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The Effects of an Acute Bout of Continuous versus Accumulated Exercise of Isocaloric Energy Expenditure on Blood Lipids, Lipoproteins and Related Enzyme Activities

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THE EFFECTS OF AN ACUTE BOUT OF CONTINUOUS VERSUS ACCUMULATED EXERCISE OF ISOCALORIC ENERGY EXPENDITURE ON BLOOD LIPIDS, LIPOPROTEINS AND RELATED ENZYME ACTIVITIES

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ABSTRACT

Effects of acute bouts of continuous versus accumulated exercise of isocaloric energy expenditure on blood lipids, lipoproteins and related enzyme activities. **Purpose:** The purpose of this study was to determine if exercise, whether continuous (CE: completed all in one session) or intermittent (completed in either two (IE 2) or three (IE 3) exercise sessions) expending the same number of calories produced similar changes in the lipid/lipoprotein profile as well as transport enzymes. **Methods:** Sixteen healthy (22±2.1 year old) men (VO_2 max = 37.0±3.3 $mL \cdot kg \cdot min^{-1}$) randomly completed three exercise trials, CE, IE 2 and IE 3, expending 450 calories. Baseline data were collected in the evening and included anthropometric measurements, diet records and venous blood samples. The CE trial was done during one continuous time period and the intermittent trials were separated by 4-5 hrs all over the course of one day between the hours of 7 am and 9 pm. In addition to baseline blood samples were drawn immediately post exercise (IPE) and 24 and 48 hours following exercise. Each exercise trial sample was analyzed for total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) and subfractions ($HDL\text{-}C_2$, $HDL\text{-}C_3$). Samples were also analyzed to determine LDL-C particle size, lecithin cholesterol acyl transferase activity (LCATa) and cholesterol ester transfer protein activity (CETPa). **Results:** While no significant alterations in HDL-C and LDL-C were observed $HDL\text{-}C_2$ was shown to increase compared to baseline by 44% for CE 48 hours post exercise, 44% for IE 2 48 hours post exercise, 39% for IE 3 IPE and continued to rise for IE 3 48 hours post exercise by 66%. Furthermore, LCATa was significantly increased compared to baseline by 12% for CE 48 hours post exercise and 12% IE 3 48 hours post exercise. Furthermore, there was a 10% increase when comparing CE IPE to CE 48 hours post exercise, a 3% increase between IE 2 24 hours post exercise and IE 2 48 hours post exercise, a 2% increase between IE 3 IPE and IE 3 24 hours post exercise and an 11% increase when comparing IE 3 24 hours post exercise and IE 3 48 hours post exercise. No other significant differences were found. **Conclusions:** The results of this study indicate that whether the exercise is continuous or intermittent, keeping calorie expenditure the same, causes significant changes in the $HDL\text{-}C_2$ subfraction, which was augmented by an increase in LCATa.

CHAPTER 1

INTRODUCTION

Early research has shown that reducing low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) while increasing high density lipoprotein cholesterol (HDL-C) is associated with a reduced risk for cardiovascular disease (CVD; Kannel et al., 1971). Furthermore, the larger and less dense particles of each lipoprotein are also associated with a reduced CVD risk. Regular aerobic exercise has been shown to exert favorable influences over the lipid and lipoprotein profile. More specifically, the effects of acute exercise are consistent showing reductions in TG and increases in HDL-C reflected in the HDL-C_2 subfraction hence exercise is typically prescribed for those individuals with elevated TG or decrease HDL-C concentrations (Kannel et al., 1971).

Additional data have provided researchers with information that show a positive change in the lipid and lipoprotein profiles with an acute bout of exercise. In sedentary men elevations in HDL-C and decreases in TG concentrations have been reported after expending between 350 and 500 kcals by either cycling or treadmill walking (Alhassan et al., 2001; Crouse et al., 1997; Grandjean et al., 1996). These changes can persist for up to 48 hours post exercise and are mediated by increased lipoprotein lipase (LPL) activity. LPL activity acts to facilitate the hydrolysis and clearance of TG rich lipoproteins and will furthermore influence the transfer of the TG to the HDL-C for clearance. Hence, after exercise as LPL activity increases it will move greater concentrations of TG into the HDL-C, making a denser HDL-C_2 particle which allows for greater clearance and lower total TG concentrations in the plasma. This process can also be influenced by decreasing the activity of hepatic lipase (HL) and cholesterol ester transfer protein (CETP), but changes in these enzymes are reported less frequently after acute exercise then those of LPL.

Recent research on the particle size and density of LDL-C suggests that long, intense exercise as well as chronic exercise training can favorable alter particle size, influencing them to become larger and less dense (Frey et al., 1993; Houmard et al., 1994). Changes in particle size have not been well documented with acute exercise and this study intends to investigate the implications of a 450 kcal bout of exercise on the acute changes in LDL-C particle size and density.

Addressing the issue of intermittent bouts of exercise on fitness is not new. It has been established that intermittent bouts of exercise over the course of several months can show positive changes in cardiorespiratory fitness, as reflected by maximal oxygen uptake (VO_2 max), but few studies have examined its effects on the lipid/lipoprotein profile and the enzymes related to lipid transport. A study by Ebisu (1985) used untrained, college-aged men and was designed to evaluate aerobic fitness and blood lipids. There were three exercise groups; each group ran the same distance differing only in the number of sessions per day, one, two or three sessions. The authors failed to report exact times taken to complete each session, but the exercise was considered complete when each subject attained their given distances. Results showed that all the exercise groups significantly improved aerobic fitness and that there were no significant differences in VO_2 max between the groups. However, a side note of this study was that HDL-C levels only improved in the subjects who exercised three times per day.

Despite surmountable evidence stating that physical activity has a variety of positive health benefits on the lipid profile as well as enhancing glucose tolerance and insulin sensitivity, Americans are sedentary and reports show that 40% of individuals are inactive contributing to 250,000 deaths per year (Hahn et al., 1986; McGinnis et al., 1993). A striking reality is that most Americans simply state lack of time as the number one reason why they do not participate in regular activity (Martin et al., 1982). Researchers have diligently tried to conjure up exercise programs designed to fit a busy lifestyle and also provide sufficient stimulus to elicit health benefits. Given this dilemma a group of experts from the Centers for Disease Control (CDC) and the American College of Sports Medicine (ACSM) released a recommendation for adults on physical activity, which states that every US adult should accumulate 30 minutes or more of moderate-intensity physical activity (approximately 50-70% VO_2 max) on most, preferably all, days of the week (Pate et al., 1995).

This recommendation was designed to emphasize the benefits of moderate-intensity exercise and that this exercise can be accumulated over short bouts through out the day rather than one long continuous bout. The recommendation reflects that it is not necessarily the intensity, mode or duration of the activity bouts, but rather the total amount of activity performed that is linked to a decrease in CVD mortality. Research to support these claims comes from Leon et al. (1987), who showed a significantly lower death rate as a result of CVD in individuals who performed 47 minutes of activity versus 15 minutes of activity per day. Furthermore, Paffenburger et al. (1986) demonstrated that men who expended an estimated 2000 kcal/week also had a lower death rate compared to those who expended 500 or less kcal/week.

The most important aspect of this recommendation is that unlike the previous statements, the CDC/ACSM acknowledged the use of short intermittent bouts of exercise as being as effective as one continuous bout. A pair of studies were referenced in the recommendations that made a credible argument for adherence to intermittent exercise (DeBusk et al., 1990; Ebisu, 1985).

The study by DeBusk et al. (1990) examined the effects of three 10-minute bouts of moderate to vigorous intensity exercise compared to a single 30-minute bout for 8 weeks and they found that the intermittent bouts showed a higher retention rate than the single 30-minute bout. Jacobsen et al. 2003 supported these results when evaluating adherence to exercise during a 72-week program. They found that overall retention was higher amongst the intermittent exercise groups as compared to the single continuous bout group. Together these studies suggest that using intermittent exercise may be an effective tool in improving adherence in addition to altering the lipid/lipoprotein profile.

Statement of the Problem

To date there are few studies that have examined the acute effects of intermittent exercise bouts on the lipid/lipoprotein profile. Furthermore, none have examined the full lipid/lipoprotein profile including lipid transport enzymes. There are important health implications in validating multiple daily sessions of accumulated exercise. Multiple bouts of exercise may encourage more people to exercise and it has been shown that adherence may be better in this type of program. Therefore, the purpose of this study was to ascertain the effects of continuous versus accumulated, intermittent acute exercise, of similar calorie expenditure on lipids and lipoproteins as well as enzymes related to lipid transport.

Research Hypotheses

Hypothesis 1: The total caloric expenditure of 450 kcal will be sufficient enough that subjects will show favorable changes to the lipid profile for both the continuous and intermittent exercise groups. These changes will include increasing HDL-C and lowering TG concentrations.

Hypothesis 2: After an acute intermittent and continuous exercise bout there will be an increase in the total HDL-C cholesterol that will be reflected in an increased HDL-C_2 subfraction. Furthermore, the LDL-C particle density will decrease allowing for a larger more buoyant particle that is not implicated in heart disease. These changes will occur in both groups due to the fact that total calorie expenditure will be kept constant.

Hypothesis 3: The effects of the two exercise protocols will produce changes in plasma lecithin cholesterol acyl-transferase (LCAT) activity with no changes in cholesterol ester transfer protein (CETP) activity. Finally, these changes should occur in both groups, again due to the equivalent calorie expenditure.

Hypothesis 4: The changes to the lipoproteins, specifically reduced TG and increased HDL-C, will exists for up to 48-hours post exercise regardless of the patterning of exercise sessions.

Operational Definitions

The following terms have been identified to aid in the comprehension of the following study.

Lipid – Particles that are insoluble in water and include free and esterified cholesterol, triglycerides, and free fatty acids.

Lipoprotein – A lipid protein complex that is responsible for transporting lipids in the plasma, making them water soluble.

Apolipoprotein – This is the protein portion of a lipoprotein.

Continuous exercise bout – This can be defined as one uninterrupted period of daily exercise and for this study will expend a total of 450 kilocalories.

Intermittent exercise bout – This can be defined as either two 225 kilocalorie or three 150 kilocalorie interrupted exercise sessions, over the course of one 10 hour day, separated by a minimum of four hours and no more than five hours, used to expend a total of 450 kilocalories.

Maximal Oxygen Consumption (VO_2 max) – The maximal amount of oxygen taken up in one minute during a maximal exercise test.

Lipoprotein Lipase (LPL) – The enzyme that catalyzes the reaction responsible for hydrolyzing lipids from lipoprotein. It is found in the endothelial walls of the adipose and muscle capillaries.

Hepatic Lipase (HL) – A liver enzyme that catalyzes the reaction that will convert HDL-C_2 into HDL-C_3.

Lecithin Cholesterol acyl-transferase (LCAT) – This enzyme is responsible for catalyzing the reaction forming cholesterol esters and then transfers these esters into the HDL-C particle core.

Cholesterol Ester Transfer Protein (CETP) – This enzyme is thought to be responsible for mediating the exchange of TG and cholesterol esters between HDL-C, VLDL-C, LDL-C and chylomicron remnants.

Hematocrit – Percentage of blood that is the red blood cells.

Plasma volume shifts – The relative increase in red blood cell percentage of total blood volume due to the increase in the liquid percentage of the blood.

Assumptions

The following assumptions were made in this study:

1. All subjects were equally motivated and performed to the best of their abilities on all tests.
2. All subjects followed pre-test rules as they related to fasting, alcohol consumption, exercise, and medication.
3. All individual observations were independent of each other.
4. Analysis of variance is robust to variables that are not normally distributed and have unequal variances.

Delimitations

The following limitations are applicable to this study:

1. The results can only be applied to those individuals who are sedentary, defined as VO_2 max ≤ 40 ml·kg·min^{-1}
2. The subjects are limited to the Florida State University and surrounding Tallahassee area.
3. Subject age was limited to 18-35 years old

Significance of the study

The significance of this study has broad implications in that it will provide important information on the reactivity of blood lipids and lipoproteins, and particle size to an acute bout of continuous and intermittent exercise. Furthermore, the results on the lipid and lipoprotein transport enzymes should help clarify mechanisms responsible for mediating cholesterol balance as a result of an exercise intervention. This study will generate scientific knowledge that can be used by practitioners prescribing exercise for CVD risk reductions among the population.

Another benefit of the proposed study is that it will provide the necessary framework to propose long-term studies and evaluate the chronic effects of a large volume (450 kcals) of exercise on blood lipid and lipoprotein markers. This acute bout of exercise was designed to evaluate the changes that occur not only with 450 kcals of energy expenditure but also determine if continuous and intermittent exercise stimuli produce similar results.

CHAPTER 2

REVIEW OF LITERATURE

Atherogenesis

The process of atherogenesis can be described as the gradual closing of the arterial lumen due to deposition of lipids, calcification and the proliferation of smooth cells. Fatty streaks appear within the intima of the artery, at a young age and gradually worsen with the aging process. These fatty streaks are a result of an oxidized LDL-C, which is produced because of free radical formation due to the lipid peroxidation process. This oxidized LDL-C particle traverses the endothelial lining and becomes embedded producing macrophages and foam cells.

After the LDL-C becomes lodged in the endothelial lining the development of the fibrous plaque is initiated. This plaque is formed by the enlarging fatty streaks that push against the endothelial cells, causing them to rupture, which exposes the smooth muscle to the blood stream. This exposure causes platelets to aggregate at the injured tissue site and a paracrine factor known as platelet derived growth factor is released, which will cause smooth muscle cell proliferation. In addition, excess amounts of fibrous tissue will develop around the macrophages. Furthermore, the amount of lipid deposited, smooth muscle cell proliferation and connective tissue formation are the three critical components determining how narrow the arterial lumen will become.

The final stage of the atherosclerotic process is the development of the lesion. This lesion can lead to serious complications as the plaque may cause deformation of the artery and ultimately leading to rupture and bleeding. This bleeding may calcify causing the fibrous plaque to crack and a thrombus may develop. Ultimately, the atherosclerotic plaque formation will result in a diminished blood flow through the artery and further reduce oxygen delivery.

Cardiovascular Disease, Lipoproteins and Health

Cardiovascular disease is the leading cause of mortality and morbidity in the United States, claiming approximately one million (440,175 males and 505,661 females) lives per year and 6.3 million worldwide (US Department of Health and Human Services, 2001). Cardiovascular disease can be attributed to both modifiable and non-modifiable risk factors. The non-modifiable factors are those that cannot be changed and include genetic predisposition to the disease, age, and gender. Modifiable risk factors are ones that can be altered by lifestyle intervention, and include obesity, hypertension, hypercholesterolemia, diabetes, lack of physical activity, psychological stress and cigarette smoking.

Since the inception of the National Heart Institute and the American Heart Association in 1948 there have been several large cohort studies, which have examined the effects of the modifiable risk factors on CVD. The Framingham, Oslo, and Bogalusa Heart Studies as well as the Multiple Risk Factor Intervention Trials (MRFIT) were all designed to examine the etiology of CVD and furthermore how to reduce its prevalence (Holme et al., 1982; Kannel et al., 1964; MRFIT Research Group, 1986; Srinivasan et al., 1982). The Framingham Heart Study was among the first to reveal that the incidence of atherosclerosis is proportional to higher plasma cholesterol levels, and in fact, was among the strongest predictors of mortality (Kannel et al., 1964). The MRFIT studies showed an independent contribution to increased mortality for cholesterol and a substantial

escalation in risk when elevated cholesterol was combined with other risk factors (MRFIT Research Group). These results are common to other epidemiological studies with diverse populations (Lipid Research Clinics, Oslo and Bogalusa Heart Studies).

Eventually, investigations broadened to include the examination of the various classes of lipoproteins and their contribution to CVD (Kostner, 1983). Results of the Framingham heart studies reveal that the incidence of atherosclerosis and CVD is proportional to higher plasma cholesterol levels, more specifically the level of LDL-C in plasma (Kannel, et al., 1971). Generally speaking incidence of heart disease rises dramatically when total cholesterol levels approach 280 mg/dL and LDL-C concentrations are above 200 mg/dL (Kannel et al., 1971). When concentrations reach these levels the thickening of the arterial intima accelerates resulting in a blockage that can inhibit coronary blood flow. The development of these lesions can be directly attributed to the accumulation of LDL-C cholesterol on the artery wall. This accumulation is due to LDL receptor saturation and suppression. Basically, the receptors become saturated and the rate of LDL-C removal is significantly reduced because without receptors to remove the LDL-C they must remain in circulation or rely on alternate pathways of degradation that operate in a diminished capacity (Brown et al., 1986; Kovanen et al., 1981).

The increase in LDL-C in circulation increases the propensity for the particle to become oxidized and further display a cytotoxic effect on the artery primarily by releasing paracrine factors that attract platelets to the site of damage. The aggregation of the platelets results in the characteristic foam cell that will eventually become the atherosclerotic plaque. Brown et al., (1986) also found an association between very low density lipoprotein (VLDL-C) and CVD mortality, but that it was not as strong as the relationship with LDL-C.

