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The purpose of this study was to compare the effects of a single session of exercise versus accumulated sessions of exercise on plasma lipid and lipoprotein concentrations. Twelve young (22.5±2.5 years, mean±SEM), overweight (body mass index=29.7±3.9 kg·m^{-2}), sedentary participants performed, in random order, a single exercise session (treadmill exercise at 60% maximal oxygen consumption for 90 minutes), accumulated exercise sessions (the same protocol as above for three consecutive days), and a control session (no exercise for six consecutive days). Blood samples taken immediately before each exercise/control session, and at 24, 48 and 72 after exercise were analyzed for high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations. Data were analyzed using a repeated measures ANOVA, based upon *a priori* analyses (i.e., pre versus post-exercise time points) both within and across protocols. Significant interactions and main effects were analyzed using post-hoc paired samples T-tests. Statistical significance was set at the 0.05 level.

The three exercise session protocol produced significant elevations in HDL-C concentrations 24 and 48 hours post-exercise, compared to the control. Additionally, there was a significant time by trial interaction for HDL-C, with concentrations elevated at 48 hours post-exercise for the three exercise session protocol compared to the single session. A significant percentage change reduction in TG concentration was observed after the three exercise session protocol compared to the control. These results suggest that, in young, overweight, sedentary individuals, accumulating three consecutive 90-

minute sessions of exercise is more effective in improving plasma HDL-C and TG concentrations when compared to a single session of exercise. In summary, these results support the additive benefits of exercise and justifies future exercise training studies aimed at determining the exact manner in which to accumulate exercise sessions (e.g., every day, every other day, etc.) in an effort to most effectively improve an individual's lipid and lipoprotein profile.

EFFECTS OF EXERCISE ACCUMULATION ON PLASMA LIPID AND

LIPOPROTEIN CONCENTRATIONS

By

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A Dissertation Submitted to
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APPROVAL PAGE

This dissertation was approved by the following committee of the Faculty of

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CHAPTER I

INTRODUCTION

Increased physical activity levels have been shown to reduce the risk of coronary

artery disease (CAD). However, due to the complexity of processes stimulated by

physical activity, the exact metabolic mechanisms responsible for the reduced CAD risks

have not been fully identified (Haskell, 1994). Physical activity interventions, in the

form of exercise training and single exercise sessions, have been shown to positively

modify certain lipoprotein factors. Specifically, plasma high density lipoprotein-

cholesterol (HDL-C) and triglyceride (TG) concentrations have been shown to be the

most modifiable by exercise. Also, research has not determined whether exercise training

changes in HDL-C and TG are the result of an accumulation of effects from individual

exercise sessions or mainly a result of the last session of exercise completed.

Despite not having a precisely defined dose-response curve charting physical activity and

CAD risk, professional organizations have implemented physical activity

recommendations. With the primary goal of improving overall health, adults are

recommended to engage in at least 30 minutes of moderate intensity physical activity

(e.g., 40-60% VO_{2max}) on most days of the week (Pate et al., 1995; Haskell et al., 2007).

However, the Institutes of Medicine (IOM) has recommended that sedentary adults

attempting to lose weight should accumulate as much as 90 minutes of moderate intensity

exercise every day (Brooks et al., 2004). While many health-related benefits may be

expected, the primary goal of the latter recommendation is to increase caloric

expenditure. Aerobically-trained individuals tend to exhibit anti-atherogenic blood lipid

profiles compared to their inactive counterparts (Halbert et al., 1999; Thompson, 1990;

Thompson, 1991). There is limited longitudinal evidence to support exercise induced

changes in total cholesterol (TCHL) and/or low density lipoprotein cholesterol (LDL-C)

concentrations (Leon et al., 1996; Leon et al., 2002; Kraus et al., 2002; Nieman et al.,

2002). Moreover, the majority of cross-sectional studies indicate smaller, non-significant

differences for TCHL and LDL-C concentrations between exercise-trained and inactive

individuals (Durstine and Haskell, 1994, Superko, 1991). In contrast, both observational

studies and randomized clinical trials provide stronger evidence for significantly reduced

TG and elevated HDL-C baseline concentrations in physically active individuals

(Durstine et al., 1994; Durstine et al., 2000; Halbert et al., 1999; Thompson, 1990).

However, it is currently unknown what exercise "frequency" will produce the most

prolonged and clinically significant TG and HDL-C concentration changes.

Most exercise training studies resulting in improved HDL-C and TG

concentrations have utilized training doses of 3-5 exercise sessions per week for 6-8

months. These findings are congruent with the traditional approach to the physical

activity dose-response curve, which indicates multiple sessions of exercise must be

performed over an extended period of time to be of benefit (e.g., reduced CAD risk)

(Haskell, 1994). However, an alternative hypothesis is that some of the blood lipid

changes resulting from an exercise training program are due to the acute metabolic

responses elicited both during and shortly after the most recent session of activity. Few

studies to date have analyzed the effects of aerobic exercise training (i.e., a possible *accumulated* or *additive* effect) compared to a single session of physical activity regarding exercise-induced blood lipid changes. Fully defining the effects of *accumulating* sessions of exercise will constitute an important step in determining an optimal exercise frequency for favorable alteration of one's blood lipid profile.

It has been demonstrated in numerous investigations that a single session of exercise can result in acute lipid and lipoprotein modifications (Ferguson et al., 1998; Gordon et al., 1998; Grandjean et al., 2000; Jafari et al., 2003; Park and Ransone, 2003; Thompson et al., 2001; Wooten et al., 2008). Similar to exercise training, single sessions of exercise appear to primarily modify HDL-C and TG concentrations. These studies have included various exercise modes, intensities and durations. However, few studies to date have analyzed the effect of accumulating single sessions of exercise. For example, it is unknown if exercise performed on a day-to-day basis is more beneficial than exercise performed every other day. Examining the effect of accumulated daily exercise sessions can lead to an eventual definition of the most effective exercise regimen [i.e., frequency of exercise (everyday versus every other day, etc.)] for improving one's plasma lipid profile. This will result in more accurate exercise recommendations for optimally reducing an individual's risk for cardiovascular disease (CVD).

Although professional organizations have produced physical activity recommendations, the amount and timing (i.e., duration per session and time between each exercise session) of physical activity required to optimally elevate HDL-C and reduce TG plasma concentrations has not been determined. As a first step in addressing

this problem, the purpose of the proposed dissertation was to investigate the effects of a

single session of exercise versus the potential additive effect of three sessions of exercise

on the blood lipid profiles of young, sedentary men and women. Results from this study

will provide important baseline information for future investigations assessing more

specific components of exercise prescription (i.e., frequency, caloric expenditure/exercise

time, etc.) in an effort to decrease CVD risk.

Purpose of the Study

The purpose of the study was to compare the effects of a single session of

exercise versus accumulated sessions of exercise on plasma lipid and lipoprotein

concentrations in young, sedentary obese participants.

Specific Aims

The Specific Aims of this investigation are as follows:

1. To compare the effects of a single 90-minute treadmill exercise session performed

   at $60\%VO_{2max}$ versus no exercise (control group) on lipid and lipoprotein

   concentrations in young, sedentary obese adults.

   Working Hypothesis (a): A single session of exercise will produce improved

   plasma

   triglyceride concentrations 24-72 hours post-exercise compared to the control

   group.

Working Hypothesis (b): A single session of exercise will produce improved plasma

HDL-C concentrations 24-72 hours post-exercise compared to the control group.

2. To compare the effects of three 90-minute treadmill exercise sessions performed at 60% VO_{2max} (completed on consecutive days) versus no exercise (control group) on lipid and lipoprotein concentrations in young, sedentary obese adults.

Working Hypothesis (a): Three consecutive days of exercise will produce improved plasma triglyceride concentrations 24-72 hours after the last session of exercise compared to the control group.

Working Hypothesis (b): Three consecutive days of exercise will produce improved plasma HDL-C concentrations 24-72 hours after the last session of exercise compared to the control group.

3. To compare the effects of a single 90-minute treadmill exercise session performed at 60% VO_{2max} versus three 90-minute exercise sessions performed at 60% VO_{2max} (completed on consecutive days) on lipid and lipoprotein concentrations in young, sedentary obese adults.

Working Hypothesis (a): Three consecutive days of accumulated exercise will produce a greater improvement in plasma triglyceride concentrations 24-72 hours after the last session of exercise compared to the improvement in plasma triglyceride concentrations 24-72 hours after a single session of exercise.

Working Hypothesis (b): Three consecutive days of accumulated exercise will produce a greater improvement in plasma HDL-C concentrations 24-72 hours

after the last session of exercise compared to the improvement in plasma HDL-C concentrations 24-72 hours after a single session of exercise.

Significance of the Study

It has not been determined whether the favorable blood lipid and lipoprotein changes observed with exercise training occur as an accumulation of small effects from individual exercise sessions, or if the "training" effect is primarily a result of the last session of exercise completed. This study attempted to clarify the difference between the stimulatory effects of a single session versus accumulated sessions of exercise on blood lipid and lipoprotein concentration changes. The accumulated sessions of exercise (3 consecutive days of treadmill exercise at $60\%VO_{2max}$ for 90 minutes) were expected to simulate a short-term exercise training effect. The post-exercise effects of this exercise training protocol will be compared to the post-exercise effects of a single session of exercise (90 minutes of treadmill exercise at $60\%VO_{2max}$). These data will aid future investigations assessing more specific components of exercise prescription (i.e., frequency, caloric expenditure, etc.) in an effort to decrease CVD risk.

Limitations

1. Volunteers served as subjects and, therefore, did not make up a random sample.

2. Total caloric output for each experimental exercise session did differ slightly. Although specific caloric expenditure was not assigned for each exercise session, it

was calculated from expired gases. Also, this study investigated a published physical

activity recommendation that was based upon time rather than caloric expenditure.

3. Dietary intake was not directly controlled. However, subjects completed three day

    dietary intake logs prior to each experimental exercise protocol and maintained

    dietary logs throughout each day of all three protocols.

4. Failure of subjects to comply with the instructions of the researchers to refrain from

    exercise during the week/weeks in between experimental protocols may have

    produced variability in the results. However, only physically inactive subjects were

    recruited. As $\geq$300 kcal of energy expenditure is typically needed to elicit significant

    acute changes in plasma lipids, it was unlikely that any impulsive physical activity

    would have been sufficient to alter the results of this study (Grandjean et al., 2001).

Delimitations

1. Twelve apparently healthy males and females between the ages of 18-30 years, who

    were non-smokers (also not using tobacco products), did not drink more than an

    average of 2 alcoholic beverages per day, and did not use lipid altering medications,

    were recruited as subjects (as smoking, alcohol consumption, and lipid altering

    medications could have affected blood lipid concentrations independent of any

    exercise induced changes). Young subjects were recruited due to a higher likelihood

    of being able to complete the required 90 minutes of exercise per session on three

    consecutive days.

2. Subjects were tested following a 12 hour fast to minimize dietary effects on blood lipid and lipoprotein concentrations.

3. Subjects were not engaging in any abnormal and/or significant physical activity during the time periods between exercise protocols.

4. Subjects performed a single session (treadmill exercise 60% VO_{2max} for 90 minutes), accumulated sessions (three consecutive treadmill sessions 60% VO_{2max} for 90 minutes), and control session (no exercise for six consecutive days), respectively, in a random crossover design.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Coronary heart disease (CHD) is the leading cause of death (about 35% of all deaths) in the United States (Gordon et al., 1998; Jafari et al., 2003). However, over the past few decades, a substantial body of evidence has demonstrated an inverse relation between physical activity and CHD risk. In response, many professional organizations have recommended that all adults should accumulate at least 30 minutes of moderate-intensity physical activity on most, if not all, days of the week. However, there is compelling evidence that obese individuals attempting to lose weight should perform 90 minutes of moderate intensity activity (Brooks et al., 2004). While it is well documented that regular physical activity reduces CHD risk, research has not defined the mechanisms responsible for this cardioprotective effect.

While all mechanisms for CHD risk are not defined, the beneficial effects of physical activity may be, partially, due to modified blood lipids and lipoproteins (Durstine, 2001). Independent of other major cardiovascular risk factors, observational studies suggest that physically active individuals tend to exhibit an anti-atherogenic blood lipid profile when compared to their inactive counterparts (Durstine et al., 2001; Pate et al., 1995). Experimental data suggests that the favorable blood lipid profiles of physically active individuals are primarily due to increased HDL-C and reduced TG

concentrations (Halbert, 1999; Grandjean, 2000; Crouse, 1999; Kraus, 2000). These blood lipid and lipoprotein modifications are, within limits, based upon a dose-response relationship.

This dose-response relationship suggests that as the dose of physical activity increases the beneficial response of plasma lipid and lipoproteins will also increase. Exercise dose is generally defined as the type, intensity, and duration of physical activity required to produce plasma lipid benefits. However, the exact dose of physical activity required for the most optimal blood lipid and lipoprotein response has not been defined. Also, the most optimal frequency (i.e., time allotted between exercise sessions) in which to perform the exercise dose has not been determined (Durstine and Haskell, 1994). Since blood lipid concentrations can be transiently (i.e., 24-72 hours) altered after one exercise session it is important to assess exercise frequency. It is not only a matter of how many times per week exercise should be performed, but how much time should be allotted between each exercise session. Therefore, in order to properly produce a more optimal blood lipid response it is vital that all aspects of exercise frequency (i.e., days per week and time between each exercise session) be fully defined. A more thorough understanding of how daily exercise accumulation affects short-term blood lipid concentrations will aid in producing more optimal long-term exercise prescriptions, thereby decreasing CVD risk.

This chapter presents a review of related literature in the area of blood lipid and lipoprotein changes produced from exercise. It is organized into the following sections: Cholesterol Transport, Effects of Physical Activity on Lipoprotein Metabolism, Aerobic

Exercise Training, Single-Sessions of Exercise, and the Acute Augmented Exercise Response.

Cholesterol Transport

The movement of cholesterol and lipids throughout the body is primarily conducted by lipoprotein particles. A lipoprotein is defined as a globular biochemical assembly containing both lipids and proteins. The outer core, or "shell", of the structure is composed of phospholipids and apolipoproteins. These components allow the lipoprotein to be water-soluble and aid in determining the action and core content of the lipoprotein. The core is composed of lipid, primarily in the form of triglyceride (TG) and cholesteryl ester (CE). All cells of the body require both TG and CE for normal metabolic functions. Most lipoproteins carry TG and CE to peripheral cells, while other lipoproteins carry TG and CE away from peripheral cells. These two transport systems maintain a homeostasis for TG and CE concentrations within the blood stream.

This homeostatic environment is maintained via two separate lipoprotein transport systems which work together. The forward cholesterol transport system (FCT) transports both exogenous (i.e., dietary intake) and endogenous (i.e., liver production) lipids to the liver and peripheral tissue. The reverse cholesterol transport (RCT) system transports TG and CE back to the liver for catabolism. Table 1 provides a full list of the apolipoproteins for both the forward and reverse cholesterol transport systems (Davis and Wagganer, 2005).

Table 1

Apolipoproteins

Apolipoprotein	Site of Synthesis	Associated Lipoprotein	Function
Apo A-I	Liver, Intestine	Chylomicron, HDL	Structural protein for HDL. Cofactor for LCAT. Ligand for putative HDL receptor.
Apo A-II	Liver	HDL	Inhibits Apo-E binding to receptors (through the E-AII complex.)
Apo A-IV	Intestine	Chylomicron, HDL	May facilitate cholesterol efflux from cells. Activator of LCAT.
Apo B-100	Liver	VLDL, IDL, LDL	Structural protein for VLDL and LDL. Ligand for LDL receptor.
Apo B-48	Intestine	Chylomicron	Structural protein.
Apo C-I	Liver	VLDL, HDL	Modulates remnant binding to receptors. Activates LCAT.
Apo C-II	Liver	Chylomicron, VLDL, HDL	Cofactor for LPL.
Apo C-III	Liver	Chylomicron, VLDL, HDL	Modulates remnant binding to receptors
Apo E2	Liver, brain, skin, testes, spleen	Chylomicron, VLDL, HDL	Ligand for LDL receptor. Preferentially bind with HDL.
Apo E3	Liver, brain, skin, testes, spleen	Chylomicron, VLDL, HDL	Ligand for LDL receptor. Preferentially bind with HDL.
Apo E4	Liver, brain, skin, testes, spleen	Chylomicron, VLDL, HDL	Ligand for LDL receptor. Preferentially bind with VLDL.

