavin adenine dinucleotide) that carry the hydrogen atoms (H_2) to the ETC (electron transport chain). And then the atoms are split into protons and electrons within the ETC. At the end of the ETC, the H^+ combines with O_2 to form water, thus preventing acidification. The electrons that were split from the hydrogen pass through the ETC and finally provide energy for the phosphorylation of ADP to form ATP referred to as oxidative phosphorylation (Willmore & Costill, 1994). Therefore, when adequate amounts of energy are available from oxidative metabolism, removal of lactate is achieved in two ways: First, a small portion of it is converted back into pyruvate and then metabolized oxidatively by the all the body tissues. Second, the remaining lactate is reconverted into glucose mainly in the liver, and the glucose in turn is used to replenish the glycogen stores of the muscles as an energy source (Guyton & Hall, 1996). In this reaction, 1 mmol glucose results in the production of ≈39mmol ATP (Spriet, Howlett, & Heigenhauser, 2000).

Lactate threshold for Exercise Intensity

The expression of exercise intensities relative to percent $VO_{2\ max}$ or percent maximal heart rate has been commonly used in exercise-related studies as a criterion for intensity although variability in physiological responses to submaximal exercise may occur due to wide ranges of exercise intensities compared with the LT (Dwyer & Bybee, 1983; Weltman et al., 1989). McLellan and Gass (1989) reported that individuals with a similar $VO_{2\ max}$ showed different metabolic responses to exercise at the same relative intensity. Coyle, Coggan, Hopper, and Walters (1988) also observed individuals with a similar $VO_{2\ max}$ can vary in glycogen use and exercise performance during exercise at similar work rates and percentages of $VO_{2\ max}$. These findings indicate that although individuals exercise with the same relative oxygen uptake, their metabolic and physiological responses may vary (Baldwin, Snow, & Febbraio, 2000). Therefore, although training prescriptions based on percent $VO_{2\ max}$ or maximal heart rate allow a simple constant workload (i.e., same % $VO_{2\ max}$ or % maximal HR) for all individuals, they are not accounted for individual metabolic and physiological differences. In other words, although the LT or AnT is not a constant percent of $VO_{2\ max}$ (i.e., vary from 40 to 85% $VO_{2\ max}$) or heart rate max for all individuals, it may be considered as a precise training prescription with respect to individualized metabolic stress for each individual. In addition, LT may provide less variability in the metabolic and physiological responses between individuals than percentages of $VO_{2\ max}$ or maximal heart rate. Wasserman, Whipp, Koyl, and Beaver (1973) suggested that the AnT or LT, reflecting changes in cellular metabolism, may be a more sensitive indicator of metabolic adaptations to

exercise than some optional percentage of the $VO_{2\ max}$ because percent $VO_{2\ max}$ does not distinguish between workload above and below the AnT or LT. Consequently, exercise prescriptions at a specific intensity within the commonly recommended range of 50 to 85 % $VO_{2\ max}$ (Dwyer & Bybee, 1983) or 60 to 90% of heart rate max may result in metabolically different workloads in individuals but similar $VO_{2\ max}$ or % heart rate.

Lactate threshold means that highest workload without a gradual increase in blood lactate can be maintained over a long duration (Stegmann & Kindermann, 1982). During exercise blood lactate concentration has been used to determine anaerobic threshold (AnT) as the point at which lactate rise above the resting level (Davis, Vodak, Wilmore, Vodak, & Kurtz, 1976; Yoshida, Suda, & Takeuchi, 1982) or it reaches a fixed value (i.e., 4.0 mmol/L). This fixed value, 4.0 mmol/L, was considered as also AnT or OBLA (Kindermann, Simon, & Keul, 1979).

The LT and OBLA have been shown to be accurate predictors of endurance performance and submaxial fitness as well as may decrease the individual physiological and metabolic variability rather than $VO_{2\ max}$. Therefore, LT as exercise intensity has been accepted as useful tools for exercise prescription and appropriate criterion to normalize the exercise intensity between individuals with different aerobic capacities (Baldwin et al., 2000; Padilla, Mujika, Orbananos, & Angulo, 2000; Tanaka & Matsuura, 1984; Yoshida et al., 1990).

Lactate Threshold and Anaerobic Threshold (AnT)

The AnT is the highest oxygen uptake (VO_2) that can be maintained during

prolonged exercise with no accumulation of lactic acid (Wasserman & McIlroy, 1964). Anaerobic threshold (AnT) has been used to define the peak work rate or O_2 uptake at which aerobic metabolic processes can no longer meet the skeletal muscle requirements for ATP (Wasserman, Van Kessel, & Burton, 1967). Consequently, as workload is increased above the AnT, progressive increases in anaerobic glycolysis to sustain adequate availability of ATP and this acceleration in glycolysis leads to an elevated muscle lactic acid concentration that causes metabolic acidosis (Sahlin, 1978).

The AnT is an important functional division because the physiologic responses to exercise are different between above the AnT and below the AnT. Above the AnT, metabolic acidosis are developed and therefore, exercise endurance reduced due partially to the slowing VO_2 kinetics so that a steady state and minute ventilation is delayed and increases, respectively (Wasserman, 1987).

The AnT can be measured either directly from lactate concentration by measuring blood lactate at frequent intervals during a period of increasing work rate where blood lactate begins to increase or the gas exchange consequences of metabolic acidosis by visual inspection of graphical plots of gas exchange indexs such as ventilatory equivalents, end-tidal gas concentrations, and respiratory exchange ratio known as conventional techniques (Beaver, Wasserman, & Whipp, 1986; Wasserman, 1987).

Simonton, Higginbotham, and Cobb (1988) reported that the use of coplotted ventilatory equivalents for O_2 and CO_2 yielded ventilatory threshold values comparable to values obtained by using multiple parameters ($r = 0.94$) and have also shown high test-retest reproducibility in experienced laboratories. Although the ventilatory threshold did not precisely predict lactate level for individual subjects, the lactate increment at the

ventilatory threshold occurred within a narrow range in both normal subjects and patients with cardiac heart failure (Simonton et al., 1988). Caiozzo et al. (1982) also reported that use of VE/VO_2 and VE/VCO_2 as a criterion to detect AnT yielded highly correlated with determined from the break point in blood lactate and is the most frequently used methods (Dickstein, Barvik, Aarsland, Snapinn, & Karlsson, 1990). In addition, this detection of AnT using visual plots of the ventilatory equivalents of O_2 and CO_2 against time has shown high test-retest reproducibility (Eldridge, Ramsey-Green, & Hossack, 1986).

CHAPTER III

METHODS AND PROCEDURES

Exercise training has been associated with decreases in total cholesterol and increases in HDL-c (Crouse et al., 1997; Miller et al., 1979; Ratliff et al., 1978; Stein et al., 1990). However, the effects of a single session of aerobic exercise at the different exercise intensities (70% of LT and LT) on changes in lipid and lipoprotein subfractions have not been defined among college-age men. Accordingly, the purpose of this study was to determine the effect of a single bout of aerobic exercise at different exercise intensities (70% LT and LT) measured from the evaluation of lactate concentration on lipid and lipoprotein cholesterol subfractions in college-age men, assuming each subject equally expend 350 kcal of energy during different exercise intensities.

Subjects

Eighteen subjects drawn from college age men aged 18 to 30 who were apparently healthy, non-smoking, and asymptomatic volunteered for this investigation. Smokers and subjects who drink alcohol were excluded from this study because smoking and alcohol appear to have effects on plasma lipids (Hartung, Reeves, Foreyt, Patsch, & Gotto, 1986; Moffatt, 1988; Stamford, Matter, Ronald, & Stanley, 1984). Subjects on lipid-altering

medication, including hormone replacement therapy, also were excluded from the study.

Each subject was monitored using a health history and demographic questionnaire (Appendix A) and subjects with more than one major CVD risk factor or any disease symptoms as defined by the ACSM' guidelines for Exercise Testing and Prescription (Kenney, 1995) were eliminated from the study group. Body composition (skinfolds) was measured for all subjects. Subjects who were obese (over 25% body fat) as determined by American College of Sports Medicine (1995) were excluded from this investigation. Additionally, a capillary blood sample from the subject's fingertip was analyzed to select participants whose TC was less than 239mg/dl. All subjects provided informed consent for participation (Appendix B) as approved by the Oklahoma State University Institutional Review Board (IRB).

Procedures

Pre-Screening

Eighteen subjects were recruited from the university population from fliers placed throughout campus. A phone interview with each subject served as initial screening for subjects who met the criteria and verbally agreed to participate, and the second screening was scheduled. Prior to the first visit, subjects were asked to abstain from alcohol, lipid-altering medication, and exercise for 48-hrs.

Second Screening

Following the phone interview, subjects signed the consent form (Appendix B) and filled out the health history and demographic questionnaire (Appendix A). Each subject was measured for height, body weight, and body fat (skinfolds). Three skinfold sites were used for estimating percent body fat in men (abdominal, chest, and thigh) and percent body fat was calculated according to the Jackson and Pollock equations (Jackson & Pollock, 1985). Additionally, a fingertip blood sample from each subject was collected to select participants whose TC was less than 239 mg/dl because over 240 mg/dl is considered as hypercholesterolemia (Laboratory Standardization Panel, 1988). The capillary blood sample using fingertip was collected with a disposable transfer pipet (Lifescan Inc., Milpitas, CA) and analyzed for cholesterol concentration using a cholesterol analyzer (Accu-chek Instantplus®, Roche Diagnostics Co., Indianapolis, IN).

Exercise test for determining $Vo_{2\ max}$ and Lactate Threshold (LT) - Subjects reported to the OSU Wellness Center laboratory for initial treadmill test without consuming food, alcohol, or caffeine in the 3 hours preceding $VO_{2\ max}$ test. Subjects also restrained from any medications to alter lipid metabolism and strenuous exercise in the 48 hours preceding the test.

Each subject's $VO_{2\ max}$ was determined using a continuous, incremental, level treadmill protocol to determine the oxygen uptake (VO_2), heart rate (HR), and velocity of treadmill associated with LT and 70% of LT. During the $VO_{2\ max}$ test, 12-lead ECG, blood pressure, rate of perceived exertion (RPE), and lactate were measured or recorded.

The 12-lead ECG, blood pressure, RPE and lactate measurements were recorded during the last 30 second of each 3 min stage, and heart rate at the end of every minute.

Prior to placement of electrodes (Quinton Instrument Co., Bothell, Washington) and respiratory equipment, participants warmed up by stretching major muscle groups for 5 min. Following the stretching routine, subjects conducted a warm-up walk at 3.4 to 3.7mph for 4 min. After warmed up, subjects started the exercise test on the treadmill under the supervision of the investigator.

During the test, the subjects exercised on a motor driven treadmill (Quinton Co., Seattle, WA) while respiration, EKG and blood pressure were analyzed via a Cardio-Pulmonary Exercise System (Quinton Instrument Co.), EKG monitor (Q5000 Exercise Test Monitor, Quinton Co.), and blood pressure monitor (Model 412 Blood Pressure Monitor, Quinton Instrument Co.), respectively. The treadmill protocol was a continuous, incremental, level running test to determine the $VO_{2\ max}$ and velocity associated with lactate threshold (LT). Each stage was 3 min in duration with an initial velocity of 130 m/min (≈ 4.9mph) that increased 10 m/min (≈ 0.4 mph) for each subsequent stage and the grade of treadmill remained at 0 % (Stoudemire et al., 1996). During the test, verbal encouragement was given and the test was voluntarily terminated when the subject could no longer continue.

During the $VO_{2\ max}$ test, 12-lead ECG, blood pressure, heart rate, RPE, and blood lactate were recorded during the last 30 second of each 3 min stage, and the 12-lead ECG and heart rate at the end of every minute. Metabolic data were collected using a standard open circuit system with respiratory metabolic measurements recorded automatically every 30 seconds. The gas analyzers were calibrated using commercial gases of known

concentrations before and after each test. The following respiratory measurements were measured: volume of expired air (VE; 1/min), volume of carbon dioxide produced (VCO_2; 1/min), relative volume of oxygen consumed (VO_2; ml/kg/min), respiratory exchange ratio (RER), ventilatory equivalent for oxygen (VE/VO_2), and carbon dioxide (VE/VCO_2). During the testing period, a noseclip and mouthpiece attached to a two-way valve supported by a headset (Erich Jaeger GmbH & Co. KG., Germany) were attached to the subjects. $VO_{2\ max}$ was determined by the following criteria: 1) an exercise heart rate within 10 bpm of age-predicted maximum (220-age), 2) respiratory exchange ratio $\geq$ 1.1, 3) a plateau in VO_2 values despite a further increased work intensity, or 4) lactic acid $>$ 8 mM/L (Hughes, Thorland, Housh, & Johnson, 1990).