Kostner (1983) reported that HDL-C demonstrated an inverse relationship with CVD. This information provided additional evidence and helped to solidify the connection between HDL-C and CVD that was originally proposed by the Framingham, Tromoso and Honolulu Heart Studies of the 1970's (Castelli, 1977; Kannel et al., 1979; Miller et al., 1977). The cardioprotective nature of HDL-C is due to its association with the process known as reverse cholesterol transport, which will be discussed in detail later. The general idea is that the HDL-C particles are responsible for taking cholesterol esters to the liver for catabolism and excretion via the bile. This process rids the body of excess cholesterol that could potentially be reincorporated into the LDL-C; remain in circulation and cause atherosclerosis. This inhibition or delay of the atherosclerotic process as well as the enhancement of HDL-C in circulation has been a focus of a plethora of research aimed at "figuring out" how to alter the lipid profile to be more cardioprotective and hence reduce the occurrence of CVD. These ideas have focused on exercise, diet alone or a combination of the two interventions.

The Lipid Profile

Lipid and lipoprotein metabolism is a complex process that involves several enzymes, co-factors and transportation pathways. They are designed to allow for the exchange of cholesterol and TG between particles for maintenance of cholesterol homeostasis as well as normal functioning of several cholesterol dependent activities, such as hormone formation and function. These particles are named for their density and their respective composition of varying amounts of cholesterol, TG, phospholipid and

apolipoprotein (apo). These lipoproteins are a necessary part of a complex transport system designed to move exogenous and endogenous lipids between the liver, peripheral tissues and intestine. The four major types of lipoproteins are: chylomicrons, VLDL-C or pre-β-lipoprotein, LDL-C or β-lipoprotein, and HDL-C or α-lipoprotein.

Normal lipid metabolism at rest requires free fatty acids (FFA) to generate adenosine triphosphate (ATP) to support basal functioning. The primary source for these FFA is TG stores within the adipose tissue and the muscle. These FFA are liberated by LPL, which produces three FFA and glycerol. The glycerol is transported thru the blood to the liver and metabolized whereas the process for obtaining energy from the FFA is more complicated. The FFA must be transported into the mitochondria, which is a complex process that involves specialized transporter enzymes known as carnitine-acyl transferase I and II (CAT-I and CAT-II). These enzymes are used to bring the FFA from the outer membrane to the inner membrane where they are then subjected to oxidative phosphorylation to produce ATP.

The normal process of lipid metabolism is disrupted during exercise, specifically it is accelerated in order to provide enough ATP to sustain physical activity of low to moderate intensity (25-75% VO_2max) and increased duration (>30 minutes of activity). The acceleration is aided by the release of catecholamines stimulating β-adrenergic receptors, specifically β_2-receptors. These receptors are typically found on adipocytes and in the muscle, both containing increased amount of TG, which is convenient for energy liberation and production. During low intensity (25-55% VO_2max) exercise the main source of FFA comes from the adipocytes and thus as intensity of exercise increases (60-80% VO_2max) intramuscular TG (IMTG) becomes the primary source of FFA (Romijn et al., 1993).

Chylomicrons are the largest lipoprotein and the least dense; they are responsible for transporting the fat, as TG, derived from intestinal absorption to the peripheral tissues for storage. The chylomicron is associated with the apolipoprotein B-48, which will govern its metabolism. As chylomicrons remain in circulation and are acted upon by LPL, present in the peripheral tissue, via apolipoprotein C-II. This causes the release of the FFA and a hydrolization of the TG making a chylomicron remnant. The chylomicron remnants are scavenged by the HDL-C, which recognize the apolipoprotiens C and E that are associated with the particle.

A slightly denser particle known as VLDL-C is also involved in moving TG to the peripheral tissue, but more importantly is heavily involved with LPL in circulation. These reactions are aimed at producing LDL-C (associated with apolipoprotein B-100) via continuous TG hydrolysis, which now is the primary way cholesterol is transported to the peripheral tissues. This has already been discussed because this is the initiation of the atherosclerotic process.

Finally, the smallest and most dense particle known as HDL-C (associated with apolipoprotein A-I and A-II) has a cardioprotective role in the maintenance of cholesterol balance. Studies have supported a role for HDL-C particles in reverse cholesterol transport, an important modulating mechanism to transport cholesterol from the peripheral tissue back to the liver for breakdown (Durstine et al., 2000, Grandjean et al., 1998).

These individual particles can further be divided within each class based on density. For example, there are four distinct LDL-C species that exist in circulation.

They typically have different diameters which will govern how dense they are as measured by units of nanomoles of particle per liter. They are classified as IDL-C or LDL-C_4 (23-27 nm), LDL-C_3 (21.3-23 nm), LDL-C_2 (19.8-21.2 nm) LDL-C_1 (18.3-19.7nm). LDL-C_{1-4} can be generalized by the nomenclature of Pattern A particles (>20.5 nm) or Pattern B particles (<20.5 nm). Similarly the HDL-C particles can also be divided into HDL-C_2 and HDL-C_3 also by density as units of mg/dL cholesterol. The HDL-C_2 is the larger particle ranging from 8.8 to 13 nm whereas the HDL-C_3 particles are smaller ranging from 7.3 to 8.7 nm. Both HDL-C particles seem to play important roles in CVD. Research has found that the smaller, more densely packed LDL-C particles are more likely to cross the endothelial barrier and become lodged in the arterial wall (Ranallo et al., 1998). An important side note is that although having larger LDL-C particle size is more favorable than smaller LDL-C particles, high concentrations of LDL-C regardless of size is unfavorable to the lipid/lipoprotein profile. A high concentration of HDL-C_2 particles may have a protective effect on CVD whereas a larger circulating concentration of HDL-C_3 may suggest an inefficiency of cholesterol collection and catabolism.

Reverse Cholesterol Transport

Reverse cholesterol transport is a process that involves several steps intended to move plasma cholesterol from the peripheral tissues back to the liver for catabolism. The process of reverse cholesterol transport is mediated by HDL-C acting in association with lecithin cholesterol acyl transferase (LCAT) through apo A-I. Research has supported a role for both HDL-C particles playing a role in cholesterol removal, but the pre-beta HDL-C particle (HDL-C_2) has a special ability to remove cholesterol more efficiently. To complete the picture other key enzymes contribute to cholesterol flux during reverse transport, these include HL, CETP, and LPL.

The activity of each of these enzymes is extremely important in maintenance of internal cholesterol balance. Lipoprotein lipase is utilized to breakdown the chylomicrons and VLDL-C particles, with the intent of removing TG to produce an HDL-C particle. This reaction will esterify free cholesterol and shift it to the core of the HDL-C_3 particle. With the continual influx of cholesterol ester the HDL-C_3 particle now becomes a less dense HDL-C_2 particle. This influx is due to CETP, which takes cholesterol ester from the HDL-C particle gives it to the chylomicron and VLDL-C in exchange for TG, which are being given to the HDL-C_2. Once the HDL-C_2 particle reaches a sufficient size it will accumulate multiple copies of apo E. This apo E is then recognized by the scavenger receptor B-1 on the liver and via HL the HDL-C particle is degraded and the cholesterol is catabolized and excreted in the bile.

Cross-Sectional Data on Lipid Profiles (Total cholesterol and LDL-C)

Limited evidence exists supporting exercise induced changes in total cholesterol (TC) and LDL-C, in addition there is little evidence for differences in trained versus sedentary individuals (Hartung et al., 1980; Kokkinos et al., 1995; Kokkinos et al., 1995; Lakka et al., 1992; Martin et al., 1977; Wood et al., 1977). These particular studies have demonstrated that exercise may perhaps have an affect on the lipid profile but upon further analysis these observational studies have failed to control for several key extraneous variables. These include things like differences in body weight, body fat, calorie intake and lifestyle habits such as alcohol consumption and smoking. All of these factors are known to affect the lipid profile and when they are included in statistical models, there is no difference between active and sedentary individuals (Lakka et al.,

1992; Leclerc et al., 1985; Williams et al., 1998). Epidemiological data also fail to consistently find evidence to support the notion that exercise alters TC and LDL-C levels (Gordon et al., 1983; Gordon et al., 1984).

High Density Lipoproteins and Triglycerides

Present literature suggests that the HDL-C particles and plasma TG concentrations can be altered with exercise and can be a target for intervention when an individual's goal is to lower cholesterol and reduce the risk of CVD (Durstine et al., 1994; Durstine et al., 2000). These studies furthermore support a role for lower TG and higher HDL-C levels in trained individuals compared to their sedentary counterparts. Significant TG differences between groups have been demonstrated in several studies and these differences range from 18 to 77 mg/dL (Kokkinos et al., 1995; Wood et al., 1977; Martin et al., 1977; Hartung et al., 1980; Lakka et al., 1992; Kokkinos et al., 1995; Williams et al., 1998; Lehtonen et al., 1978; Hagan et al., 1983; Williams et al., 1986; Thompson et al., 1991; Blessing et al., 1996; Giada et al., 1991; Reaven et al., 1990; Williams et al., 1997). In addition, HDL-C levels are significantly higher in trained individuals, 4 to 24 mg/dL, compared to sedentary individuals (Wood et al., 1977; Martin et al., 1977; Hartung et al., 1980; Enger et al., 1998; Lehtonen et al., 1978; Lehtonen et al., 1978; Aedner et al., 1980; Rotkis et al., 1982; Hagan et al., 1983; Thompson et al., 1983; Herbert et al., 1984; Stevenson et al., 1997; Giada et al., 1991; Reaven et al., 1990; Williams, 1993, 1996, 1997). These changes are very important due to the fact that increases in HDL-C will facilitate an increase in reverse cholesterol transport to rid the body of excess cholesterol. Furthermore, decreasing TG in circulation will reduce the incidence of incorporation into other lipoproteins as well as being stored as adipose tissue increasing body fat percentage and body weight.

Cross-sectional data have also provided information regarding an approximate threshold of exercise to induce these changes in HDL-C and TG. Research has shown that those previously sedentary individuals exposed to an exercise intervention will experience increases ranging from 3.5 to 6 mg/dL in HDL-C by having an energy expenditure equivalent to 1500 to 2200 kcal/week (Williams, 1996, 1997, 1998; Kokkinos et al., 1999). In addition there appear to be results that support that there are additional increases in HDL-C, 1.5 to 3 mg/dL, with every increase in energy expenditure. This energy expenditure needs to be equivalent to 1100 kcal/week or approximately 10 miles in volume, in addition to the 1500 to 2200 kcal already suggested, to attain these values. Currently there is no literature to specify whether or not changes occur beyond a certain threshold of energy expenditure, which is another area that needs to researched. Similarly TG concentrations will decrease, by 7 to 20 mg/dL, with expenditures of 1500 to 2000 kcal/week and again with an additional 1100 kcal/week TG can be further decreased by 3 to 8 mg/dL (Williams, 1996, 1997, 1998, Kokkinos et al., 1999).

Despite these positive changes that occur with the lipid profile, one must be cautious about the findings from cross-sectional data. This is primarily due to the fact that this type of research is observational and may tend to over exaggerate the results and relationships between the lipids and lipoproteins. To fully appreciate the changes that might occur to the lipid profile with exercise it is important to also consider the longitudinal data, which will specifically examine the results of the lipid profile after a specific intervention.

Longitudinal Studies (Total cholesterol and Low density lipoproteins)

The longitudinal study literature provides support for the cross-sectional data in that there seems to be no changes in TC and LDL-C despite various types of training interventions. There are some studies that show at best a modest change in TC and LDL-C ranging from 4 to 7% in both men and women (Kiens et al., 1980; Raz et al., 1988; Binder et al., 1996; Boyden et al., 1993; Ready et al., 1995). Furthermore, the changes that are reported are typically in subjects who were previously sedentary and who are trained to expend 1200 or more kcal/week (Altekruse et al., 1973; Ponjee et al., 1995; Nye et al., 1981; Despres et al., 1991; Kiens et al., 1980; Ready et al., 1995; Shepard et al., 1979; Baker et al., 1986; Despres et al., 1988, 1990; Peltonen et al., 1981). Finally, there seems to be no association between body weight or fat changes, and exercise intensity with the changes seen in TC or LDL-C (Hill et al., 1989; Lopez et al., 1974; Nye et al., 1981; Kiens et al., 1980; Wood et al., 1988; Baker et al., 1986; Peltonen et al., 1981; Rotkis et al., 1984; Moll et al., 1979; Bassett-Frey et al., 1996; Franklin et al., 1979; Houmard et al., 1994; Lewis et al., 1979; Leon et al., 1979; Milesis et al., 1976; Whitehurst et al., 1991; Schwartz et al., 1987; Van der Eems et al., 1985; Wirth et al., 1985; Wood et al., 1983). Furthermore, the data support that changes in TC and LDL-C with an exercise intervention are usually due to diet interventions that are also placed on the subjects.

High density Lipoproteins and Triglycerides

In contrast to the results found with TC and LDL-C, the majority of the longitudinal research does support a role for exercise in increasing HDL-C and lowering plasma TG. Only a few studies do not support these findings (Leon et al., 1979; Gaesser et al., 1984; Barr et al., 1991). The reason for the difference in results can be due to the fact that there are differences in training regimens, baseline subject characteristics and changes in body weight and fat that are not always taken into consideration Despite some disparities the changes in HDL-C and TG are typically seen with both genders, however it is important to note that changes in TG are more commonly reported in men than women (Gaesser et al., 1984; Barr et al., 1991).

There are certain factors that are going to be important when analyzing HDL-C and TG data; this includes initial HDL-C status, body fat distribution and body weight. Research has suggested that those individuals with normal or high HDL-C levels (>38 mg/dL) show more favorable changes in HDL-C than those with levels lower than 37 mg/dL (Houmard et al., 1994; Williams et al., 1994; Zmuda et al., 1998; Nicklas et al., 1997), although this may not always be the case a suggested by a meta-analysis (Tran et al., 1983). Therefore, it seems prudent to evaluate an individual's HDL-C level prior to testing in order to establish a baseline value.

The notion that baseline values (for HDL-C) must be considered prior to intervention is not true for TG, the literature does not support that baseline TG levels will influence how they will respond to exercise. In addition, there are several studies that support that changes in HDL-C and TG among men can only occur when body weight and body fat are reduced (Raz et al., 1988; Wood et al., 1988; Baker et al., 1986; Despres 1988, 1990; Houmard et al., 1994; Leon et al., 1979; Schwartz, 1987; Wood et al., 1983, Schwartz et al., 1992; Huttunen et al., 1989; Wynne et al., 1980). However other studies have shown that changes in HDL-C and TG can occur independent of body weight reduction (Tomiysau et al., 1996; Kiens et al., 1980; Stein et al., 1990; Zmuda et al.,

1998; Aellen et al., 1993; Dressendorfer, 1982; Higuchi et al., 1984; Pollack et al., 1969; Sutherland et al., 1983; Thompson et al., 1988, 1997; Weintraub et al., 1989). Again, this is similar for women. Most of the studies support a role for increasing HDL-C with exercise despite body weight changes (Goodyear et al., 1986; Rotkis et al., 1984; Van der Eems et al., 1985; Blumenthal et al., 1988, 1991; Duncan et al., 1991).

It seems that regular exercise can increase HDL-C by 2 to 8 mg/dL and reduce TG by 5 to 38 mg/dL in both men and women. Research supports that the training threshold seems to be a minimum of 1200 kcal/week energy expenditure to elicit these changes. Several studies have found that energy expenditures less than 1000 kcal/week is not enough to alter HDL-C in both men (Boyden et al., 1993; Brownwell et al., 1982; Bassett-Frey et al., 1996; Franklin et al., 1979; Manning et al., 1991; Suter et al., 1992; Szmedra et al., 1998; Williford et al., 1988; Woods et al., 1986) and women (Binder et al., 1996; Whitehurst et al., 1991; Van der Eems et al., 1985; Hardman et al., 1989; Blumenthal et al., 1991). Triglycerides are altered with a similar caloric expenditure threshold as HDL-C. Research has shown that a weekly energy expenditure of greater than or equal to 1200 kcal will produce changes in TG regardless of baseline levels (Tomiysau et al., 1996; Baker et al., 1996; Despres et al., 1988; Schwartz et al., 1992; Dressendorfer et al., 1982; Thompson et al., 1988; Thompson et al., 1997; Weintraub et al., 1989; Despres et al., 1990). Additionally, TG concentrations have been also shown to decrease with expenditures of only 1000-1200 kcal/week (Lopez et al., 1974; Kiens et al., 1980; Raz et al., 1988; Wood et al., 1988; Houmard et al., 1994; Huttunen et al., 1979; Wynne et al., 1980; Pollack et al., 1969; Lapman et al., 1985) but this is not consistent and therefore, it is recommended that there be a minimum of at least 1200 kcal/week to produce favorable changes.

Research using Total Caloric Expenditure as an Intervention

The recent direction of the literature has been to have a consistent independent variable to manipulate. The independent variable that most studies have used is calorie expenditure. Several studies have utilized this methodology and have observed beneficial changes in lipid and lipoprotein profiles (Aellen et al., 1993; Crouse et al., 1995, 1997, 1997a; Davis et al., 1992; Grandjean et al., 1998; Kim et al., 2001; Leon et al., 2002; Marrugat et al., 1996; Pronk et al., 1995; Woolf-May et al., 1999; Williams, 1998). These projects have all focused on extended populations including men, women, trained, untrained, normocholesterolemic, hypercholesterolemic as well as examining menstrual status. Most of the studies exercised their population at various intensities ranging from ~50% to 85% VO_2max. The goal of each of these studies was to keep calorie expenditure equivalent regardless of the intensity used within a certain study.