Forward Cholesterol Transport

It is well understood that cholesterol is involved in several biologic processes. In normal biological processes cholesterol is primarily synthesized by the liver, with smaller portions coming from other various bodily tissues. Along with this internal synthesis, cholesterol can also be obtained from exogenous (e.g., dietary) sources. Cholesterol functions as a stabilizing component for the cellular membranes of all body cells and acts as a major precursor in the production of steroid hormones.

The dietary intake of lipid generally consists of TG, which are three long-chain fatty acids attached to a glycerol backbone. These fatty acids pass through the digestive track, where they are eventually absorbed by the small intestine. The small intestine packages these fatty acids into a large lipoprotein micelle called a chylomicron. The outer surface is naturally hydrophilic consisting of phospholipids (9%), cholesterol (3%) and apolipoproteins (1%) with the core content of TG (86%) and CE (1%) (Hussain, 2005; Hultin and Olivecrona, 1998). The outer shell of the chylomicron contains apolipoprotein B-48, apo C-II, apo E, and apo A-1. Apolipoprotein B-48 is the only apolipoprotein formed in the small intestine. The general functions of these apolipoprotein's are to stabilize and identify the chylomicron in circulation. The density of a chylomicron is low because it is very large ($\geq$100nm) and has a high core lipid content (especially buoyant triglycerides).

Chylomicrons are too large to penetrate the capillary membrane; therefore they are secreted into the lymphatic system and enter the bloodstream by way of the thoracic duct. Once in the blood, chylomicrons acquire apo E and apo C-II and are progressively

reduced in size by the action of lipoprotein lipase (LPL), which is bound to the surface of capillary endothelial cells and catalyzes the removal of free fatty acids from the chylomicron triglyceride pool (Cooper, 1997). LPL is activated by the presence of apo-CII on the outer "shell" of the chylomicron. While LPL can hydrolyze triglycerides from any lipoprotein particles, it primarily acts upon chylomicrons and very-low-density lipoproteins (VLDL) due to their high TG content and the presence of apo-CII. The action of LPL hydrolyzing TG from the core of the chylomicron also causes surface fragments of the outer shell to break off and be transferred to maturing HDL particles. Primarily apo A-1 ligands from the chylomicron will be transferred to HDL particles, benefiting the RCT system.

The fatty acids released by the action of LPL can be utilized by peripheral tissues as an energy source or stored intracellularly as a triglyceride for later use. The action of LPL will quickly deplete the chylomicron TG content producing a smaller chylomicron remnant. These chylomicron remnants are taken up by hepatocytes through a receptor-mediated process, with apo E serving as the primary binding ligand (Hultin and Olivecrona, 1998; Redgrave, 2004; Rustaeus et al., 1999). The two primary liver receptors responsible for chylomicron remnant clearance from the blood are LDL receptors (LDL-R) and LDL receptor-related proteins (LRP). While both receptors contribute to chylomicron remnant clearance, the LDL-R is normally predominant (Redgrave, 2004). This entire process occurs quickly, which is why chylomicrons and their remnants are generally only observed in postprandial plasma samples.

The endogenous cycle of the FCT system begins with the hepatic production of lipoprotein particles containing TG and cholesterol. The production of VLDL's is stimulated by an increased delivery of free fatty acids (e.g., high intake of dietary fat) to hepatocytes. Triglycerides and phospholipids to be used in the formation of VLDL are synthesized in the liver, whereas VLDL cholesterol can be synthesized *de novo* or reutilized from LDL particles (discussed below). Acyl coenzyme A: cholesterol acyltranferase (ACAT), is believed to be the primary hepatic enzyme responsible for the production of VLDL molecules (Shelness and Sellers, 2001). ACAT, located at the endoplasmic reticulum, acts to esterify free cholesterol delivered to the liver by other lipoproteins. The cholesteryl esters produced by the action of ACAT can be stored for later use (intracellular cholesteryl ester pool), or packaged with triglycerides, phospholipids and an apo-B100 protein to form a VLDL particle (Shelness and Sanders, 2001).

The major protein constituent of VLDL is Apo B-100, with smaller amounts of apo-E and apo-C proteins. The apo B-100 ligand functions in VLDL to mediate binding with the LDL-R. Although apo B-100 is also a constituent of intermediate density lipoprotein (IDL) molecules, it does not appear to play a major role in the binding of IDL to LDL-R. Similar to the chylomicrons, the apo-E ligands are primarily responsible for most of the receptor-mediated clearance of VLDL and IDL. VLDL particles have two potential fates: receptor-mediated endocytosis or conversion to LDL particles. Both are determined by the apolipoproteins of the VLDL particle and the presence of plasma lipases. Approximately half of the apo-E enriched VLDL particles will be taken up by

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LDL-R endocytosis. The other half will be converted to LDL particles through the action of plasma lipases (Havel, 1984; Shelness and Sellers, 2001).

The TG content of VLDL particles are primarily hydrolyzed by the action of LPL and hepatic lipase (HL). As previously discussed, LPL is located on the capillaries of endothelial cells. As the VLDL particle comes in contact with the endothelial cells, LPL hydrolyzes the TG content causing the VLDL particle to shrink in size. HL is a member of the same TG lipase gene family as LPL and is synthesized primarily by hepatocytes. This lipase stays localized to the hepatic sinusoid capillaries bound to heparin-sulfate proteoglycans (HSPG) (Wang and Briggs, 2004). HL primarily hydrolyzes triglycerides and phospholipids in VLDL and IDL, leading to the production of LDL particles (Jin et al., 2002). HL can further hydrolyze LDL generating a smaller, more dense particle. While HL may produce a small, dense LDL particle, which is considered to be more atherogenic, it also positively alters the RCT system. The interaction of HL with HSPG has been shown to be involved with the uptake of HDL cholesteryl esters by scavenger receptor class-B type I (SR-BI) located on the liver (Lambert et al., 1999). The hydrolysis of triglyceride-rich HDL_2 by HL generates a lipid-poor pre-B HDL particle that recirculates in the RCT system. Based upon past research it appears that HL primarily acts to benefit the RCT system, however it does affect the FCT system as well (Jansen et al., 2002). The continuous action of plasma lipases will cause the VLDL to become smaller and smaller, eventually being transformed into an IDL particle.

IDL particles are typically recognized as a "transition" lipoprotein in the conversion of VLDL to LDL. IDL particles still retain the apo B-100 and apo-E

constituents but have lost most of the apo-C proteins. The apo-E ligand, not the apo B-100, of the IDL particle primarily acts to strongly bind the particle to LDL-R, clearing the particle from plasma. While some IDL particles are cleared via receptor mediated endocytosis, most of the particles stay in the blood stream where LPL and HL continue to hydrolyze core triglycerides. Both VLDL and IDL particles are rapidly cleared (e.g., half-life of minutes to a few hours) from plasma due to their high binding affinity to LDL-R (Takashi et al., 2004; Rustaeus et al., 1999) and the rapid hydrolysis action of LPL and HL (Havel and Kane, 1995).

LDL particles are the end products of lipase-mediated hydrolysis of VLDL particles and transport the majority of total plasma cholesterol (i.e., ~70%) (Rapp, 2002). Moreover, as the triglyceride-rich core of the larger VLDL particles is hydrolyzed by LPL and HL, the surface lipids and proteins are also remodeled. The majority of outer shell protein constituents are transferred to HDL particles, producing a small, cholesterol rich LDL particle devoid of almost all apolipoproteins except apo B-100 (Havel, 1984). While some peripheral tissues are able to take up small amounts of LDL, the majority is metabolized by the liver via LDL-R (Rapp, 2002). Unlike VLDL and IDL, the LDL particle does not have any apo-E constituents. The primary protein constituent of LDL particles is apo B-100, which has a lower affinity for LDL receptors than apo-E (Schneider, 1991). Due to this poor binding affinity, the half-life of LDL particles is approximately 2 to 3 days. Thus, the difference in the affinities of apo B-100 and apo-E plays a role in plasma cholesterol homeostasis. Figure 1 demonstrates the mechanisms involved in FCT.

Figure 1

Forward Cholesterol Transport
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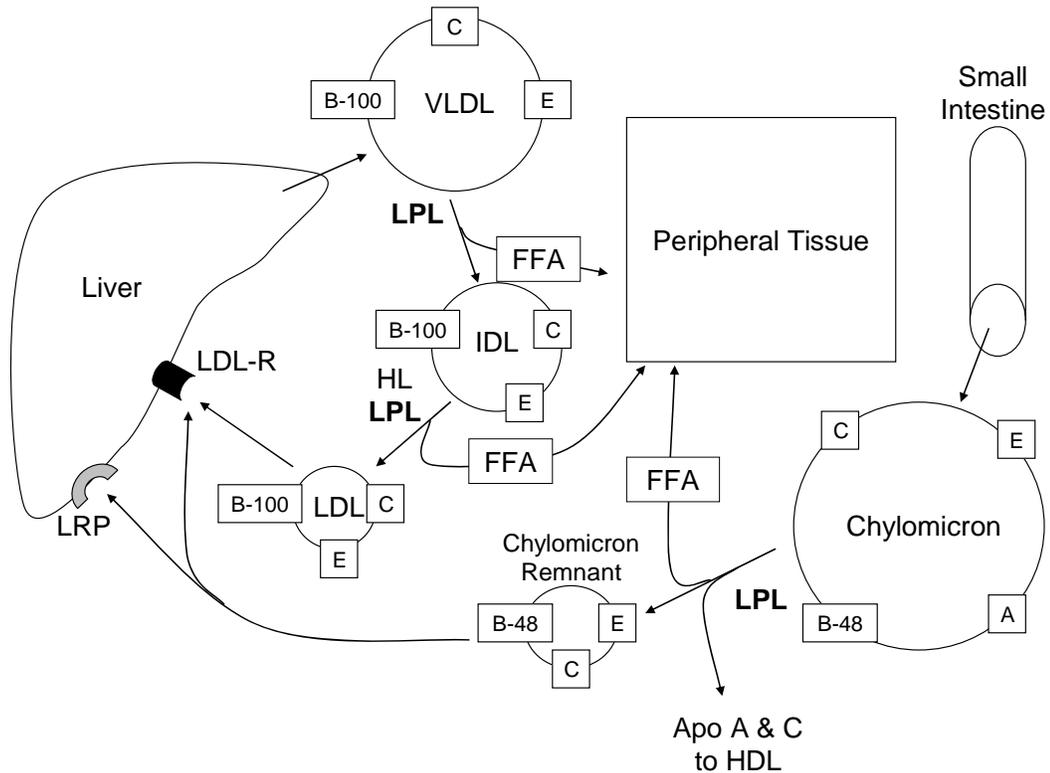
Forward Cholesterol Transport. Dietary lipid is secreted from the small intestine in chylomicron particles. The triglyceride content of the chylomicron is hydrolyzed by the action of lipoprotein lipase (LPL). Free fatty acids (FFA) are taken up by peripheral tissues while the smaller chylomicron remnants are catabolized by both the hepatocyte low-density lipoprotein (LDL) receptor (LDL-R) and the LDL receptor-related protein (LRP) mainly through recognition of apolipoprotein (apo) E. The endogenous hepatic production of lipid is primarily packaged in very low-density lipoprotein (VLDL). The VLDL particle is reduced to an IDL particle via the action of LPL. The IDL particle is subsequently reduced to an LDL particle by the actions of LPL and hepatic lipase (HL). Hepatic catabolism of the LDL particle primarily occurs through apo-B100 recognition by the LDL-R. The major apolipoproteins associated with each particle are indicated and their characteristics and functions are listed in Table 1 (Davis and Wagganer, 2005).

While the primary function of LDL is to transport cholesterol to the liver for catabolism, metabolic problems can occur when plasma LDL concentrations are elevated. Plasma LDL concentrations can become elevated 1) due to an insufficient number of hepatic LDL receptors, 2) the receptors do not possess proper affinity for the apo B ligand of the LDL particle, or 3) when dietary fat intake is high (causing LDL receptor synthesis to be down-regulated) resulting in elevated plasma cholesterol concentrations (Kwiterovich, 2000). This excess of plasma cholesterol can induce and promote endothelial injury, inflammation, and ultimately the development of atherosclerosis. Specifically, the excess cholesterol carried by LDL can be internalized by macrophages in the vascular intima by scavenger receptors. When the LDL particle enters the arterial wall it is oxidized, which increases its affinity to macrophage scavenger receptors. These scavenger receptors can then be transformed into foam cells which secrete growth factors that stimulate the proliferation and migration of smooth-muscle cells, resulting in the formation of an atherosclerotic lesion. Hence, excess cholesterol depositions from circulating sources (e.g., LDL particles) are currently believed to be the most frequent contributing factor for the development of atherosclerosis.

Reverse Cholesterol Transport

All cells of the body require cholesterol for the synthesis and maintenance of cell membranes. However, body cells do not possess an analogous (i.e., catabolic) mechanism for the metabolic disposal of cholesterol. This is problematic because research indicates cholesterol may be atherogenic in vascular wall cells at some

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physiological level and of exposure and is outright cytotoxic at higher levels (Tulenko et al., 2002). Therefore, the metabolic balance of cholesterol is accomplished by the RCT pathway. The RCT pathway functions to extract accumulated cholesterol from atherosclerotic plaque (and extrahepatic tissues, as well), and deliver it back to the liver for elimination as bile salts or biliary cholesterol. The delivery of cholesterol to the liver is primarily performed by HDL particles, which have been shown to protect against atherosclerosis (Fielding and Fielding, 1995; Leaf, 2003; Masson et al., 2008; Rader, 2003). The entire RCT pathway involves the availability and interaction of apolipoproteins, enzymes, transfer proteins, and receptors.

The formation of HDL particles involves the accumulation of lipid-poor apo A-I particles, which originate from three major sources (Fielding and Fielding, 1995). First, the liver secretes an apo A-I phospholipid disc called nascent HDL. Second, the intestine directly synthesizes a small apo A-I containing HDL particle. Third, small HDL discs are derived from excess material shed from chylomicrons and VLDL as they are hydrolyzed by LPL (Ribalta et al., 2003). Apo A-I is essential for various cellular activities including the correct assembly and overall stability of HDL particles (Rye and Barter, 2004; Wang and Briggs, 2004), activation of the enzyme lecithin: cholesteryl acyltransferase (LCAT) (Sviridov et al., 2000), and the binding of phospholipid transfer protein to HDL particles (Huuskonen et al., 2001). Most of the nascent or immature apo A-I immediately begin removing free cholesterol and phospholipids from peripheral tissues including atherosclerotic plaque (Wang and Briggs, 2004). This process of lipid transfer from the plasma membrane of cells to apo A-I containing lipoproteins (e.g., small and large HDL

particles) is mediated by the cell membrane transporter ATP-binding cassette transporter 1 (ABCA1) (Tall, 2008).

Both immature and more mature forms of HDL particles primarily obtain free cholesterol and phospholipids via ABCA1. However, the immature HDL particle has the ability to freely transverse the endothelium, making it the major acceptor of cholesterol and phospholipids from peripheral cells and atherosclerotic plaque formations (Tulenko et al., 2002). As the HDL particle becomes more mature (i.e., spherical) it has the ability to obtain lipids from other lipoproteins in circulation (Wang and Briggs, 2004; Sviridov and Nestel, 2002). The free cholesterol on the surface of the lipidated apo A-I is esterified by lecithin: cholesteryl acyltransferase (LCAT) (Sviridov et al., 2000).

LCAT is a soluble enzyme primarily synthesized by the liver and activated in the plasma by the presence of apo A-1. As the apo A-I esterifies free cholesterol it will begin to mature and grow into a more disc-like shape, generally called a preB$_2$-HDL (Mahley et al., 1984; Sviridov and Nestel, 2002). This growth occurs because the esterification of the free cholesterol with a long-chain fatty acid decreases its hydrophilicity, and the newly formed cholesteryl esters move away from the surface of the disc into the core of the lipoprotein. As the core of the disc fills with cholesteryl esters it will change into a more mature and spherical shape. Therefore, LCAT plays a pivotal role in promoting cholesterol efflux from peripheral cells to HDL after the initial cholesterol efflux mediated by ABCA1 to apo A-I, through which cholesterol molecules enter the RCT pathway.