LT was determined by visual inspection of plots of the lactate-velocity relationship observed on the $VO_{2\ max}$ test (Stoudemire et al., 1996). The highest velocity attained that is not associated with an elevation in blood lactate above baseline is designed as velocity-LT. This occurs just prior to an abrupt and non-linear increase in blood lactate (Poole & Gaesser, 1985) that is observed with subsequent exercise intensities. Velocity associated with blood lactate was determined from the plot of blood lactate against running velocity during incremental test (Figure 5). However, a clear break point was not always occurred to determine the LT, so, the alternative, ventilation threshold, was used for a reference to determine it (Figure 6). The ventilatory threshold was determined from computer-generated printouts of expired gas variables measured breath by breath.

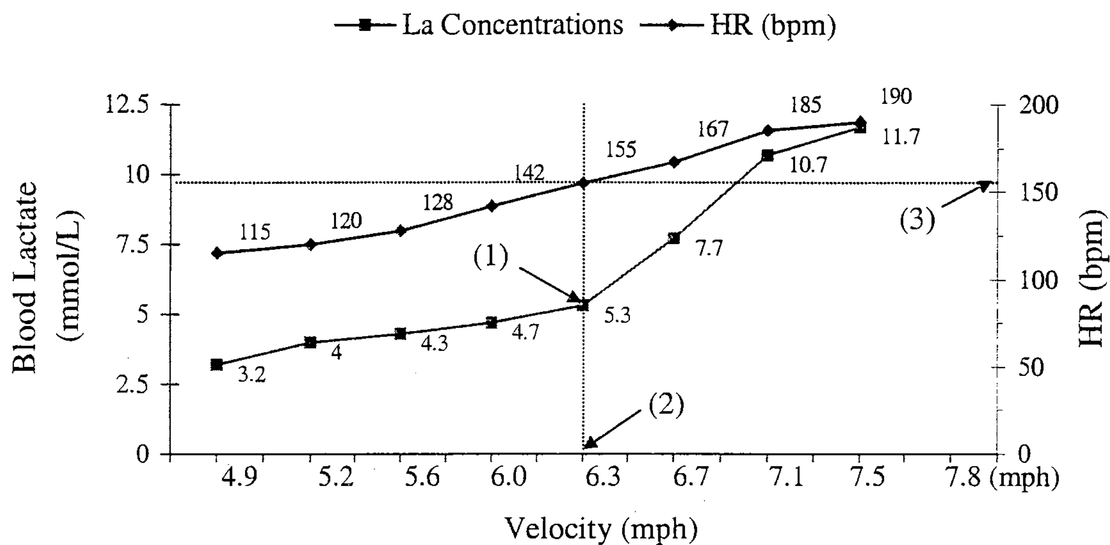


Figure 5) Example of schematic determination of heart rate and treadmill velocity at LT using one subject in this investigation. (1) represents value for LT, (2) represents velocity projected from (1), and (3) represents heart rate matched from LT.

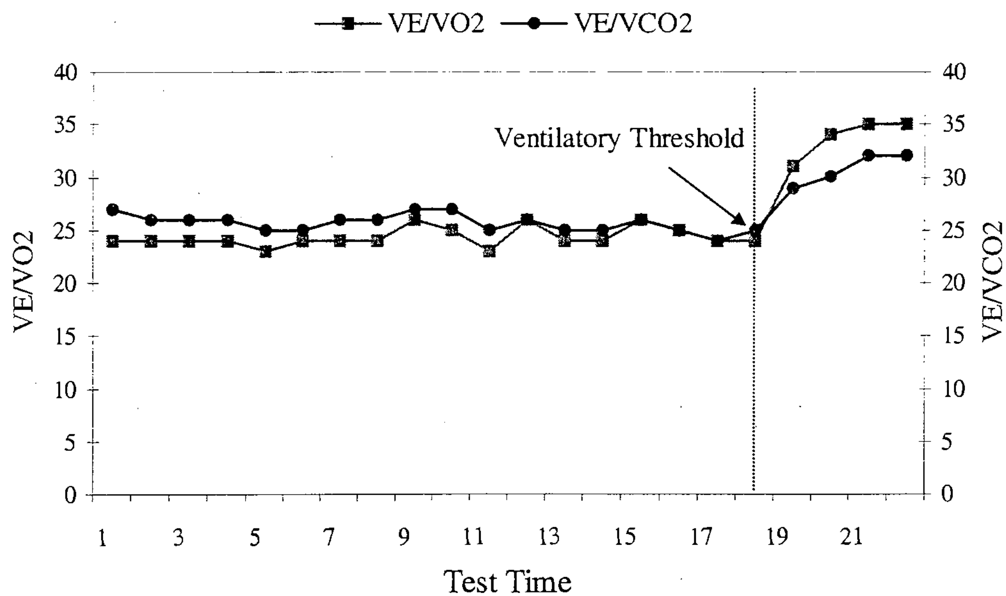


Figure 6) Schematic determination of ventilatory threshold (VT) using coplots of the ventilatory equivalents of O_2 and CO_2 against time using one of subject' responses to the $VO_{2\ max}$ test. The VT is closely related to initial increments in lactate concentration that is consistent with early buffering and increased CO_2 production (Dickstein et al., 1990).

Figure 5 is an example of HR corresponding to velocity-LT from individual plots of HR against velocity. The HR corresponding to velocity-LT from individual plots of HR against velocity was determined and designated as the HR associated with the LT (HR-LT) (Weltman et al., 1989). These HR-LT and treadmill velocity-LT were used as references to maintain constant blood lactate concentration during exercise trials while the Vo_2-LT was used as a reference to calculate the caloric expenditure during exercise trials at LT intensity.

Exercise Trials

Subjects were randomly assigned into one of two different intensities (i.e., 70% of LT and LT), counterbalanced running or walking test determined basis of subject' training status, for the exercise trials one week after the initial visit for $VO_{2\ max}$ test. The running or walking test for exercise trials was conducted following a 4 min warm-up at 3.4-3.7 mph.

The velocities for the LT and 70% of LT were determined by examining the blood lactate observed during $VO_{2\ max}$ test (Figure 6). For the LT intensity, the velocity associated with LT was used and continuously monitored by measurements of blood lactate every 5 min and heart rate within ± 5 bpm associated with LT until each subject expended 350 kcal of energy. However, if the velocity was difficult to maintain predetermined blood lactate level, the velocity was adjusted by reduction of treadmill speed: i.e., 0.2 mph of treadmill speed was decreased when predetermined either heart rates or blood lactate concentrations were over 5 bpm or 1.0 mmol/L, respectively. For

the 70% of LT intensity, however, the workload was set at the velocity determined during the $VO_{2\ max}$ test, but this intensity was held during the trial until each subject expended 350 kcals of energy. Subjects were also shown the Borg's 6-20 point scale (Borg, Hassemen, & Lagerstrom, 1987) during exercise trials at both intensities (70% LT and LT).

After a completion of the first exercise trial for either 70% LT or LT intensities, subjects were automatically assigned into the remaining intensity of two different trials at least two days after the first trial and the exercise trial was performed following exact protocol previously described.

Energy Cost for Exercise Sessions

During the $VO_{2\ max}$ test, energy cost was automatically calculated by summing kcals measured per minute based on the absolute oxygen consumed per minute and kcal expenditure per liter oxygen consumed from the minute RER value (Robergs & Roberts, 1997):

Kcal expenditure/min = VO_2 L/min $\times$ Kcal$\cdot$ L/O_2 (consumed based on RER value).

However, during the exercise trials at the 70% of LT and LT intensities, the caloric expenditure was determined using metabolic equations for gross VO_2 in metric units as following (Franklin, 2000):

For walking $VO_2 = (0.1 \cdot S) + (1.8 \cdot S \cdot G) + 3.5$

For running $VO_2 = (0.2 \cdot S) + (0.9 \cdot S \cdot G) + 3.5$

* VO_2 is gross O_2 consumption in ml/kg/min; S is speed in m/min; G is the percent grade as a fraction.

Each liter of O_2 consumed represents an energy expenditure of 5 kcal (Durstine, King, Painter, Roitman, & Zwiren, 1993). Therefore, exercise duration to spend 350 kcal was determined before sub-maximal exercise sessions (70% LT and LT intensities) as following equation: Kcal expenditure/min = VO_2 L/min × 5 Kcal· L/O_2. Energy cost was calculated by summing kcals measured per minute indirectly by the gross absolute oxygen consumed per minute and kcal expenditure per liter oxygen consumed (5 kcal/O_2 L/min).

Blood Sampling and Analysis

Subjects reported to the laboratory approximately prior to exercise testing and following a 12 to 14 hours fast. Before each blood draw, the subjects were asked to follow a 12 to 14 hours overnight fast by maintaining dietary adherence and abstaining from physical activity.

A 15-ml sample of blood (two 8.5-ml vacutainer tubes) at each time point were obtained for the determination of lipid and lipoprotein 6 times throughout the investigation (6 times × 15-ml = 90-ml for each participant): 1) before exercise (BE: for baseline at both 70% LT and LT intensity), 2) immediately post-exercise (IPE: for immediate exercise response at both 70% LT and LT intensity), and 3) 24 hours post-exercise (24 h: for 24 h post-exercise response at both 70% LT and LT intensity).

A catheter was insert into an antecubital vein of seated subjects for collecting blood samples and the tourniquet were removed within 1 min during blood collecting. To obtain serum, two 8.5-ml vacutainer tubes containing SST® gel and clot activator (Becton

Dickinson Vacutainer System, Franklin Lakes, NJ) were used to draw blood and allowed to stand 30 min at room temperature for clotting. Serum was isolated by centrifugation at 1000 × g for 15 min within 2 h of veinpuncture. Serum was then separately put into 1.5-ml microcentrifuge tubes (No. 0540810, Fisher Scientific Co., Pittsburgh, PA) for storage in a freezer at –80°C until analysis. In addition, capillary blood samples using fingertip were collected into 5 μL microhematocrit tubes (no.8889-301506, Sherwood Medical Industries, St. Louis, MO) before and after exercise to determine hematocrit for plasma volume changes.

Lactate Analysis - Capillary blood samples using the fingertip were collected via disposable transfer pipet (Lifescan Inc., Milpitas, CA) at each 3 min interval of exercise during $VO_{2\ max}$ test to determine LT and 5 min interval to check maintaining LT during exercise trials at LT intensity, respectively. The blood samples were analyzed for blood lactate concentration using an Accusport Potable Lactate Analyzer (Boehringer Mannheim Co., Indianapolis, IN) via a drop of blood soaking through a yellow protective portion of the test pad maintaining the red blood cells. Only the plasma reaches the detection film. Lactate is determined by reflectance photometry via a colorimetric lactate oxidase mediator reaction (Roche Diagnostics Ltd., Bell Lane, BN7 1 LG, Lewes, GB).

Isolation and Analysis of HDL_3 Subfractions - HDL_3 was measured in a supernate from an aliquot of serum after precipitation of HDL_2, LDL, and VLDL (Rifai & Warnick, 1994). The HDL_2 was calculated as the difference in cholesterol between the two supernates (HDL-c – HDL_3-c = HDL_2) (Cloey & Bachorik, 1989). Serum, control

materials, and precipitation reagents were equilibrated to room temperature and were transferred 0.4-ml serum to 1.5-ml microcentrifuge tubes for HDL_3-c subfraction. The 0.4-ml serum was introduced into appropriately labeled tubes and I pipetted an aliquot for HDL_3 determination. For the HDL_3, I weighed out 1.91g dextran sulfate, 39.74g $MgCl•6H_2O$, and 50 mg NaN_3 which were dissolved and mixed well in deionized water to make 100-ml solution. The final working reagent (WR) contains 19.1mg/mL dextran sulfate, 1.95mol/L $MgCl_2$, and 0.05% NaN_3 (Rifai & Warnick, 1994). The final working reagent, 120 μl, and 800 μl of 0.15 mol/L NaCl solution were then added into each tube for HDL_3 and votex for 5 sec.