The earliest of these studies by Davis et al. (1992) examined trained runners (mean VO_2max 62±4 ml/kg/min^{-1}) who were exercised for either 60 minutes at 75% of VO_2max or 90 minutes at 50% VO_2max so that approximately 950 kcal were being expended. Their results were somewhat disappointing since no differences were seen in any of their blood lipid parameters including HDL-C and HDL-C_2. The authors speculated that the lack of significance could have been due to plasma volume changes, duration or intensity of the exercise. Furthermore, they suggested that these subjects had very high values to begin the study and perhaps their stimulus was not enough to produce significant changes in their measured values.

Subsequent studies conducted by Crouse et al., (1995, 1997, 1997a) examined two groups of approximately nineteen middle-aged (47 yrs), unfit (VO_2max 31.1±1 mL/kg/min^{-1}) hypercholesterolemic (TC = 258 mg/dL) men who exercised at either 50 or 80% VO_2max at expended 350 kcal per session. The purpose of these experiments was to quantify changes in lipid and lipoprotein profiles, differentiate between acute and long-term effects and analyze apolipoprotein concentrations. The findings of these studies suggest that expending 350 kcal was sufficient to reduce TG levels 24 and 48 hours post exercise compared to baseline (Baseline= 195±17, 24 hrs= 159±15*, 48 hrs= 164±18* mg/dL; *$p<0.05$, significantly different from baseline). In addition increases in HDL-C (Baseline= 44±2, 24 hrs.= 48±3*, 48 hrs.= 49±2* mg/dL; *$p<0.05$, significantly different from baseline) and HDL-C_2 (Baseline= 6.2±.9, 24 hrs.= 6.4±1.1*, 48 hrs.= 7.2±1.1* mg/dL; *$p<0.05$, significantly different from baseline) were seen and this will tend to cause an increase the HDL-C to TC ratio, all of which are known to reduce the risk of CVD.

Chronic and acute apolipoprotein responses seem to vary and are dependent upon the changes seen in the lipoproteins they are associated with, for example if HDL-C increases there is typically an increase in apolipoprotein A-1. In addition, it has been shown that 24 weeks of training is sufficient to reduce TC by -5.5% in hypercholesterolemic men whereas normocholesterolemic men do not react the same way (Davis et al., 1992; Gordon et al., 1994). This may be due to the fact that hypercholesterolemic individuals tend to be overweight and have bad eating habits and therefore regular exercise may promote weight loss and better eating, both factors contributing to lowering TC. Another important finding within this group of data were that there seemed to be transient changes seen with the acute bouts of exercise which led the experimenters to suggest that expending 350 kcal every other day is adequate due to the elevated HDL-C levels 48 hours after one exercise session.

Kokkinos et al., (1995) furthermore suggested that there is a dose response relationship between miles run per week and HDL-C levels. They found that there was a 0.008 mmol$\cdot$L^{-1} increase in HDL-C with each mile run per week, an average of 1670 kcal/week, above sedentary individuals' levels. This was supported by the US National Runners' Health Study (1997) that found that the most important determinant of HDL-C change was with the number of miles run per week. More recent research including the HERITAGE study (Leon et al., 2002) examined 675 sedentary, healthy, white and black men and women aged 17 to 65 years old. The subjects were followed for 20 weeks and asked to perform supervised cycle ergometry at the same relative intensity, starting at 55% VO_2max and progressing to 75% VO_2max a weekly volume of approximately 328 kcal/session or about 984 kcal/week. Results of this study showed that there were significant increases in HDL-C (1.4 mg/dL or +3.6%), HDL-C_2 and decreases in HDL-C_3 ($p<0.001$). The research (Kokkinos et al., 1995; Crouse et al., 1995, 1997, 1997a; Leon 2002) suggests that the minimum amount of calories an individual must expend in one exercise session is approximately 350 kcal.

Acute Exercise, Lipoproteins and Transport Enzymes

The literature examining acute exercise sessions has flourished due to the curiosity surrounding the question asking "how much exercise is necessary?" The primary rationale behind studying a single exercise session is so that exercise prescriptions may be tailored to an individual's goals for altering the lipid and lipoprotein

profile. Results of acute studies on lipid and lipoprotein changes and how long these changes persist can allow clinicians to recommend exercise programs that can best fit their client's schedule. The acute lipid/lipoprotein response can be summarized by stating that TG decreases by an average of 14-50% and HDL-C increases on average by 4-18%. No other lipid/lipoprotein variables change with acute exercise, with the exception that TC may change, but only if the exercise is of a prolonged period of time (3 hours or greater). Furthermore, the transport enzyme literature suggests that there are changes in LPLa, but that this can take up to 4 hours post-exercise to fully activate, and LCATa increases.

Early studies (Durstine et al., 1983; Lennon et al., 1983; Kantor, 1984, 1987) all show that acute exercise sessions increase HDL-C. Durstine et al (1983) examined ten physically active male subjects (VO_2max 60.39±6.53 mL/kg/min^{-1}) during low intensity exercise (45% VO_2 max) until exhaustion on plasma lipids. Furthermore, the researchers described the time segments with their changes in the profile. Results showed that subjects exercised on average 268±11 minutes and that HDL-C increased within 2 hours of the exercise bout compared to baseline, 47.4±1.8 to 50.1±1.7 mg/dL. HDL-C values continued to increase as exercise continued into the 3rd and 4th hour as well as post exercise (51.6±2.1, 52.5±2.3, 52.7±2.4 mg/dL). Values from 2 hours up until recovery were all significantly different than pre-exercise levels ($p<0.05$). This increase in HDL-C in turn increased the HDL-C/LDL-C ratio producing a more favorable lipid profile. These were significant findings and the authors suggested that one low intensity prolonged exercise session can increase HDL-C.

A study by Lennon (1983) supports Durstine's findings (1983). Lennon used 28 subjects (14 male, 14 female) who were classified as well trained (M-52.6±3.81; F-47.9±6.92 ml/kg/min^{-1}) or moderately trained (M-42.6±4.67; F-38.1±2.2 mL/kg/min^{-1}). These subjects exercised for forty minutes at 55% VO_2 max on the cycle ergometer. The researchers found that within 10 minutes of the start of the exercise session and regardless of gender or training status, HDL-C increased compared to baseline (53.1±13.4 vs. 58.8±13.9 mg/dL). These changes persisted over the course of the forty minute exercise bout, but declined by the 15-minute post-exercise reading. Lennon et al. commented that these are positive changes in the profile, but they are transient as they had diminished within 24 hours. The diminished values may be due to a low caloric expenditure compared to the study by Durstine (1983).

Kantor et al (1984), similar to Durstine (1983), used trained runners and examined the effects of one prolonged exercise session on LPLa, HDL-C and their relationship. Subjects performed at 42-kilometer foot race and had cholesterol levels measured the day before and after the race. Results of this study showed a decrease in TC and LDL-C immediately post exercise, but similar to Lennon, stated that these were transient changes as they normalized by the 48 hour blood draw. Further, they found that there was a decrease in TG that persisted for 48 hours as well as an increase in HDL-C, reflected in HDL-C_2, at 48 hours post exercise. In addition, LPLa was increased after exercise, which can explain the increase in HDL-C_2 and the decrease in TG. Kantor later reported (1987) increases in HDL-C and decreases in TG in trained men that again were present immediately post exercise and persisted 48 hours after exercise.

Acute exercise studies persisted through the 1990's, but tended to focus now on untrained individuals. Previously most research was completed on highly trained

subjects, but with the prevalence of CVD studies were needed to examine how to decrease an individual's risk via exercise. Pay et al (1992) compared 22, normolipidemic, healthy, trained and untrained men (61.3±1.2 vs. 53.9±2.3 mL/kg/min^{-1}) and women (54.9±2.4 vs. 37.3±1.8 mL/kg/min^{-1}) who were asked to walk at 30% VO_2 max on a treadmill for two hours. Results showed that there was a significant increase in HDL-C in both trained and untrained subjects regardless of gender (T- .88±.06 vs. 1.10±.08 mg/dL and UT- .73±.09 vs. .76±.08 mg/dL). It is important to note that the untrained individuals showed an interesting pattern over the 2-hour exercise bout. The first hour of exercise was marked by a more dramatic increase compared to the 2-hour reading (.92±.13 vs. .76±.08 mg/dL). Pay et al (1992) suggest that this may be due to the training adaptations that occur within individuals to enhance cholesterol metabolism, but points out that one session can alter HDL-C profile. The problem with this study is that they did not quantify energy expenditure so exercise prescriptions were limited to very low intensity exercise for long durations.

A later study by Crouse et al (1995) set out to characterize the short-term changes in blood lipid concentrations in hypercholesterolemic men and to compare the effects of intensity on post-exercise lipid responses. This study utilized two groups of approximately nineteen middle-aged (47 yrs.), unfit (VO_2max 31.1±1 mL/kg/min^{-1}) hypercholesterolemic (TC = 258 mg/dL) men who exercised at either 50 or 80% VO_2max and expended 350 kcal per session. Results of this study showed that both TC and LDL-C decreased significantly immediately post exercise, but normalized within 48 hours, similar to Kantor et al (1984). In addition, TG was significantly decreased at 24 and 48 hours post exercise (Baseline= 195±17, 24 hrs= 159±15, 48 hrs= 164±18 mg/dL), in addition HDL-C (Baseline= 44±2, 24 hrs= 48±3, 48 hrs= 49±2 mg/dL) and HDL-C_2 (Baseline= 6.2±.9, 24 hrs= 6.4±1.1, 48 hrs= 7.2±1.1 mg/dL) were significantly increased by 24 hours post exercise and persisted through the 48 hour reading. This occurred regardless of intensity and the authors suggested that a re-distribution of cholesterol was likely to have occurred and that there were perhaps roles for CETP, LCAT, LPL or HL, but since they did not test for these markers suggested that future research might elucidate a mechanism.

A recent study by Grandjean et al (2000) took the next step and analyzed the lipid transport enzymes. Their study set out to examine the responses of blood lipid/lipoproteins as well as LPLa, CETPa, HTGLa, and LCATa to a single session of aerobic exercise. Subjects were physically inactive hypercholesterolemic (VO_2max 31.3±1 mL/kg/min^{-1}) and normocholesterolemic (VO_2max 35.4±1.6 mL/kg/min^{-1}) men (TC- 252±5 vs. 179±5 mg/dL). They were asked to exercise at 70% VO_2 peak to expend 500 kcal on a treadmill. This study concluded that prior cholesterol status did not govern the magnitude of change either immediately post exercise (IPE), 24 or 48 hours post exercise. For example TG levels in normocholesterolemic men were reported as baseline, IPE, 24 and 48 hours 132±17 and 131±18, 115±14 and 117±13 mg/dL, respectively compared to hypercholesterolemic men at baseline 155±13 and then 148±8, 140±12, and 136±11 mg/dL, respectively. Each group showed decreases that persisted 48 hours post exercise. Similar changes were reported for HDL-C, which showed an increase IPE, 24 and 48 hours post exercise compared to baseline. A threshold for exercise should be based on the individual's tolerance level or aerobic capacity. Additionally, the authors postulated that an increase in CETPa was directly related to an

increase in TC and LDL-C and inversely related to HDL-C and HDL-C_2. Furthermore, similar to Crouse (1997) and Kantor (1987), they found that TC and LDL-C decreased immediately post exercise and then normalized within 24 hours. Again, similar to other studies TG decreased and HDL-C increased and these changes persisted for 48 hours post exercise. Enzyme results showed that there was no change in LCATa, and that HTGLa or CETPa did not change contributing to a stable HDL-C particle. LPLa did increase and this was thought to be responsible for the increase in HDL-C and decrease in TG, the authors suggest that perhaps LPL can regulate the conversion of the two particles.

In summary, the response of the lipid/lipoprotein profile and lipid transport enzymes after an acute bout of exercise support a more positive profile, in that HDL-C increases and TG decreases. Furthermore, research supports that acute exercise can change the activity of critical lipid metabolism enzymes to facilitate these positive changes.

Lipids/Lipoproteins and Intermittent Exercise Bouts

Previously, the major focus on lipid research had been the effects of acute bouts of exercise on lipid changes and how long these changes persist. Furthermore, another focus had been to establish the amount (kcal of energy expenditure) of exercise necessary to elicit positive changes in the lipid/lipoprotein profile. The early debate between volume of exercise and intensity of exercise was settled by numerous studies that stated while the intensity of exercise is important for type of substrate utilized (Romjin, 1993) the total volume (kcal) of exercise was most efficient in producing consistent positive changes in the lipid/lipoprotein profile. Crouse et al (1995) sought to characterize the short-term changes in blood lipids in hypercholesterolemic men. They found that energy expenditure of approximately 350 kcals caused significant immediate post exercise decreases in TC and LDL-C, which were normalized within 24 hours (Crouse et al., 1995). More importantly they reported a significant decrease in TG at 24 and 48 hours as well as increased HDL-C by 24 hours that persisted thru 48 hours. Subsequent research (Crouse et al., 1997; Grandjean et al., 2000; Kraus et al., 2002) validated these changes and changed the way exercise was prescribed for various populations including hypercholesterolemic men, normocholesterolemic men and women and premenopausal women. These changes however were all a result of one continuous bout of exercise.

To date there have only been six published studies examining the effects of intermittent exercise on blood lipids (Ebisu et al., 1985; Snyder et al., 1997; Woolf-May et al., 1998; Woolf-May et al., 1999; Altena et al., 2006; Mestek et al., 2006 Table 1).

Table 1. Summary of Intermittent Exercise Studies and Lipoproteins

Reference	Subjects	Exercise Intervention	TC	TG	HDL-C	LDL-C
Ebisu, 1985	53 Males	Group A- One session/day Group B- Two sessions/day Group C- Three sessions/day 10 weeks TM Running (80% HR max) for 3-6 miles/day Group D- Sedentary control	NSD	NSD	All Groups increased HDL, but only Group C increased significantly from baseline	NSD
Snyder et al., 1997	13 Females	Walking at 50-65% HRR for 10 min, 3x/day for 32 weeks	NSD	NSD	NSD	NSD

Table 1. Continued

Woolf-May et al., 1998	49 Males/ Females	Group A- long walkers (20-40 min sessions/day) Group B- Repetitive short walkers (10-15 mins/session, no more than 3 sessions per day) 18 weeks, 70-75% VO_2max start at 60 mins work up to 200 mins Group C- sedentary control	NSD	NSD	NSD	NSD
Woolf-May et al., 1999	56 Females	Group A- Long walkers (20-40 min/sessions) Group B- Intermediate Walkers (10-15 min/sessions) Group C- Short Walkers (5-10 min/sessions) 18 weeks walking 70-75% VO_2 max start at 60 mins work up to 200 mins Group D- sedentary control	NSD	NSD	NSD	Long walkers and intermediate walkers significant decrease from baseline. Control group significantly increase from baseline.

Only two of the six studies included kcal quantification as part of their methodology, and this appears to be a central focus when examining changes in the lipid/lipoprotein profile. In addition, all but one of these studies was long-term, the shortest study being 10 weeks in length and two studies used middle-aged overweight or obese individuals.

Ebisu et al. (1985) examined 53 untrained college-aged males (21 yrs) and exercised them for 10 weeks at 80% maximum heart rate for 3 days per week starting at 3 miles and progressing to 6 miles. Their subjects were divided into four groups, the first group running once per day, the second group running twice per day, the third group running three times per day and a fourth sedentary control group. The number of miles run was consistent between each of the exercising groups, but, there was no comment on kcal expenditure, fasting time between sessions and all sessions were conducted outside of the lab and based on exercise logs completed by each participant. Furthermore, this intensity would seem to be impractical for the general sedentary population, as one may not be able to sustain this intensity for more than several minutes, but Ebisu et al. reported pre-VO_2 max values ranging from 53.8±1.5 to 58.2±1.5 ml/kg/min^{-1}, suggesting their subjects could be considered as highly fit according to aerobic capacity.

Results of this study showed that all exercise groups, regardless of the number of sessions per day, showed significant increases in both VO_2 max (from 55.90 to 60.23 mL/kg/min^{-1}) and running time (from 11 mins/1.5 mile to 9:30 mins/1.5mile) during the 1.5 mile run trial. In addition, there was a significant increase in HDL-C (46.67 vs. 51.17 mg/dL) only in the group who exercised for 3 bouts per day. The other groups did not show significant improvements, Group 1, 46.0 vs. 47.5 mg/dL; Group 2, 45.56 vs. 47.72 mg/dL and control group 50.28 vs. 52.0 mg/dL. The authors concluded that perhaps three exercise sessions are more effective than one or two for changing HDL-C levels and failed to comment on why they thought the non-exercising control group showed fluctuations in HDL-C values. These results however, provide an incentive to further investigate one continuous session versus multiple exercise bouts in one day. Furthermore, these results were reported over a 10-week study, the benefit of the proposed study would be that it would allow researchers to quantify the acute effects of these types of exercises and use them for future chronic studies.