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The small, spherical mature HDL particles produced from the esterification of free fatty acids are called HDL_3 particles. As more free cholesterol is accepted and esterified the HDL_3 particle will grow in size to form an HDL_2 particle. Therefore, the HDL_3 particle will have a more triglyceride-rich core, while the HDL_2 particle will possess a more cholesteryl-ester rich core. These two particles can quickly be converted back and forth, primarily due to the action of cholesteryl ester transfer protein (CETP) and secondarily due to HL (described above) and endothelial lipase (EL). The primary action of CETP facilitates the transfer of cholesteryl esters from HDL to lower-density, more triglyceride-rich lipoproteins (e.g., VLDL, IDL, LDL, and chylomicron remnants).

CETP is a glycoprotein that is primarily secreted by the liver, and then binds to HDL particles in the blood stream. The basic function of CETP is to elicit the redistribution of triglycerides and cholesteryl esters, and, to a lesser extent, phospholipids between plasma lipoproteins. This redistribution primarily occurs by transferring cholesteryl esters from HDL_2 particles to triglyceride-rich lipoproteins in exchange for triglycerides. As the HDL_2 particle losses cholesteryl esters and gains triglyceride content it will shrink in size and density, eventually becoming an HDL_3 particle. The HDL_3 particle will begin esterifying free cholesterol from extrahepatic tissues until it once again gains enough size and density to be classified as an HDL_2. The cholesteryl esters moved from the HDL particles to triglyceride-rich lipoproteins is delivered back to the liver for metabolism. Therefore, CETP acts to provide an indirect pathway by which HDL cholesteryl esters (i.e., esterified extrahepatic free cholesterol) are delivered to the liver and removed from the bloodstream.

As previously mentioned, EL also plays a role in HDL metabolism. This lipase shares a similar identity to both LPL and HL. However, unlike LPL and HL, EL is synthesized by endothelial cells and functions at the site where it is synthesized. Compared to LPL and HL, EL is more active in hydrolyzing HDL lipids, in particular phospholipids, generating free fatty acids (McCoy et al., 2002; Badellino and Rader, 2004). EL primarily shows phospholipase activity and relatively little triglyceride lipase activity (Borggreve et al., 2003). Therefore, it is possible that EL is a physiological regulator of HDL metabolism, but more research is needed in this area to characterize its exact role.

At this point in the RCT system it is important to discuss the role of phospholipid transfer protein (PLTP), which also possesses the ability to mediate the exchange of products between the FCT and RCT pathways. It is synthesized in various tissues of the body (e.g., liver, lungs, and adipose tissue), however its main source in human plasma is unknown (Eckardstein et al., 1996). PLTP facilitates phospholipid and free cholesterol transfer between lipoproteins during lipolysis (Albers and Cheung, 2004), and is believed to promote cholesteryl ester transfer toward TG-rich lipoproteins (Borggreve et al., 2003). It is also believed to play a role in the conversion of HDL into larger and smaller particles (i.e., HDL remodeling) (Huuskonen et al., 2001). During this remodeling process, pre B-HDL particles are produced that act as initial acceptors of free cholesterol from peripheral tissues (Eckardstein et al., 1996; Huuskonen et al., 2001). Both the lipid transfer and HDL remodeling processes of PLTP are believed to aid in the indirect pathway of the RCT system.

This indirect pathway of cholesterol removal is just one aspect of the RCT system. As previously mentioned, the primary precursor for the formation of HDL particles is the apo A-I protein. Apo A-I has a strong affinity to the SR-BI located on the cell surface of the liver. Specifically, the SR-BI prefers the apo A-I associated with spherical, more mature HDL particles (e.g., HDL_3 and HDL_2) as opposed to the apo A-I found in $preB_2$-HDL (Liadaki et al., 2000). Therefore, some mature HDL particles will bind with the SR-BI, which selectively takes up cholesteryl esters located within the HDL particle. This will act to shrink the size and density of the particle until it is released and allowed to re-circulate in the blood stream once again. The particle can then start the entire process again, collecting free cholesterol and potentially circulating back to the SR-BI for cholesteryl ester clearance from the blood stream. This is generally considered to be a more direct pathway for the delivery of cholesterol to the liver for metabolism; however both the indirect (as described above) and the direct pathway comprise the entire RCT system. Figure 2 demonstrates the mechanisms involved in reverse cholesterol transport.

Effects of Physical Activity on Lipoprotein Metabolism

Physical activity can be defined as bodily movement performed by the contraction of large muscle groups, which results in energy expenditure beyond resting energy requirements. The general benefits of habitual physical activity include increased exercise capacity, endurance, and muscle strength. While physical activity has many healthful benefits, epidemiological studies have shown that it may prevent, and help treat,

Figure 2

Reverse Cholesterol Transport

Peripheral Tissue or Macrophage:

FC / PL

ABCA1

Liver

SR-BI

TG-rich lipoproteins

TG
CE

preß₁-HDL

preß₂-HDL

HDL₂

LCAT

HDL₃

CETP

TG   CE

CE   TG

LCAT

A

A
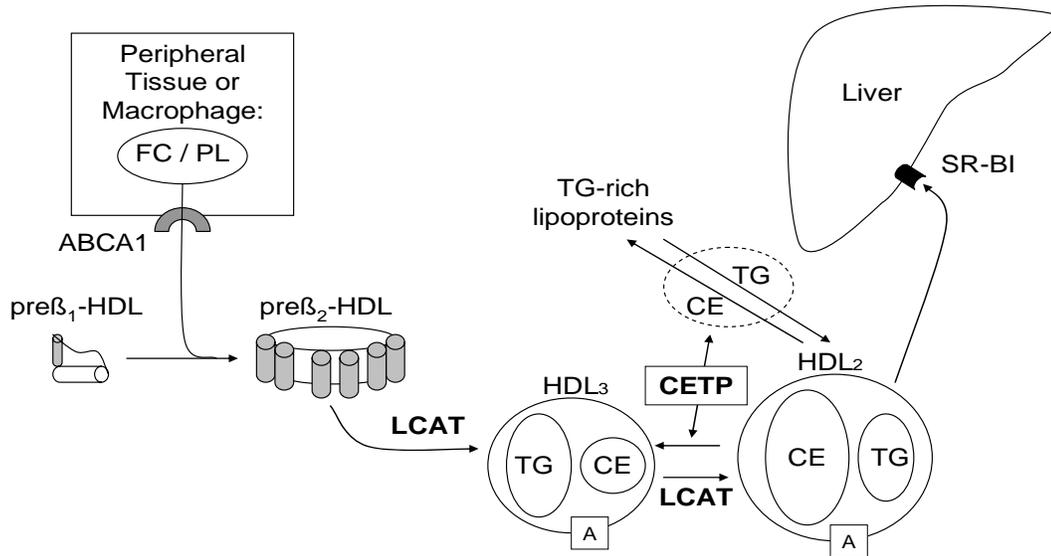
Reverse Cholesterol Transport. Lipid-poor pre-beta₁ high-density lipoprotein (preß₁ HDL), composed primarily of apolipoprotein (apo) A-I acquires free cholesterol (FC) and phospholipids (PL) from peripheral tissue or arterial macrophages. This efflux process is facilitated by the cellular protein adenosine triphosphate-binding cassette protein A1 (ABCA1) and allows the preß₁ HDL particle to take on a more discoidal shape. Esterification of cholesterol in preß₂ HDL by lecithin-cholesterol acyltransferase (LCAT) leads to the formation of spherical HDL₃ particles. The continued action of LCAT transforms HDL₃ particles into larger, more cholesterol-rich HDL₂ particles. Cholesteryl ester transfer protein (CETP) facilitates the transfer of CE from HDL to triglyceride (TG)-rich lipoproteins in exchange for TG. Adding and removing CE to/from HDL, LCAT and CETP, respectively, cause HDL particles to be in constant flux between HDL₂ and HDL₃. Hepatic catabolism of HLD primarily occurs through apo A-I and apo A-II recognition by the scavenger receptor B-I (SR-BI) (Davis and Wagganer, 2005).

many of the risk factors associated with the development of coronary artery disease (CAD) (Durstine et al., 2001). This cardioprotective effect may be partially explained by a favorable influence on plasma lipid and lipoprotein concentrations (Durstine and Haskell, 1994; Welk and Blair, 2000). It has been suggested that the demands of exercise modify the action of lipid regulatory enzymes and transfer proteins, producing post-exercise changes in plasma lipid and lipoprotein concentrations. The enzymes and transfer proteins most often implicated in this beneficial effect on lipid metabolism primarily include LPL and HL, with a smaller emphasis on LCAT and CETP.

LPL is a rate-limiting enzyme for the catabolism of triglyceride-rich lipoproteins and its action is believed to be directly modified by muscular activity levels (Leaf, 2003). Current data suggests that even a small increase in physical activity (i.e., slow treadmill walking compared to physical inactivity) can beneficially increase muscle LPL activity, independent of gender (Perreault et al., 2004; Bey & Hamilton, 2003; Tall, 2002). More specifically, an acute bout of moderate intensity exercise increases muscle tissue LPL more than adipose tissue LPL (Grandjean et al., 2000; Lambert et al., 1999; Thompson et al., 2001; Seip et al., 1993). The prevailing thought is that muscular contractions associated with acute exercise act to deplete intramuscular triglyceride (IMTG) stores, which stimulates the production and secretion of muscle tissue LPL (Durstine and Haskell, 1994; Thompson, 1991; Zderic & Hamilton, 2006). The increased LPL moves to endothelial cells in capillary beds of muscle tissue where it will hydrolyze more triglycerides from VLDL and chylomicrons in order to restore the IMTG stores (e.g., those depleted during exercise). While this may restore the IMTG concentrations within

the muscle it may also reduce the triglyceride content of VLDL and chylomicron particles, resulting in lower plasma triglyceride concentrations (Mead et al., 2002; Durstine and Haskell, 1994). The hydrolysis of triglyceride-rich lipoproteins can also result in the production of more remnants to be converted into HDL. The increased activity of LPL is believed to be one of the major factors for lipid changes seen with an increased physical activity level. However, while being a vital factor, LPL may only partially explain how the increased metabolic demands associated with physical activity may decrease plasma triglyceride concentrations.

Similar to LPL, HL may also serve as a ligand to mediate the interaction of lipoproteins with cell surface proteoglycans and/or receptors, facilitating the uptake of lipoprotein triglycerides and "recycling" of HDL. At best, there is weak evidence indicating HL activity decreases or remains unchanged with an increase in physical activity levels (Magkos et al., 2006; Lambert et al., 1999; Visich et al., 1996; Sady, et al., 1986). If physical activity decreased HL activity it could result in a beneficial increase in plasma HDL concentrations. HDL concentrations would increase due to an increase in the half-life of HDL_2 particles. However, the interaction of HL and HDL particles is very complex and not fully understood by researchers. Therefore, HL may at least partially explain why triglyceride concentrations decrease and HDL concentrations increase with physical activity, but it is likely that LPL plays a more vital role.

The two lipid transport proteins that have been linked to physical activity and lipid transport changes are LCAT and CETP. LCAT is a plasma glycoprotein responsible for most of the esterification of cholesteryl esters in HDL particles. Data indicates that a

single session of exercise may increase LCAT activity (Frey et al., 1991; Olchawa et al., 2004), but not always (Durstine et al., 1994; Rapp, 2002). It is possible for LCAT activity to increase without an increase in plasma concentration. If LCAT activity increased post acute exercise, it would theoretically lead to a higher plasma concentration of HDL-C (Borggreve et al., 2003). However, more is known about the effects that physical activity has on CETP. CETP catalyzes the net flux of esterified cholesterol from cholesterol ester-rich particles (i.e., HDL) to Apo-B containing lipoproteins (i.e., VLDL, chylomicrons, and LDL). CETP activity has been shown to decrease following acute exercise (Foger et al., 1994) and physical exercise training (Seip et al., 1993). While some authors dispute the cardioprotective effect of low CETP (Inazu et al., 1990), it is believed that decreased CETP activity increases plasma HDL-C and apo A-1 levels, and concomitantly reduces HDL-triglyceride content by triglyceride transfer to apo-B containing lipoproteins (Leaf, 2003). With the primary exercise-induced increases in HDL-C being attributed to changes in plasma lipase activities, CETP level changes are viewed as an additional or secondary factor for the exercise-induced increases in plasma HDL-C concentrations (Seip et al., 1993). While these two transport proteins have the potential to affect lipid metabolism, research has not fully defined their primary role or association with increased physical activity levels.

Aerobic Exercise Training

It is well established that aerobic exercise training (e.g., walking, jogging, cycling, etc.) is beneficial for reducing both the morbidity and mortality rates associated

with CAD. Numerous excellent review articles have been published in reference to the beneficial effects of aerobic exercise training on plasma lipid and lipoprotein concentrations (Durstine and Haskell, 1994; Hardman, 1999; Kelley et al., 2004; Kodama et al., 2007; Kokkinos and Fernhall, 1999; Leon and Sanchez, 2001; Leaf, 2003; Thompson et al., 2001). However, varied exercise interventions, protocols, and subject baseline characteristics have hindered the determination of the most optimal exercise dose required to obtain the most beneficial blood plasma concentration changes. The following text will explore some of the relevant findings, in particular those related to the study of total cholesterol (TCHL), LDL-C, HDL-C and triglyceride concentration changes with aerobic exercise training.

Changes in TCHL and LDL-C Concentrations

Regarding endurance exercise training and TCHL and LDL-C changes, few studies have shown statistically significant results. Longitudinal studies report infrequent TCHL and LDL-C changes, with the majority (~80%) showing no statistically significant reductions after exercise training (Halverstadt et al., 2003; Leon et al., 2002; Kraus et al., 2002; Nieman et al., 2002; Vislocky et al., 2009). The small amount of data showing TCHL and/or LDL-C reductions (Altena et al., 2006; Halbert et al., 1999; Spate-Douglas & Randall, 1999) are largely unreliable due to not accounting for confounding variables [e.g., natural fluctuations (no control group), body weight, caloric intake, nutrient composition of diets, alcohol intake, and/or smoking)]. When these confounding variables are controlled, the reductions in TCHL and/or LDL-C concentrations are

generally found to be non-significant (Williams, 1998; Grandjean et al., 1996).

Therefore, the anti-atherogenic blood profile of aerobically trained individuals is not

primarily due to low TCHL and LDL-C concentrations. Instead, exercise training

appears to produce an anti-atherogenic blood lipid profile by beneficially modifying

HDL-C and TG concentrations.

Changes in HDL-C and TG Concentrations

Research has reported more frequent changes in HDL-C and TG following

aerobic exercise training. In fact, more than 50% of the exercise training studies show

HDL-C improvements, while approximately 33% show TG reductions (Durstine and

Haskell, 1994; Durstine et al., 2000; Leon et al., 1996). This increased frequency of

reported changes indicates that HDL-C and TG are more responsive to exercise training

compared to TCHL and LDL-C. While it appears that HDL-C and TG are more

responsive to exercise training, some studies exist showing small, non-statistically

significant concentration changes. Suggested reasons for these disparate findings include

different training regimens, baseline characteristics, and loss of body weight from pre- to

post-exercise training (Bassuk and Manson, 2003). However, when these factors are

properly controlled, HDL-C and TG concentration changes may be observed following

an exercise training regimen (Durstine et al., 2000).

Compared to any other lipoprotein, physical activity has the most consistent and

significant effect on HDL-C concentrations (Durstine et al., 2001; Leon et al., 2002). In

prospective cohort studies, physically active men and women have significantly higher

concentrations of HDL-C compared to their sedentary counterparts (Skoumas et al., 2003). Moreover, cross-sectional studies reveal that physically active individuals exhibit a 4 to 24 mg/dL higher HDL-C concentration compared with their less active counterparts (Durstine et al., 2001; Thompson et al., 2001).

Numerous reviews have been conducted on exercise training, supporting HDL-C changes. A review conducted by Leon and Sanchez (2001), analyzed 51 studies (28 randomized controlled clinical trials that assessed the effects of 12 weeks of aerobic exercise training on lipids in adult men and women. Significant changes in HDL-C concentrations were observed in 28 of the 51 studies (47%; $P<0.05$). The subset of studies also showed that HDL-C concentrations increased significantly (4.6%; $P<0.05$) when dietary factors were held constant. The data also suggested that neither sex nor age were predictors of responsiveness of HDL-C concentration to aerobic exercise training. The Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study, a 20-week intervention study conducted on men and women, resulted in a mean increase in HDL-C concentrations of 3.6% ($P<0.05$) (Leon et al., 2000). More importantly, the observed increase in HDL-C concentrations was similar for males and females, blacks and whites, and offspring and parents. This information supports the fact that exercise training alone can elicit HDL-C concentration changes in most individuals independent of gender, age, or race.