The tubes were allowed to stand at room temperature for 30 min before centrifugation. The centrifugation was conducted for 30 min with a refrigerated centrifuge at 1500 × g at 4 °C (CR3I, Jouan, Inc., Winchester, Virginia) (Warnick, Benderson, & Albers, 1982). Tubes were then removed from the centrifuge and inspected supernates for turbidity. Any turbidity or cloudiness in the supernates indicates incomplete sedimentation of LDL/VLDL, and consequent contamination and overestimation of HDL (Warnick et al., 1982). Following the mixing of solution and reagent, supernatant cholesterol was multiplied by appropriate ratio (in this case, multiplied by 3.3) to correct for dilution. Finally, the clear supernate was removed by pipetting from the pellet for HDL_3-c analysis, transferred with pipet to sample cup and performed for $HDL\text{-}_3$ analysis on the supernate, or a second labeled vial for later analysis (Warnick et al.).

Enzymic Cholesterol Assay - The spectrophotometer was adjusted to 500 nm wavelength. Tubes were labeled for water blank, calibrators, control materials, and specimens. A total of 2.0-ml enzymic cholesterol reagent was dispensed into each of the tubes and placed them in an ice bath. Additionally, 100μl of water, calibrator, control material, or supernate was added to the appropriate tubes and mixed thoroughly. Tubes were transferred to a water bath at 37 °C for 20 min and cooled to room temperature and absorbance within 15 min after adjusting spectrophotometer to zero with the water blank. Cholesterol in specimens was calculated and materials were controlled in relation to the calibrator (Warnick et al., 1982).

$$\text{Cholesterol unknown} = \frac{\text{absorbance of unknown}}{\text{absorbance of calibrator}} \times \text{cholesterol}$$

Cholesterol Analysis (TC, FC, CE, HDL, HDL_2-c, LDL, and VLDL) - Total serum cholesterol (TC), free cholesterol (FC), high-density lipoprotein (HDL), and triglycerides (TG) were enzymatically measured using a Clinical Analyzer, CABAS FARA II, (Roche Diagnostic Systems, Inc., Montclair, NJ). All blood samples were analyzed by mixing 4μL of serum and 300μL of cholesterol reagent in a tube for total cholesterol. The tube was mixed well and warmed at 37°C in a water-bath for 5 min. The total amount of cholesterol in the test samples was determined by measurement of the absorbance of the red color at its maximal absorption wavelength of 505 nm (Wako Cholesterol CII, Enzymatic colorimetric method, Code No. 276-64909).

The chemical principle of this test is that cholesterol esters (CE) in the serum were principally first hydrolyzed by cholesterol esterase to free cholesterol and free fatty acids.

The cholesterol produced and the free cholesterol already present in the serum are oxidized in a reaction catalyzed by cholesterol oxidase that generates hydrogen peroxide. The hydrogen peroxide formed paticipates in the quantitative oxidative condensation between p-chlorophenol and 4-aminoantipytine in the presence of peroxidase. The product of the reaction is a red quinone pigment. The total amount of cholesterol in the test samples was determined by measurement of the absorbance of the red color at its maximal absorption wavelength of 505nm. The absorbances were measured spectrophotometrically at 505 nm. The intensity of the color produced was directly proportional to the total cholesterol concentration in the sample (Wako Cholesterol CII, Enzymatic colorimetric method, Code No. 276-64909).

The serum free cholesterol (FC) in all blood samples was determined by mixing 5μL of serum and 300μL of cholesterol reagent in a tube for FC. The enzymatic method (Wako Free Cholesterol C, Enzymatic colorimetric method, Code No. 997-64909) employed no irritant reagents and all reactions were accomplished at 37°C within 15 min. In this test, FC in the serum is oxidized by cholesterol oxidase to cholesterone and produces simultaneously hydrogen peroxide that causes phenol and 4-aminoantipytine to undergo quantitatively an oxidative condensation in the presence of peroxidase to produce a red color. The amount of FC in the test samples was determined by measurement of the absorbance of the red color at maximal absorption wavelength of 505nm (Wako Free Cholesterol C, Enzymatic colorimetric method, Code No. 997-64909). The amount of CE was obtained by subtraction of the amount of FC from the amount of cholesterol (CE = TC - FC).

For HDL, all blood samples were analyzed by mixing 2.4μL of serum and 240μL of cholesterol reagent in a tube. Unimate HDL Direct (Roche Diagnostic System, Inc., Art. 0764272, Somervile, NJ) allows for the direct determination of HDL-c in the presence of LDL, VLDL, and chylomicrons. The amount of HDL-c in the test samples was determined by measurement of the absorbance of the red color at maximal absorption wavelength of 550nm. The increase in absorbance at 550nm is directly proportional to the HDL-c concentration of the sample.

HDL_2 was calculated from the difference between HDL-c and HDL_3. LDL-c and VLDL-c were calculated from the TC, TG, and the HDL-c according to the procedure of Friedewald, Levy, and Frederickson (1972) as following TABLE IV.

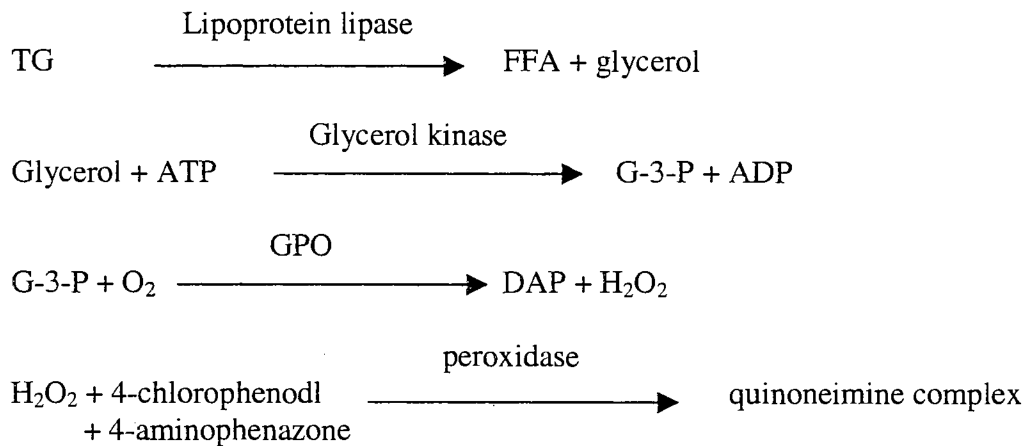
TABLE IV

EQUATIONS FOR SELECTED LIPOPROTEIN CHOLESTEROL.

Lipoproteins	Equations
HDL_2-c	HDL-c – HDL_3
LDL-c	TC – [HDL-c + (TG/5)]
VLDL-c	TC – (HDL-c + LDL-c)
CE	TC - FC

For triglycerides (TG), all blood samples were analyzed by mixing 4μL of serum and 300μL of cholesterol reagent in a tube for triglycerides. In this procedure, triglycerides are hydrolyzed by LPL to free fatty acids and glycerol. Glycerol is then phosphorylated to glycerol-3-phosphate (G-3-P) by adenosine 5'-triphosphate (ATP) in a reaction catalyzed by glycerol kinase (GK). The oxidation of glycerol-3-phosphate is catalyzed by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate and

hydrogen peroxide. Hydrogen peroxide (H_2O_2) reacts with 4-chlorophenodl and 4-aminophenazone in the presence of peroxidase to form a quinoneimine complex, which can be read at 490-550nm (Roche Diagnostics Systems, Inc. Nutley, New Jersey 07110-1199) as shown below. The increase in absorbance is proportional to the concentration of triglycerides in the sample.



Analysis of Plasma Volume Changes and Hematocrit

Analysis of Plasma Volume Changes - Relative plasma volume changes (%) that resulted from exercise were calculated from hematocrit (Hct) measurement (Van Beaumont, 1972). The plasma volume changes in different time-points such as immediately post-exercise (IPE) and 24 h post-exercise (24 h), compared to the baseline (BE) blood sample were calculated using equation that was demonstrated by Van Beaumont (1972). The subscripts B and A refer to before exercise for baseline blood sample (ΔPV %) and post-exercise, respectively.

$$\Delta PV\ \% = \frac{100}{100 - Hct_B} \times \frac{100\,(Hct_B - Hct_A)}{Hct_A}\ \%$$

$$\text{where: } Hct_B = (RCV_B / RCV_B + PV_B) \times 100$$

$$Hct_A = (RCV_B / RCV_B + PV_A) \times 100$$

Hematocrit (Hct) Analysis - Hct was determined in duplicate by capillary blood samples collected in 5μL microhematocrit tubes (no.8889-301506, Sherwood Medical Industries, St. Louis, MO). The microhematocrit tubes were sealed with critoseal (no. 89-215003, VWR Scientific Inc., Westchester, PA) and centrifuged for 5 min at 1500 × g. The blood volume in the microhematocrit tubes and the packed red cell volume were measured with a metric ruler to the nearest 0.25 mm. Hct at each time point was taken as the average of the corrected ratios obtained for the 2 tubes.

Statistical Analyses

All baseline dependent variables were tested. SPSS software was used for statistical analysis. As primary statistical analysis, differences in both treatments (LT and 70% LT) over time among all lipid and lipoprotein variables were identified using repeated-measure MANOVA in a complete within-subjects design. However, HDL_2-c, LDL-c, VLDL-c, and CE were calculated from the equations (TABLE IV) and these blood variables are linearly dependent (i.e., a = 2b) on one of the measured variables such as HDL_3-c, HDL-c, TC, TG, and FC. This relationship between calculated and measured variables that lead to singularity matrix prevents from running MANOVA. Hence, two

separate repeated-measure MANOVA were conducted to prevent the singularity. After finding significant differences in both multivariate and univariate tests, simple main effect comparisons as a post-hoc analysis were used to determine a location of significant differences.

For secondary analysis, two separate repeated-measure MANOVA at each level of intensities are used to detect a significant difference in the time sequence (BE, IAE, and 24-h) if the main effect, intensity condition, is not significant at the multivariate tests. This design could increase statistical power for a significant difference over time condition by reducing error term rates. Therefore, it could be worth to run separate repeated-measure MANOVA at each level of intensity condition if there is no significant main effect for the intensity condition. After finding a significant difference in both multivariate and univariate tests, simple main effect comparisons as a post-hoc test were used to determine the location of significant differences.

Pearson correlations were also examined to determine whether HR -LT determined from $VO_{2\ max}$ test are correlated with mean heart rates determined during exercise trial at LT intensity for each individuals. In addition, paired-samples *t*-tests were used to find out whether the lactate concentrations predetermined from $VO_{2\ max}$ tests were maintained during exercise trial tests at the LT intensity for each individual.

The level of significance was $p < 0.05$ for all statistical tests. The results are presented as means ± SE.

CHAPTER IV

RESULTS

The purpose of this study was to determine the effect of a single bout of aerobic exercise at different exercise intensities measured from evaluation of lactate concentration on lipid and lipoprotein cholesterol subfractions in college men, assuming each subject equally expended 350 kcal of energy during different exercise intensities. Therefore, lipid variables such as TG, TC, FC, and CE as well as lipoprotein variables such as HDL-c, HDL_2-c, HDL_3-c, LDL-c and VLDL-c were measured over time (BE, IPE, and 24 h PE) at each different exercise intensity (70% of LT and LT).

Subject's Characteristics

Eighteen healthy college-age males participated in this investigation and their characteristics were shown in the TABLE V. However, two of eighteen subjects were dropped out due to an absence of subsequent exercise trails after $VO_{2\ max}$ test.

TABLE V

DESCRIPTIVE CHARACTERISTICS OF SUBJECTS

Variable	Range	Mean ± SD
Age, yr	19 – 30	25.17 ± 3.49
Weight, kg	54.30 – 105	78.97 ± 11.74
Height, cm	167.6 – 191	180 ± 7
Body fat, %	7.9 – 24.9	15.58 ± 4.66
BMI, weight (kg) ÷ height^2 (meter^2)	19.33 – 28.78	24.41 ± 2.98
Total cholesterol, mg/dl	117.50 – 184.00	152.03 ± 19.37
$VO_{2\ max}$, ml/kg/min	32.3 – 56.47	44.95 ± 6.07

* SD: standard deviation.