Later studies by Snyder et al. (1997) and Woolf-May et al. (1998) found no significant differences in any blood lipid parameters following 32 and 18 weeks of exercise, respectively. The study by Snyder et al. implemented an intermittent exercise program requiring their subjects to exercise for 10 minutes per session, 3 sessions per day for 32 weeks. The exercise protocol was brisk walking (50-65% heart rate reserve, HRR) and with this they estimated energy expenditure via the ACSM equation. In addition, they used 24-hour diet recalls to monitor energy intake, which research has shown that obese subjects, when asked to perform a 24-hour recall, underestimate kcal consumption (Johnson et al., 1994). This in concert with the subjects only expending 610 kcal/week, equaling roughly 122 kcal/day, and may not have been enough of a stimulus to elicit the necessary changes needed as suggested by Crouse, Grandjean, Krauss, and Gordon. The study by Woolf-May et al. (1998) expended approximately 887 kcal/week (long walk group) and 756 kcal/week (short walk group), this is still only equivalent to 177 kcal and 151 kcal/day, respectively, again not sufficient to cause changes to the lipid/lipoprotein profile as previously suggested. These results may be due to the fact that neither study controlled for energy intake and told their subjects to maintain their normal diet for the duration of the study.

Woolf-May et al. (1999) examined the effects of single and accumulated short bouts of walking during an 18-week trial on 56 men and women, aged 40 to 66, who were considered unfit (VO_2max ranging from 22.6±6.6 to 34.7±11.6 mL/kg/min^{-1}). The researchers implemented four groups walking at differing frequencies and durations. The first group designated the long walkers (LW) walked 20-40 min/bout. The second group was the intermediate walkers (IW) and they were told to walk 10-15 min/bout, the third group was the short walkers (SW) and they were instructed to walk 5-10 min/bout, and the fourth group was designed to be the control. The exercise was designed to start at 60 minutes per week and gradually increase to 200 minutes per week of walking at 70-75% VO_2max. The results showed that there were significant decreases in pre- versus post-intervention values for LDL-C (LW 4.95±1.54 vs. 4.66±1.52; IW 4.94±0.68 vs. 4.53±0.83 mmol/L) and Apo-II (LW 0.46±0.08 vs. 0.41±0.08; IW 0.45±0.08 vs. 0.43±0.08 mmol/L) in both the long walk and intermediate walk groups and hence an increase in the Apo I/Apo-II ratio in the long walk group only. There were no other changes in any other lipid/lipoprotein variables, which included Apo A-I, Apo-B, TC, TG and HDL-C. The researchers failed to report kcal consumption during the study, which is known to have a greater affect on LDL-C levels than exercise. There are a few studies which report decreases in LDL-C with exercise, but this is usually seen in highly trained athletes expending large numbers of kcals. As in the other studies the subjects in this experiment only expended on average 541 kcal/week, equivalent to 108 kcal/day. Again, the problem with this study, as with the previous three, is that they may have provided enough stimuli to improve aerobic fitness but not to change the lipid/lipoprotein profile.

Recently there have been two additional studies published contributing to the intermittent body of literature. The first by Altena et al (2006) is yet another training study examining the effects of continuous versus intermittent exercise at 60% VO_2 max (75% HR max). The training consisted of either jogging or walking at the appropriately prescribed exercise intensity to expend approximately 245 kcal/session for a total of 1225 kcal/week. The intermittent sessions were performed in three 10-minute bouts separated by 20-minute rest periods, during which the subject were seated quietly reading or doing

paperwork. Both the continuous and intermittent sessions totaled 30 minutes and heart rate was monitored to ensure intensity. Results showed that post training TC was significantly decreased in both groups (continuous -4.7% and intermittent -11.3%), however the groups were not significant from each other suggesting both continuous and intermittent exercise exerted the same influence. No group differences were reported for HDL-C and its subfractions. LDL-C was significantly decreased post-training for both groups (continuous -2.9%, intermittent -11.2%), but similar to TC the difference was not significant between groups. LDL-C particle size did not change significantly in either group post training.

These results suggest that training of this magnitude may alter both TC and LDL-C, but have no effect on HDL. Results of Altena et al (2006) are similar to Woolf-May et al (1999) who found training decreased LDL-C, but similar to Woolf-May did not report calorie intake over the training period so that we may consider the effects that diet may have had over the lipid and lipoprotein response.

The most recent and perhaps most similar study to the proposed project was completed by Mestek et al (2006) who compared the effects of one continuous 500 kcal exercise session versus three accumulated (167 kcal/session) exercise sessions. This study used 9 males participants of similar age and anthropometric characteristics, one difference was that this study proposed to use subjects whose VO_2 max was less than or equal to 40 ml/kg/min^{-1} and the Mestek study had participants who ranged from 40.0 to 45.3 ml/kg/min^{-1}. Results showed that TC was significantly lower in the continuous session compared to the accumulated sessions but were subsequently unaltered by exercise. Both TG and LDL-C showed no significant differences as a result of either exercise intervention, however there was an increase in HDL-C for both the continuous session (2 mg/dL) and the accumulated sessions (7 mg/dL). Furthermore, there was a significant difference between conditions, specifically the accumulated sessions produced a greater increase in HDL-C compared to the continuous session (50±7 vs. 52±7 mg/dL for continuous; 49±8 vs. 56±7 mg/dL for accumulated). The authors concluded that three intermittent exercise sessions separated by at least 4 hours was more effective at increasing HDL-C compared to one continuous session. Unfortunately, both Ebisu (1985) and Mestek et al. (2006) state that they cannot offer an explanation for those findings.

Previous research on intermittent exercise has provided sufficient evidence to support an increase in aerobic capacity, but its effects on lipids and lipoproteins are ambiguous. This is perhaps due to the striking fact that the previous research has not provided a sufficient stimulus (kcal expenditure/session) to elicit these changes. Ebisu (1985) and Woolf-May et al. (1999) both reported changes in different lipoproteins after their prescribed exercise intervention. Ebisu (1985) and Mestek et al. (2006) reported an increase in HDL-C, but only in the three exercise sessions per day group as compared to one and/or two exercise sessions per day and suggested that it might take three sessions to stimulate a change. Furthermore, Woolf-May et al. found changes in LDL-C, which is typically the lipoprotein least cited for changing due to an exercise stimulus. Finally, no one has analyzed the lipid transport enzymes in order to try and explain why HDL-C changes with one stimulus and not another. The variability of results and failure to quantify kcal expenditure, an important factor governing lipid/lipoprotein changes, merit

research examining the effects of an acute bout of exercise delivered in one continuous session and either 2 or 3 multiple sessions on lipid and lipoprotein profiles.

Practical Public Health Implications of Study

Healthy People 2010 was established to promote health awareness in the United States population. Their goals were twofold, first to improve the quality of life and years of healthy life and secondly to eliminate health disparities within the population. The report listed physical activity as one of the leading health indicators that required improvement, followed by a need to reduce the occurrence of CVD. Specifically, the report called for a decrease in physical inactivity and an increase in moderate physical activity for at least thirty minutes a day (US Dept Health and Human Services, 2000). Furthermore in an attempt to curb the increases in the incidence of CVD the report called for decreases in total cholesterol and decreases in the number of individuals who have an unfavorable lipid profile (US Dept Health and Human Services, 2000).

Despite epidemiological evidence stating that physical activity has a variety of positive health benefits on the lipid profile as well as enhancing glucose tolerance and insulin sensitivity, Americans are sedentary and reports show that 40% of individuals are inactive contributing to 250,000 deaths per year. A striking reality is that most Americans simply state lack of time as the number one reason they do not participate in regular activity. Researchers have diligently tried to conjure up exercise programs designed to fit a busy lifestyle and also provide sufficient stimulus to elicit health benefits. Given this dilemma a group of experts from the CDC and ACSM released a recommendation for adults on physical activity, which states that every US adult should accumulate 30 minutes or more of moderate-intensity physical activity (approximately 50-70% VO_2 max) on most, preferably all, days of the week (Pate et al., 1995).

This recommendation was designed to emphasize the benefits of moderate-intensity exercise and that this exercise can be accumulated over short bouts rather than one long continuous bout. The recommendation reflects that it is not necessarily the intensity, mode or duration of the activity bouts, but rather the total amount of activity performed that is linked to a decrease in CVD mortality. This comes at a crucial time when overweight and obese individuals are at an all time high and this weight problem continues to increase. Experts have suggested that researchers need to develop ways to assess the physical activity patterns of people and advocate exercise programs that the American population will follow and that will provide the necessary health benefits. In addition, as a scientific community we need to make our research available to individuals so that they can participate in exercise regimens specifically to tackle the problems they are experiencing.

Research has shown that accumulating exercise is effective for weight control by reducing body weight, body mass index, body composition and waist to hip ratios (Donnelly et al., 2000; Jakicic et al., 1999; Schmidt et al., 2001). Furthermore, several of these studies report that adherence is equally successful or greater with intermittent bouts compared to continuous bouts of exercise (Jakicic et al., 1999; Jacobson et al., 2003; Jakicic et al., 1995). Jakicic et al (1995) reported that short-bout exercise groups reported exercising on a greater number of day compared to long-bout groups (87.3±29.5 days vs. 69.1±28.9 days) and for a longer total duration (223.8±69.5 min/week vs. 188.2±58.4 min/week).

This brief synopsis finds value in intermittent exercise bouts and the goal of this study is to add to the breadth of literature that exists, as well as contribute valuable information regarding the influence of these intermittent bouts on an important CVD risk factor, the lipid and lipoprotein profile.

CHAPTER 3

METHODS

Subjects

Subjects were sixteen (N=16) healthy males between the ages of 18-35 years old. Participants met all of the following criterion for admission into the study: 1) free of major medical problems such as cardiovascular disease, heart attack, angina, hypertension, lipid abnormalities, diabetes, or any injury or limitation that may have prevented them from completing the exercise protocols, 2) non-smokers, 3) sedentary, having a VO_2 max between 25 and 40 ml·kg·min^{-1}, 4) body fat no higher than 25% and no lower than 5% 5) baseline HDL-C levels greater than 37 mg/dL and 6) were willing to have blood drawn and refrain from alcohol consumption during the testing period. Subjects were recruited from Florida State University and the surrounding community by posted flyers, newspaper advertisements as well as announcements made in classes of students on the Florida State University campus (Appendix A). Subjects were made aware of the nature of the study as well as the risks and benefits of participation.

Initial Screening

All subjects signed an informed consent, approved by the Florida State University Institutional Review Board (Appendix B), as well as completed a health history and physical activity questionnaire (Appendix C).

During the first laboratory visit, subjects had resting blood pressure taken manually with a sphygmomanometer and stethoscope. Subjects were excluded from the study if the arterial blood pressure was greater than 140/85 mm/Hg. Height was assessed using a stadiometer (Medart, St. Louis, MO) to the nearest 0.5 cm and weight, in kilograms, on a Seca scale (Model 707, Seca Corporation, Columbia, MD), while the subject was wearing exercise clothing (shorts and T-shirt). Using the height and weight measurements body mass index (BMI) was calculated by taking pounds in kilograms and dividing that by height in meters squared (kg/m^2). Body composition, using the seven-site skinfolds was taken using Lange calipers (Beta Technology Inc., Santa Cruz, CA) and a VO_2 max test was performed (Appendix D). Finally, the researchers used the finger stick procedure to sample the subject's blood to immediately assess HDL-C values using the Quick Medical Bio Scanner2000 and HDL-C specific test strips (CM:585).

Determination of Maximal Aerobic Capacity (VO_2 max)

Subjects completed a modified Balke graded exercise test on a motor driven treadmill for the determination of VO_2 max during their initial screenings. The modified Balke protocol consists of the subjects walking at 3.3 – 3.6 miles per hour for three-minute stages. The test began at 2.5% grade and increased by 2.5% per stage, when the test reached 10% grade the stages thereafter increased by 0.2 miles per hour and 2.5% grade until the subject reached volitional exhaustion (Appendix E). This protocol was selected because it allows unfit men (VO_2 max < 45 ml/kg/min) to achieve maximal aerobic capacity while allowing ventilatory threshold (VT) to be established. Aellen et al (1993) suggested that exercising individuals above VT may adversely affect the lipid profile, specifically by lowering both HDL-C and HDL-C_2 acutely. VT was established using the Dmax methodology described by Kara et al (1999), and the linear regression of the ventilatory equivalents. This information was used to determine the subjects exercise intensity during their experimental trials sessions and kept all subjects below their VT.

During the test expired gases were collected and recorded as 30-second averages. Ratings of perceived exertion (RPE) using the Borg scale (1970) were collected at the end of every stage. The criteria for attainment of VO_2 max were as follows: 1) VO_2 plateau between the last two stages of <2.0 ml/kg/min; 2) a heart rate within ±10 beats per minute of age predicted maximum heart rate (220-age); 3) a respiratory exchange ratio (RER) of ≥1.15 and 4) volitional fatigue described by the subject.

Measurement of Body Composition

Body composition was measured using the seven-site skinfold method. Lange skinfold calipers (Beta Technology Inc., Santa Cruz, CA) were used to ascertain subcutaneous fat deposits (millimeters) at the triceps, abdominal, suprailiac, thigh, chest, midaxilla and subscapular regions of the body. Measurements were taken in duplicate by the same individual trained in taking body composition measurements. All measurements were within 1 mm, and if they were not that region was tested a third time after which the closest two were averaged to ensure accuracy. The seven sites were added together and then used to calculate body fat percentage as per the Siri equation (1956). Subject's body fat was not to be higher than 25% or lower than 5% to qualify for entrance into the experiment.

Experimental Design

Subjects were required to complete all phases of the experiment. All trials were randomized, occurring during the same time of day and separated by at least one week from each other to avoid any residual effects of the previous exercise session. Baseline testing took place in the evening and then any subsequent exercise sessions were based on that evening time. For example if the baseline measurement was completed at 6 pm, then exercise trial one would occur at 5 pm, the following day to ensure that they finished exercise and could have blood drawn at 6 pm. This was done to standardized blood draw timing to account for any daily fluctuations that might be seen in the lipid profile. Furthermore, the subjects then reported to the lab 15 minutes prior to 6 pm for both 24 and 48 hours post exercise blood draws. Intermittent sessions were based on the evening session going backwards. Again if their blood draws began at 6 pm, the 2 times per day trial would occur before the blood draw at 5 pm and 1 pm for every subject and the three times per day trials would occur at 5:30 pm, 1:30 pm and 9:30 am. Subjects were required to come the to lab for each exercise session after at least a 4-hour fast so that blood could be drawn for the determination of baseline measurements of TC, TG, HDL-C, HDL-C_2, HDL-C_3, and TG levels as well as enzyme (CETP, LCAT) activity. The rationale behind only a 4 hour fast was to mimic the testing sessions, so that the study examined the effect of the exercise without the confounding effects of diet.

Subjects underwent one of three trials each consisting of exercise at 65% of the previously established VO_2 max to expend 450 kcals. If 65% was over the VT for a subject the assignment of exercise intensity was decreased by 5% in an attempt to standardize all subjects. For example if 65% was above the VT then the workload for the subject was reduced to 60%.

The three trials consisted of: 1) a single continuous exercise session to expend 450 kcals, 2) two intermittent exercise sessions designed to expend 225 kcals/session for a total of 450 kcals and 3) three intermittent exercise sessions designed to burn 150 kcals/session for a total of 450 kcals. The intermittent sessions took place a minimum of

four hours apart. All exercise sessions were completed within one 12 to 14 hour period. Table 2 represents the exercise testing and blood draw schedule for the subjects.

Table 2. Schedule of Exercise Sessions and Blood Draws

Group	Exercise Intervention	Blood Draws
Continuous Exercise (CE)	TM Walking/Running at 65% VO_2 max. One Continuous Session to expend 450 kilocalories	Baseline blood draw Immediately post-exercise (IPE) (450 kcal) 24 hours post exercise 48 hours post exercise
Two Intermittent Sessions (IE 2)	TM Walking/Running at 65% VO_2 max. Two sessions of 225 kilocalories each separated by a minimum of 4 hours.	Baseline blood draw IPE of 2nd exercise session (450 kcal) 24 hours post exercise 48 hours post exercise
Three Intermittent Sessions (IE 3)	TM Walking/Running at 65% VO_2 max. Three sessions of 150 kilocalories each separated by a minimum of 4 hours.	Baseline blood draw IPE of 3rd exercise session (450 kcal) 24 hours post exercise 48 hours post exercise

Dietary Assessment

Subjects were asked to complete a seven-day food diary one week prior to testing to establish the subject's eating habits. The subjects were then asked to maintain this eating pattern during the weeks they were participating in the experimental trials. This was for the purpose of ensuring that subjects maintained their normal diets throughout the testing periods to eliminate the confounding effects of food intake on blood parameters. Diet records were analyzed using Nutritionist Five™ (First DataBank, Inc., San Bruno, CA).