Recent randomized controlled clinical trials testing the effects of at least a 12 week aerobic exercise training on lipid response, with dietary factors held constant, have also shown significant beneficial HDL-C and TG concentration changes. One such study

conducted by Kraus et al. (2002) sought to determine the effects of varying levels of amounts and intensities of exercise on plasma lipids and lipoproteins. Overweight, sedentary men and women (n = 111) were randomly assigned to participate for 6 months in a control group or 8 months in one of three exercise groups: 1) high-amount/high intensity exercise (caloric equivalent of jogging 20 miles per week at 65-80% of peak oxygen consumption); 2) low-amount/high-intensity exercise (caloric equivalent of jobbing 12 miles per week at 65-80% peak oxygen consumption); or 3) low-amount/moderate-intensity (caloric equivalent of walking 12 miles per week at 40-55% of peak oxygen consumption). Final data showed subjects in the high-amount/high-intensity group had significantly increased HDL-C concentrations (4 mg/dL) and decreased TG concentrations (-35 mg/dL) compared to the low-amount/high-intensity, low-amount/moderate-intensity, and control groups. This indicates that the highest amount of exercise produced beneficial changes in HDL-C and TG independent of exercise intensity. Conclusions from this study also indicate that the amount of exercise, even in the absence of clinically significant weight loss, can significantly improve HDL-C and TG concentrations (Table 2).

These findings by Kraus et al. (2002) are important, especially when considering the dose-response relationship of physical activity and blood lipid profile changes. Overall, there appears to be a dose-response relationship that only achieved statistical significance at the highest dose. With regard to intensity of activity, this and other studies have not shown a consistent relationship for improvements in plasma HDL-C and TG concentrations (Kraus et al., 2002; Crouse et al., 1997). Therefore, it appears that the

Table 2

The Effects of Aerobic Exercise Training on TG and HDL-C Concentrations

Source	Subj	Study Design	Int	Time (min)	Kcal	Timing	TG	HDL-C
Altena et al., Med Sci Sport Exerc 38(2): 367-372, 2006	18 32 UT	TM 4 weeks 5x/week	60	30	NR	IPre IP	83 -7	47 3
Bounds et al., Int J Sport Nut Ex Metab10(2): 114-27, 2000	14 M 28 ModT	TM 2 weeks 3x/week	65	NR	350	IPre 24HrP 48HrP	183 -45* -45*	45 5* 5*
Couillard et al., Arterio Thromb Vasc Biol 21: 1226-32, 2001	200 M 35 UT	Cycle 20 weeks 3x/week	75	50	NR	IPre 24HrP	82 -3	31 0
Crouse et al., J Appl Physiol 83:2019-28, 1997	26 M 47 UT	Cycle 24 weeks 3x/week	50-80	NR	350	IPre IP	207 -36*	43 1
Grandjean et al., J Sports Med Phys Fit 36(1):54-9, 1996	20 F 45 UT	TM/Cycle 24 weeks 3x/week	65	30	NR	IPre IP	150 -1	40 6
Halverstadt et al., Metab 52 (11): 1505-11, 2003	83 M/F 57 UT	TM/Cycle 24 weeks 3x/week	70	40	NR	IPre IP	160 -18*	46 3*
Kraus et al., NEJM 347(19): 1483-92, 2002	111 M/F 52,UT	TM & Cycle	40-55	12 mi Per week	NR	IPre IP	197 -35*	40 4*
Leon et al., J Cardiopulm Rehabil 16(3): 183-92, 1996	16 M 33 UT	TM 12 weeks 5x/week	60	45	2000 per week	IPre IP	160 -25*	35 9*

Table 2

The Effects of Aerobic Exercise Training on TG and HDL-C Concentrations (cont.)

Source	Subj	Study Design	Int	Time (min)	Kcal	Timing	TG	HDL-C
Leon et al., Metab 49(4): 513-20, 2000	675 M/F 36,UT	Cycle 20 weeks 3x/week	75	50	328 per bout	IPre 24HrP 72HrP	112 -8 0	41 0 1
Leon et al., Int J Sports Med 23:1-9, 2002	675 M/F 36 UT	Cycle 20 weeks 3x/week	75	50	328 per bout	IPre 24HrP 72HrP	110 -2 -5	41 4* 4*
Nieman et al., J Am Coll Nutr 21(4):344 -50, 2002	91 F 45 UT	TM 12 weeks 5x/week	60-80	45	NR	IPre 3-wk IP	145 3 12	50 2 2
Spate-Douglas et al., Arch Phys Med 80: 691-5, 1999	13 F 40 NR	Walking 12 weeks 3x/week	60	6 miles per week	NR	IPre IP	32 8*	NR NR
Vislocky et al., J of Nutr Biochem 20: 26-34, 2009	11 M/F 24 UT	Walk/Jog 6 weeks 4x/week	65	60	NR	IPre IP	93 12	60 6*
Williams et al, Metabol 43 (7):917-24, 1994	46 M 44 UT	Jogging 12 weeks 3x/week	65	30	NR	IPre IP	NR NR	43 5*

Table 2

The Effects of Aerobic Exercise Training on TG and HDL-C Concentrations (cont.)

Source	Subj	Study Design	Int	Time (min)	Kcal	Timing	TG	HDL-C
Zmuda et al., Atheroscl 137: 215-21, 1998	17 M 39 UT	Walk/Jog/ Cycle 12 month 4x/week	60-80	50	NR	IPre IP	132 -4	38 4

Subj = subject; n, gender = Male (M) or Female (F); subject average age in years; training status = Trained (T) or Untrained (UT). Study Design: Treadmill (TM). Int = Intensity in $\%VO_{2max}$. Time (min) = duration of exercise in minutes. Kcal = calories expended per session. Timing = timing of blood sample collection; I = Immediate, P = Post, Ex = Exercise, Pre = Pre-exercise. TG = triglyceride plasma concentrations. HDL-C = high density-lipoprotein-cholesterol plasma concentrations. Not Reported (NR). Baseline lipid and lipoproteins are given as mg/dL, then mg/dL change from baseline. * Significantly different from baseline, $P < 0.05$.

overall amount of exercise is more important in eliciting blood lipid profile changes compared to exercise intensity.

While mean HDL-C concentrations generally improve consistently across entire treatment groups in interventional studies, some have reported significant individual variability. One study suggests that baseline HDL cholesterol concentrations may affect responsiveness to physical activity (Kokkinos and Fernhall, 1999; Kelley et al., 2005). Data regarding this variation is inconsistent when considering whether greater benefits occur with low- (Leon et al., 2000; Leon et al., 2002) versus normal- to high-baseline HDL-C cholesterol concentrations (Zmuda et al., 1998; Couillard et al., 2001; Thompson et al., 2001). It may be reasonable to assume that less physically fit individuals, who tend to display lower baseline HDL-C concentrations, may be more responsive to physical activity. However, information obtained by Williams and colleagues (1994) found that

exercise training increased HDL-C to a lesser extent in men with low versus normal baseline HDL-C cholesterol. Similar data has been collected for female subjects (Halbert, 1999), suggest little if any between gender difference in the HDL-C response to physical activity. Moreover, a study conducted by Couillard et al. (2001) found that endurance exercise can improve the lipid profile of obese men with initially low HDL-C and elevated TG concentrations. However, the blood lipid profiles of similarly obese men with initially low HDL-C as an isolated trait (i.e., normal TG concentrations) were less responsive to endurance exercise training. Therefore, the beneficial HDL-C cholesterol response elicited by physical activity may not be similar independent of baseline concentrations.

Triglycerides are increasingly being recognized as an independent cardiovascular risk factor (Cullen, 2000) and may be modified by regular exercise training. While exercise training appears to modify TG levels, significant reductions are reported less frequently than increases in HDL-C. Specifically, reductions in TG concentrations have been found in approximately 33% of the related literature (Leon et al., 2001; Durstine et al., 2000). Studies reporting significant TG reductions have ranged from 5 to 38 mg/dL reductions, observed 18 to 72 hours post-exercise. While not reported as frequently, TG concentration changes occur in a similar post-exercise timeline as HDL-C changes (Bounds et al., 2000; Crouse et al., 1997; Cullinane et al., 1982; Foger et al., 1994) (Table 2).

Although some studies fail to show reduced TG following exercise training, most of these studies involve subjects with low baseline TG concentrations. Furthermore, a

relationship exists between pre-exercise TG concentration and the magnitude of the post-exercise change. Therefore, subjects with elevated pre-exercise TG values typically show the greatest post-exercise changes compared to subjects with lower initial TG values (Crouse et al., 1995). Also, research has shown that a reduction in body weight (i.e. adiposity) is not a prerequisite for exercise to produce significant post-exercise changes in TG concentrations (Durstine et al., 2001).

In summary, it is evident that aerobic exercise training can often be effective in raising HDL-C and lowering TG concentrations. These changes generally occur during similar post-exercise time frames (i.e., 24-72 hours) due to the role of HDL molecules in the delivery of TG to other lipoproteins (i.e., VLDL and LDL) and the action of other metabolic factors (i.e., LCAT, CETP, and LPL). The beneficial HDL-C and TG changes elicited by exercise training appear to occur independent of age, sex, or race. More importantly, the beneficial post-exercise blood lipid changes can be elicited due to a metabolic response to exercise rather than a reduction in body weight (i.e., adiposity). While research has proposed a general recommendation for exercise training (e.g., 12-weeks of at least 1200 kcal expenditure per week), the exact dose-response relationship has not been established. Determining this relationship has proven to be difficult due to the wide variance in exercise protocols (i.e., dose per session, time between each exercise session). In order to define the exercise training dose-response relationship it is necessary to understand the effects of a single session of exercise.

Single-Sessions of Exercise

Exercise training involves the accumulation effects from a single exercise sessions. Fuel substrates required for the performance of aerobic exercise primarily comes from the mobilization and oxidation of carbohydrate and lipid stores. Therefore, it can be reasonably expected that a single session of exercise can modify lipid and lipoprotein metabolism. In fact, it is possible that the anti-atherogenic lipid profile of an exercise trained individual is, at least in part, a consequence of a delayed (hours) response to the last session of exercise independent of the training response (Thompson et al., 2001).

While many exercise-induced metabolic changes (i.e., decrease in insulin resistance, reductions in blood pressure, increased coronary blood flow, etc.) contribute to the reduction in CAD risk, plasma lipid profile changes appear to be among the most modifiable (Haskell, 2001). Although not defined, previous research suggests that some of the blood lipid effects elicited by exercise training may occur as an acute response to single sessions of exercise, some only as a training response, and some as the result of an interaction between the two. Defining these interactions has proven to be difficult for researchers, and is the primary reason there is not a defined dose of exercise required to optimally modify an individuals' blood lipid profile. However, researchers agree that defining a dose-response relationship should begin by assessing the effects of single sessions of exercise followed by testing the proper accumulation of these individual sessions into an optimal exercise training program (Haskell, 1994).

Total work performed during a single session of exercise is generally reported as either total time or calories expended, with exercise intensity typically defined as percentage of maximal oxygen consumption. Physical activity recommendations utilize total time, while blood lipid specific research has primarily reported caloric expenditure. Therefore, the majority of the research presented in this section reports caloric expenditure. However, it is the aim of this study to utilize the IOM's physical activity recommendation of 90 minutes of moderate intensity exercise to assess the interaction between acute and short-term training responses on blood lipid profile changes.

Past intervention protocols analyzing the blood lipid and lipoprotein response to a single session of exercise vary widely. This is not surprising when considering various confounding factors (i.e., age, gender, training status, body composition, baseline lipid concentrations, and plasma volume expansion). Along with these subject-based factors, previous research has varied in mode, duration, intensity, and volume (energy expenditure) of the exercise stimulus. All of these factors have the potential to affect lipid and lipoprotein concentrations and should be considered when interpreting published research related to the lipid response to a single session of exercise. Also, since exercise-induced alterations in plasma volume can affect lipid and lipoprotein concentrations, all of the studies discussed report their concentrations after correction for plasma volume change.

Changes in TCHL and LDL-C Concentrations

Studies examining the TCHL and LDL-C response to a single session of exercise generally show no changes, however some variability is noted. Many well-controlled studies have shown that a reduction in TCHL may occur after a single session of exercise. However, these small TCHL reductions are very short-lived (<24 hours) and rarely reach statistical significance (Bounds et al., 2000; Crouse et al., 1997; Crouse et al., 1995; Grandjean et al., 2000; Hughes et al., 1990; Kantor et al., 1987). Moreover, exercise requiring a higher caloric output (i.e., >1,000 kcal) has been weakly associated with a longer lasting post-exercise TCHL reduction (i.e., up to 120 hours post-exercise) (Foger et al., 1994). While these data weakly support a post-exercise TCHL reduction, there are an equal number of studies showing no post-exercise changes (Davis et al., 1992; Ferguson et al., 1998; Imamura et al., 2000; Sady et al., 1996; Visich et al., 1996). Moreover, increases in plasma TCHL concentrations have been observed 24 to 48 hours after exercise in untrained men with normal and elevated blood cholesterol (Crouse et al., 1997; Crouse et al., 1995; Kantor et al., 1987). In summary, it appears that even a prolonged (i.e., marathon) session of exercise may not produce consistent and/or significant reductions in post-exercise TCHL concentrations.

Similar to the findings noted for TCHL, LDL-C exhibits a highly variable response to a single session of exercise. This is not surprising when considering that the primary lipoproteins responsible for carrying the bulk of circulating cholesterol are LDL molecules. Reductions in LDL-C that range from zero to 38% have been reported in trained men immediately and up to 72 hours after completing marathon type exercise

(>800 kcal energy expenditure) (Bounds et al., 2000; Ferguson et al., 1998; Foger et al., 1994; Sady et al., 1986; Visich et al., 1996). However, small increases (5%) and decreases (8%) in post-exercise LDL-C concentrations have been observed in hypercholesterolemic men post-exercise (energy expenditure ranging from 350 to 500 kcal) (Crouse et al., 1997; Crouse et al., 1995; Grandjean et al., 2000; Hughes et al., 1990). The conflicting LDL-C changes after exercise are likely due to the variation in energy expenditure and the training status of the hypercholesterolemic men. These data indicate the possibility that hypercholesterolemic men may react to exercise differently than normocholesterolemic men, but this hypothesis requires more research.

Data on LDL-C changes in women is sparse, but reductions have been reported 24 hours after performing marathon type exercise (Pronk et al., 1995; Goodyear et al., 1990). However, data collected utilizing smaller amounts of caloric expenditure (i.e., 300 to 500 kcal) have shown no post-exercise LDL-C concentration changes in young vs. old or normal vs. hypercholesterolemic women (Crouse et al., 1999; Imamura et al., 2000). These trends are similar to those found in male subjects. Overall, it appears that the stimulus of an acute session of exercise (e.g., 350-500 kcal) is not enough to significantly reduce TCHL and/or LDL-C concentrations in either male or female subjects (Thompson et al., 2001).

Changes in HDL-C and TG Concentrations

Since HDL-C is inversely related to the risk of developing CAD, this lipoprotein has received a significant amount of research attention. Plasma TG concentrations have

also been extensively studied, with recent prospective cohort studies establishing it as a significant and independent risk factor for CAD (Assmann et al., 1998; Hokanson and Austin, 1996). The general consensus of research findings indicates that single sessions of exercise are effective stimuli for temporarily elevating HDL-C and reducing TG concentrations. These temporary effects observed after a single session of exercise are believed to, at least partially, account for the anti-atherogenic lipid profile of an aerobically trained individual. Concentration increases in HDL-C range from 3 to 14 mg/dL and appear to peak 24 to 48 hours post-exercise before returning to pre-exercise concentrations by 72 hours (Thompson et al., 2001). In a similar time frame, reductions in TG range from 5 to 26 mg/dL and appear to peak 18 to 24 hours post-exercise and may persist for up to 72 hours (Thompson et al., 2001). Past research has utilized exercise protocols with intensities ranging from 50 to 90% VO_{2max} and durations from 12 to 244 minutes (i.e., 350 to 1500 kcal energy expenditure), respectively (Angelopoulos et al., 1993; Bounds et al., 2000; Crouse et al., 1997; Crouse et al., 1995; Davis et al., 1992; Ferguson et al., 1998; Foger et al., 1994; Frey et al., 1993; Goodyear et al., 1990; Gordon et al., 1998; Grandjean et al., 2001; Hughes et al., 1990; Imamura et al., 2000; Kantor et al., 1987; Kantor et al., 1984; Lee et al., 1991; Lennon et al., 1983; Park and Ransone, 2003; Swank et al., 1987; Tsetsonis & Hardman, 1995; Visich et al., 1996). Therefore, numerous articles and abstracts have examined the acute effect of exercise on lipids and lipoproteins, as shown in Table 3. However, the wide ranges of exercise intensities and durations have made it difficult to define an optimal exercise dose required to produce a metabolic response.