Lactate, HR, and RPE Responses during $VO_{2\ max}$ Test and Exercise at LT Intensity

Mean blood lactate threshold value determined from $VO_{2\ max}$ test was close to 4.0 mmol/L (4.06 ± 0.786 mmol/L) in this investigation. However, individual lactate threshold values varied from 2.2 to 5.3 mmol/L. In addition, the range of blood lactate concentrations at the end of continuous, incremental, level treadmill test to determine LT for individuals was from 5.00 to 14.7 mmol/L (average 9.33 ± 2.87 mmol/L) as shown in TABLE VI.

TABLE VI

RANGE AND AVERAGE LACTATE CONCENTRATION, HR, AND RPE AT LT

Variable	Tests	Range	Mean ± SD
[Lactate] at LT, mmol/L	$VO_{2\ max}$ test	2.2 – 5.3	4.06 ± 0.786
[Lactate] at LT intensity, mmol/L	Exercise trial	2.4 – 7.21	4.59 ± 1.06
HR at LT, bpm	$VO_{2\ max}$ test	145 – 193	164.50
HR at LT intensity, bpm	Exercise trial	144 – 187	161.56

The blood lactate threshold values determined from $VO_{2\ max}$ test for individuals were used to maintain lactate threshold values during the exercise trials. The average blood lactate threshold value was 4.06 ± 0.786 mmol/L (TABLE VI). However, the average lactate concentration during exercise trial at LT intensity was 4.59 ± 1.06 mmol/L that indicates higher average lactate concentration than average lactate concentration determined from the $VO_{2\ max}$ test. However, the average correlation between lactate concentrations and heart rates during $VO_{2\ max}$ test was $r = .86 \pm 0.096$. The correlation indicates that a strong correlation existed between lactate concentrations and heart rates. However, this correlation does not indicate that heart rates associated with LT (HR-LT) predetermined from the $VO_{2\ max}$ test was useful reference to maintain constant lactate concentration during exercise trial at LT intensity because this HR-LT was determined during $VO_{2\ max}$ test and this high correlation was expected; the higher the lactate concentration, the higher heart rate will be. Therefore, Pearson correlations were again examined to determine whether HR-LT determined from $VO_{2\ max}$ test are still highly correlated with heart rates determined during exercise trial at LT intensity. The correlations results indicated that heart rates from $VO_{2\ max}$ test were similar to that from

exercise trials (TABLE VI) and heart rates (correlation = .751, $p < .001$) still has a strong correlation between two situations. In addition, the results of paired-samples *t*-tests were that although mean lactate concentration determined from $VO_{2\ max}$ tests was higher than that of determined during exercise trial at LT intensity, there was no statistical difference (df = 17, $p > .151$) between the two situations.

Metabolic and Exercise Performance Data

Caloric expenditures for the LT and 70% of LT intensities were 372.55 ± 28.96 kcal and 365.89 ± 75.97 kcal respectively, except for a warm-up and cool-down. Total kcal including warm up and cool down, total time, distance, % of $VO_{2\ max}$, HR, and speed (mph) for each trial were also listed in TABLE VII.

TABLE VII

METABOLIC AND EXERCISE PERFORMANCE DATA

Parameter	LT intensity (range)	70 % of LT intensity
Total Kcal	411.27 ± 32.71	404.60 ± 74.80
(warm up + cool down)	(357.52 – 454.44)	(259.34 – 520.55)
Kcal	372.55 ± 28.96	365.89 ± 75.97
	(322.91 – 413.40)	(226.05 – 485.06)
Time (min)	29.22 ± 5.78	52.06 ±18.33
	(21.00 – 45.00)	(28.00 – 100.00)
Total Distance (mile)	2.75 ± .34	3.43 ± .91
	(2.10 – 3.74)	(2.13 – 5.92)
% of $VO2_{max}$	76.86 ± 8.63	45.10 ± 14.07
	(61.34 – 94.58)	(26.39 – 67.53)
HR (beats/min)	160.45 ± 12.13	112.77 ± 14.81
	(144.10 – 188.43)	(96.00 – 150.17)
% Maximal Heart Rate	82.32	57.88
	(74.28 – 96.14)	(48.73 – 76.62)
Speed (mph)	5.74 ± .73	4.08 ± .49
	(4.59 – 7.10)	(3.40 –5.00)

Average Plasma Volume Changes with Exercise at LT and 70% of LT Intensities

The acute changes in plasma volume at each time point after exercise that occurred (TABLE VIII) altered the blood lipid and lipoprotein concentrations as shown in TABLE VIII and X. Repeated measure MANOVA was used to find out whether there are significant differences in Hct with lipid and lipoprotein variables between the intensities (70 % LT and LT intensities) and among time sequences (before, IPE, and 24 h). In addition, interaction effect also was analyzed from these two independent variables (2 intensities and 3 levels over time).

TABLE VIII

PLASMA VOLUME CHANGES WITH EXERCISE AT LT AND 70% LT, BASED ON THE HEMATOCRIT MEASUREMENTS (MEAN ± SD) OF EIGHTEEN MALES AT EACH TIME POINT.

Parameter		BE (range)	IPE (range)	24 h PE (range)
Hct (%)	LT	44.31 ± 2.02	43.56 ± 2.06	43.41 ± 2.23**
		(41.00 – 47.00)	(39.50 – 47.00)	(40.00 – 46.00)
	70 % LT	44.25 ± 1.90	43.53 ± 2.33	43.47 ± 2.27
		(39.50 – 47.00)	(39.50 – 48.00)	(39.50 – 47.00)
PVC (%)	LT	0	3.25 ± 5.74	3.86 ± 4.54
			(-7.76 – 12.86)	(-3.97 – 12.92)
	70 % LT	0	3.19 ± 6.82	3.52 ± 7.85
			(-7.76 – 15.38)	(-7.80 – 20.03)

** $p < 0.01$ compared to BE (before exercise) value.

The results of this statistical analysis revealed no significant differences between intensities and interaction effect (intensities × time sequences) on Hct changes, but there was a statistical difference in time sequences ($F(2, 14) = 5.431$, $p < .05$; power = .756).

The simple main effect as a post-hoc analysis revealed that Hct of 24 h was significantly ($F(1, 15) = 11.52$, $p < .01$) lower than BE at LT intensity.

Plasma volume changes based on Hct at IPE and 24 h for the LT intensity were 3.25 ± 5.74 % and 3.86 ± 4.54 %, respectively and at IPE and 24 h for the 70% of LT intensity were 3.19 ± 6.82 % and 3.52 ± 7.85 %, based on the hematocrit measurements, respectively as following TABLE VIII.

Plasma Volume Unadjusted and Adjusted Lipid and Lipoprotein Concentrations Over Time College-Age Men Exercising at LT and 70% of LT Intensities

The acute changes in plasma volume at each time point after exercise that occurred altered the blood lipid and lipoprotein concentrations. In other words, this increased plasma volume after aerobic exercise at LT and 70% LT intensities caused to magnify the lipid and lipoprotein concentration. Plasma volume unadjusted and adjusted lipid and lipoprotein concentrations over time college-age men exercising at LT and 70% LT intensities were as shown in the TABLE IX and X.

TABLE IX

PLASMA VOLUME UNADJUSTED LIPID AND LIPOPROTEIN CONCENTRATIONS OVER TIME COLLEGE-AGE MEN EXERCISING AT LT AND 70% LT INTENSITIES.

Parameter	BE	IPE	24 h PE
HDL-c (mg/dl)			
LT	47.56 ± 11.33	48.38 ± 11.80	48.63 ± 9.24
70% LT	48.71 ± 8.79	48.71 ± 9.93	48.53 ± 9.96
HDL_2-c (mg/dl)			
LT	21.78 ± 8.41	21.36 ± 8.62	22.23 ± 8.30
70% LT	18.82 ± 8.13	19.01 ± 9.61	19.02 ± 9.73
HDL_3-c (mg/dl)			
LT	25.78 ± 6.29	27.02 ± 6.40	26.40 ± 4.82
70% LT	29.89 ± 2.97	29.70 ± 3.69	29.51 ± 4.28
LDL-c (mg/dl)			
LT	80.85 ± 20.19	82.94 ± 19.02	81.40 ± 19.12
70% LT	81.74 ± 19.62	82.68 ± 17.45	81.64 ± 19.28
VLDL-c (mg/dl)			
LT	23.21 ± 9.08	24.50 ± 10.37	19.85 ± 7.08**
70% LT	22.61 ± 5.69	23.08 ± 8.15	22.13 ± 6.11
TG (mg/dl)			
LT	116.06 ± 45.39	122.50 ± 51.84	99.25 ± 35.38**
70% LT	113.06 ± 28.46	115.41 ± 40.76	110.65 ± 30.55
TC (mg/dl)			
LT	151.63 ± 23.04	155.81 ± 22.22	149.88 ± 21.81
70% LT	153.06 ± 20.09	154.47 ± 19.22	152.29 ± 18.26
FC (mg/dl)			
LT	40.44 ± 14.08	44.00 ± 11.92	44.56 ± 8.83
70% LT	43.18 ± 6.20	45.94 ± 6.73	44.12 ± 7.19
CE (mg/dl)			
LT	111.19 ± 13.89	111.81 ± 16.93	105.31 ± 15.47
70% LT	109.88 ± 17.01	108.53 ± 13.63	108.18 ± 14.34

* $p < 0.05$ compared to BE value.

** $p < 0.01$ compared to BE value

TABLE X

PLASMA VOLUME ADJUSTED LIPID AND LIPOPROTEIN CONCENTRATIONS OVER TIME COLLEGE-AGE MEN EXERCISING AT LT AND 70% LT INTENSITIES.

Parameter	BE	IPE	24 h PE
HDL-c (mg/dl)			
LT	47.56 ± 11.33	50.21 ± 13.77	50.45 ± 11.21*
70% LT	48.71 ± 8.79	50.34 ± 10.35	50.18 ± 10.02
HDL_2-c (mg/dl)			
LT	21.78 ± 8.41	22.24 ± 9.67	23.19 ± 9.26
70% LT	18.81 ± 8.13	19.74 ± 10.11	19.53 ± 9.57
HDL_3-c (mg/dl)			
LT	25.78 ± 6.29	27.97 ± 7.09	27.48 ± 5.40
70% LT	29.89 ± 2.97	30.60 ± 3.05	30.65 ± 5.19
LDL-c (mg/dl)			
LT	80.85 ± 20.19	85.87 ± 21.01*	84.77 ± 21.00
70% LT	81.74 ± 19.62	85.09 ± 16.45	84.75± 21.55
VLDL-c (mg/dl)			
LT	23.21 ± 9.08	25.24 ± 10.54	20.54 ± 7.08**
70% LT	22.61 ± 5.69	24.10 ± 9.20	22.96 ± 6.71
TG (mg/dl)			
LT	116.06 ± 45.39	126.21 ± 52.70	102.68 ± 35.39**
70% LT	113.06 ± 28.46	120.50 ± 45.99	114.78 ± 33.55
TC (mg/dl)			
LT	151.63 ± 23.04	161.32 ± 27.02**	155.98 ± 25.58
70% LT	153.06 ± 20.09	159.68 ± 22.44	157.89 ± 21.92
FC (mg/dl)			
LT	40.44 ± 14.08	45.67 ± 13.13	46.31 ± 9.53*
70% LT	43.18 ± 6.20	47.49 ± 7.53	45.66 ± 7.66
CE (mg/dl)			
LT	111.19 ± 13.89	115.65 ± 19.70	109.67 ± 18.52
70% LT	109.88 ± 17.01	112.04 ± 13.71	112.23 ± 17.31

* $p < 0.05$ compared to BE value.