Subject Compliance

Subjects were instructed to refrain from strenuous physical activity, alcohol, medications and exposure to environmental tobacco smoke 36 hours prior to exercise sessions. Subjects also had both written and verbal instructions on how to maintain their normal diets, as reported in their weekly diaries. Every time the subject returned to the lab for testing verbal confirmation of compliance with these procedures was obtained. Subjects were warned that they would be removed from the study if large deviations from the protocol occurred, including a subject's inability to comply with diet, alcohol consumption and/or being exposed to smoke.

Exercise and Control Trials

All exercise testing began in the evening hours, after a fast of at least 4 hours. The intermittent trials containing two or three sessions per day had a minimum of four to five hours between each session. For example if subject one had their first trial at 6:00pm, they were asked to come back no earlier than 1 pm but no later than 2 pm. During the "break" between sessions subjects were asked to eat a meal, consistent with

what each subject reported in their diet records. This procedure of exercise, 4-5 hour break and eating a meal was repeated between trials two and three for the three-intermittent exercise sessions.

Prior to exercise the subject sat quietly for 15 minutes for the determination of heart rate and blood pressure followed by blood collection. The subject then walked on the treadmill at the previously determined 65% VO_2 max to expend the pre-determined number of kilocalories (450, 225, 150) that was randomly assigned. Kilocalorie expenditure was determined by the use of indirect calorimetry for the first 20 minutes of each trial. This 20-minute period was designed to confirm that the subject had met steady state at the appropriate intensity. The speed and grade of the exercise trials was determined from their VO_2 max test, furthermore minor adjustments to speed and grade were made during the exercise trial to bring their VO_2 closer to their predetermined exercise intensity of 65% (±5%). After the first 20 minutes the subject removed the mouthpiece for 10 minutes, at 30 minutes and then every 10 minutes thereafter the mouthpiece was placed back in the mouth of the subject for 5 minutes to measure VO_2 ensuring the continuation of steady state exercise. This procedure was repeated for all exercise trials, including ensuring that the subject's appropriate speed and grade was maintained throughout each exercise session.

Testing Equipment

Gas exchange and ventilatory parameters was measured by open circuit spirometry using a Truemax 2400 metabolic measurement system (Consentius Technologies, Sandy, UT). This metabolic system provided data in 30-second intervals on all metabolic parameter relevant to this study including VO_2, volume of carbon dioxide (VCO_2), respiratory exchange ratio (RER), and kcal calculations. This was used to estimate relative substrate contribution to the exercise bout. Prior to the testing sessions the metabolic system was calibrated as per the specifications supplied by the manufacturer. These briefly are a flow calibration using a 3L calibration syringe (no. 5530, Hans Rudolf, Inc., Kansas City, MO) and a gas calibration using tanks containing a known mixture of 16% oxygen and 4% carbon dioxide (16% O_2, 4% CO_2, Scott Medical Products, Plumsteadville, PA). Temperature, barometric pressure and relative humidity of the testing environment were measured using an indoor hygrometer (Perception II TM, Davis Instruments, Hayward, CA) and then input into the metabolic system for data collection adjustments.

A mouthpiece (no. 1003, Vacumed, Ventura CA), attached to a two-way non-rebreathing valve and six-foot tubing (no. 112263 and 666120, Hans Rudolph, Inc., Kansas City, MO) was used to collect expired air and deliver it to the metabolic system. Heart rate data were collected using a polar heart rate monitor (Polar CIC Inc., Port Washington, NY). During exercise, heart rate measurements were recorded using the interface with the metabolic systems as a way to also validate exercise intensity. Blood pressure was taken before exercise using a sphygmomanometer and stethoscope (General Medical Corp., Richmond, VA). The graded exercise test as well as exercise trial sessions were performed on a motor driven treadmill (Q65/Series 90, Quinton Instruments Co., Bothell, WA).

Blood Sampling and Storage

Blood samples were drawn in the evening after at least a 4 hour fast at baseline, 24, and 48 hours post exercise. During intermittent sessions, the minimum time between

testing sessions was 4 hours. All samples were taken following 15 minutes of seated rest and collected from an antecubital vein using a Vacutainer brand collection set (no. 367251, Becton Dickinson, Franklin Lake, NJ) into a vacutainer tube. A total of 12 ml of blood was collected for the determination of hematocrit and hemoglobin (3 ml, sodium heparin, no. 366387, Becton Dickinson, Franklin Lakes, NJ), plasma TG, TC (3 ml, Sodium heparin, no. 366387, Becton Dickinson, Franklin Lakes, NJ), and plasma HDL-C, HDL-C_3 and CETPa (6 ml, spray-dried EDTA (K2), no. 367861, Becton Dickinson, Franklin Lakes, NJ).

Hematocrit and hemoglobin were determined within 48 hours of collection, and the remaining blood was centrifuged (2,800 g) within 10 minutes of collection, at 4˚C for 20 minutes using a refrigerated centrifuge (Sorvall RT7, DuPont Sorvall Products, Newton, CT). Following centrifugation, plasma samples for the determination of TG and TC were placed into 2.0ml microcentrifuge tubes (no. CN1700-GTS T, National Scientific Supply Co, Inc., Claremont, CA). Then, 2ml of plasma containing the anticoagulant EDTA was used for the separation of HDL-C and HDL-C_3. Supernatent for the determination of HDL-C and HDL-C_3 was transferred to microcentrifuge tubes for storage. All microcentrifuge tubes were then stored at -70˚C until a time that all samples for each parameter may be analyzed at the same time in an attempt to reduce inter- and intra- assay variation.

Biochemical Analysis

Total Cholesterol and Lipoproteins

Total cholesterol was assayed according to the technique developed by Allain et al (1974) with the modifications provided by Roeschlau (1974) (Infinity™, Thermo DMA, Louisville, CO). To summarize the procedure, cholesterol esters are enzymatically hydrolyzed by cholesterol esterase to cholesterol and FFA. Free cholesterol is then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide then combines with HBA and 4-aminoantipyrine to form a chromophore (quinoeimine dye), which can be read spectophotometrically at 500 nm.

Determination of plasma HDL-C and HDL-C_3 was performed based on the procedures developed by Warnick and Albers (1978) and Gidez et al (1982). A heparin-manganese reagent was prepared first to precipitate the Apo-B containing lipoproteins, leaving behind a supernatant containing the HDL-C fraction. A small amount of this supernatant was set aside for the determination of HDL-C. Next, the less dense HDL-C_2 particles were precipitated using a dextran sulfate solution (Dextralip 15, Genzyme Corp., Cambridge, MA) leaving behind the HDL-C_3 particle. These samples were then analyzed using the same technique as described above for TC. Plasma HDL-C_2 was then calculated as the difference between HDL-C and HDL-C_3. Plasma LDL-C was calculated according the Friedwald equation (1972).

Triglycerides

Determination of TG was performed by a commercially available kit (Infinity™, Thermo DMA, Louisville, CO). This procedure is based on the work of Bucolo and David (1973) with modifications provided by McGowan (1983) and Fossati (1982). This procedure allows for the TG to be enzymatically hydrolyzed by lipase to FFA and glycerol. The glycerol is then phosphorylated by ATP and glycerol kinase to produce

glycerol-3-phosphate and ADP. Glycerol-3-phosphate is oxidized by dihydroxyacetone phosphate (DHP) by glycerol phosphate oxidase producing hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and 3,5-dichloro-2-hydrobenzene sulfonate to produce a red dye. The absorbance of this dye at 520 nm is proportional to the concentration of TG present in the sample.

Lipid Enzymes

The measurement of CETPa was determined by the procedures of Tollefson and Albers (1994), later modified by Tato et al. (1995). This procedure (CETP activity kit, Roar Biomedical, New York, NY) uses a labeler substrate that is introduced and measured as the percent transferred from HDL-C_3 to LDL-C, donator to acceptor respectively. Donor and acceptor lipoproteins were prepared from normolipidemic samples by preparative ultracentrifugation prior to analysis. This sample was incubated at 37˚C for 1-3 hours to allow for the formation of ^{3}H-CE. After the 1-3 hour incubation the sample was read at a fluorescence intensity of 465 nm and 535 nm. The expressed sample as read in picomoles (pmol) represents the transfer value.

LCATa was measured by the procedure of Stokke and Norum (1971) using a LCAT activity kit (Roar Biomedical, New York, NY) in which trace amounts of labeled cholesterol are added to plasma and incubated. Samples are incubated for 4 to 8 hours at 37˚C during this incubation time LCAT is inhibited by a disulfide, this inhibition allows the tracer to equilibrate with the endogenous lipoprotein cholesterol. The sample is then read at emission intensities of 390 nm and 470 nm, the activity of LCAT is then given as a ratio of hydrolyzed (390 nm) and non-hydrolyzed (470 nm) substrate. This ratio will indicate the increase in concentration of the 390 nm emitter and simultaneous decrease in concentration of 470 nm emitter when in the presence of LCAT.

The size and distribution of the LDL-C particle subclasses was determined by electrophoretic separation utilizing non-denaturing 2-16% polyacrylamine gradient gels (Rainwater, 1999). The relative distribution of the LDL-C subclasses are described as an integrated diameter corresponding to the weighted mean size of all LDL-C subclasses in one sample. In addition, the relative proportion of LDL-C subclasses <25.5 nm or >25.5 nm are reported for each sample (St. Pierre et al., 2001; Tchernof et al., 1996).

Hematocrit and Hemoglobin

Hemoglobin was assessed in duplicate using the cyanomethehemoglobin method (Pointe Scientific, Inc., Lincoln Park , MI). Hematocrit was determined in triplicate using the microcapillary method where whole blood is placed into a heparinzed micro-hematocrit capillary tube (no. 22-362-566, Fisher Scientific, Pittsburgh, PA) and centrifuged using an IEC Micro MB centrifuge (Model 3411, International Equipment Company, Needham Heights, MA) for seven minutes. Hematocrit was then determined using a circular microcapillary tube reader (Model 2201, International Equipment Company, Needham Height, MA). Hematocrit and hemoglobin values were used to estimate changes in plasma volume relative to the baseline of each trial and according to Dill and Costill (1974). All blood samples taken during the exercise period were adjusted for plasma volume shifts that occurred during aerobic activity.

Calculations and Statistical Analysis

Data were analyzed using a 3 x 4 (group x time) repeated measure analysis of variance (ANOVA). Groups were the continuous exercise group (450 kcal), two-session exercise group (225 kcal/each) and three-session exercise group (150 kcal/each).

Dependent variables were measured in mg/dL for TC, TG, HDL-C, HDL-C_2, HDL-C_3, LDL-C, furthermore, LCATa and CETPa were measured in activity levels. Once significance was detected a Tukey's post hoc test was employed to determine where significance occurred, using the SPSS 10.0 statistical package.

Sample size estimation was determined as a function of effect size (ES), the significance criterion (α) and the statistical power. ES was calculated using the following formula: $ES = (\mu_1 - \mu_0)/\sigma_0)$. Using this formula ES was calculated for HDL-C based on previous literature using similar subject criteria and calorie expenditure, ES= 43-41/2, resulting in an ES of 1.0. For this experiment an ES of 0.80 was used as a modest value based on this literature (Grandjean et al., 2000). Significance was accepted *a priori* at the $p<0.05$ and with the accepted ES of 0.80 a minimum of sixteen subjects are required (Cohen, 1988). Values were reported as mean ± standard deviation. Coefficient of variations were established for TC, TG and HDL-C as less than 2%. In addition, in an attempt to establish relationships that may exist between the dependent variables a Pearson product-moment correlations were completed to determine if relationships existed between the dependent variables. Correlations were tested specifically for enzyme activity and lipid/lipoprotein variables. For example correlations were tested between CETP activity and TC, LDL-C and HDL-C and LCAT activity with HDL-C. All significance was accepted at $p<0.05$.

CHAPTER 4

RESULTS

Baseline Data

Thirty seven males were recruited from Florida State University and completed both informed consent and a health history form prior to any exercise testing. Of the thirty seven recruited, sixteen (N=16) were accepted into the study because they met all the prerequisites for participation in this project, the other participants were dismissed because of failure to meet those same prerequisites. Subject's descriptive characteristics are presented in Table 3.

All participants achieved a VO_2 max using standard criteria with, 14 of 16 subjects achieving a plateau, during the graded treadmill exercise test with the mean VO_2 max equal to 37.0±3.3 mL.kg.min-1 (range 31.4 – 41.6 mL/kg/min^{-1}). Corresponding maximum RPE (19.0±0.8), maximum RER (1.10±0.10) and maximum HR (189±9) were indicative of a maximal effort. Subjects could be generally described as recreationally active with most participating in regular resistance training regimens with little or no emphasis on cardiovascular training as indicated by their moderate to low VO_2 max values (37.0±3.3 mL/kg/min^{-1}).

Table 3. Descriptive Characteristics of Subjects

	Mean	**Range**
Age (years)	22.1 ± 2.1	19 – 28
Height (m)	1.8 ± 0.1	1.66 – 1.91
Weight (kg)	86.4 ± 14.6	68.5 – 104.2
BMI (kg/m^2)	26.9 ± 4.0	20.0 – 32.1
HR (bpm)	71.9 ± 12.1	50.0 – 88.0
VO_2 max (mL/kg/min^{-1})	37.0 ± 3.3	31.4 – 41.6
Body fat (%)	16.7 ± 6.8	6.9 – 25.0

BMI= Body mass index; HR= Heart rate; VO_2 max= maximal oxygen consumption

Baseline lipid and lipoprotein values are reported in Table 4 and serve as an initial point of reference. Comparing the baseline data of the subjects in this study with reported data from NCEP-ATP III showed that 75% (12 of 16) of the subjects fall within normal limits for TC (TC <200 mg/dL) as well as for TG (<200 mg/dL). LDL-C results showed that 37.5% (6 of 16) of the subjects had optimal values (LDL-C <100 mg/dL), 50% (8 of 16) had near optimal values (100-129 mg/dL), 1% (1 of 16) had borderline high values (130-159 mg/dL) and an additional 1% (1 of 16) had very high values (>190 mg/dL). Finally, HDL values for 13 of the 16 (81.25%) subjects were above 37 mg/dL, considered normal, while three subjects (18.75%) had low values ≤30 mg/dL. It is important to comment that despite these subjects being below the previously established criteria for minimum HDL-C levels, due to testing equipment problems, it did not affect the results of the study. Additional statistics were done without these subjects to validate the data. When separating the HDL-C particle into its subfractions HDL-C_2 and HDL-C_3

values were 13.7±4.8 mg/dL (range 8.8 to 17.2 mg/dL) and 26.5±12.1 mg/dL (range 15.1 to 38.8 mg/dL) respectively. These results therefore, suggest that the majority of the subjects in this study are considered normocholesterolemic.

Table 4. Subject Baseline Lipid and Lipoprotein Values

Lipid or Lipoprotein Variable	Mean ± SD	Range
Total Cholesterol (mg/dL)	178.6 ± 33.8	123 – 252
Triglycerides (mg/dL)	137.5 ± 63.8	61 – 239
Low Density Lipoprotein (mg/dL)	106.0 ± 34.0	59.1 – 194.3
High Density Lipoprotein (mg/dL)	39.4 ± 10.5	26.0 – 68.9
High Density Lipoprotein$_2$ (mg/dL)	13.7 ± 4.8	8.8 – 17.2
High Density Lipoprotein$_3$ (mg/dL)	26.5 ± 12.1	15.1 – 60.1

Lipid transport enzymes CETP and LCAT were analyzed for activity levels. Baseline CETP activity were 25.4±9.1 pmol of cholesterol ester transferred over three hours, while baseline LCAT activity was 1.69±0.138 μmol chol ester·L^{-1}·hr^{-1} of substrate hydrolyzed.

Experimental Values

A repeated measures ANOVA was employed to identify differences between means. There were no exercise effects for TC and LDL-C (Tables 5 and 6 respectively), when compared to their baseline values as these lipid and lipoprotein values are typically more affected by diet as opposed to exercise.

Table 5. TC Response to Continuous and Intermittent Exercise

Treatment	Mean ± SD (mg/dL)			
	Baseline	**Immediately Post**	**24 hrs post**	**48 hrs post**
CE	178.6±33.9	184.7±45.1	183.1±41.8	185.5±35.4
IE 2	178.6±33.9	175.0±39.2	173.2±37.0	172.4±33.0
IE 3	178.6±33.9	179.4±43.5	177.6±41.4	177.6±44.6

Table 6. LDL-C Response to Continuous and Intermittent Exercise

Treatment	Mean ± SD (mg/dL)			
	Baseline	**Immediately Post**	**24 hrs post**	**48 hrs post**
CE	106.0±33.9	109.1±39.5	110.8±36.1	115.1±29.4
IE 2	106.0±33.9	99.1±32.8	101.1±31.0	105.1±32.3
IE 3	106.0±33.9	102.6±35.0	107.8±31.0	113.1±38.9

With regards to total HDL-C and TG no significant differences were observed between baseline values and subsequent times, which included immediately post exercise, 24 and 48 hours post exercise. An important observation to note however is that despite TG values not demonstrating a significant time difference there was a continual decrease over the various time intervals following exercise through 48 hours post exercise (Table 7).