Table 3

The Effects of Single Sessions of Exercise on TG and HDL-C Concentrations

Source	Subj	Mode	Intensity (% max)	Time (min)	Kcal	Timing	TG	HDL-C
Angelopoulos & Robertson, J Sp Med Phys Fitness 33:264-267, 1993	7M 22 UT	TM	65	30	NR	IPre 24HrP 48HrP	93 -11 -1	42 0 0
Angelopoulos & Robertson, Int J Sp Med 14:196-201, 1993	7M 22 UT	TM	65	30	NR	IPpre 24HrP 48HrP	123 48 5	39 1* 0
Bounds et al., Exerc. Metab 10: 114-127, 2000	14M 28 T	TM	70	~108	1000	24HrPre 24HrP 48HrP	113 -16 -25*	48 5* 4*
Crouse et al., Med Sci Sports Exerc 33(5) (S1): May, 2001	15M 44 UT	TM	60 -70	NR	500	Low		
						24HrPre 24HrP 48HrP	190 -25* -25*	35 3* 3*
						Moderate		
						24HrPre 24HrP 48HrP	103 -13* -13*	51 5* 5*
Crouse, et al. JAP 83:2019-2028, 1997	26M 47 UT	Cycle	50	NR	350	IPre 24HrP 48HrP	207 -44* -34*	43 5* 6*
Crouse et al., JAP 79:279-286, 1995	39M 46 UT	Cycle	50 & 80	NR	350	IPre 24HrP 48HrP	177 -33* -26*	45 4* 5*
Davis et al., JAP 72(3): 914-919, 1992	10M 28 T	TM	50	90	950	IPre 24HrP 48HrP 72HrP	79 -10 -7 -2	56 0 0 3
Ferguson et al., JAP 85(3):1169-1174, 1998	11M 32 T	TM	70	60 to 112	800 to 1500	IPre 24HrP 48HrP	110 -40* -19*	42 12* 10*

Table 3

The Effects of Single Sessions of Exercise on TG and HDL-C Concentrations (cont.)

Source	Subj	Mode	Intensity (% max)	Time (min)	Kcal	Timing	TG	HDL-C
Frey et al., Euro J Appl Phys 66:521-25, 1993	13M 31 T	Run	78	130	NR	IPre 20HrP	97 -30*	56 0
Gordon et al., Br J Sports Med 32: 63-67, 1998	12F 27 T	TM	75	NR	800	IPre 24HrP 48HrP	97 -21 -23	60 0 5*
Grandjean et al., JAP 89:472-480, 2000	25M 45 UT	TM	70	NR	500	Normocholesterolemic		
						IPre 24HrP 48HrP	140 -16* -16*	43 4* 6*
						Hypercholesterolemic		
						IPre 24HrP 48HrP	155 -15* -19*	41 2* 5*
Hughes et al., J Sports Med Phys Fitness 30:37-44, 1990	24M 25 T	TM	20% below LT	15 30 45	NR	IPre 24HrP 48HrP	108 -20 15	50 3* -1
Imamura, et al. ClinExPathPharm, 2000	7F 22 UT	Cycle	60	30 60	170 321	Ipre 24HrP	62 -6	54 0
Kantor et al., Metabol 36(2): 188-192, 1987	21M 31 UT	Cycle	80	60	NR	24HrPre 24HrP 48HrP 72HrP	97 8 13 9	39 3* 3* 3*
Kantor et al., Metabol 33(5): 454-457, 1984	10M 29 T	Run	85	188	NR	24HrPre 18HrP 42HrP	95 -34* -26*	65 10* 13*
Park, et al. Int.J.SpMed 24: 2003	18M 25 UT	TM	70% LT, LT	NR	350	IPre 24HrP	266 -27*	46 4*

Table 3

The Effects of Single Sessions of Exercise on TG and HDL-C Concentrations (cont.)

Source	Subj	Mode	Intensity (% max)	Time (min)	Kcal	Timing	TG	HDL-C
Swank et al., Atherscl 63:187-192, 1987	9M 25 T	TM	70	40	NR	IPre 24HrP 48HrP 96HrP	91 -7 -6 -3	62 1 0 2
Tsetsonis & Hardman, Eur J Appl Physiol 70:329-36, 1995	5M/7F 28 UT	TM	30 & 60	90	NR	IPre 24HrP	74 -14	50 1
Visich et al., EJP 72:242-248, 1996	12M 26 T	TM	74	28 41 55	400 600 800	24HrPre 24HrP	67 -13*	40 6*
Wooten & Biggerstaff, Eur J Appl Physiol 104: 19-27, 2008	11F 24 UT	TM	65	NR	500	IPre 24HrP 48HrP	103 -20 -26*	51 -1 -5*
Zhang et al., Am J Endocrinol Metab 283:E267-E274, 2002	16M 29 UT	TM	60	60	~500	IPre 24HrP	198 -18*	34 0

Subjects: n = subject number; gender = Male (M) or Female (F); age in years; training status = Trained (T) or Untrained (UT). Mode: Treadmill (TM). Duration: minutes (min). Not Reported (NR). Timing: I = Immediate, P = Post, Ex = Exercise, Hr = Hour(s). TG = Triglycerides. HDL-C = High Density Lipoprotein-Cholesterol. Baseline lipids and lipoprotein-lipids are given as mg/dL, then mg/dL change from baseline.
* Significantly different from baseline, $P < 0.05$.

Many experimental studies have reported simultaneous increases in HDL-C and

reductions in TG concentrations. Crouse et al., (1995) utilized 39 hypercholesterolemic,

untrained men (~45 years of age; BMI=28 kg/m^2) to compare the effects of a single cycle

ergometer exercise session at moderate (i.e., 50% VO$_{2max}$) versus high (80% VO$_{2max}$)

intensity exercise. Subjects were required to expend 350 kcals, which took 60 minutes at the moderate intensity and 35 minutes at the high intensity, respectively. Both exercise intensities showed similar statistically significant elevations (+4 mg/dL) in HDL-C and reductions in TG (-25 mg/dL) concentrations 24 and 48 hours post-exercise. Grandjean et al., (2000) reported similar findings when comparing hypercholesterolemic (n= 13) to normocholesterolemic (n=12) men (~45 years of age; BMI=27.8 kg/m^2). Subjects performed a treadmill session, requiring 500 kcal, at 70% VO$_{2max}$. While total exercise time was not reported, it can be calculated that exercise time was 45-60 minutes. Subjects demonstrated a concomitant 14% increase (+11 mg/dL) in HDL-C concentrations and a 10% reduction (-11.1 mg/dL) in TG concentrations 24 and 48 hours post-exercise. Crouse and colleagues (2001) recruited sedentary middle-aged men (~44 years of age) with initially moderate (e.g., ~50 mg/dL) or low (e.g., 35 mg/dL) HDL-C concentrations. After expending 500 kcal during a treadmill run at 60 to 70% VO$_{2max}$, all subjects demonstrated an increase in HDL-C (+3.2 mg/dL) and reduction in TG concentrations (-24.7 mg/dL) 24 and 48 hours post-exercise.

Taken together, the evidence indicates that a single session of exercise can stimulate concomitant elevations in HDL-C and reductions in TG concentrations. Most research indicates that initial blood lipid profile will not affect the extent of post-exercise blood lipid profile changes. However, individuals with initially low HDL-C levels as an isolated trait (i.e., without elevated TG concentrations) may be less responsive to exercise induced blood lipid profile changes (Couillard et al., 2001). A single session of exercise lasting between 35 and 60 minutes was reported to be sufficient for stimulating post-

exercise lipid metabolism changes, regardless of exercise intensity. Since these studies utilized middle-aged caucasian males, the results cannot be generalized to other populations. However, there are similarities between these findings and the results of single session exercise in younger, untrained individuals.

Research on young untrained individuals has produced mixed results in terms of the effects of a single session of exercise on post-exercise HDL-C and TG concentrations. Since most untrained individuals are not able to perform long duration exercise, most studies involve a moderate amount of exercise at a moderate intensity. With the exception of a few studies (Angelopoulos and Robertson, 1993; Imamura et al., 2000, Magkos et al., 2006, Wooten et al., 2008), numerous studies indicate that a single session of exercise can produce beneficial HDL-C and/or TG concentration changes in young, untrained individuals. A study conducted by Kantor and colleagues (1987) assessed the lipid profiles of young (25-39 years of age) men before and after a cycle ergometer session at 80% VO_{2max}. Untrained subjects (n=10) cycled for one hour while trained (n=11) subjects cycled for two hours, implemented to represent a hard daily workout. Although exercise time was not matched, both trained and untrained subjects experienced similar statistically significant elevations in HDL-C concentrations (~ 5 mg/dL) from pre to 24, 48, and 72 hours post-exercise. Similar changes have been reported by Alhassan et al. (2001; abstract) for HDL-C concentrations following a treadmill bout at 60 to 70%VO_{2max}, requiring 350 kcal. Utilizing 14 sedentary, African-American females (~32 years of age), HDL-C was significantly elevated immediately through 48 hours post-exercise. While HDL-C concentrations were beneficially altered, no significant TG

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concentration changes were observed. However, other studies assessing young, untrained individuals have shown TG concentrations to be more responsive than HDL-C levels.

A study conducted by Lee and colleagues (1991) recruited 24-45 year old women (n=12) who were untrained and mildly obese. Data results showed significant reductions (-5mg/dL) in TG concentrations ~24 hours after performing a 45 minute treadmill walk at 60% VO_{2max}. Tsetsonis & Hardman (1995) found similar results in young (~28 years old) untrained subjects (five men; seven women). In random order, subjects performed treadmill exercise for 90 minutes at 30%VO_{2max} and 60%VO_{2max}. Significant TG concentration reductions (-3 mg/dL) were observed in all subjects, independent of intensity, 24 hours post-exercise. Zhang and colleagues (2002) pre-screened TG concentrations for 16 sedentary male subjects (~38 years old), classifying 10 as normal and six as hypertriglyceridemic. All subjects performed an hour treadmill session at 60% VO_{2max}, with hypertriglyceridemic subjects presenting a decreased TG concentration (~37 mg/dL) 24 hours post-exercise. While both the normal and hypertriglyceridemic subjects experienced similar LPL increases, the subjects with normal pre-exercise TG concentrations showed no post-exercise lipid changes (See Table 3).

In summary, research conducted on young, untrained individuals is somewhat discrepant. Some studies report only increased post-exercise HDL-C concentrations, while others report only reduced post-exercise TG concentrations. In support of all previously reported single session articles, it appears that exercise intensity alone does not affect post-exercise lipid metabolism. From these studies it appears that a young, untrained individual needs to perform a minimum of 45 to 60 minutes (e.g., >350 kcal

energy expenditure) of exercise to elicit post-exercise HDL-C and/or TG concentration changes, independent of gender or race. Although, there is no guarantee that both HDL-C and TG concentrations will concomitantly improve, these plasma markers still appear to be independently modifiable in young, untrained individuals.

Apart from a few studies (Davis et al., 1992; Hughes et al., 1990; Imamura et al., 2000; Lennon et al., 1983; Swank et al., 1987), exercise of longer duration (i.e., higher caloric expenditure) generally elicits beneficial post-exercise HDL-C and TG concentration changes (See Table 3). However, similar to the data presented on young, untrained individuals, there is no guarantee that exercise will significantly modify both HDL-C and TG concentrations post-exercise. For example, Hughes et al. (1990) required 24 moderately trained normolipidemic males (19-31 years of age) to perform a 30 and 45 minute treadmill run, in a random crossover design, at 20% below lactate threshold. While both exercise durations produced a decreasing trend in post-exercise TG, concentrations did not reach statistical significance. However, data from the 45 minute session showed significantly higher HDL-C concentrations at 24 hours post-exercise compared to data from the 30 minute session. Results also showed these HDL-C increases were returning toward baseline levels by 48 hours post-exercise.

Utilizing similar subjects, Gordon and colleagues (1998) required young (18-35 years of age) moderately trained females to perform a treadmill run at 75% VO_{2max} for approximately 72 minutes (800 kcal). TG concentrations showed a non-significant trend toward a decrease, while HDL-C concentrations increased 24 hours post-exercise, becoming significant (5 mg/dL) at 48 hours post-exercise. Additionally, Bounds et al.,

(2000) recruited 14 moderately trained men (~28 years of age) and had them exercise at 70% VO_{2max} for approximately 108 minutes (~1,000 kcal). Results showed a significant (e.g., 11 mg/dL) increase in HDL-C 24 hours after exercise and remained significantly elevated (e.g., 8 mg/dL) until 48 hours after exercise. Moreover, TG concentrations showed a significant (e.g., 16 mg/dL) decrease 24 hours post-exercise and remained significant (e.g., 25 mg/dL) through 48 hours post-exercise. These studies indicate that young, moderately trained individuals may be able to significantly increase HDL-C concentrations, and potentially reduce TG concentrations, by performing an exercise bout lasting between 30 and 72 minutes.

To test this exercise duration range, Ferguson and coworkers (1998) assessed HDL-C and TG concentrations following 70% VO_{2max} treadmill runs in trained males. Eleven trained men (21-44 years of age), in a random crossover design, performed treadmill bouts requiring 800, 1,100, 1,300, and 1,500 kcal. Final data indicates that an energy expenditure of 1,500 kcal was the most effective in significantly decreasing post-exercise TG concentrations (e.g., 31 mg/dL decrease 24 hours post-exercise and 13 mg/dL decrease 48 hours post-exercise). Moreover, 1,500 kcal expenditure produced statistically significant HDL-C concentration changes immediately post-exercise (e.g., 6 mg/dL increase), 24 hours post-exercise (e.g., 9 mg/dL increase), and 48 hours post-exercise (e.g., 7 mg/dL increase). Similar to the 1,500 kcal exercise bout the 1,100 and 1,300 kcal exercise bouts elicited statistically significant HDL-C and TG concentrations 24 hours post-exercise. However, these significant changes returned to non-significant levels by 48 hours post-exercise. Therefore, this study indicates that a 1,500 kcal

exercise bout is required to elicit statistically significant HDL-C and TG concentration changes both at 24 and 48 hours post-exercise.

An earlier study conducted by Kantor et al., (1984) supports the findings of Ferguson. Kantor assessed the effects of a 42 kilometer race (~188 minutes of exercise) on ten trained male runners (~30 years old). This prolonged session of exercise elicited a significant increase (e.g., 10 mg/dL) in HDL-C 18 hours after exercise, remaining elevated 42 hours post-exercise (e.g., 13 mg/dL). Moreover, TG concentrations were significantly decreased (e.g., 34 mg/dL) 18 hours post-exercise, remaining significantly decreased through 42 hours post-exercise (e.g., 26 mg/dL). A more recent prolonged exercise study conducted by Frey et al., (1993) also supports the findings of Ferguson. Frey had 13 endurance trained males (~31 years of age) perform a 30 kilometer run (~130 minutes) at ~80% VO_{2max}. Results showed a statistically significant decrease (e.g., 30 mg/dL) in TG concentrations 20 hours post-exercise. However, this study did not examine blood samples beyond 20 hours post-exercise, limiting the results of post-exercise lipid changes.

Taken together, these findings suggest that acute bouts of aerobic exercise can increase HDL-C concentrations in young, trained males and females. The increase in HDL-C and decrease in TG concentrations appear to peak approximately 24 to 48 hours post-exercise then begin to return to baseline levels thereafter. This time line for HDL-C and TG change, as well as the magnitude of change (e.g., percent increase from pre to post-exercise) appears to be similar for both genders. Moreover, these studies utilized subjects with normal to high baseline HDL-C concentrations (~40-56 mg/dL) who still

exhibited the ability to temporarily modify HDL-C levels via a single session of exercise. This is vital in that more sedentary individuals (i.e., untrained) may not have to perform as much exercise to elicit blood lipid profile changes compared to more trained individuals. Finally, these studies suggest that HDL-C concentration changes are primarily related to exercise duration rather than exercise intensity (Ferguson et al., 1998; Hughes et al., 1990).