** $p < 0.01$ compared to BE value.

Statistical Analyses

Primary Statistical Analysis

This investigation was conducted to determine significant differences existed in blood concentrations of lipid and lipoproteins over time (BE, IPE, and 24-h) following exercise trials at 70% LT and LT intensities for eighteen subjects. The independent variables were intensity (LT and 70% LT) and time (BE, IPE, and 24 h). The dependent variables were blood concentrations of lipids (TC, TG, FC, and CE) and lipoproteins (HDL-c, HDL_2-c, HDL_3-c, LDL-c, and VLDL-c). Two separate multiple repeated-measure MANOVA was conducted to determine differences in both intensity and time conditions as well as interaction effect (time × intensity). However, the main effect (intensity condition: LT and 70% LT, Hotellings = .737, $p > .05$) and interaction effects (Hotellings = .364, $p > .05$) on the multivariate tests were not significant, whereas the main effect for time condition was significant on the measured variables (HDL-c, HDL_3-c, TG, TC, FC and Hct; Hotellings= 1.32, $p < .01$) and on the calculated variables from the equations (HDL_2-c, LDL-c, VLDL-c and CE; Hotellings= 1.12, $p < .01$).

Secondary Statistical Analysis

Therefore, two separate repeated-measure MANOVA at each level of intensities were used to detect a significant difference in the time sequence (BE, IAE, and 24 h). Consequently, four separate multiple repeated-measures MANOVA were performed as following: HDL-c, HDL_3-c, TC, TG, FC and Hct at both intensities (1= LT and 2= 70%

LT) as well as HDL_2-c, LDL-c, VLDL-c and CE at both intensities (3= LT and 4= 70% LT), respectively.

After finding significant differences in multivariate tests, then univariate F-tests were checked. If both multivariate and univariate tests were significant, simple main effects for the time condition were used to determine the location of significant differences.

Significant differences were detected between BE-24 h on HDL-c ($F(1, 15) = 4.71$, $p < .05$, ↑6.08%), BE-24 h and IPE-24 h on VLDL-c ($F(1, 15) = 8.79$, $p < .01$, ↓13%; 18.16, $p < .01$, ↓22.88%, respectively), BE-24 h and IPE-24 h on TG ($F(1, 15) = 8.79$, $p < .01$ and 18.16, $p < .01$, respectively), BE-IPE on TC ($F(1, 15) = 11.43$, $p < .01$), and BE-24 h on FC ($F(1,15) = 6.07$, $p < .05$) at only LT intensity (Figure 10).

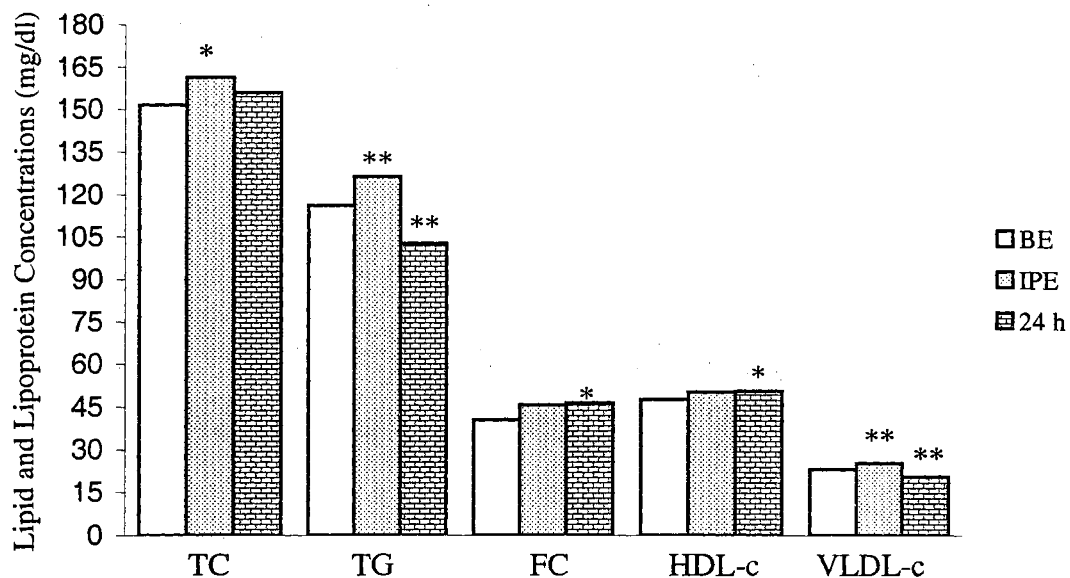


Figure 7) TC, TG, FC, HDL-c, and VLDL Responses to Exercise over Time at LT Intensity. * $p < 0.05$, ** $p < 0.01$.

One of the null hypotheses stated that there were no significant differences between intensity conditions (LT and 70% LT) in lipids and lipoprotein concentrations with response to a single session of aerobic exercise in men. The results failed to reject this null hypothesis. It was also hypothesized that no interaction between the intensity condition (LT and 70% LT) and time condition (BE, IPE, and 24 h) would occur. This hypothesis was also failed to reject. Also, the null hypotheses stated that there were no significant differences over time in lipids and lipoprotein concentrations immediately post-exercise (IPE) and 24 h post-exercise (24 h) compared to the baseline (before exercise; BE) with response to a single session of aerobic exercise in men. There were significant changes over time in lipids and lipoprotein concentrations IPE and 24 h compared to the baseline. Therefore, this hypothesis was rejected.

In addition, to determine the pattern changes of HDL-c over times the ratios of FC/CE and HDL_2-c/HDL_3-c were analyzed using repeated-measures MANOVA. However, there was no significant difference in both ratios of FC/CE and HDL_2-c/HDL_3-c over time condition (BE, IPE, and 24 h; Hotellings = .567, $p > .05$). The result indicated that although the decreases in the ratio of HDL_2-c/HDL_3-c IPE (0. 832) and 24 h (0.876) compared to the BE (0.900) at the LT intensity were observed, the significant increase in 24 h HDL-c ($p < 0.05$) at LT intensity was due to the increases in both HDL_2-c (6.47%) and HDL_3-c subfractions (6.59%). In addition, although there was significant increase in 24 h FC, there was also no significant difference in the ratio of FC/CE at 24 h (0.425) compared to BE (0.363).

TABLE XI

THE RATIOS OF HDL_2/HDL_3-c AND FC/CE.

Parameter	Intensities	BE	IPE	24-h PE
HDL_2/ HDL_3-c	LT	.900 ± .411	.832 ± .357	.876 ± .405
(mg/dl)	70 % LT	.627 ± .291	.647 ± .358	.659 ± .398
FC/CE	LT	.363 ± .120	.400 ± .113	.425 ± .069
(mg/dl)	70 % LT	.394 ± .060	.422 ± .040	.422 ± .050

Values listed as mean ± SD.

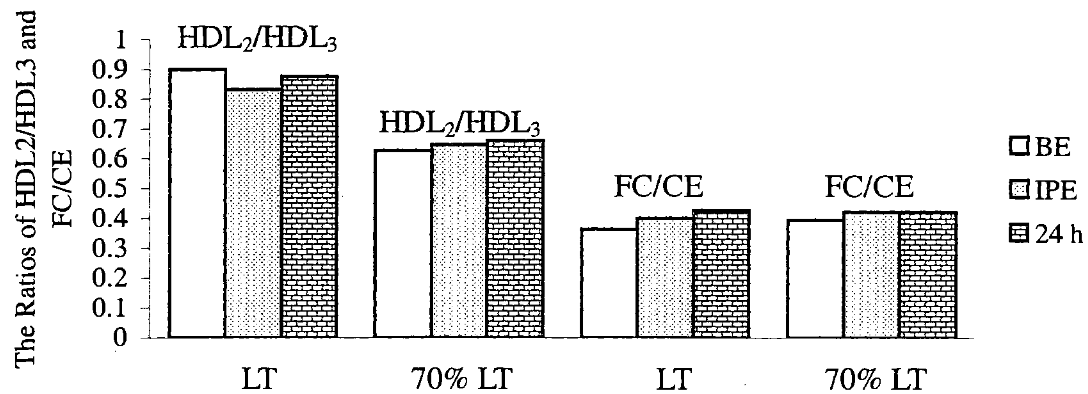


Figure 8) The Ratios of HDL_2-c/HDL_3-c and FC/CE over Time.

Summary

The purpose of this study was to determine the effect of different exercise intensities measured from the evaluation of lactate concentration on lipid and lipoprotein cholesterol subfractions in college men, assuming each subject equally expend 350 kcal of energy during different exercise intensities (70% of LT and LT intensities).

Based on the results of this study, although the mean blood lactate threshold value determined from $VO_{2\,max}$ test was 4.06 ± 0.786 mmol/L is close to the 4.0 mmol/L,

individual lactate threshold values varied from 2.2 to 5.3 mmol/L. On the contrary, the mean lactate concentration during exercise trial at LT intensity was 4.59 ± 1.06 mmol/L that indicates higher than mean lactate concentration determined from $VO_{2\ max}$ test. However, Pearson correlations revealed that heart rate still has a strong correlation between two situations (.751, $p < .001$). Therefore, heart rate was appropriate reference to monitor maintaining constant lactate concentration during exercise trial at the level of lactate concentration determined from $VO_{2\ max}$ test in this investigation. In addition, the results of paired-samples *t*-tests indicated that although mean lactate concentration determined from $VO_{2\ max}$ tests was higher than that of determined during exercise trial at LT intensity, there was no statistical difference (df = 17, $p > .151$) between the two situations. Therefore, LT predetermined form $VO_{2\ max}$ test could be maintained during the exercise trials at LT intensity for expending 350 kcal by either remaining or decreasing treadmill velocity associated with LT based on monitoring HR although mean blood lactate concentration during the exercise trial was higher than that of $VO_{2\ max}$ test (4.06 ± 0.786 vs. 4.59 ± 1.06).

Furthermore, repeated-measures MANOVA revealed that there was only significant difference in Hct changes over time. The post-hoc analysis, simple main effect, revealed that Hct of 24 h was significantly lower than BE. That refers to increase of plasma volume, magnifying the lipid and lipoprotein concentrations. However, these plasma volume changes at IPE and 24 h for the LT and 70% of intensities were similar based on the hematocrit measurements, respectively.

In assessing the lipid and lipoprotein variables, the increase in HDL-c at the 24 h was due to the increase in both HDL_2-c and HDL_3-c. The increase in IPE TC at the LT

intensity occurred while the decreases in 24 h TG and VLDL concentrations at the LT intensity also occurred at different time point, respectively. These decreases in the concentrations of TG and VLDL-c were significantly contributing to change in 24 h HDL-c concentration. In addition, to determine the pattern changes of HDL-c over times the ratios of FC/CE and HDL_2-c/HDL_3-c were analyzed using repeated-measures MANOVA. However, there were no significant differences in both ratios of FC/CE and HDL_2-c/HDL_3-c over times. Therefore, the significant increase in 24 h HDL-c ($p < 0.05$) at LT intensity was due to the increases in both HDL_2-c (6.47%) and HDL_3-c subfractions (6.59%) even though 24 h FC was increased significantly.

Consequently, exercise at LT intensity can favorably alter the lipid profile by the increase in 24 h HDL-c concentration in combination with decreases in TG and VLDL at 24 h post-exercise.

CHAPTER V

CONCLUSIONS, DISCUSSION AND RECOMMENDATIONS

Introduction

It is generally accepted that endurance trained athletes have higher HDL-c concentrations than sedentary counterparts (Hartung et al., 1980; Huttunen et al., 1979). It may be partially attributed to the therapeutic effect of regular aerobic exercise on the lipid profile. Many of the lipid changes after a single bout of exercise are similar to those reported after chronic exercise training as well. If so, it's worth to measure acute changes of blood lipid and lipoprotein profiles with response to a single bout of aerobic exercise. Because this measurement with response to acute aerobic exercise may also provide some pattern changes of lipid and lipoprotein profiles if there is an effect of acute aerobic exercise on lipid and lipoprotein profiles. However, acute increases in HDL-c with response to a single bout of aerobic exercise have been observed in some studies (Crouse et al., 1997; Gordon et al., 1998) but not in others (Angelopoulos & Robertson, 1993; Cullinane et al., 1981; Nikkila et al., 1987). These inconsistent results with response to acute aerobic exercise might be from differences of exercise intensity, duration and caloric expenditure. In other words, caloric expenditure, duration, and intensity of exercise might be directly related to the degree of changes in HDL-c. Therefore, if

caloric expenditure is held constant (i.e., 350 kcal), the response of HDL-c might be mainly influenced by exercise intensity. However, many investigators use percentages of $VO_{2\ max}$ or of maximal heart rate in metabolic or hormonal studies as a criterion for intensity although variability in physiological and metabolic responses to submaximal exercise may occur due to wide ranges of exercise intensities compared with the LT (Tim et al., 1999).