Table 7. The Effect of Exercise on Blood Triglycerides Across the Experimental Time Points

Treatment	**Mean ± SD (mg/dL)**			
	Baseline	**Immediately Post**	**24 hrs post**	**48 hrs post**
CE	137.5±63.8	144.7±67.8	139.2±57.1	125.8±67.4
IE 2	137.5±63.8	154.5±65.8	138.0±66.7	120.7±50.0
IE 3	137.5±63.8	161.6±83.4	135.9±59.0	117.2±46.7

So as can be seen in Table 7 immediate post exercise TG values continued to decrease for all treatment groups up to 48 hours.

While there were no significant changes observed for total HDL-C concentration (Table 8) analysis of the HDL-C particles revealed significant differences in the concentration of HDL-C_2 and HDL-C_3 during the experiment (Figures 1 and 2 respectively)

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Table 8. Post Exercise HDL-C Levels

Treatment	**Mean ± SD (mg/dL)**			
	Baseline	**Immediately Post**	**24 hrs post**	**48 hrs post**
CE	39.4±10.5	39.9±9.7	40.2±11.8	41.8±13.0
IE 2	39.4±10.5	40.0±6.2	40.5±7.1	40.6±7.1
IE 3	39.4±10.5	41.4±9.8	41.5±10.6	42.9±14.2

Specifically significance was found for HDL-C_2 when comparing baseline to specific subsequent time points for all three trials (Figure 1).

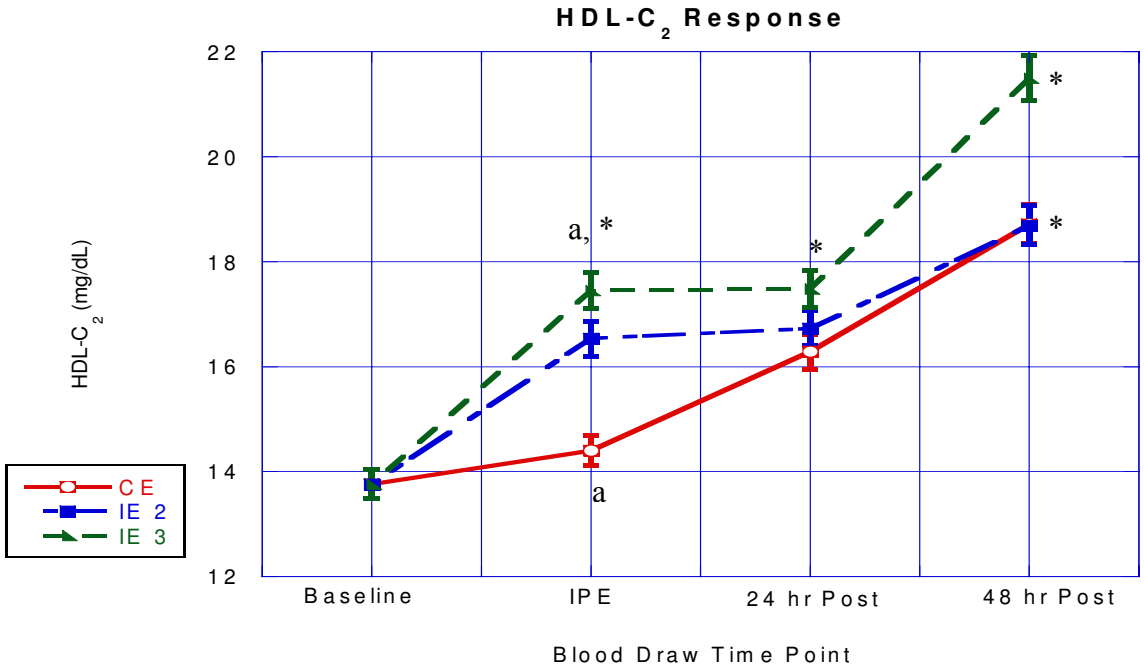

Figure 1. HDL-C_2 Response to Continuous and Intermittent Exercise
* denotes a significant difference from baseline, "a" denotes significantly different from each other ($p < 0.05$)

Significant increases of 44% were observed when comparing baseline (12.9±4.8 mg/dL) to CE 48 hours post exercise (18.7±5.3 mg/dL) and to IE 2 48 hours post exercise (18.6±4.1 mg/dL) respectively. Furthermore HDL-C_2 was found to be significantly elevated at all three post exercise time points. Resulting from the IE 3 exercise session HDL-C_2 rose 39% immediately post exercise (17.8±4.5 mg/dL), and continued to rise to 21.5±3.9 mg/dL (66%) by 48 hours post exercise. Furthermore, significance was found for HDL-C_2 when comparing CE immediately post exercise (14.5±4.9 mg/dL) and IE 3 immediately post exercise (17.5±3.9 mg/dL).

Along with the increases in HDL-C_2 there are also significant decreases in HDL-C_3 (Figure 2).

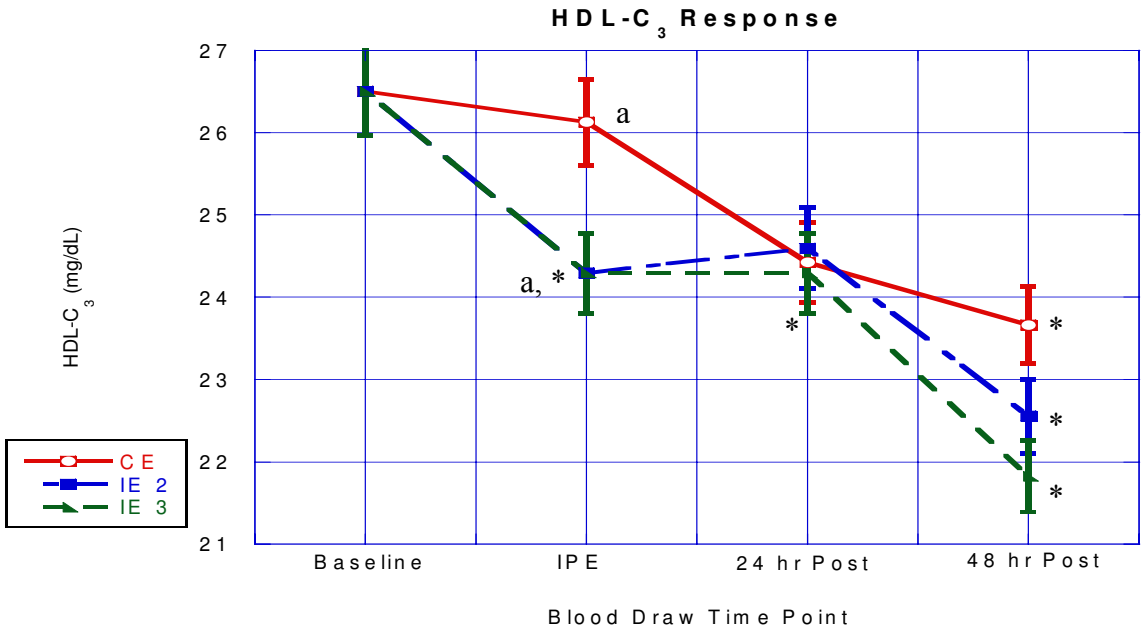

Figure 2. HDL-C_3 Response to Continuous and Intermittent Exercise
* denotes a significant difference from baseline, "a" denotes significantly different from each other.

First, when comparing baseline (26.5±12.1 mg/dL) HDL-C_3 dropped significantly by 10% at CE 48 hours post exercise (23.6±11.1 mg/dL). The IE 2 trial also carried a significant 15% reduction from baseline values (26.5±12.1 mg/dL) at 48 hours post exercise (22.5±8.9 mg/dL). Finally significance was found when comparing baseline (26.5±12.1 mg/dL) to each subsequent time point for the IE 3 trial. Specifically, a 8% reduction was found for IE 3 immediately post exercise (24.2±10.1 mg/dL), IE 3 24 hours post exercise (24.2±11.3 mg/dL) and a 17% reduction for IE 3 48 hours post exercise (21.9±14.2 mg/dL). Furthermore, HDL-C_3 was found to be significantly reduced when comparing CE immediately post exercise (26.1±10.6 mg/dL) and IE 3 immediately post exercise (24.2±10.1 mg/dL).

The activity of CETP did not change over time for CE, IE 2 and IE 3 nor were there differences between treatments at any time during post exercise (Table 9).

Table 9. Cholesterol Ester Transfer Protein Activity

Treatment	**Mean ± SD (%CE transferred/4h)**			
	Baseline	**Immediately Post**	**24 hrs post**	**48 hrs post**
CE	25.4±9.1	24.2±9.2	24.6±9.3	23.9±8.9
IE 2	25.4±9.1	24.9±10.6	23.4±8.6	22.7±8.7
IE 3	25.4±9.1	25.4±11.6	23.5±11.0	23.3±11.5

Conversely, there were significant changes observed for LCATa. As can be seen in Table 10 observed during the CE trial LCATa increased by 12% from baseline (1.69±.13 μmol chol est·L^{-1}·h^{-1}) to 48 hours post exercise (1.89±.25 μmol chol est·L^{-1}·h^{-1}). A similar increase was also seen when comparing baseline activity (1.69±.13 μmol chol est·L^{-1}·h^{-1}) to IE 3 48 hours post exercise (1.89±.39 μmol chol est·L^{-1}·h^{-1}).

Table 10. Lecithin Cholesterol Acyl Transferase Activity

Treatment	**Mean ± SD (μmol chol est·L^{-1}·h^{-1})**			
	Baseline	**Immediately Post**	**24 hrs post**	**48 hrs post**
CE	1.69±0.13[a]	1.72±0.21[b]	1.83±0.29	1.89±0.25[a,b]
IE 2	1.69±0.13	1.69±0.26	1.66±0.18	1.71±0.18
IE 3	1.69±0.13[c]	1.69±0.26[d]	1.73±0.24[d,e]	1.89±0.39[c,e]

(Values sharing common superscripts are significantly different $p<0.05$)

There were no treatment by time differences observed for IE 2. No significant changes occurred immediately post exercise for any treatment. The CE trial also showed a significant 10% increase in LCATa from CE immediately post exercise (1.72±.21 μmol chol est·L^{-1}·h^{-1}) to CE 48 hours post exercise (1.89±.25 μmol chol est·L^{-1}·h^{-1}). This observation was made for the other trials including the IE 2 trial where there was a 3% increase in activity from the 24 hour post exercise time point (1.66±.18 μmol chol est·L^{-1}·h^{-1}) to the 48 hour time point (1.71±.18 μmol chol est·L^{-1}·h^{-1}) and again noted in the IE 3 trial. A significant 2% increase was found between IE 3 immediately post exercise (1.69±.26 μmol chol est·L^{-1}·h^{-1}) and IE 3 24 hours post exercise (1.73±.24 μmol chol est·L^{-1}·h^{-1}) and then again activity showed another significant 11% increase from IE 3 24 hours post exercise (1.73±.24 μmol chol est·L^{-1}·h^{-1}) to IE 3 48 hours post exercise (1.89±.39 μmol chol est·L^{-1}·h^{-1}). The data suggests that exercise, whether continuous or intermittent, was sufficient to cause LCATa to increase continuously throughout the time immediately post exercise through 48 hours post exercise.

No significant time by treatment changes for LDL peak particle size were observed (Table 11).

Table 11. LDL peak particle size

Treatment	**Mean ± SD (nm)**			
	Baseline	**Immediately Post**	**24 hrs post**	**48 hrs post**
CE	25.0±0.80	25.3±0.7	25.1±0.74	25.2±0.85
IE 2	25.0±0.80	25.1±1.0	25.2±0.87	25.0±0.92
IE 3	25.0±0.80	25.3±1.0	25.2±0.88	25.4±0.86

Correlational analysis revealed significant relationships ($p<0.05$) between LCATa and the HDL-C subfractions. Specifically, there was a significant positive correlation ($r = .62$) between LCATa and HDL-C_2 and a significant negative correlation ($r = -.57$) between LCATa and HDL-C_3. These results suggest that as LCAT activity increases the concentration of HDL-C_2 will also increase, while the concentration of HDL-C_3 decreases. Furthermore, CETP was not found to be significantly correlated to TC, LDL-C or HDL-C.

CHAPTER 5

DISCUSSION

The purpose of this study was rooted in practicality, simply designed to examine whether exercising once a day compared to two or three shorter sessions in one day was affective in modifying the lipid and lipoprotein profile. Recognizing the difficulty that people have in dedicating time to exercise each day this information should allow the implementation of a more effective exercise regime for individuals who are unable to dedicate extended time (60-90 mins.) to exercise, but could easily incorporate shorter sessions throughout the day, with a particular goal of altering lipid and lipoprotein profiles.

With that goal in mind this study proposed four main hypotheses related to whether one continuous exercise session per day or intermittent sessions (either 2 or 3 per day), keeping calorie expenditure the same, was more effective at altering the lipid/lipoprotein profile including transport enzymes. The first hypothesis posits that 450 kcal of energy expenditure would be sufficient in both continuous and intermittent groups to increase HDL-C and decrease TG. Hypothesis two stated that the change in HDL-C would be reflected as an increase in the HDL-C_2 subfraction and that the size of the LDL-C particle size would increase thereby making the LDL-C more buoyant. The third hypothesis was that all exercise protocols would be sufficient to increase LCAT activity but produce no changes in CETP activity. Finally, hypothesis four stated that these changes, regardless of protocol, would persist for up to 48 hours post exercise. As such, this discussion will be centered on relevant findings related to the previously stated hypotheses with an emphasis on integration of the mechanism of change.

The present study was the first to analyze the effects of continuous versus intermittent exercise on the HDL-C subfractions, LCTAa, CETPa and LDL-C particle size. Likewise, this is the first study to report significant changes in the HDL-C subfractions and LCATa as a result of intermittent exercise.

The Effect of Continuous versus Intermittent Exercise on TG and HDL-C

Results of this study do not support hypothesis one, in that the exercise stimulus of 450 kcal was not sufficient to produce changes in TG or HDL-C (Table 5 and 6). It is well-documented that continuous acute exercise (Crouse et al., 1995; Crouse et al., 1997; Grandjean et al., 2000) will lead to decreased TG concentrations while HDL-C concentrations increase. The findings of this study were similar to those in another documented intermittent study by Mestek et al. (2006). Mestek et al. (2006) found no changes in TG concentration however, they did report an increase in HDL-C but only after three intermittent exercise sessions, which was also similar to Ebisu (1985). No explanation is offered by the authors of these studies (Mestek et al 2006; Ebisu 1985) as to the lack of change in TG. It is however plausible to suggest that these findings (Mestek et al., 2006; Ebisu 1985) along with the results of the present study may be related to the timing of blood collection to the ingested meal. Since blood was collected in both the present study and the Mestek et al. (2006) study after four to six hours of fasting the remnants of the meal may have influenced the TG concentration. TG levels may have still been elevated prior to the exercise sessions contributing to the non-significant decline seen in this study (Table 5). Research examining the effects of exercise on TG clearance in the postprandial state suggest that TG clearance is most

effective when exercise is done from 16 hours before a meal to 1.5 hours after a meal, is of moderate intensity and is a minimum of 500 kcals or greater (Katsanos, 2006). The present study only fulfills one of the three requirements, exercising at a moderate intensity, to optimize TG clearance in the postprandial state and furthermore, was not designed to meet those requirements.

HDL-C concentrations for the three exercise sessions (IE 3) were unaltered in this study unlike previous research (Ebisu, 1985; Mestek et al., 2006). However, the present study is similar to those studies in that HDL-C concentrations did not change with the continuous exercise bouts. At present there is no mechanism to explain why this occurred with the HDL-C and further why it seemed to only occur in the three exercise session trial. It is possible that HDL-C may be influenced by food intake and subsequent timing of blood draw, however this has not been examined and may provide an area of future research.

HDL-C Subfraction and LDL-C Particle Size Response to Continuous and Intermittent Exercise

Whether exercise was continuous or intermittent, 450 kcals of energy expenditure was sufficient to increase concentrations of HDL-C_2 at 48 hours post exercise for all the trials (31% for CE, 31% for IE 2, 46% for IE 3), while HDL-C_3 was significantly decreased during these times. In addition, HDL-C_2 was also significantly increased immediately post (13%) and 24 hours (13%) after the IE 3 exercise session (Figures 2 and 3).

Changes in the HDL-C subfractions are well documented after continuous acute exercise (Crouse et al., 1995, Crouse et al., 1997, Grandjean et al., 2000). Typically, acute exercise is responsible for an increase in HDL-C_3 amongst untrained individuals, but is not always the case (Crouse et al., 1995). Crouse et al. (1995) reported a non-significant 27% increase in HDL-C_2 concentrations 48 hours after a single 350 kcal bout of exercise. It was suggested that in these hypercholesterolemic men there may be a time delay necessary to alter lipid metabolism. Similar to the present study both the continuous and IE 2 session trials showed a significant increase in HDL-C_2 at 48 hours and the IE 3 session trial showed increases immediately post exercise as both 24 and 48 hours. The exercise stimulus in this study was 100 kcal greater than Crouse et al (1995) reported and perhaps the distribution of the exercise stimulus over the course of the day contributed to the changes seen in the IE 3 trial. This was supported by the result that found HDL-C_2 concentrations significantly higher at the IE 3 immediately post exercise value compared to the CE immediately post exercise value. It seems however that 450 kcals whether expended in one continuous bout or divided over intermittent sessions throughout the day elicit increases in HDL_2 at 48 hours. Furthermore, three intermittent sessions throughout the day show significant differences in immediately post exercise and 24 hours post exercise as well.