Acute Augmented Exercise Response

The majority of the literature presented in this review indicates that both a single session and exercise training can beneficially alter post-exercise plasma lipids and lipoproteins. Significant increases in HDL-C concentrations are reported after a single session (i.e., moderate intensity session lasting from 30 to 188 minutes) of exercise, ranging from 2 to 13 mg/dL (5-20%). These beneficial changes in HDL-C were typically observed from 1.5 to 48 hours post-exercise. Similarly, significant reductions in TG concentrations range from 5 to 37 mg/dL (4-36%), and are seen within a similar post-exercise timeframe as HDL-C changes. Exercise training studies [typically based upon a physical activity recommendation (i.e., >20 weeks, ≥3 sessions per week, moderate intensity, ≥30 minutes per session)] show significant increases in HDL-C ranging from 2 to 8 mg/dL (4-22%), reaching peak concentrations by 24-48 hours post-training (Table 2). Likewise, significant reductions in TG concentrations range from 5 to 38 mg/dL (4-37%), and are seen within a similar post-study timeframe as compared to HDL-C. For

acute exercise and exercise training, HDL-C and TG concentrations returned to pre-study/exercise concentrations within 72 hours post-exercise.

Therefore, both acute and exercise training have been shown to increase HDL-C and decrease TG concentrations to a similar magnitude and post-exercise time frames. However, there are internal differences between the two methods of assessing post-exercise lipid and lipoprotein concentrations, which have not been answered. Firstly, the effect of exercise frequency on post-exercise blood lipid changes has not been directly assessed. Most exercise training studies implement multiple weeks of training during which subjects are required to perform a set number of weekly exercise sessions. The specific frequency of these weekly exercise sessions is generally not defined (i.e., every day, every other day, etc.). This set up does not allow the researcher the ability to conclude on any short-term (i.e., one to three sessions) exercise frequency based hypotheses. Secondly, most studies assess pre- and post-exercise training (i.e, immediate, 24, 48, 72, and/or 96 hours after the last individual exercise session of the training protocol). With approximately 12 weeks between these two blood samples, any mid-study (i.e., daily or weekly) blood concentration fluctuations are missed. By not measuring multiple time-points throughout an exercise training study, little data exists assessing the "acute augmented response".

The acute augmented response suggests that accumulating repeated sessions of exercise produces an additive effect beyond that of a single session of exercise (Grandjean et al., 2001). It is hypothesized that accumulated sessions of exercise will produce an additive effect (i.e., greater reduction in CAD risk) on an individual's blood

lipid profile. This additive effect occurs because subsequent exercise sessions are performed while the blood lipid profile is still favorably altered due to the previous exercise session (Figure 3). Therefore, this augmented response may occur briefly after 1 to 2 exercise sessions, then reach a natural limit (i.e., plateau in beneficial effects) (Durstine et al., 1994; Thompson et al., 2001). Chronic exercise training shows beneficial results as well as acute, however, it is not know if these beneficial results are due to the accumulation of exercise or the effects of one exercise stimulus.

Regarding single session exercise and the acute augmented response, few data are presently available. Angelopoulos et al. (1993) studied the lipid response of nine untrained men (~22 years of age) after 30 minute treadmill bouts at 65% VO_{2max}. Nine young (e.g., 22 years old) male subjects performed three exercise patterns (each pattern separated by a seven day period): (1) single session, (2) two sessions (separated by 48 hours), and (3) three sessions (each separated by 48 hours). Blood samples were drawn at five minutes, 24 and 48 hours after the last exercise session within each experimental pattern. Final results showed that HDL-C was significantly increased 24 hours post-exercise for all exercise patterns, then quickly returned to pre-exercise concentrations by 48 hours post-exercise. Exercise pattern(1) elicited a 1 mg/dL increase; pattern (2) elicited a 2 mg/dL increase, while pattern (3) elicited a 4 mg/dL increase. This pattern of post-exercise HDL-C change supports the position that accumulating single sessions of exercise in a set frequency may produce an additive effect. Moreover, an additive effect which produces greater health benefits beyond those observed after a single session of

Figure 3

Acute Augmented Exercise Response
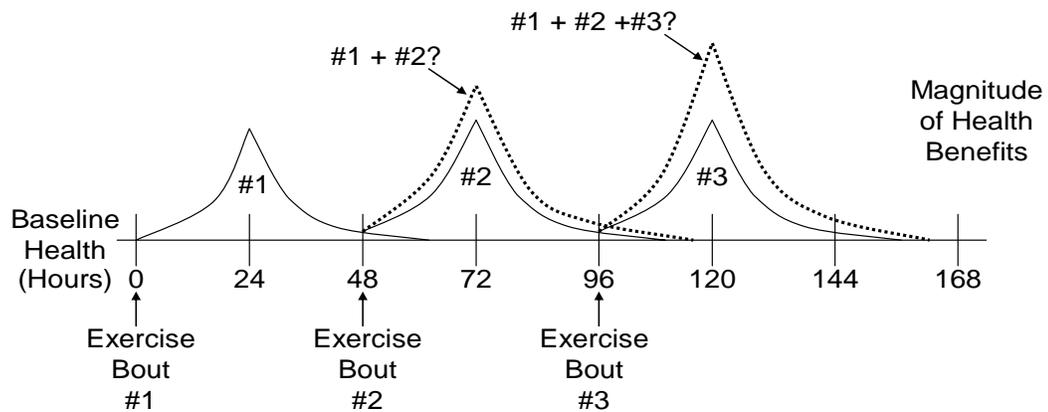
Acute Augmented Exercise Response. Performing a bout of exercise (exercise bout #1, above) generally produces a known magnitude of health benefits, as seen with the solid black lines, which return to baseline health concentrations after a set amount of time (i.e., 48-72 hours post-exercise). Currently, it is assumed that this acute response to a single bout of exercise will be observed with each additional exercise bout (i.e., exercise bout #2 and #3). However, it is hypothesized that accumulating repeated bouts of exercise will produce an additive effect beyond that of a single bout of exercise. This phenomenon may occur because accumulated sessions of exercise will produce an additive effect on the magnitude of health benefits (dotted lines) because subsequent exercise bouts are performed while health benefits are still favorably altered from the previous exercise bout.

exercise. It is important to note that blood samples were not obtained before the second [i.e., pattern (2)] exercise session or before the second and third [i.e., pattern (3)]. Interestingly, the results did not show HDL-C concentrations staying significantly elevated beyond 24 hours post-exercise for any exercise pattern. This study did not show any statistically significant TG changes, although a substantial decline (~28 mg/dL) in TG concentrations was observed 24 hours post-exercise in pattern three. Therefore, it is possible that some of the benefits of accumulating two and three exercise sessions were not fully measured.

A study attempting to answer the acute augmented response question was recently conducted by Biggerstaff et al. (2005-abstract). This study utilized a similar exercise protocol as compared to Angelopoulos et al. (1993). Eleven young (e.g., 25 years old) female subjects performed three exercise sessions, each session separated by 48 hours. Treadmill exercise was performed at 65% VO_{2max} for a duration requiring 500kcal (~45 minutes). Blood samples were obtained based upon the first exercise session as follows: immediately pre, 24 hours post, 48 hours post (coordinating with exercise session #2 conducted 48 hours after the first exercise session), 96 hours post (coordinating with exercise session #3 conducted 96 hours after the first exercise session), and 144 hours post (acting as a 48 hour post-exercise session #3). Therefore, unlike Angelopoulos et al. (1993), this study did attempt to assess blood lipid and lipoprotein concentration changes throughout an accumulated exercise protocol. It is also important to note that this study did have all subjects perform a resting protocol in which subjects followed the same blood sampling protocol, but simply relaxed in a chair for ~45 minutes instead of

exercising. A significant reduction in TG concentrations (i.e., 20 mg/dL) was observed 48 hours after the third exercise session. HDL-C concentrations were trending upward at the 96-hour blood sampling, but were not significant, while appearing to begin to return to pre-exercise concentrations by 48 hours post-exercise session #3.

Grandjean and colleagues (2001) conducted a study to determine the serum lipid and lipoprotein responses to repeated sessions of exercise on successive days (abstract). Fifteen hyperlipidemic men (~50 years old) expended 350 kcals via treadmill walking/jogging at 70% VO_{2max} for four consecutive days (24 hours between each session). Blood samples were obtained prior to all four exercise sessions, then 24 and 72 hours after the fourth exercise session. Final results showed one session of exercise significantly raised HDL-C concentrations by 1.5 mg/dL and significantly lowered TG concentrations by 40 mg/dL. Moreover, as successive exercise sessions were performed both HDL-C and TG levels continued to improve in an additive manner. These concentration improvements persisted for three days after the last exercise session. This indicates that four days of successive exercise produces an additive effect that beneficially alters HDL-C and TG concentrations, while also increasing the amount of time in which these benefits persist (i.e., post-exercise).

A recent study conducted by Mestek et al. (2006) partially assessed the acute augmented response to exercise. This study compared the effects of one 500 kcal exercise session to three 167 kcal exercise sessions, both completed within one day. Nine moderately trained male subjects (20-30 years of age) completed one-500 kcal exercise session and three-167 kcal exercise sessions, separated by at least one week. Both

exercise protocols were performed at 70% VO_{2max} on a treadmill. For the three-167 kcal exercise protocol, a minimum four hours of rest was required between each session. Therefore, subjects completed all three exercise sessions (i.e., morning, mid-day, and evening) within one day. The most significant finding was the accumulated (i.e., three sessions of 167 kcals each) was more effective in terms of raising HDL-C than the single 500 kcal session of exercise. Compared to pre-exercise HDL-C concentrations, the three-167 kcal exercise protocol elicited significant increases at 24 (5 mg/dL) and 48 hours post-exercise (7 mg/dL). While the 500 kcal exercise session did not elicit significant increases in HDL-C, there was a slight upward trend at the 48 hour (2 mg/dL) post-exercise time point. TG concentrations were also assessed, but no significant changes were elicited. However, the accumulated exercise session did elicit a downward trend in TG concentrations from pre- to 24-hours post-exercise (31 mg/dL), and pre- to 48 hours post-exercise (25 mg/dL). The single session of exercise did not cause any changes in post-exercise TG concentrations. Even though TG concentration changes did not reach statistical significance, this study does support previous data suggesting accumulating exercise in a set pattern may be more effective in modifying lipid and lipoprotein concentrations compared to one session. While this study does not directly match the Angelopoulos or Grandjean articles, it still provides insight to the effects of accumulating exercise in a set pattern.

Although the data on the acute augmented exercise response is limited, vital information is gained from these studies, listed in Table 4. While each author utilized

Table 4

The Effects of Acute Accumulated Sessions of Exercise on TG and HDL-C
Concentrations

Source	Subj	Study Design	Int. (% max)	Time (min)	Kcal	Session	Timing	TG	HDL-C
Angelopoulos et al., Int J Sports Med 14: 196-201, 1993	9M 22 UT UT	TM 1, 2, & 3 bouts ex 48hr	75	30	NR	1 Ex	IPre	123	39
							24HrP	48	1*
							48HrP	4	0
						2 Ex	IPre	114	37
							24HrP	-14	2*
							48HrP	5	0
						3 Ex	IPre	135	39
							24HrP	-28	4*
							48HrP	-5	1
Biggerstaff et al., Res Q Exerc Sport 76(1): 76, 2005	11F 25 ModT	TM 3 bouts of ex 48hr	65	~45	500		Ipre	103	51
							24HrP	-10	1
							48HrP	-10	1
							96HrP	-13	2*
							144HrP	-20*	1
Grandjean et al., Med Sci Sport Exerc 33(5): S215, 2001	15M 50 UT	TM 4 cons. days of ex 24hr	70	NR	350		Day 1	266	32
							Day 2	-40*	0
							Day 3	-60*	2*
							Day 4	-59*	3*
							24HrP	-80*	4*
							72HrP	-75*	6*
Mestek et al., Int J Sport Nutr & Ex Metabol 16: 245-54, 2006	9M 30 ModT	TM 1-500 kcal	70	NR	500	Cont.	IPre	156	50
							24HrP	-1	0
							48HrP	-2	2
		3-167 kcal				Accum.	IPre	159	49
							24HrP	-31	5*
							48HrP	-25	7*

Subjects: n = subject number; gender = Male (M) or Female (F); age in years; training
status = Trained (T) or Untrained (UT). Mode: Treadmill (TM). Duration: minutes (min).
Not Reported (NR). Timing: I = Immediate, P = Post, Ex = Exercise, Hr = Hour(s). TG =
Triglycerides. HDL-C = High Density Lipoprotein-Cholesterol. Baseline lipids and
lipoprotein-lipids are given as mg/dL, then mg/dL change from baseline.
* Significantly different from baseline, P < 0.05.

different age groups, previous research indicates that age does not significantly impact post-exercise blood lipid changes. These studies also utilized different exercise protocols, although the outcomes of each provide direction for this proposal. The studies conducted by Angelopolous et al (1993), Biggerstaff et al. (2005) indicate that performing three non-consecutive days of moderate intensity exercise can be more effective than one exercise session in reducing TG concentrations and concomitantly increasing (potentially significantly) HDL-C concentrations in young untrained or moderately trained individuals. Findings by Mestek et al. (2006) support this finding even if total kcal expenditure is split into three sessions versus one continuous session. Most importantly, results from Grandjean et al. (2001-abstract) indicate that repeated bouts of exercise on successive days can produce an *additive* effect on raising HDL-C concentrations and lowering TG concentrations. These exercise-induced changes in HDL-C and TG appear to persist for up to 3 days after the last exercise session. This information supports the proposed exercise protocol, which implemented three successive days of exercise.

In summary, both single session and exercise training appear to improve an individual's blood lipid profile, primarily by affecting HDL-C and TG concentrations. While this beneficial effect on blood lipid profile has not been shown in all experimental settings, the potential for modifying plasma lipid markers is indeed present. This assumes that the exercise is of sufficient duration (e.g., $\geq$30 - 60 minutes), which appears to be based upon individual training levels. It is believed that this exercise duration stimulates metabolic factors (i.e., LPL, CETP, and LCAT) which affect plasma lipid

concentrations. However, the beneficial effects of lipid metabolism following an exercise session may be due to the acute response of the single session or a delayed response from multiple sessions is presently unknown. Clearly, more work is necessary in order to ascertain the exact duration of exercise and proper exercise accumulation (i.e., frequency per week) required to produce the most optimal post-exercise blood lipid response. Thereby effectively reducing the risks associated with the development of atherosclerosis and related CHD.

Based on the current state of lipid and lipoprotein knowledge, a direct comparison of a single session to accumulated sessions of exercise is needed. While final data did not define the most optimal exercise duration or frequency, it tested an exercise recommendation (i.e., 90 minutes of moderate intensity) and assessed a specific exercise frequency (i.e., three consecutive days of exercise). Results will be utilized for future exercise studies designed to investigate various elements of exercise dose (including exercise frequency) on blood lipid markers in how they relate to cardiovascular disease risk factors.

CHAPTER III

METHODS AND PROCEDURES

This chapter presents the methods that were used to investigate the effect of a single session compared to accumulated sessions of exercise, matched for time, on plasma lipid and lipoprotein concentrations. It is organized according to the following subsections: Subjects, Pre-Study Screening, Maximal Exercise Testing, Submaximal Exercise Protocols, Dietary Records, Handling and Analysis of Blood Samples, and Statistical Analysis.

Subjects

Twelve apparently healthy, sedentary subjects, males (n=6) and females (n=6) of 18-26 years were recruited to participate in this study through both posted flyers (Appendix A) and via word of mouth. Sample size was determined in order to observe statistically detected effect given 80% statistical power (Appendix B). Because we expected the greatest subject variability to occur in the measurement of HDL-C concentration (females typically have higher HDL-C concentrations), this dependent variable was used in the power calculation.

Subjects were non-smokers and sedentary (e.g., performed less than one exercise session per week over the previous six months). In addition, subjects had a resting blood pressure < 140/90mmHg and a waist circumference $\geq$ 102 cm (males) or $\geq$ 88 cm (females). Subjects were not using lipid or lipoprotein altering medications or supplements (e.g., fish oil capsules) during the six months leading up to participation in this study. Only normally

menstruating females were allowed to participate in the study. More specifically, only females who had three consecutive, normal length menstrual cycles were allowed to participate in the study. Also, female subjects were not pregnant nor had they breastfed within the past six months prior to participation in the study. A health history questionnaire (Appendix C), physical activity questionnaire (Appendix D), and informed consent (Appendix E) were completed by all subjects to determine eligibility.