Therefore, the purpose of this study was to determine the level of exercise intensity, assuming subjects equally expended 350Kcal of energy during aerobic exercise at different intensities (70% of LT and LT intensities), which would promote a significant increase in blood lipoproteins (HDL-c, HDL subfractions such as HDL_2 and HDL_3-c). I analyzed data with multiple repeated-measures MANOVA to determine the difference in lipids and lipoprotein cholesterol subfractions after a single session of aerobic exercise. Paired-samples *t*-tests were used to find out whether the lactate concentrations predetermined from $VO_{2\ max}$ tests were maintained during exercise trial tests at the LT intensity for each individual. In addition, Pearson correlations were also examined to determine whether HR -LT determined from $VO_{2\ max}$ test are correlated with mean heart rates determined during exercise trial at LT intensity for each individuals.

Conclusion

The following conclusions were made from the present study:

1) The LT intensity can be the threshold intensity of acute aerobic exercise necessary to promote a significant increase in HDL-c in the college age men (25.72 ± 4.55 yrs) with a baseline HDL-c of 47.56 ± 11.33.

2) The increase in 24 h PE HDL-c (2.89 mg/dl; 6.08%) compared to the baseline appeared to be positively related to the exercise intensity (LT).
3) The acute increase in HDL-c at the 24 h was due to the increase in both HDL_2-c (1.19 mg/dl; 6.47%) and HDL_3-c (1.7 mg/dl; 6.59%) although there were no statistical changes in both HDL_2-c and HDL_3-c concentrations.
4) The increase in IPE TC ($p < .01$; 5.73%) at the LT intensity occurred while the decreases in 24 h TG ($p < .01$; -13.03%) and VLDL concentrations ($p < .01$; -13.03%) at the LT intensity also occurred at different time point. These decreases in the concentrations of TG and VLDL-c were significantly contributing to change in 24 h HDL-c concentration. However, the increase in IPE TC ($p < .01$; 6.39%) at the LT intensity was due to the increases of HDL_3-c, LDL-c and VLDL-c.
5) The hematocrit changes only at 24 h of LT intensity were significantly different from the baseline value (TABLE VIII). This significant plasma volume change (PVC) based on the Hct could significantly magnify not only 24 h lipid and lipoprotein profile but also IPE lipid and lipoprotein profile (TABLE X).

Discussion

To date, no investigation has examined lipid and lipoprotein metabolism using LT or % of LT as a criterion for exercise intensity. The expression of exercise intensities relative to % $VO_{2\ max}$ or percent maximal heart rate has been commonly used in exercise-related studies as a criterion for intensity, although variability in metabolic and

physiological responses to submaximal exercise may occur due to wide ranges of exercise intensities compared with the LT (Dwyer & Bybee, 1983; Weltman et al., 1989). McLellan and Gass (1989) reported that individuals with a similar $VO_{2\ max}$ showed different metabolic responses to exercise at the same relative intensity. Coyle et al. (1988) observed individuals with a similar $VO_{2\ max}$ can vary in glycogen use and exercise performance during exercise at similar work rates and percentages of $VO_{2\ max}$. These findings indicate that although individuals exercise with the same relative oxygen uptake, their metabolic and physiological responses may vary (Baldwin et al., 2000). Therefore, although training prescriptions based on percent $VO_{2\ max}$ or maximal heart rate set a constant workload for all individuals, they do not account for individual metabolic and physiologic differences. Although the LT or AT is not a constant percent of $VO_{2\ max}$ or maximal heart rate for all individuals, it may be considered as a precise training prescription with respect to individualized metabolic stress for each individual. Therefore, lactate threshold or % of LT may provide less variability in the metabolic and physiological responses between individuals than percentages of $VO_{2\ max}$ or percentages of maximal heart rate. Consequently, exercise prescriptions at a specific intensity within the commonly recommended range of 50 to 85 % $VO_{2\ max}$ (Dwyer & Bybee) or percentages of heart rate max may result in metabolically different workloads in individuals with similar $VO_{2\ max}$ or % heart rate. Therefore, to reduce the variability and provide more precise prescription, the 70% of LT and LT intensities were used as independent variables. During the exercise trial at LT intensity, HR was utilized as a reference to maintain constant blood lactate concentration predetermined from $VO_{2\ max}$ test for each individual. The results of this investigation indicated that constant blood

lactate concentrations for individuals were maintained during exercise trial at LT intensity by adjusting velocity via monitoring HR predetermined from $VO_{2\ max}$ test. However, LT was defined that highest workload without a gradual increase in blood lactate can be maintained over a long duration (Stegmann & Kindermann, 1982). In addition, MLSS represents the upper limit of blood lactate concentration that can be maintained as a lactate steady state during constant workload (Heck et al., 1985). However, a lactate steady state could not be maintained if the velocity was not adjusted (decreased) by HR monitoring in this investigation. This result is in agreement with the findings of Beneke (1995) and Orok, Hughson, Green, and Thomson (1989). They reported that lactate concentrations during constant workload exercise were increased compared with those measured at the same workload during an incremental exercise test.

Changes in plasma volume (PV) based on the hematocrit (Hct) measurements occur immediately post-exercise and 24 h post-exercise in this investigation compared with baseline plasma volume. Harrison, Edwards, and Leitch (1975) reported that a reduction in plasma volume occurs during exercise and returns to pre-exercise levels during recovery. In the study of Harrison et al. six male subjects exercised for 50 min at 25% and 55% of $VO_{2\ max}$ at various temperatures (24, 30, 35, and 42 C°). Changes in the Hct, hemoglobin (Hb), plasma protein concentrations, and the activity of injected albumin were each used to calculate PV changes occurring during exercise and recovery. There was a 1% reduction in red blood cell volume during moderate exercise and recovery (Harrison, et al., 1975), suggesting that exercise increases plasma protein levels (i.e., albumin) during recovery by promoting the rate of protein movements from extravascular compartments to the intravascular compartment. However, there are many contradictory

reports; during recovery acute increases in PV have been recorded in some studies (Gillen et al., 1991; Green et al 1984; Harrison et al.) while no changes occurred in others (Pivarnik, Leeds, & Wilkerson, 1984; Novosadova, 1977). Human RBC have been shown to increase, decrease, or remain constant in volume during physical stress depending on the combined interactions of plasma osmolarity and blood pH (Van Beaumont, Underkofler, & Beaumont, 1981).

After and during exercise, changes of intravascular pressure, protein content, or pH may alter the plasma volume. In this investigation, acute changes in plasma volume occurred at each point following exercise bout (TABLE VIII) as well as resulting increased in blood lipid and lipoprotein concentrations as shown in TABLE IX and X. Hematocrit (Hct) was measured for the plasma volume change and the concentrations of the measured lipid variables were adjusted according to the magnitude of the plasma volume changes at each point by calculating the PV adjustment IPE and 24 h samples. These reductions of Hct during recovery (IPE and 24 h) magnified significant increases in lipid and lipoprotein concentrations.

The present study observed a significant increase in the 24 h HDL-c concentration (2.89 mg/dl; 6.08 %; $p < .05$) compared to before exercise (BE) for baseline at LT intensity. The present response of increased HDL-c is in agreement with earlier reports measuring untrained subjects during a single bout of moderate exercise of similar intensity and duration but different time points (Angelopoulos, Robertson, Goss, Metz, & LaPorte, 1993; Kantor, Cullinane, Sady, Herbert, & Thompson, 1987; Swank, Robertson, Deitrich, & Bates, 1987).

For instance, Angelopoulos et al. (1993) reported total HDL-c was higher at 5 min than pre-exercise in seven sedentary collage aged men running submaximal treadmill: HDL_3-c levels were significantly higher at 5 min and 48 h post-exercise than pre-exercise. However, total HDL-c did not differ from pre-exercise at 24 and 48 h post exercise. Unfortunately, the lipid measurements were not corrected for plasma volume changes.

Swank et al. (1987) also reported that HDL-c and HDL_3-c were significantly elevated ($P < 0.01$) only at 5 min after exercise compared to pre-exercise values in 9 healthy female subjects running at 70% of $VO_{2\ max}$. Additionally, HDL_2-c showed no significant change at any of the post-exercise time points compared with pre-exercise. Results indicated that the rise in HDL-c following acute exercise was due to an increase in the subfraction HDL_3-c. The change in total HDL-c and HDL_3-c was transient to pre-exercise values that were re-attained within 24 h following acute submaximal exercise. However, the lipid measurements in the studies of Swank et al. and Angelopoulos et al. (1993) were not corrected for plasma volume changes, which can either increase or decrease the HDL-c concentration as previously discussed.

Kantor et al. (1987) demonstrated that total HDL-c increased in both trained and untrained subjects only at 48 h post bicycle-exercise. Fortunately, the lipid measurements were corrected for plasma volume changes. In the study of Kantor et al. post-exercise expansion of plasma volume magnified the increase in concentration of HDL-c and HDL-c subfractions in both groups. Acute exercise contributed to increase HDL levels by significantly raising the HDL_2-c subfraction in trained subjects and the HDL_3-c subfraction in untrained subjects. In terms of adjusted PVC, this result is

comparable with the present investigation that found a significant increase in 24h HDL-c.

In contrast, although similar intensity and duration were administered to untrained subjects, the increase of HDL-c in this study does not agree with the investigation of Cullinane et al. (1981) studying lipid profiles in eight sedentary men pre-exercise and post-exercise. A reduction in estimated LDL-c was observed 66 hours after the exercise and all other serum lipid (TG, TC, and HDL-c) and hematocrit measurements did not differ from pre-exercise concentrations. However, Cullinane et al. did not account for individual body weight differences that effect caloric expenditure individually. Different caloric expenditure could act a variation in this study.

Exercise-induced changes in HDL-c are the result of the interaction among exercise intensity, duration, frequency of each exercise session and length of the exercise training period (Kokkinos and Fernhall, 1999). In addition, baseline HDL-c level, training status, and gender also affect the HDL-c response to the exercise (Zumuda et al., 1998; Durstine & Haskell, 1994). Stein et al.(1990) demonstrated that a minimum training intensity that is required to the increase HDL-c level was equal to 75% maximal heart rate. Significant increase in HDL-c occurred only in the 75% and 85% maximal heart rate groups without correcting for plasma volume changes. Hicks et al. (1987) reported that high intensity (90% $VO_{2\ max}$) of aerobic exercise is more likely to increase in HDL-c concentration compared with moderate intensity (60% $VO_{2\ max}$) of aerobic exercise.

In contrast, Aellen et al. (1993) studied the effects of anaerobic and aerobic training on lipoprotein concentrations in 45 healthy untrained men. Training with intensity below the AnT had a significant increase in HDL, HDL_2, LDL/HDL, HDL_2/HDL_3, and cholesterol/HDL. This investigation concluded that training above the AnT has no effect

on blood lipoprotein profiles. As a result of this reported study, beneficial adaptations in lipoprotein profile should be achieved with moderate training intensities below the AnT.

If caloric expenditure for an exercise session (i.e., 350 kcal) is held constant, the response of HDL-c might be mainly influenced by exercise intensity. However, almost all reported studies employed percentages of $VO_{2\ max}$ or heart rate as a criterion for intensity even though variability in metabolic and physiological responses to submaximal exercise may occur. On of the reason is that the contribution of aerobic and anaerobic systems differs considerably as a function of HR_{max} and VO_{2max} due to wide ranges of exercise intensities compared with the LT (Tim et al., 1999). This variability in physiological responses and wide ranges of exercise intensities of $VO_{2\ max}$ or heart rates as a factor affecting lipid metabolism may cause different response of HDL-c concentration to acute exercise.