The lack of change in LDL-C particle size is perhaps due in part to the low caloric expenditure in this study. Most documented changes in LDL-C particle size have been observed with high levels of total energy expenditure and also after prolonged exercise in highly trained subjects (Lamon-Fava et al., 1989). Lamon-Fava et al. (1989) reported that in subjects who completed an endurance triathlon the change seen in LDL particle size was associated with a decline in TG concentrations. It was hypothesized that decreases in plasma TG levels allow for a compositional modification of the LDL-C

particle. The increase in lipolysis via LPL would decrease the TG content of the core of the LDL-C particle making it larger and less dense. There were no changes in TG concentrations in the present study and therefore the size of the LDL-C particle would not alter its composition or density. Currently, there are no studies that report a caloric expenditure threshold for LDL-C particle size change in untrained individuals. Furthermore there is no previous research on the effects of intermittent exercise on LDL-C particle size. Given this information it is difficult to compare the results of this study to those of others, it seems reasonable to conclude however that the 450 kcal stimulus in this study was not sufficient to alter LDL-C particle size.

The Role of Continuous and Intermittent Exercise on LCATa and CETPa

The results of the present study showed that exercise, whether continuous or intermittent, was sufficient to increase LCATa 48 hours post exercise for the CE (12%) and IE 3 (12%) trials (Table 8). Furthermore, LCATa for IE 2 increased by 3% from 24 hours to 48 hours post exercise and IE 3 continually increased from immediately post exercise by 2% at 24 hours and 11% at 48 hours post exercise. Additional analysis revealed a significant and positive correlation ($r = 0.62$) to the HDL-C_2 subfraction as well as a significant negative correlation ($r = -0.57$) to the HDL-C_3 subfraction. The results of the correlation analysis suggest that as LCATa increases HDL-C_2 also increases and furthermore, when LCATa increases HDL-C_3 will decrease, thereby supporting the notion that LCATa directly influences the amount of cholesterol influx to the HDL-C core allowing for the conversion of HDL-C_3 to HDL-C_2.

Furthermore, the lack of change seen with CETPa also supports this hypothesis in that Pearson product moment correlations revealed a small non-significant relationship between CETPa and HDL-C_2 ($r = -0.26$) thereby suggesting that the activity of this enzyme does not apparently influence HDL-C subfraction distribution.

It has been shown that both exercise training (Taskinen et al., 1981) and acute exercise (Dufaux et al., 1986, Frey et al., 1991) can increase LCATa which will facilitate an increase in HDL-C_2 and further regulate the HDL-C_2:HDL-C_3 ratio. LCAT in the presence of apolipoprotein A-I, esterifies free cholesterol into a cholesterol ester that is moved into the HDL-C_3 core. This reaction causes a chemical gradient that generates a cholesterol supply for LCAT. Additional cholesterol supply fuels the internalization of these cholesterol esters into the HDL-C_3 core and as a result when the core expands it is converted to the HDL-C_2 particle (Durstine et al., 2002). Results of this study support those of the previous studies and suggest that the changes in HDL-C_2 and HDL-C_3 are due to the significant increases in LCATa. The observed changes in HDL-C subfractions could also be due to changes in the activity of the lipid transport enzymes. While HL and LPL were not measured in the present study the activity of HL has been shown the decrease after acute exercise (Gordon et al., 1994). It seems likely decreased HL activity contributed to the observed increase in HDL_2 in circulation. As a further result, HDL_2 to HDL_3 ratio becomes altered due to decreased HL and lessens the conversion of HDL-C_2 to HDL-C_3 at the liver.

The results of this study were similar to previous research in that no changes in CETPa were found following an acute bout of exercise (Grandjean et al., 2000). Again, since the role of CETP has not been fully researched it is hard to speculate why changes do not occur although it has been suggested that possible influential factors may include TG composition of VLDL-C (Bagdade et al, 1991). However the CETP concentrations

in this study fall within normal range as reflected by normal HDL-C levels. Furthermore, research has not examined whether baseline enzyme levels play a role in their ability to become modified as a result of an exercise intervention. Regardless, the correlation analysis revealed that there were no significant relationship between CETPa and the lipid/lipoprotein variables suggesting that the activity of this enzyme was not critical in regulating the changes observed in the HDL-C subfractions.

Intermittent Study Comparisons

There have only been a few chronic studies examining a similar protocol, continuous versus intermittent exercise (Ebisu, 1985; Snyder et al., 1997; Woolf-May et al., 1998; Woolf-May et al., 1999) and only one examining the effects of acute exercise in a continuous bout versus intermittent bouts (Mestek et al. 2006). Similar to all of these previous studies no changes in TG were seen in any exercise intervention across time. This may be a result of several different factors including inconsistent reporting of dietary intake and low calorie expenditure as seen in the Woolf-May studies (1998, 1999) or the differences in subject characteristics as seen in the Ebisu study (1985). TG are influenced by diet and typically are measured after a 12-hour fast, despite control over diet like in the current study and the Mestek study (2006), it was simply not possible to get blood TG measurements during intermittent sessions over the course of one day allowing for a 12-hour fast. Therefore, TG concentrations may have been influenced by previous food consumption. It is however important to mention that in the present study there is a non-significant decline in TG concentration in all three trials from the immediate post exercise reading to the 48 hour post exercise reading (see Table 5).

In addition, the results of this study are similar to those of Mestek et al. (2006) who reported no changes in TC or LDL-C as a result of the exercise intervention. These results are also supported by the vast body of acute continuous exercise literature that also shows no significant differences in TC and LDL-C (Davis et al., 1992; Durstine et al., 1996; Durstine et al., 1983; Ferguson et al., 1998; Kantor et al., 1984). The subject population in this study could generally be described as normocholesterolemic and as reported by the health history questionnaire had no underlying metabolic conditions that contribute to high cholesterol. Given these characteristics a single exercise session may not influence an apparently normal cholesterol profile. TC and LDL-C changes are typically observed when dietary interventions are made, such as reducing fat intake or when a reduction in body composition occurs (Durstine et al., 2001). The present study did not require weight loss or a change in the participants' diet, therefore we did not expect nor see changes in TC or LDL-C.

The difference between the present study and the other intermittent studies, particularly Ebisu (1985) and Mestek et al (2006) is that the present study reported no significant changes in total HDL-C values at the three exercise session time point (see Table 6). Both Ebisu (1985), utilizing a chronic protocol and Mestek et al (2006) utilizing an acute protocol found significantly higher HDL-C values for those participants in their three exercise session group. Neither research group can provide an explanation for this finding merely reporting that it exists. The present study did demonstrate a very small non-significant increase (8%) in HDL-C values but not comparable to those of Ebisu (1985) or Mestek et al. (2006). HDL-C has been shown to change with acute continuous exercise in hypercholesterolemic men after expending 350 kcals and normocholesterolemic men after expending 500 kcals (Crouse et al., 1995, 1997;

Grandjean et al., 2000), it is possible to speculate that perhaps the 450 kcals in the present study may be below the necessary threshold of energy expenditure for change in a normocholesterolemic population. It is interesting that in the present study total HDL-C did not change but the subfraction concentration did. One possibility for this finding is that total HDL-C concentrations are governed not only by the enzymes related to lipid transport but also by other mediators of reverse cholesterol transport such as transporter proteins and receptors whereas the subfraction concentrations in circulation may be more susceptible to the enzymes also present in circulation contributing to the changes seen in the present study.

Summary and Conclusions

Specifically, this study indicates that IE 3 produced greater changes in the HDL-C subfractions compared to CE or IE 2. In addition, LCATa values seemed to increase in both CE and IE 3 compared to IE 2. It can be speculated that perhaps performing three sessions over the course of one day, despite energy expenditure remaining constant, provides an "all-day" stimulus that allows the body to carry out lipid/lipoprotein metabolism more efficiently than one or even two sessions alone. The IE 3 session was designed to expend 150 calories every 4 to 5 hours and was timed around meals. This may suggest that eating and exercising at set intervals may allow for enhanced HDL-C metabolism that is facilitated by an increase in LCATa. Most importantly from a practical standpoint the changes in HDL-C subfractions and LCATa are seen in all three interventions suggesting that if an individual is looking to alter the lipid/lipoprotein profile it can be done employing various types protocols.

Future Research

The results seen not only in this study but other intermittent lipid studies warrant further research designed to replicate the findings as well as continue to examine the effects of intermittent exercise on other markers of health. There is still no intermittent data on the effects of the lipase (HL and LPL) enzymes, which may help to provide a rationale for why three sessions appear to be better than one session. Further, the concept of a threshold is a very important underlying variable that still needs to be evaluated not only in the acute continuous exercise research but now within the intermittent data as well. This can further help clinicians to provide more accurate exercise prescriptions for their clients or patients based on specifics such as gender, cholesterol status, and even fitness level. It would be important to conduct this study using a female population to see if the effects are similar between men and women so that gender specific exercise prescriptions can be made. Finally, the literature on baseline HDL-C levels and how they are affected by exercise stimuli are still vague, research designed to look at intermittent exercise stimuli and participants with varying HDL-C levels is also warranted.

APPENDIX A

Study Recruitment Flyer

EXERCISE STUDY!!!
PARTICIPANTS NEEDED!!!

You can participate if you ARE:

♦MALE
♦between 18-35 years old
♦a NON-smoker
♦NOT participating in regular exercise

BENEFITS:

♦FREE Blood test
- ♥Cholesterol
- ♥Triglycerides
- ♥Low Density Lipoprotein
- ♥High Density Lipoprotein

♦FREE diet analysis
♦FREE fitness test

CALL or E-MAIL SARA CHELLAND at 850-443-4843 or schelland@yahoo.com

APPENDIX B

Human Subjects Approval and Informed Consent

Florida State
UNIVERSITY

Office of the Vice President For Research
Human Subjects Committee
Tallahassee, Florida 32306-2763
(850) 644-8633 · FAX (850) 644-4392

APPROVAL MEMORANDUM

Date: 12/3/2004

To:
Sara Chelland
MC 1493

Dept.: **NUTRITION FOOD AND MOVEMENT SCIENCES**

From: **John Tomkowiak, Chair** John Tomkowiak M.D.

Re: **Use of Human Subjects in Research**
The effects of an Acute Bout of Continuous versus Accumulated Exercise of Isocaloric Energy Expenditure on blood lipids, lipoproteins and related enzyme activities

The forms that you submitted to this office in regard to the use of human subjects in the proposal referenced above have been reviewed by the Human Subjects Committee at its meeting on **11/10/2004**. Your project was approved by the Committee.

The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals which may be required.

If the project has not been completed by **11/9/2005** you must request renewed approval for continuation of the project.

You are advised that any change in protocol in this project must be approved by resubmission of the project to the Committee for approval. Also, the principal investigator must promptly report, in writing, any unexpected problems causing risks to research subjects or others.

By copy of this memorandum, the chairman of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols of such investigations as often as needed to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

This institution has an Assurance on file with the Office for Protection from Research Risks. The Assurance Number is IRB00000446.

cc: R.J. Moffatt
HSC No. 2004.772

Florida State
UNIVERSITY

Office of the Vice President For Research
Human Subjects Committee
Tallahassee, Florida 32306-2763
(850) 644-8633· FAX (850) 644-4392

REAPPROVAL MEMORANDUM

Date: 10/26/2005

To:
Sara Chelland
MC 1493

Dept.: **NUTRITION FOOD AND MOVEMENT SCIENCES**

From: **Thomas L. Jacobson, Chair**

Re: **Reapproval of Use of Human subjects in Research:**
The effects of an Acute Bout of Continuous versus Accumulated Exercise of Isocaloric Energy Expenditure on blood lipids, lipoproteins and related enzyme activities

Your request to continue the research project listed above involving human subjects has been approved by the Human Subjects Committee. If your project has not been completed by 10/18/2006 please request renewed approval.

You are reminded that a change in protocol in this project must be approved by resubmission of the project to the Committee for approval. Also, the principal investigator must report to the Chair promptly, and in writing, any unanticipated problems involving risks to subjects or others.

By copy of this memorandum, the Chairman of your department and/or your major professor are reminded of their responsibility for being informed concerning research projects involving human subjects in their department. They are advised to review the protocols of such investigations as often as necessary to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

Cc: R.J. Moffatt
HSC No. 2005.754-R

All subjects who sign an informed consent will be assigned a code. Information for subjects will be kept within a personal folder identified by their respective codes. A master list will be kept in a locked filling cabinet in Dr. Robert J. Moffatt's office (406 Sandels Building) and will be the only way to link coded subject information to the subject. Following completion of the project, the principle investigators will keep all files in a locked file drawer (406 Sandels Building) for a period of five years, at this time the data will be destroyed.

6. **IS THE RESEARCH AREA CONTROVERSIAL AND IS THERE A POSSIBILITY YOUR PROJECT WILL GENERATE PUBLIC CONCERN? If SO, PLEASE EXPLAIN.**

No controversy is anticipated over this research.

7. **DESCRIBE THE PROCEDURE TO BE USED FOR SUBJECT DEBRIEFING AT THE END OF THE PROJECT. IF YOU DO NOT INTEND TO PROVIDE DEBRIEFING, PLEASE EXPLAIN.**

Data collected will not be discussed with subjects until they have completed the project. Following completion the subjects will be provided with all information and a detailed explanation of the results. Subjects will have the option of providing a mailing address where their results can be sent following analysis of the data.

INFORMED CONSENT FORM

I, _____ freely and voluntarily and without any element of force or coercion, consent to be a participant in the research project entitled "The effects of an Acute Bout of Continuous versus Accumulated Exercise of Isocaloric Energy Expenditure on blood lipids and lipoproteins and the lipid related transport enzyme activities." This research is being conducted by Sara Chelland, a graduate student in exercise physiology under the supervision of Dr Robert J. Moffatt at Florida State University. I understand that physical activity has a variety of positive health benefits on the lipid profile. I understand the purpose of the research project is to determine if two or three intermittent exercise session has the same effect as one continuous exercise session on lipids, lipoproteins and lipid transport related enzymes. Secondly, if any changes occur, how long do they persist after the last exercise session?

I understand that I will be asked to report to the laboratory on four separate occasions. The first lab visit will consist be a familiarization where I will be asked to sign an informed consent form, provide a basline blood draw and perform a treadmill maximal oxygen consumption test. I understand that I will then be asked to perform three separate trials each consisting of exercise at 65% of the previously established VO_2 max, to expend 600 kcals. The three trials will consist of 1) a single continuous exercise session to expend 600 kcals, 2) two intermittent exercise sessions designed to expend 300 kcals/session for a total of 600 kcals and 3) three intermittent exercise sessions designed to burn 200 kcals/session for a total of 600 kcals. The intermittent sessions will take place a minimum of four hours apart, with the subjects being encouraged to eat immediately after each session and then not again until after the next session. **Prior to the start of each collection period a baseline blood draw will be performed. Blood draws will be taken immediately post exercise, 24 and 48 hours post exercise. The total time commitment to the study, including post exposure blood draws will vary by day depending on the trial, but in total will require of twenty (20) hours of laboratory time.**

I understand that the benefits I will get from participating in this project will include: 1) total cholesterol value, 2) high-density lipoprotein value, 3) low-density lipoprotein levels, and 4) assessment of my dietary practices. I understand that at the conclusion of the investigation all my information and the group results will be made available to me upon request.

I understand the risks associated with performing a maximal exercise test or exercise sessions include fainting, irregular heart beats, and in rare instances heart attack or death. I also understand that it is my responsibility to report my health status, changes in status during exercise or any other feelings of discomfort to aid the investigator in providing you with the proper attention. The Thagard Student health center phone number is 644-8055.

I understand that the risks associated with venipuncture (blood sampling) include the possibility of fainting, bruising of the skin, and local infection (if the puncture site is not kept clean). I also understand that heparin will be infused into my blood, which may cause bleeding or stimulate an allergic reaction, in order to study the activity of enzymes related to lipid metabolism. The risk of bleeding from a heparin infusion is small since IV heparin is fairly short acting and that allergic reactions to heparin are extremely uncommon. The total amount of blood being collected over the course of the entire study will be 30 milliliters (mL) and that this can be replenished naturally by the body. I understand that these risks are minimal, as the principal investigators have been trained to draw blood using correct sterile techniques and that a licensed nurse will be present during the heparin infusions. Additionally I understand that I may experience some pain as the needle pierces my skin. The principal investigators will attempt to minimize the possibility of injury or risk through detailed explanation of procedures and keen attention to my well-being.

I understand that the information obtained in this investigation will be regarded as privileged and confidential to the full extent allowed by law. All my results and information will be kept confidential and identified by a subject code. The link between the code and my information will be properly stored in Dr. Moffatt's office in a cabinet during the investigation period. All data will be destroyed within five (5) years upon the completion of this project. My name will not appear on any of the results. No individual responses will be reported. Only group findings will be reported.

I understand there is a minimal level of risk involved if I agree to participate in this investigation. I am able to stop my participation at any time I wish without prejudice, penalty or loss of benefits to which I am otherwise entitled. I have been given the right to ask and have answered any inquiry concerning this investigation. Questions if any, have been answered to my satisfaction.