Prior to participation, each subject was informed of all procedures, potential risks, and benefits associated with the study through both verbal and written form in accordance with the procedures approved by the University of North Carolina at Greensboro Institutional Review Board for Human Subjects Research.

Pre-Study Screening

The researcher only conducted the following pre-study screening on potential subjects who reported that they were sedentary. Potential subjects reported to the exercise physiology laboratory to have baseline assessments conducted. Waist circumference was measured to the nearest half centimeter, a minimum of two times at the lateral border of the iliac crest, as described in The Third National Health and Nutrition Examination Survey (NHANES III) (NIH, 1998). A third measurement was obtained if the first two measurements were not within $\pm$ 1 cm. Body weight was also measured at this session, to the nearest half kilogram.

During a 10-minute (seated) rest period subjects completed a health history questionnaire, physical activity questionnaire, and an informed consent. After this 10-minute rest period, resting heart rate (HR) and blood pressure (BP) were assessed on both arms using

an automated blood pressure cuff (Omron, HEM-70SCP, Vernon Hills, IL) following the guidelines of the Seventh Report of the Joint Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (Chobanian et al., 2003). Duplicate BP readings were taken from both arms in a rotating order (i.e., left, right, left, right). If duplicate readings were not consistent ($\pm$5 mm Hg systolic, $\pm$3 mm Hg diastolic), a third reading was obtained and all data was averaged. Also, if duplicate resting HR readings were not within $\pm$5 beats per minute, respectively, a third measurement was obtained and all data was averaged. A 5-minute, 12-lead resting electrocardiogram (ECG) (Quinton Instruments Q3000, Seattle, WA) was then conducted to test for any heart abnormalities. All subjects were required to have a medical release form (Appendix F) signed by a physician prior to their involvement in this study.

At the end of the pre-study screening, subjects were given three food diary data forms (Appendix N), along with serving size examples. Subjects completed these dietary data forms (e.g., two weekdays and one weekend day) during the days leading up to their next laboratory visit. All future dietary record information was provided to the subject at the end of each laboratory visit (as described in the Dietary Records section).

Maximal Exercise Testing

Upon entering the exercise physiology laboratory, subjects were seated in a quiet environment for at least 10 minutes. Following the 10-minute rest period, resting heart rate and blood pressure were measured by an automated blood pressure cuff (Omron, HEM-70SCP, Vernon Hills, IL), utilizing the same procedures as described above. Height and

weight were assessed using a Seca standiometer and a calibrated physician's scale (DETECTO), respectively. Additionally, body fat percentage was assessed via a three site skinfold test using Harpenden calipers (Creative Health Products, Ann Arbor, MI) via the technique described by Jackson and Pollock (1978) for male subjects and Jackson et al. (1980) for female subjects. Male subjects had chest, abdominal, and thigh measurements taken, while female subjects' had tricep, suprailiac, and thigh measurements taken. All skinfold measurements were taken on the right side of the subjects' body and performed a minimum of two times at each site by the same researcher. The researcher rotated through the measurement sites in order to allow time for the skin to regain normal texture and thickness. If the two measurements of each site were not within 2mm, a third measurement was taken. The intra-site average was then calculated for each skinfold site. Each site average was then added together to obtain a total skinfold thickness for all three sites. This number was utilized for the determination of body density. Based upon ethnicity, the proper percent body fat equation was utilized. For Caucasian subjects, the Siri equation was utilized to convert body density to percent body fat as follows: % body fat = [(4.95/body density) – 4.5] x 100. For African-American male subjects, the Wagner & Heyward equation was utilized to convert body density to percent body fat as follows: % body fat = [(4.37/body density) – 3.93] x 100. For African-American female subjects, the Ortiz equation was utilized to convert body density to percent body fat as follows: % body fat = [(4.85/body density) – 4.39] x 100. Separate equations were utilized in order to account for the natural difference in bone density amongst different ethnicities (ACSM, 2009). Therefore, the above

equations were utilized based upon ethnicity and age. The form that was utilized for subject data collection is presented in Appendix G.

All graded maximal exercise tests were conducted in the Exercise Physiology Laboratory at The University of North Carolina at Greensboro. Prior to the initiation of each test, both the flow meter and gas analyzers on the Ametek metabolic cart (Pittsburgh, PA), or Parvo Medics TrueOne®2400 metabolic cart (Sandy, UT) were calibrated to known gases. Subjects who tested on a given cart remained on that cart throughout all exercise sessions. The environmental conditions in the laboratory [temperature and relative humidity using a Fisher Scientific®(Model: 11-661-13) digital readout (Pittsburgh, PA) and barometric pressure using a Precision barometer (Southhampton, PA)] were measured in order to standardize all metabolic measurements.

Prior to the initiation of the maximal exercise test a full explanation was given to each subject in regard to the rating of perceived exertion (RPE) scale (Appendix H). This scale was utilized during the maximal test as an indicator of subject perceived exercise intensity. Heart rate and rhythm were monitored before, during, and following exercise testing via a twelve lead ECG. Subjects were then fitted for a one-way valved mouthpiece, which was utilized for expired gas analysis. A nose clip was then positioned in order to ensure no air escaped through the nose during testing procedures. With the subject standing on the treadmill, heart rate and ECG tracings were recorded and blood pressure was monitored by auscultation utilizing a stethoscope and mercury sphygmomanometer.

A maximal graded exercise test on a standardized institutional treadmill (Quinton Q55, Quinton Instruments, Seattle, WA) was then performed in order to determine maximal

oxygen consumption (VO_{2max}). The graded exercise protocol utilized was adaptable to an individual's fitness level and designed to elicit volitional exhaustion within 8-12 minutes. Exercise was performed until one of the following occurred: subject could no longer continue due to fatigue, VO_{2max} was achieved, technical difficulties, and/or subject showed signs or symptoms that indicate the test be terminated. The criteria for achieving VO_{2max} had to include at least two of the following: oxygen consumption plateau with an increased work rate, obtainment of $\pm$ 10 beats per minute of age-predicted maximum heart rate (220-age), a respiratory quotient $\geq$ 1.1. During each two minute stage, heart rate, rhythm, VO_2, respiratory quotient, and RPE were monitored and recorded (Appendix I).

Following the test, subjects performed a cool-down period for several minutes until heart rate fell below 120 beats per minute or stabilized. The peak (or max) VO_2 value was determined by calculating the mean of the three highest consecutive twenty-second VO_2 values. This VO_{2max} value was utilized to calculate workload during the submaximal aerobic exercise protocols. After the conclusion of this test, subjects were provided with study instructions and food diary data forms and, then scheduled for the remaining experimental visits. A stepwise description of all maximal exercise procedures is provided in Appendix J.

<center>Submaximal Exercise Protocols</center>

At the end of the maximal exercise laboratory visit all subjects received a detailed schedule outlining the time and dates of future exercise protocols. Male subjects returned to the exercise physiology laboratory within one week, while females returned within the first seven days of their next menstrual cycle. Subjects were instructed to maintain their

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sedentary lifestyle throughout the length of data collection. The weight of each subject was then obtained to determine any weight variations which may have occurred since the previous laboratory visit. Following a 10-minute rest period, resting BP and HR data were collected (as described above). Approximately 28mL of blood was obtained by a trained phlebotomist, and the same sterile procedures were used for all blood samples taken throughout the study. A Polar heart rate monitor (Woodbury, NY) was then placed on the upper torso for determination of exercising heart rate and post-exercise assessment of average heart rate throughout the entire session.

Male subjects then performed all three exercise protocols in random order with at least one week in between the last and first day of each protocol. Female subjects performed all three exercise protocols in random order with each protocol beginning within the first seven days of the menstrual cycle. All treadmill exercise sessions were matched for time (e.g., 90 minutes).

As with the maximal aerobic exercise testing procedures, prior to each exercise protocol described below, both the flow meter and the gas analyzers on the metabolic carts were properly calibrated. Also, the environmental conditions of the laboratory were analyzed and entered into the metabolic cart as previously indicated. Subjects were also allowed to consume 4 ml/kg body weight of water every 30 minutes to help maintain hydration and plasma volume (Ferguson et al., 1998).

The three experimental protocols are presented in Figure 4. Protocol one required a single session of exercise followed by three days of post-exercise blood samples. Protocol

Figure 4

Experimental Protocols
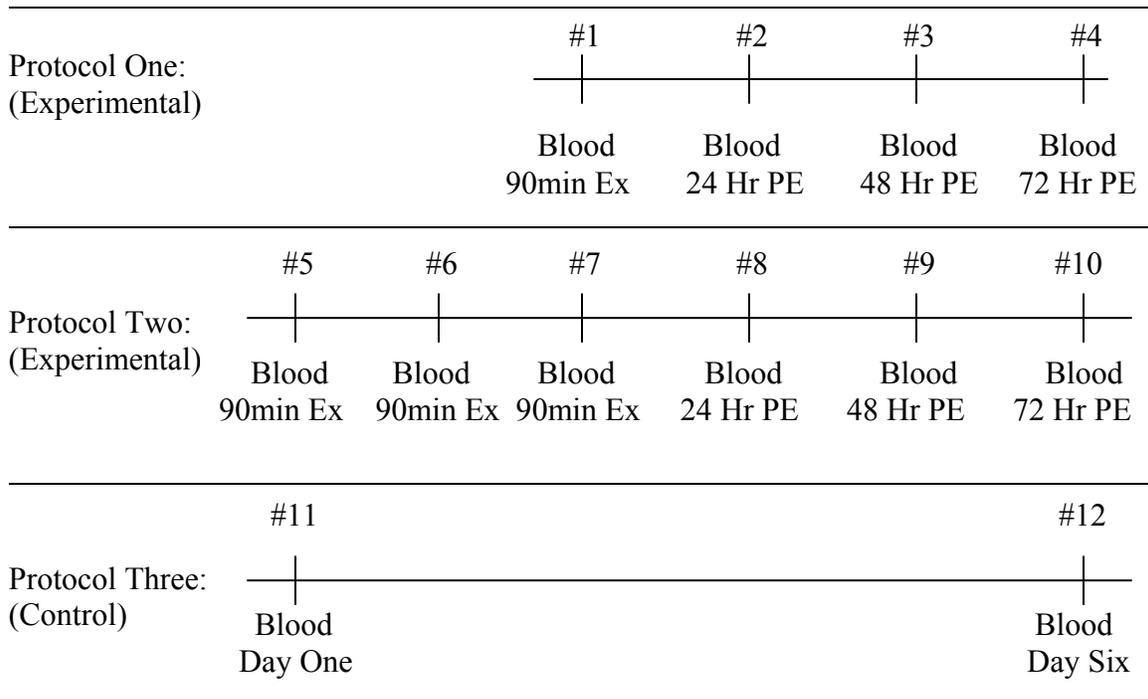
Experimental Protocols. Protocol One required a blood sample (Blood, see above) prior to a single session of aerobic exercise (e.g., 90 minutes; see session #1 above) with additional blood samples taken every 24 hours post-exercise (e.g., 24 Hr PE) until 72 hours post-exercise. Protocol Two required subjects to accumulate three days of exercise (session #5, #6, and #7; 90 minutes per session), then assessed blood lipid concentration changes in the same time frame as Protocol One (i.e., 24, 48, and 72 hours post-exercise). Within each of these two protocols, blood lipid concentrations were assessed from the pre-exercise time-points to all other time-points. Protocol Three (control) required two blood samples six days apart, equal to the number of days between the first and last session of protocol two. These time-points provided information regarding any natural fluctuations which may have occurred during the same amount of time required to perform protocol two.

two required three consecutive days of exercise, followed by three days of post-exercise

blood samples after the third exercise session. Protocol three acted as the control,

requiring subjects to provide two blood samples separated by a six-day time period. Each

exercise session within a protocol required 90 minutes of exercise at a moderate intensity

(i.e., 60% VO_{2max}). The exercise intensity (60% VO_{2max}) was at the upper end of the moderate intensity range, which is now widely recommended for the attainment of health-related benefits. The exercise duration (90 minutes) was selected to 1) be of sufficient duration to stimulate a post-exercise lipid and/or lipoprotein response, and 2) meet the criteria of a physical activity recommendation for obese individuals attempting to lose weight (Brooks et al., 2004). Using these exercise parameters, this study directly assessed the post-exercise blood lipid and lipoprotein responses to one session of exercise versus the accumulation of three sessions of exercise.

Similar to the maximal exercise test, subjects were breathing into a mouthpiece interfaced with the metabolic cart. The beginning of each exercise session required the subject to walk at a comfortable pace for three minutes. After this warm-up period, treadmill speed was increased until the heart rate range coinciding with 60% VO_{2max} was achieved. At the five minute mark gas analysis, via the metabolic cart, began. Gas exchange was measured until 15 minutes of exercise was completed. During this time, VO_2 values were cross referenced with the subject's VO_{2max} information to ensure proper intensity (e.g., 60% VO_{2max}). While time of exercise was the primary goal of this study, caloric expenditure was also estimated as the product of the absolute oxygen consumption and the caloric equivalent for oxygen according to the specific respiratory exchange ratio (Park & Ransone, 2003). Caloric expenditure was calculated every fifteen minutes. After caloric expenditure was calculated, the subject was then allowed to remove the mouthpiece and nose clip until the next 15-minute caloric expenditure reading. During the time period when oxygen consumption was not being measured, exercise intensity was maintained at a constant level,

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cross-referenced via heart rate and RPE. Total energy expenditure was extrapolated from the

15-minute oxygen consumption readings. Heart rate was monitored throughout the entire

test and utilized as a gauge of exercise intensity. This process was conducted in order to

continuously monitor caloric output while also allowing the subject to maintain a certain

level of comfort, until 90 minutes of exercise was complete.

Protocol One required subjects, in a single exercise session, to perform 90 minutes of

treadmill exercise at 60% VO_{2max}. As previously indicated, prior to this exercise protocol

a blood sample was obtained. Additional blood samples were obtained 24, 48, and 72 hours

post-exercise. Protocol Two required three sessions of treadmill exercise at 60% VO_{2max}.

Each exercise session was separated by 24 hours and followed the same guidelines as

previously described for Protocol One. A pre-exercise blood sample was obtained prior to

each exercise session, followed by 24, 48, and 72 hours after the third exercise session. Each

of the 90-minute exercise sessions in Protocols One and Two allotted two 5-minute rest

periods (seated behind treadmill) between the three 30-minute exercise periods in order to

ensure completion of each session and compliance with the protocols. However, subjects

could continue exercising if they did not require this allotted break. Since intermittent

exercise is advocated for health-related benefits, these brief periods of rest were not expected

to affect the study's results (Durstine et al., 2001; Quinn et al., 2006). Protocol Three

constituted the control trial, requiring no exercise with blood sampling at day one and day

six, corresponding to the first and last days of Protocol Two. Only two blood samples, rather

than daily samples, were obtained in order to decrease the burden on the subjects and to

increase compliance. While daily samples would have been optimal, two samples still

allowed assessment of any non-exercise related fluctuations in plasma variables, particularly in female subjects as they advanced through the follicular phase of their menstrual cycle.

Dietary Records

All subjects were instructed to maintain their normal pre-study diet during the study period and complete food diary data forms (Appendix N) to allow for nutrient intake assessment. At the end of the pre-study screening visit to the laboratory, subjects were given three food diary data forms. The same researcher provided instructions regarding portion sizes and recording of foods and beverages consumed. Dietary records were kept for the three days prior to each exercise protocol (two weekdays and one weekend day). Subjects continued to keep dietary logs throughout each day of all three protocols, ending the day before the final blood sample. Records were analyzed for total calories, protein, carbohydrate, fat, and cholesterol (Diet Analysis Plus, Wadsworth Publishing Co., Salem, OR) (Table 6).

Handling and Analysis of Blood Samples

Within each subject, all blood sampling occurred at approximately the same time of day ($\pm$ 1 hour). Approximately 28 mL of blood were obtained with each sample, with a small portion being utilized to test hemoglobin (Drabkin et al., 1935) and hematocrit (Dill et al., 1974) levels. The remainder of the blood sample was immediately centrifuged at 1,500 rpm for 20 minutes at 4°C in a Beckman Coulter (Allegra 6KR) centrifuge (Palo, Alto, CA). Blood plasma was then pipetted into microcentrifuge tubes and stored at -80°C. Plasma

samples were analyzed with a Bio-Tek (Powerwave 340, Winooski, VT) microplate for

TCHL, LDL-C, HDL-C, and TG concentrations, using commercialized assays from Wako

Chemicals USA Inc. (Richmond, VA) commercially prepared reagents. All samples were

assayed in triplicate on the first thaw. Intrassay coefficients of variation were 2.4% for TG,

1.6% for TCHL, and 2.7% for HDL-C. To reduce the effects of interassay variations, all

samples for a given subject were analyzed at the same time. Interassay coefficients of

variation were 2.6% for TG, 1.1% for TCHL and 5.8% for HDL-C.