This investigation found an increase in HDL-c 24 h following single bout of aerobic exercise at LT intensity due to an increase in both HDL_2-c (6.47%) and HDL_3-c subfractions (6.59%) although there were no statistically significant differences in both HDL_2-c and HDL_3-c subfractions over time. In addition, there were no pattern changes of HDL-c over times by observing the ratios of FC/CE and HDL_2-c/HDL_3-c although there was significant increase in 24 h FC. This investigation is agreement with the findings of Gordon et al. (1998) although the time point of change in the HDL-c concentration was different compared with the present study. Gordon et al. reported that although 48 h HDL_2-c and HDL_3-c were not significantly different from baseline, the rise in 48 h HDL-c after running at 75% $VO_{2\ max}$ (72 ± 9 min) in trained women was attributed to an increase in both HDL_2 and HDL_3. However, at 48 h after exercise, the

increase in HDL-c correlated highly with changes in HDL_2-C (Gordon et al.). Based on this result, we can assume that the increase in HDL_2-c may require either the time to convert from HDL_3-c to HDL_2-c or amount of exercise performed to cause increase in HDL_2-c concentration.

In the assessing lipid variables, the present investigation revealed a significant decrease in 24 h TG in comparison to BE (116.06 ± 45.39 vs. 102.68 ± 35.39 mg/dl) at the LT intensity. Lipoprotein lipase (LPL) is a key enzyme required for the hydrolysis of triglyceride-rich particles. The lipolysis of TG via LPL has been suggested to be the main cause of an increase in HDL-c and this reduction in TG concentration mainly due to a decrease in TG concentration in the VLDL (Annuzzi, Jansson, Kaijser, Holmquist, & Carlson, 1987). Consequently, TG and VLDL responses of this study were expected. The present investigation showed reduced 24 h TG and VLDL concentrations at the LT intensity. This reduced TG concentration after the exercise may be due to the replenishment of intramuscular stores reduced during the exercise (Annuzzi et al., 1987). In addition, this decrease in TG concentration may be closely related to the intensity of exercise. In fact, glycogen depletion has been suggested as one factor responsible for the increase in muscle vasculature LPL activity after prolonged exercise (Jacobs, Lithell, & Karlsson, 1982). A higher intensity may lead to a reduction in muscle glycogen that could significantly decrease the TG concentration via increased LPL activity in this investigation.

In contrast, TC concentration at IPE was significantly higher than before exercise (BE) at LT intensity while increased IPE TC was decreased 24 h (TABLE X). This response was similar to other report of Hughes et al. (1990). However, reports of acute

change after a single bout of exercise have been inconsistent. Variations in TC may result from different duration of exercise, subject characteristics, not individualized utilization of intensities (i.e., $VO_{2\ max}$ or heart rate criteria as intensity) in a study. However, increased post-exercise TC value is more likely related to intensity of exercise; higher intensity may cause acute increase in TC concentration by increasing HDL-c, LDL-c or VLDL-c as compared to low intensity. This relationship has been seen in several reports (Hicks et al., 1987; Hughes et al.).

This investigation also found a significant increase in FC 24 h ($p < .05$; 14.52%) at LT intensities compared to the BE (baseline) and this result may indicate increased HDL-c concentration by increase in HDL_3-c subfraction. However, there was no significant increase in FC/CE ratios over time in both intensities (TABLE XI). Frey, Baumstark, Berg, and Keul (1991) reported that baseline LCAT activity was related to FC and CE concentrations; following esterification of FC in HDL_3-c, true HDL_2-c (enriched with CE) is formed by LCAT activation (Nikkila et al., 1987). This may imply that there was no significant change in LCAT activity over time points after the exercise session. Consequently, unchanged ratio of FC to CE concentrations may lead to maintained concentration of HDL_2 subfraction after a single session of aerobic exercise.

In conclusion, although hormones and enzymes in the blood samples were not analyzed in this investigation, activity of enzymes through the secretion of hormones is closely related to exercise intensity and calorie expenditure. A major role of hormone action is to increase enzyme activity. Many hormones influence either the rate of esterification or the rate of lipolysis (Murray et al., 1996). Hormonal regulation of energy metabolism depends on both the intensity and duration of exercise that determines calorie

expenditure (Tran & Weltman, 1983). Various hormones work to ensure glucose and FFA availability for muscle energy metabolism. Stimuli from either the working muscles or motor centers via the central nervous system modify the response of the endocrine system directly via pituitary hormones or indirectly via the sympathoadrenal system (Thornton, 1985).

Consequently, these changes of enzyme activity, sympathoadrenal activity, and hormonal responses seem to be associated with an increase in HDL levels and a decrease in triglycerides concentration in the blood (Tran & Weltman, 1983). This increased enzyme activity via the increased hormone secretions is mainly affected by both intensity and duration of exercise, causing different results in HDL-c and HDL-c subfractions (increase, remain, or decrease); especially using not individualized intensity such as $\%VO_{2\,max}$ or maximal heart rate when similar calorie expenditure (i.e., 350 kcal) is hold constant. Therefore, evaluation of lactate concentrations such as LT or % of LT as a criterion for exercise intensity may be especially appropriate to study lipid and lipoprotein metabolism because it provides less variability than $\%VO_{2\,max}$ or maximal heart rate in terms of individualized exercise intensity for each individual.

Summary

The purpose of this study was to determine the effect of different exercise intensities on lipid and lipoprotein cholesterol subfractions. Based on the results of this study, the LT intensity appears to be the threshold intensity of acute aerobic exercise (expending 350 kcal) necessary to promote a significant increase in HDL-c in these

subjects. This increase in HDL-c at the 24 h was due to the increase in both HDL_2-c and HDL_3-c.

The increase in IPE TC ($p < .01$; 5.73%) at the LT intensity occurred while the decreases in 24 h TG ($p < .01$; -13.03%) and VLDL concentrations ($p < .01$; -13.03%) at the LT intensity also occurred at different time point, respectively. These decreases in the concentrations of TG and VLDL-c were significantly contributing to change in 24 h HDL-c concentration. Therefore, exercise at LT intensity can favorably alter the lipid profile by the increase in HDL-c concentration in combination with decreases in TG and VLDL at 24 h PE. In addition, these significant changes of lipid and lipoprotein profiles were magnified by the plasma volume change (TABLE VIII).

In the view of exercise intensity, evaluation of blood lactate elevation as an intensity criterion might be appropriate. Although individuals exercise with the different relative oxygen uptake, their metabolic and physiological responses may not vary as described before. Therefore, evaluation of lactate concentrations such as LT or % of LT as a criterion for exercise intensity may be appropriate to study lipid and lipoprotein metabolism because it provides less variability than $\%VO_{2\,max}$ or maximal heart rate in terms of individualized exercise intensity for each individual.

Recommendations

Based on the research findings from the present study, the following suggestions for future research will help to define the effect of aerobic exercise on acute changes in HDL-c and HDL subfractions. In the hope that future research will explore further the problem at hand, it is recommended that:

1) Obtain blood lipid and lipoprotein measurements at 48 and 72 h post-exercise to determine delayed effect of HDL-c and HDL subfractions. Additionally, determine the time that conversion from HDL_3-c to HDL_2-c over time occurs related to these phenomena.
2) Compare individuals of varying different fitness levels and caloric expenditures at the LT intensity to determine if differences exist in HDL-c and HDL subfractions as a function of caloric expenditure with changes in enzymes and hormones.
3) Investigate gender (male versus female) differences at the LT intensity to determine if differences exist in HDL-c and HDL subfractions as a function of gender with changes in enzymes and hormones.
4) Compare individuals with different baseline HDL-c (low, average, and high levels) at the LT intensity to determine if differences exist in HDL-c and HDL subfractions as a function of baseline HDL-c with changes in enzymes and hormones.

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APPENDICES

APPENDIX A

HEALTH HISTORY AND MEDICAL QUESTIONNAIRE

Name: _______________Soc.Sec. #:____________________Date: ________________
Age: _________________Date of Birth: _________________Sex:_________________

Height: _______________Weight: ______________Blood Pressure: ________/_______

Address: ____

Home Phone: ________________________________Work Phone:_________________

Personal Physician: ___________________________Phone: _____________________

HEALTH HISTORY: Have you ever had:

High Blood Pressure	_________	Low Blood Pressure	_________
Heart Disease	_________	Irregular Heart Beat	_________
Diabetes	_________	Thyroid Diseases	_________
Heart Murmurs	_________	Pulmonary Disease	_________
Chest Pain	_________	High Blood Cholesterol	_________
Arthritis	_________		

Have a parent or sibling had any heart disorders prior to age 55? _______
Have a sudden death ever occurred in a parent or sibling? _______

Do you smoke? _____ If yes, how much? ____ What sort of tobacco products do you use? ______________
What sort of people do you mix with?

Do you drink alcoholic beverages?_____If yes, please indicate the type and amount you consume per week: ____________(type)/_____________(oz.)

Do you participate in a regular exercise program? ___If yes, What activity? __________
Minutes/session? ___________Times/week ________Intensity (1=low, 10= high) ______
For how many years? ___________

Have you ever taken medication for:	Yes	Medication and Dosage	Date
High Blood Pressure	_____	____________________	_______
Low Blood Pressure	_____	____________________	_______
Heart Disease	_____	____________________	_______
Diabetes	_____	____________________	_______
Thyroid Disease	_____	____________________	_______
Pulmonary Disease	_____	____________________	_______
Arthritis	_____	____________________	_______

MEDICAL QUESTIONNAIRE:

1. Have you ever been advised by a physician to avoid exercise? _______
2. Do you ever have shortness of breath during or after exertion? _______
3. Have you ever experienced fainting or dizzy spells? _______
4. Have you ever had pain or discomfort in the chest? _______
5. Have you ever experienced swollen ankles (excluding sprains)? _______
6. Have you recently experienced heart palpitations (rapid heart beat) at rest? _______
7. Have you ever experienced claudication (unexplained lameness)? _______
8. Is there any other health condition that might limit your participation in exercise programs? ________ (e.g., bone or joint disorders, pregnancy, etc.)
9. Are you taking any medication? ______ What type? _________________
10. Have you had a medical exam in the last 12 months?_____ Date? _________

______________________________________ ____________________

Signature Date

APPENDIX B

OSU INSTITUTIONAL REVIEW BOARD CONSENT FORM

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD

Date: March 9, 2000 IRB #: ED-00-187

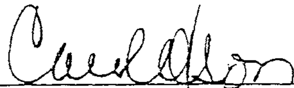
Proposal Title: "THE EFFECT OF A SINGLE BOUT OF DIFFERENT EXERCISE IMMENSITIES ON LIPID AND LIPOPROTEIN CHOLESTEROL"

Principal Investigator(s): Jack Ransone
Dong-Ho Park

Reviewed and Processed as: Expedited

Approval Status Recommended by Reviewer(s): Approved

Signature:



Carol Olson, Director of University Research Compliance

March 9, 2000
Date

Approvals are valid for one calendar year, after which time a request for continuation must be submitted. Any modification to the research project approved by the IRB must be submitted for approval with the advisor's signature. The IRB office MUST be notified in writing when a project is complete. Approved projects are subject to monitoring by the IRB. Expedited and exempt projects may be reviewed by the full Institutional Review Board.

The Effect of A Single Session of Different Exercise Intensities on Lipid and Lipoprotein Cholesterol.

I, ____________________________, voluntarily agree to participate in this investigation directed by Dong-Ho Park and Dr. Jack Ransone at Oklahoma State University. I know that while these individuals will supervise the research study, other professionals who work with them may assist or act on their behalf. I understand that at all times during the research, I will be under the supervision of the principal investigator, Dong-Ho Park. I understand that the purpose of this study is to determine whether there is a threshold, single exercise intensity, at which favorable changes in lipid and lipoprotein cholesterol subfractions occur, assuming subjects equally expend 350kcal of energy during different exercise intensities for college age men.

PROCEDURES

The procedures that I voluntarily agree to take part include:

1. A screening will be performed with a complete health-history questionnaire, a release of pertinent demographic information, and complete medical examination performed by the investigator.
2. The physiological, hematological, and chemical variables will be measured as followi ng: heart rate, electrocardiography (EKG), $VO_{2\ max}$.: hematocrit and hemoglobin: trigl yceride, total cholesterol, free cholesterol, cholesterol ester, LDL, VLDL, lactate, glucose, HDL and HDL subfractions.
3. Following tests will be performed under the supervision of a certified conditioning ins tructor: Completing two counterbalanced running or walking treadmill tests at subma ximal levels, for a duration sufficient to expend 350 kcal of energy in this study. Dur ation of exercise depends on my weight and lactate threshold, and usually it takes 20 t o 60-min on the treadmill. This study requires completing two different exercise inte nsities such as lactate threshold (LT) and 70% of lactate threshold (70% of LT).