I understand that I may contact Sara Chelland at (850) 443-3843 or **Dr Robert J. Moffatt at (850) 644-1520** for answers to any questions about this research or my rights. For additional information regarding your rights or concerns as a research subject, please contact the Florida State University Human Subjects Committee at (850) 644-8633. Most results, however, cannot be made available during participation in this study, as they may influence the outcome of following tests.

I have read and understand this consent form. The nature of this study, its possible risks, and the right to withdraw at any time without prejudice has been explained to me in the presence of the principal investigator.

(Subject)

(Date)

APPENDIX C

Health History Form

HEALTH HISTORY
(Long Form)

Name:	Age:
Address:	Sex:
Telephone Nos. (daytime):	(nighttime):
Current Weight:	Desired Weight:
Personnel Physician:	
Physician's Address:	

Directions: Please answer the following questions to the best of your knowledge about yourself. Check below any medical condition, treatment or problems that concern you.

I. HEART and CIRCULATORY

A. _____ Heart Attack, Heart disease or any other heart related problems
B. _____ Heart Valve Problems
C. _____ Heart Murmur
D. _____ Enlarged Heart
E. _____ Irregular Heart Beat
F. _____ Atherosclerosis
G. _____ Stroke
H. _____ High Blood Pressure (controlled)
I. _____ High Blood Pressure (uncontrolled)
J. _____ Rheumatic Fever
K. _____ Cardiac Surgery
L. _____ Coronary Bypass
M. _____ High Triglyceride Level
N. _____ High Cholesterol Level
O. _____ Varicose Veins
P. _____ Anemia
Q. _____ Hemophilia
R. _____ Diabetes (controlled)
S. _____ Diabetes (uncontrolled)
T. _____ Phlebitis, Emboli (blood clots)
U. _____ Other, Specify _____

II. RESPIRATORY

A. _____ Emphysema
B. _____ Bronchitis
C. _____ Pneumonia
D. _____ Asthma: _____ (childhood) _____ (currently)
E. _____ Lung Disease
F. _____ Other, Specify _____

III. OTHER DISEASE or ALIMENTS

A. _____ Back Injuries/Back Pain
B. _____ Epilepsy/Seizures (past or present)
C. _____ Allergies
D. _____ Liver Disease (Hepatitis, Jaundice)
E. _____ Kidney Disease
F. _____ Arthritis
G. _____ Orthopedic Leg, Arm or Joint Problems
H. _____ Neurologic Diseases
I. _____ Migraine Headaches/Other Frequent Headaches

Please explain any conditions you checked YES in I-III above:

_____
_____
_____
_____

IV. HAVE YOU RECENTLY HAD:

A. _____ Chest Pain
B. _____ Shortness of Breath Upon Exertion
C. _____ Heart Palpitations
D. _____ Cough on Exertion
E. _____ Cough Up Blood
F. _____ Swollen, Stiff or Painful Joints
G. _____ Dizziness
H. _____ Lightheadedness
I. _____ Fainting
J. _____ Back Problems
K. _____ Gastrointestinal Disturbances (nausea, vomiting, diarrhea, abdominal pains)

Please explain any conditions you checked in IV above:

_____
_____
_____
_____

V. FAMILY MEDICAL HISTORY (Immediate Relatives)

A. _____ Heart Attack, Heart Disease or other heart related problems
B. _____ Stroke
C. _____ Atherosclerosis
D. _____ High Blood Pressure
E. _____ Diabetes
F. _____ Lung Disease
G. _____ Respiratory Problems
H. _____ Heart Surgery or
I. _____ Heart Related Surgery
J. _____ Other, Specify: _____

VI. TOBACCO

A. Do you currently smoke or use tobacco products? _____ Yes _____ No

B. What type? _____ Cigarette
_____ Pipe
_____ Cigar
_____ Chewing tobacco

C. How long? _____

D. Amount smoked per day? _____

E. If you do not currently smoke, have you ever? _____ Yes _____ No

F. If YES, how long ago did you quit? _____

VII. EXERCISE

A. Do you exercise? _____ Yes _____ No

B. What kind of exercise do you presently engage in? _____
_____
_____

C. Is your level of effort: _____ minimal _____ moderate _____ high

D. How often do you exercise? _____ days per week

E. How long do you exercise? _____ minutes per day

Please list any prescription medications, vitamin/nutritional supplements, over-the-counter medications you are currently Taking or have taken in the last 7 days (don't forget to include birth control pills, headache/migraine medications, etc.):
_____
_____
_____

Please describe your present medical condition and anything we should be aware of concerning your health:
_____
_____
_____
_____

Date of last physical examination? _____ Results: _____
Date of last EKG _____ Results: _____

I certify that my responses to the foregoing questionnaire are true, accurate and complete:

Signature: _____ **Date:** _____

Signature of Parent/Guardian: _____ **Date:** _____
(required for participants under 18 years of age)

APPENDIX D

Anthropometric Data Sheet

ANTHROPOMETRIC DATA SHEET

Subject Name_____ Subject ID#_____

Age_____ Height(m)_____ Weight(kg)_____

BMI_____ Body fat %_____

	CHEST	AXILLA	TRICEPS	SUBSCAPULAR	ABDOMINAL	SUPRAILIAC	THIGH
TRIAL 1							
TRIAL 2							
TRIAL 3							
SUM							
AVERAGE							

RESTING DATA

Heart Rate _____

Blood pressure _____

APPENDIX E

Maximal Exercise Test Data Sheet

Subject Name_____ Subject ID Number _____

Age _____ Gender _____ Weight (kg) _____

Time (min)	Grade (%)	Pace (mph)	VO_2 (ml/kg/min)	VO_2 (L/min)	VCO_2 (L/min)	RER	HR (bpm)	RPE

Maximal Values:

HR _____ VO_2 (mL/kg/min)_____ VE (L/min)_____ RER_____

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BIOGRAPHICAL SKETCH

EDUCATION

8/01 – 4/07	The Florida State University, Tallahassee, FL PhD. Exercise Physiology Completed April 2007
DISSERTATION TOPIC:	The Effects of an Acute Bout of Continuous versus Accumulated Exercise of Isocaloric Energy Expenditure on Lipids, Lipoproteins and Lipid-related Transport Enzymes
8/99 – 8/01	Bloomsburg University, Bloomsburg, PA MS. Exercise Physiology
THESIS	The Effects of Fixed-ratio (FR) Schedules of Reinforcement on Cycle Ergometry
8/95 – 8/99	Bloomsburg University, Bloomsburg, PA BS. Exercise Physiology, Minor in Biology

GRADUATE ASSISTANTSHIPS

8/99 – 5/00 Exercise Science, Department of Health and Physical Education, Linda LeMura, PhD., FASCM, advisor.

Responsibilities:

Guest lecturing (Exercise Physiology, Fitness & Wellness, and Exercise & You), preparation of class materials, conduct exercise laboratories, proctor exams and grade papers/exams.

5/00 – 8/00 Anatomy and Physiology Laboratory, Department of Biology, c/o Margaret Till, PhD., graduate coordinator.

Responsibilities:

Set up of laboratories, preparation of solutions and laboratory practical exam set-up.

8/00 – 5/01 Exercise Science, Department of Health and Physical Education, Linda LeMura, PhD., FACSM, advisor.

Responsibilities:

Guest lecturing (Exercise Physiology and Exercise & You), preparation of class materials, conduct exercise laboratories, proctor exams and grade papers/exams.

8/01 – 4/06 Nutrition, Food and Exercise Science. Dr. Robert J. Moffatt, PhD., advisor.

Responsibilities:

Teach 3 credit units per semester. This consists of lecturing, preparation of class materials, conducting laboratory experiments, proctoring exams and grading papers. In addition, the other primary responsibility is to conduct research, write grants, IRB proposals and interact with subjects and lab procedures.

PRACTICUM

01/01 – 05/01 United States Olympic Committee
U.S. Olympic Training Center, Lake Placid, New York
Advisor: Dr. Ken Rundell

PUBLICATIONS

LeMura, L.M., Andreacci, J.L., Carlonas, R., Klebez, J.M. and **Chelland, S.A.** (2000). Evaluation of physical activity measured via accelerometry in rural fourth grade children. *Perceptual and Motor Skills,* 90: 329-337.

LeMura, L.M., von Duvillard, S.P., Andreacci, J.L., Klebez, J. M., **Chelland, S.A.**, and Russo J. (2000). Lipid and lipoprotein profiles, cardiovascular fitness, body composition, and diet during and after resistance, aerobic, and combination training in young women. *European Journal of Applied Physiology,* 82: (5-6), 451-458.

LeMura L.M., von Duvillard S.P., Cohen S.L., Root C.J., **Chelland S.A.**, Andreacci J.L., Hoover J. and Weatherford J. (2001). Treadmill and cycle ergometry testing in 5- to 6-year-old children. *European Journal of Applied Physiology.* 85(5):472-478.

LeMura, L.M., Andreacci, J.L., Russo, J. and **Chelland, S.A.** (2001). A meta-analytic review of exercise prescription components, absolute, and relative measures of functional capacity in older individuals. *Journal of Clinical Exercise Physiology*. 3(2).

Cohen, S.L., **Chelland, S.A.,** Ball, K.T., LeMura, L.M. (2002). Effects of Fixed Ratio Schedules of Reinforcement on Exercise in College Students. *Perceptual and Motor Skills.* 94:1177-1186.

Andreacci, J.L., LeMura, L.M., Urbanski, E.A., Cohen, S.L., von Duvillard, S.P., and **Chelland, S.A.** The Effects of Frequency of Encouragement on Performance during Maximal Exercise Testing. (2002). *Journal of Sports Sciences*. 20(4):345-52.

Moffatt, R.J., **Chelland, S.A,** Pecott, D.L., Stamford, B.A. (2004). Acute Exposure to Environmental Tobacco Smoke Reduces HDL-C and HDL_2-C. *Preventive Medicine*. 38:637-641

Moffatt, R.J., **Chelland, S.A**. (2004). Carnitine. In: Nutritional and Ergogenic Aids. Eds. Ira Wolinsky and Nancy Driscoll. CRC Press.

Moffatt, R.J., **Chelland S.A.** (2004). Exercise. In Nutrition and Well-Being A to Z. Ed. Delores C.S. James. Vol. 1. New York: Macmillan Reference USA. p198-201.

Moffatt, R.J., **Chelland, S.A.,** Stamford, B.A. Smoking, Heart Disease, and Lipoprotein Metabolism. (2005). In Lipid Metabolism and Health. Eds. Robert J. Moffatt and Bryant A. Stamford. CRC Press.

Austin, K.G., **Chelland, S. A.,** Cowman, J., Daigle K. (2005). Reliability of near infrared spectroscopy for determination of muscle oxygen saturation during exercise. Research Quarterly in Exercise and Sport, 76(4);440-449.

ARTICLES IN REVIEW

Campbell, S.C., Moffatt, R.J., Stamford, B.A. Smoking and Smoking Cessation: The Relationship between Cardiovascular Disease and Lipoprotein Metabolism: A Review. American Journal of Public Health, In Review

PRESENTATIONS

Chelland, S.A., Root, C., Andreacci, J.L., Klebez, J.M. and LeMura, L.M., FACSM. Maximal treadmill and cycle ergometry testing in 5-6 year old children: Variability of responses. Presented at *Mid-Atlantic Regional Meeting of the ACSM* in Ithaca, NY. Thematic poster session. November 1999.

Chelland, S.A., Russo, J., Andreacci, J.L., Kelbez, J.M. and LeMura, L.M., FACSM. Absolute versus relative measure of functional capacity on older individuals: Meta-analytic review of exercise prescription. Presented at *Mid-Atlantic Regional Meeting of the ACSM* in Ithaca, NY. Thematic poster session. November 1999.

Chelland, S.A. What type of exercise burns fat best? Presented at the *Third Annual Women's Conference* at Bloomsburg University. April 2000.

Chelland, S.A., von Duvillard, S.P., FACSM, Cohen, S.J., Andreacci, J.L., Root, C., and Weatherford, J, sponsored by Linda LeMura, FACSM. Maximal treadmill and cycle ergometry testing in 5-6 year old children: Variability of responses. Presented at *2000 National ACSM Conference* in Indianapolis, IN. May/June 2000.

Chelland, S.A., Andreacci, J., Kelbez, J., Russo, J. LeMura, L.M. and von Duvillard, S.P. Lipid profiles and cardiovascular fitness during and after resistance, aerobic and combination training in women. Presented at the *2000 Mid-Atlantic meeting of the ACSM* in Split Rock, PA. November 3rd and 4th, 2000.

Chelland, S.A., Andreacci, J., Kelbez, J., Russo, J. LeMura, L.M. and von Duvillard, S.P. Lipid profiles and cardiovascular fitness during and after resistance, aerobic and combination training in women. Presented at the *2001 National ACSM Conference* in Baltimore, Maryland.

LeMura, L.M., **Chelland, S.A.**, Root, C., Andreacci, J.L., Mazekas, M., Strohecker, K. and von Duvillard, S.P. Maximal and submaximal exercise responses during cycle and treadmill testing in 5-6 year old children. Presented at the *2001 National ACSM Conference* in Baltimore, Maryland.

Judelson D.A., Smith, S.L., **Chelland S.A.**, and Rundell K.W. Uphill cross country skiing as a determinant of overall performance in elite nordic combined athletes. Presented at the *Sixth International Olympic Committee World Congress on Sport Sciences* in Salt Lake City, UT. September 16-21, 2001.

Chelland, S.A., Cohen, S.L., LeMura, L.M., Ball K.T. The Effects of Reinforcement on Cycle Ergometry. Presented at the *2002 Florida State University Research and Creativity Day.*

Chelland, S.A., Cohen, S.L., LeMura, L.M., Ball, K. T. The Effects of Reinforcement on Cycle Ergometry. Presented at the *2002 National ACSM Conference* in St. Louis, Missouri.

Chelland, S.A., Moffatt, R.J., Pecott, D., Stamford B. The acute influence of Environmental Tobacco Smoke on HDL-C and its Subfractions in Nonsmokers. Presented at the *Florida State University Lipids Symposium*, February 20th and 21st, 2003.

Austin, K.G., **Chelland, S. A.,** Cowman, J., Daigle K. Reliability of near infrared spectroscopy for determination of muscle oxygen saturation during exercise. Presented at the *2003 National ACSM Conference* in San Francisco, California.

Greer, B.K., **Chelland, S.A.,** Bograd, B., Moffatt, R.J. The Effect of Repeated Bouts of Exhaustive Endurance Exercise on Blood Lipid and Lipoprotein Profiles. Presented at the *2004 ACSM National Conference* in Indianapolis, Indiana.

Chelland, S.A. The Effects of an Acute Bout of Continuous versus Accumulated Exercise of Isocaloric Energy Expenditure on Lipids, Lipoproteins and Lipid-related Transport Enzymes. Presented at the *2005 Florida State University College of Human Sciences Research and Creativity Day* in Tallahassee, Florida.

Greer, B.K., Bograd B., **Chelland, S.A.**, Austin, K.A., Moffatt, R.J. Markers of Myocardial Damage after Prolonged Exercise Endurance. Presented at the *2005 ACSM National Conference* in Nashville, Tennessee.

GRANTS

Andreacci, J.L., Klebez, J.M. and **Chelland, S.A.** *Bloomsburg University graduate students to present at the 2000 American College of Sports Medicine (ACSM) Conference in Indianapolis, Indiana.* Awarded a Bloomsburg University Foundation Grant ($375.00), Bloomsburg University, April 2000.

Chelland, S.A. Gatorade Sports Science Institute Grant. The Effects of an Acute Bout of Continuous versus Accumulated Exercise of Isocaloric Energy Expenditure on Lipids, Lipoproteins and Lipid-related Transport Enzymes. Awarded October 19, 2004; $1500.00**.**

Chelland, S.A Florida State University Dissertation Grant. Awarded October 19, 2004; $500.00

AWARDS

Nominated for the 2004 Outstanding Teaching Assistant Award (OTAA)
Nominated for the 2004 University Leadership Award
CHS Research and Creativity Day Presenter (’02,’03, ’04, ‘05)
Hortense Glenn Honor Society – College of Human Sciences (FSU)
Chancellor’s National Honors Society List

LEADERSHIP AND SERVICE

Vice-President of the College of Human Sciences Graduate Student Advisory Council (CHS-GSAC). Responsibilities include preparation of monthly colloquiums, and assistance in the preparation of CHS functions for graduate students. Provide leadership and council for graduate students within the college.

Moffatt, R.J. and **Chelland, S.A.** Lipids and Lipoproteins, Physical Activity and Diet: Implications for Health. Symposium held at Florida State University, February 20th and 21st, 2003. Major responsibility: Planning and coordinating the event.

Member of the Centennial Committee designed to organize and run events associated with the College of Human Sciences 100th Anniversary.

Vice-President of the Tallahassee Adult Soccer Association Executive Board (2004-2006).

PROFESSIONAL ASSOCIATIONS

American College of Sports Medicine (ACSM)
Southeast Regional Chapter ACSM (SEACSM)
American Physiological Society