Screening and Study Assignment

On the first occasion, the researchers will explain to me the purpose of the study and I will have the opportunity to ask my questions about the study. In this study, I will b e assured that my participation is completely voluntary. I am also aware that I need to co mplete a detailed medical and health history questionnaire and my fingerstick blood samp les are collected to insure that I do not have any of the conditions (total cholesterol is 135 to 239 mg/dl) that prevent me from joining the research study. The screening also includ es measuring the following variables: height, weight, and percent body fat (skinfolds mea surements). Trained personnel will conduct all testing and procedures will be explained t o me. On 2 visits, I will come to the site and my fasting blood sample before exercise an d immediately post-exercise blood samples about each 20-ml will be drawn. On 2 other v isits, my fasting blood sample of 24-hrs psot-exercise will be drawn. I should not have an y food or drink, except water, from 6: 00 p.m. the night before my blood sample visits so that I can give 20-ml (about 4 teaspoons) of blood. I will be also asked to answer certain questions about my food habits and my daily physical activity before each scheduled visit . I will be given a calendar and will be asked to come to the study site on a designed time and date.

Duration of Participation

In this study, I will conduct the exercise testing at different intensities (lactate threshold and 70% of lactate threshold) during one-month period.

Measures Made During the Study

My standing height, weight, BMI (Body Mass Index), body fat, fingerstick bloo d sample, lactate threshold and $VO_{2\ max}$ will be measured at the beginning of the study (o ne time). I agree to give 20-ml (about 4 teaspoons) of my blood to be drawn by trained p ersonnel six times (before, immediately post, and 24-hrs post-exercise at two different ex ercise intensities: LT and 70% of LT. I know that I must not consume food for 12 to 14-h rs before this blood collection except water

Costs

There will be no cost to me for the examinations performed in this study. If I ha ve health insurance, I permit the investigators to bill my health insurance for the routine p hysical in this research project. If the bill is not paid by my health insurance, I am not res ponsible for paying it. Travel and transportation costs such as bus or taxi fares, gasoline, and mileage to and from the study site will be my responsibility.

If I develop health problems during the study, I will be seen by research project physicians at no cost to me. It will be my responsibility to seek additional health-related advice/follow-up examinations. The development of health problems may be a reason fo r me to be removed from the study.

Risks

The potential risks associated with the venipuncture and fingerstick are: 1) Venip uncture and fingerstick may cause some pain or bruising, 2) Sometimes, slight risk of infe ction or clotting in the vein may occur. In any experimental study, it is possible that I will have side effects, which have not been recognized before. If such side effects should bec ome severe, I may be removed from the study.

Benefits of Participation

Subjects will get valuable information about the health status of their heart (EK G monitoring), risk of developing cardiovascular disease (blood lipids and lipoproteins) a nd the safety of hard exercise thought the health, medical history, and the physical exam (blood pressure, heart rate during exercise and rest, body composition, $VO_{2\ max}$). In additi on to, they receive free counseling on strategies to reduce coronary heart disease risk by li festyle changes or the need for medical therapy.

Compensation and Injury

If research-related injury occurs, medical treatment for the injury will be my res ponsibility for any cost that may occur. It is clear to me, that no compensation will be av ailable to me.

Subjects Assurances

I understand that: 1) my participation in this study is voluntary; 2) I may withdra w from this study at any time without penalty or loss of benefits to which I am otherwise

entitled; 3) I may be removed from the study for medical reasons or non-compliance to st udy protocol; 4) My treatment by and relations with the physicians and organizations inv olved in this research study will not be affected now or in the future if I decide not to part icipate, or if I start the study and decide later to withdraw; and 5) I have not given up any of my legal rights or released any individual or institution from liability for negligence.

I understand that I may ask questions and request information about this research project at any time. By signing this consent I acknowledge that I have been afforded the necessary opportunities to pose any questions which I may have and that they have been answered to my satisfaction. The medical terms used have been explained to me and I un derstand them. Dr. Ransone and Dong-Ho Park will be available to answer questions. Dr . Ransone may be reached in his office by calling 405-744-9439 and Dong-Ho Park at 40 5-744-1587.

I understand that no guarantees are given with regard to my participation in this project. Specifically, I understand that there is a possibility of injury or adverse reactions, as set forth above. I agree that in the event of injury or an adverse reaction, that I hereby consent that any and all appropriate emergency medical care can be given to me in respo nse to my condition.

I understand that participation is voluntary, that there is no penalty for refusal to participate, and that I am free to withdraw my consent and participation in this project at any time without penalty after notifying the project director. I may contact Dr. Jack Rans one at 405-744-9439. I may also contact Sharon Backer, IRB Executive Secretary, 305 Whitehurst, Oklahoma State University, Stillwater, OK 74078; telephone (405)-744-5700 .

I have read this consent document and fully understand the consent form. I sign it freely and voluntary. A copy has been given to me.

Research Participant: ________________________________Date:________________

Witness: ____Date:________________

I certify that I have personally explained all elements of this form to the subject before requesting the subject to sign it.

Project Director:______________________________________Date:________________

APPENDIX C

EXERCISE SUMMARY SHEET

Name: ________________Soc.Sec. #:____________________Date: _________________
Age: _________________Date of Birth: _________________Sex:__________________

Address: ____

Home Phone: _________________________________Work Phone:________________

Personal Physician: ____________________________Phone: ____________________

Exercising Testing: (**LT** **70% of LT**) Date __/____/_____ Time:__________

Blood Collection:Time (**Before**): ________ (**IPE**): _________ (**24-h**): _____________

Height: _______________Weight: _______________Blood Pressure: _______/______

Fingerstick **TC**: ________ **% Fat**: ________ **BMI** (Body Mass Index): ___________

$VO_{2\ max}$: ___________ time elapsed (**LT**) : _____________(min)
(**70%** of LT): _____________(min)

Baseline

TG: __________TC:___________ FC:___________ CE:___________

HDL: _________HDL_2:_________ HDL_3:________ LDL:_________VLDL:________

Hct:___________ Lactate:

Immediate Post-Exercise (IPE)

TG: __________TC:___________ FC:___________ CE:___________

HDL: _________HDL_2:_________ HDL_3:________ LDL:_________VLDL:________

Hct:___________

24-hs Post-Exercise (24-h)

TG: __________TC:___________ FC:___________ CE:___________

HDL: _________HDL_2:_________ HDL_3:________ LDL:_________VLDL:________

Hct:___________

APPENDIX D

RUNNING TREADMILL RESULTS DURING $VO_{2\ MAX}$ TEST

NAME: AGE: GENDER: M F

DATE: / / MEDICATIONS:

TEST: max or submax MPHR:

HR: (supine)_____ (standing)_______ BP: (supine)_____/_____ (standing) ____/____

EXERCISE DATA: speed_____(mph) at LT

Stage	Speed (mph)	Minutes	Latate	RPE	HR	VO2	BP	METS
1	4.9	0-2:59					/	
2	5.2	3:00-5:59					/	
3	5.6	6:00-8:59					/	
4	6.0	9:00-11:59					/	
5	6.3	12:00-14:59					/	
6	6.7	15:00-17:59					/	
7	7.1	18:00-20:59					/	
8	7.5	21:00-23:59					/	
9	7.8	24:00-26:59					/	
10	8.2	27:00-29:59					/	
11	8.6	30:00-32:59					/	
12	9.0	33:00-35:59					/	
13	9.4	36:00-38:59					/	

OVER

RECOVERY DATA

3 Min. (HR) (BP) /

5 Min. (HR) (BP) /

8 Min. (HR) (BP) /

EKG COMMENTS DURING RECOVERY: WNL OTHER (Explain):

REASONS FOR STOPPING: General Fatigue; Anxiety; Dyspnea; Nausea; Dizziness; Angina; Claudication; Hypotension; Hypertension; Cyanosis; Ataxia; Confusion; Pallor; Cold, Clammy Skin; EKG changes; Poor Perfusion; Other(s) (Explain):

LAST STAGE/GRADE : TOTAL TIME: MHR:

MSBP: MDBP: AEROBIC FITNESS CATEGORY:

APPENDIX E

RUNNING TREADMILL RESULTS AT LT INTENSITY

NAME: ____________________ **AGE:** ________ **GENDER: M F**

DATE: ____________________ **MEDICATIONS:** ____________________

TEST: 70% of LT **MPHR:** ________ **HR:** ________

HR: (supine) ______ **(standing)** ______ **BP: (supine)** ____ / ____ **(standing)** ____ / ____

Speed_____(mph) and Lactate level ______mM/L

Stage	Minutes	Speed (mph)	Latate	RPE	HR	VO2	BP	METS
1	0-0:59						/	
2	1:00-1:59						/	
3	2:00-2:59						/	
4	3:00-3:59						/	
5	4:00-4:59		mM				/	
6	5:00-5:59						/	
7	6:00-6:59						/	
8	7:00-7:59						/	
9	8:00-8:59						/	
10	9:00-9:59		mM				/	
11	10:00-10:59						/	
12	11:00-11:59						/	
13	12:00-12:59						/	
14	1300-13:59						/	
15	14:00-14:59		mM				/	
16	15:00-15:59						/	
17	1600-16:59						/	
18	17:00-17:59						/	
19	18:00-18:59						/	
20	1900-19:59		mM				/	
21	20:00-20:59						/	
22	21:00-21:59						/	
23	22:00-22:59						/	
24	23:00-23:59						/	
25	24:00-24:59		mM				/	
26	2500-25:59						/	
27	26:00-26:59						/	
28	27:00-27:59						/	
29	28:00-28:59						/	
30	29:00-29:59		mM				/	
31	30:00-30:59						/	
32	31:00-31:59						/	
33	32:00-32:59						/	
34	33:00-33:59						/	

Stage	Minutes	Speed (mph)	Latate	RPE	HR	VO2	BP	METS
35	34:00-34:59		mM				/	
36	35:00-35:59						/	
37	36:00-36:59						/	
38	37:00-37:59						/	
39	38:00-38:59						/	
40	39:00-39:59		mM				/	
41	40:00-40:59						/	
42	41:00-41:59						/	
43	42:00-42:59						/	
44	43:00-43:59						/	
45	44:00-44:59		mM				/	
46	45:00-45:59						/	
47	46:00-46:59						/	
48	47:00-47:59						/	
49	48:00-48:59						/	
50	49:00-49:59		mM				/	
51	50:00-50:59						/	
52	51:00-51:59						/	
53	52:00-52:59						/	
54	53:00-53:59						/	
55	54:00-54:59		mM				/	

OVER

RECOVERY DATA

3 Min. (HR) ____________ (BP)____/______

5 Min. (HR) ____________ (BP)____/______

EKG COMMENTS DURING RECOVERY: WNL OTHER (Explain):

REASONS FOR STOPPING: General Fatigue; Anxiety; Dyspnea; Nausea; Dizziness; Angina; Claudication; Hypotension; Hypertension; Cyanosis; Ataxia; Confusion; Pallor; Cold, Clammy Skin; EKG changes; Poor Perfusion; Other(s) (Explain):

LAST STAGE/GRADE : __________ TOTAL TIME:_________ MHR: ______________

MSBP:_________ MDBP:________ AEROBIC FITNESS CATEGORY:____________

APPENDIX F

LIPID RESULTS FORM

Subject #:

Name: Screening TC:

LT **Plasma Volume Change:**

Time	TG	TC	FC	CE	HDL	HDL_2	HDL_3	LDL	VLDL	Hct
BE										
IPE										
24h										

70% of LT **Plasma Volume Change:**

Time	TG	TC	FC	CE	HDL	HDL_2	HDL_3	LDL	VLDL	Hct
BE										
IPE										
24h										

VITA

Dong-Ho Park

Candidate for the Degree of

Doctor of Education

Dissertation: THE EFFECT OF A SINGLE BOUT OF DIFFERENT EXERCISE INTENSITIES ON LIPID AND LIPOPROTEIN CHOLESTEROL SUBFRACTIONS

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