Statistical Analysis

The data obtained for TCHL, LDL-C, HDL-C, and TG were analyzed using a

repeated measures analysis of variance (ANOVA). Any significant effects were further

analyzed via a paired sample t-test. Direct comparisons of pre-exercise to all other time

points were conducted, with an emphasis placed upon previous lipid and lipoprotein research

(i.e., changes usually peak one-to-two days following a prolonged exercise session and these

changes usually return to baseline by the third day) (Durstine et al., 2001). Therefore, while

a total of 12 blood draws/time points were conducted on each subject, *a priori* analyses

allowed the researcher to compare the means of certain, predetermined time points both

within each experimental protocol and across experimental protocols.

The within treatment analyses compared the two exercise protocol blood lipid

concentrations from the pre-exercise time-points to all other time-points in the same protocol.

The control protocol required two blood samples six days apart, which was the same number

of days between the first and last exercise session of protocol two (Figure 4). These time-

points provided information regarding any natural fluctuations which may occur during the same amount of time required to perform protocol two.

The across treatment analyses compared each subject's blood lipid concentrations across experimental protocols. Concentration changes between baseline and the 24-, 48-, and 72-hour post-exercise time-points were compared across the two exercise protocols. Also, the differences in each of the lipid and lipoprotein variables across the control protocol were compared to the peak changes in the two exercise protocols. This comparison assured that any changes in the exercise protocols took place due to exercise *per se* and not day-to-day variations. All analyses were performed using SPSS statistical software (SPSS Inc., Chicago, IL). Statistical significance was set at $P \leq .05$. In addition, effect sizes were calculated and are reported as small (0 – 0.19), moderate (0.2 – 0.39), or large (>0.4).

For comparison purposes the uncorrected [(U)] and corrected [(C)] (i.e., based upon plasma volume changes) experimental data ($mgdL^{-1}$) will be presented in tabular form. For the repeated measures analysis of variance, Mauchly's Test of Sphericity was first read to determine if the variances and covariances of the matrix were not significantly different. If Mauchly's Test was significant, the Hyunh-Feldt procedure was used. However, if Mauchly's Test was not significant, the Sphericity Assumed line was read to determine statistical significance.

CHAPTER IV

RESULTS

The results of this study are presented in the following sections: Subjects, Dietary Data, Submaximal Treadmill Sessions, Blood Variables, One Exercise Session Data, Three Exercise Sessions Data, Control Session Data, One Versus Three Exercise Sessions Data, and Average Post-Exercise Concentration Changes.

Subjects

All twelve subjects (3 white males, 3 black males, 4 white females, 2 black females) met previously stated recruitment criteria. Table 5 presents the subjects' descriptive statistics. As expected, above normal body mass indexes, percent body fat readings, and waist circumference readings were observed. Male subjects displayed a higher body mass index compared to female subjects due to both greater lean and fat mass per height.

Subjects fell within the normal range for resting heart rate, systolic and diastolic blood pressure. Male subjects had significantly higher resting systolic and diastolic blood pressure. The subjects exhibited average to above average VO_{2max} values, with no gender difference. Although all subjects were abdominally obese, they still displayed normal aerobic fitness levels. Also, while the specific aims of this study did not focus on race or gender, final results were not significantly affected by either of these variables.

Table 5

Descriptive Characteristics

Variable	All Subjects (n=12)	Male (n=6)	Female (n=6)	White (n=7)	Black (n=5)
Age (years)	22.5±2.5	22.8±2.8	22.2±2.5	22.6±2.8	22.4±2.4
Height (cm)	171±10	178±4†	164±9	169±12	173±7
Weight (kg)	87.7±19.3	104.6±7.5‡	70.8±9.0	83.8±22.4	93.1±14.5
BMI (kg·m^{-2})	29.7±3.9	33.0±2.1‡	26.4±1.8	28.7±4.1	31.1±3.7
Body Fat (%)	24.6±4.0	22.7±3.9	26.6±3.1	23.9±3.5	25.7±4.8
Fat Body Mass (kg)	21.3±4.4	23.7±4.5	18.8±3.0	19.5±3.6	23.7±4.7
Lean Body Mass (kg)	66.4±16.5	80.9±6.7‡	52.0±7.5	64.3±19.5	69.4±12.8
VO$_{2max}$ (ml·kg^{-1}·min^{-1})	37.9±5.5	36.7±1.5	39.1±7.7	40.0±6.0	35.0±2.9
Waist Circ.	97.7±9.5	106.6±3.2‡	88.9±1.3	95.9±9.6	100.2±9.9
Rest Heart Rate (bpm)	77.1±6.4	79.5±7.1	74.7±5.0	75.4±6.0	79.4±6.8
Rest Systolic BP (mmHg)	122.2±14.1	134.5±3.4‡	109.8±7.6	119.9±13.7	125.4±15.5
Rest Diastolic BP (mmHg)	72.2±6.6	76.2±4.4*	68.2±6.3	70.3±6.8	74.8±6.1
Mean Arterial Pressure	88.7±8.6	95.4±3.3†	81.9±6.5	86.6±8.7	91.5±8.5
TCHL	203.5±43.1	195.0±24.1	212.0±57.8	213.1±48.5	190.0±34.7
LDL-C	129.5±31.5	129.3±22.4	129.6±41.0	132.6±33.4	125.1±31.8
TG	89.0±25.6	94.9±24.7	83.1±27.4	92.2±17.5	84.5±36.1
HDL-C	56.4±17.9	46.5±12.4*	66.2±17.7	61.1±20.7	49.7±11.8
Total/HDL-C Ratio	3.61±1.0	4.19±1.1	3.20±0.7	3.49±0.9	3.84±1.3

Values are means±SD
Lipid and lipoprotein concentrations are reported as mg/dL
Different across gender (*$p<.05$; †$p<.01$; ‡$p<.001$)

Dietary Data

Each subject was required to record 22 days of dietary intake. Subjects completed dietary logs for three days prior to the beginning of each experimental protocol, and through the end of the same protocol. The average macronutrient intake of the first three days was utilized to establish pre-experiment (i.e., pre-average) levels. Eight of the 12 subjects returned all dietary records, while four subjects failed to return a total of 10 days worth of dietary information. For missing data, total averages were calculated from all other days within that experimental protocol and re-inserted for statistical analyses. All values reported were considered adequate in accordance with the Recommended Dietary Allowances, and based on these self-reports it appears as though subjects were well nourished (Dietary Guidelines, 2005). Repeated measures analysis of variance showed no significant differences across time for any measured dietary variable. Final dietary information is presented in Table 6.

Submaximal Treadmill Sessions

All subjects completed all aspects of the submaximal treadmill sessions. Performance data collected during the submaximal exercise sessions are presented in Table 7. While the 60% VO_{2max} intensity was a manageable brisk walk for most subjects, the duration of 90 minutes presented a more challenging task. In an attempt to minimize physical ailments (e.g., calf cramps, leg fatigue, heel blisters, etc.) several subjects required speed and grade modifications during the later minutes of the exercise duration. These modifications required the researcher to change speed and/or grade in an attempt to ease these physical ailments,

Table 6

Dietary Data Analysis *(Control Session)*

Variable	Pre-average	Day 1	Day 2	Day 3	Day 4	Day 5	ANOVA Statistical Sign.
Total Calories	1815.1 ± 111.1	2182.2 ± 247.9	1850.2 ± 164.2	1501.6 ± 169.8	2022.6 ± 146.2	1800.5 ± 216.5	p=.11
CHO (%)	50.2 ± 2.6	50.5 ± 2.7	48.3 ± 2.9	48.8 ± 2.5	48.8 ± 3.6	47.1 ± 4.2	p=.91
PRO (%)	18.5 ± 1.6	14.9 ± 1.5	15.9 ± 1.3	17.6 ± 1.4	19.1 ± 1.5	17.5 ± 1.8	p=.10
Fat (%)	29.7 ± 1.9	36.6 ± 2.3	35.7 ± 2.2	34.9 ± 2.4	32.8 ± 2.6	33.0 ± 3.2	p=.15
CHL (mg)	222.2 ± 24.3	229.3 ± 46.6	236.2 ± 36.2	181.1 ± 35.2	297.8 ± 59.1	349.1 ± 81.6	p=.11

Values are mean ± SEM

Pre-average = average of the three days prior to Day 1 of the control session; CHO – Carbohydrate; PRO – Protein; CHL - Cholesterol.

Table 6

Dietary Data Analysis *(1 Exercise Session)* (cont.)

Variable	Pre-average	Ex 1	24 PE	48 PE	ANOVA Statistical Significance
Total Calories	1470.8 ± 120.0	1767.1 ± 211.0	1900.9 ± 176.5	2019.6 ± 255.6	p=.14
CHO (%)	48.8 ± 2.7	46.8 ± 5.4	47.7 ± 3.4	46.1 ± 3.2	p=.89
PRO (%)	17.8 ± 1.1	20.8 ± 2.0	18.0 ± 1.9	19.3 ± 2.1	p=.40
Fat (%)	35.4 ± 2.1	32.6 ± 3.3	34.2 ± 3.4	35.2 ± 2.5	p=.69
CHL (mg)	189.7 ± 31.8	272.4 ± 74.9	215.6 ± 30.3	282.4 ± 41.7	p=.33

Values are mean ± SEM

Pre-average = average of the three days prior to the first laboratory visit of the 1-day of exercise experimental session; Ex 1 – Exercise session #1; 24PE – 24 hours post-exercise; 48PE – 48 hours post-exercise. See page above for variable descriptors.

Table 6

Dietary Data Analysis *(3 Exercise Sessions)* (cont.)

Variable	Pre-average	Ex 1	Ex 2	Ex 3	24 PE	48 PE	ANOVA Statistical Sign.
Total Calories	1596.7 ± 143.4	1919.9 ± 315.7	1461.8 ± 158.9	1473.3 ± 301.0	1617.4 ± 162.0	1547.6 ± 157.6	p=.45
CHO (%)	48.9 ± 3.0	53.3 ± 3.7	53.6 ± 4.1	51.9 ± 6.4	45.4 ± 6.5	46.9 ± 3.1	p=.48
PRO (%)	19.1 ± 2.0	18.4 ± 1.9	17.1 ± 1.7	18.5 ± 3.2	22.4 ± 3.5	18.7 ± 2.0	p=.43
Fat (%)	31.9 ± 1.9	30.5 ± 2.9	29.1 ± 3.1	30.6 ± 3.6	35.7 ± 2.1	33.0 ± 1.8	p=.44
CHL (mg)	210.5 ± 34.4	229.8 ± 50.9	187.8 ± 42.3	173.7 ± 55.9	249.0 ± 51.2	182.6 ± 23.4	p=.70

Values are mean ± SEM

Pre-average = the average of the three days prior to the first laboratory visit of the 3-days of exercise experimental session.

See page above for variable descriptors

Table 7

Performance Data during Submaximal Treadmill Exercise

Variable	1 Exercise Session	3 Exercise Sessions			ANOVA Statistical Sign. Across Protocols
		Ex 1	Ex 2	Ex 3	
%VO$_{2max}$	56.3 ± 1.5	57.3 ± 1.1	58.1 ± 1.2	57.5 ± 1.1	p=.22
VO$_2$ (ml·kg^{-1}·min^{-1})	21.1 ± 0.5	21.5 ± 0.6	21.8 ± 0.5	21.6 ± 0.6	p=.27
Total kcal Expenditure	840.0 ± 66.9	840.8 ± 57.7	863.0 ± 63.6	844.3 ± 57.2	p=.50
Kcal/kg	9.3 ± 0.3	9.6 ± 0.3	9.8 ± 0.2	9.6 ± 0.3	p=.18
RER	0.903 ± 0.01	0.908 ± 0.01	0.893 ± 0.01	0.888 ± 0.01	p=.41
Minute Ventilation (L)	124.2 ± 11.3	124.8 ± 11	127.2 ± 10	124 ± 10.4	p=.63
Heart Rate (bpm)	149.9 ± 3.8	148.6 ± 3.5	145.3 ± 3	146.4 ± 3.1	p=.11
RPE	12.9 ± 0.6	12.8 ± 0.6	12.9 ± 0.8	12.3 ± 0.7	p=.35

Values are mean ± SEM
RER = Respiratory Exchange Ratio, RPE = Rating of Perceived Exertion

while also attempting to maintain the pre-determined exercise intensity range (i.e., 58-62%VO_{2max}). However, some subjects could not maintain intensity throughout the entire 90 minute treadmill session. While final VO_{2max} percentages were slightly below originally planned, values were not significantly below the desired range, they were not significantly different across any of the exercise sessions and can still be defined as being in the upper range of moderate intensity exercise. Also, ratings of perceived exertion did not show any significant changes across exercise sessions. No other physiological responses observed within or across exercise sessions were significantly different.

Blood Variables

Plasma volume correction data from all three experimental sessions is presented in Table 8. Pre and post-exercise hemoglobin and hematocrit concentrations were inserted into a corrective equation to obtain final plasma volume percent change (Greenleaf et al., 1979). Final plasma volume changes were within normal ranges, based upon past research (Convertino, 2007; Sawka et al., 2000).

One Exercise Session Data

Specific Aim #1 addresses the comparison of the effects of a single 90-minute treadmill exercise session performed at 60% VO_{2max} versus no exercise (control group) on lipid and lipoprotein concentrations in young, sedentary obese adults. While the two working hypotheses focus only on TG and HDL-C concentration changes, all variable results will be presented in both uncorrected (i.e., not corrected for plasma volume change) and

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Table 8

Plasma Volume Correction Data

Variable	PreC	PstC
Hemoglobin (g/dL)	17.4 ± 0.4	17.5 ± 0.3
Hematocrit (%)	46.7 ± 0.7	46.5 ± 0.7
Plasma Volume Δ (%)		0.17 ± 0.5

Variable	Ex1	Ex1-24	Ex1-48	Ex1-72
Hemoglobin (g/dL)	17.1 ± 0.3	17.2 ± 0.3	17.1 ± 0.3	17.4 ± 0.4
Hematocrit (%)	46.6 ± 0.5	45.6 ± 0.7	46.7 ± 0.8	46.5 ± 0.7
Plasma Volume Δ (%)		1.38 ± 0.4	-0.11 ± 0.6	-1.41 ± 0.5

Variable	Ex3-1Pre	Ex3-2Pre	Ex3-3Pre	Ex3-24	Ex3-48	Ex3-72
Hemoglobin (g/dL)	18.0 ± 0.4	17.7 ± 0.3	17.4 ± 0.4	16.6 ± 0.3	16.7 ± 0.4	16.6 ± 0.3
Hematocrit (%)	45.0 ± 0.7	44.8 ± 0.7	45.8 ± 0.9	45.9 ± 0.9	44.5 ± 0.8	44.4 ± 0.8
Plasma Volume Δ (%)		1.97 ± 0.5	1.79 ± 0.6	6.64 ± 0.5	8.69 ± 0.5	9.18 ± 0.5

Values are mean ± SEM
PreC – Pre Control; PstC – Post Control; Ex1 – 1 exercise session; Ex3 – 3 exercise sessions; 1Pre – prior to exercise session #1; 2Pre – prior to exercise session #2; 3Pre – prior to exercise session #3; 24 – 24 hours post-exercise; 48 – 48 hours post-exercise; 72 – 72 hours post-exercise

corrected (i.e., corrected for plasma volume change) form. Moreover, the Statistical Analysis section of chapter three specifically states, "The within treatement *a priori* analyses for each of the two exercise protocols will be the comparison of blood lipid concentrations from the pre-exercise time-points to all other time-points in the same protocol." Therefore, a repeated analysis of variance was conducted to assess blood concentration differences within each exercise protocol. If any ANOVA statistical significance was noted, a subsequent post-hoc t-test was conducted to determine exact time-point significance.

Final data for the one exercise session protocol are presented in Table 9. Only the uncorrected total cholesterol variable indicated a statistical difference over time. However, when total cholesterol was corrected for plasma volume changes there was no significant time effect.

Three Exercise Sessions Data

The results of the three exercise sessions data are presented in the same manner as the results of the one exercise session data. The Statistical Analysis section of chapter three specifically states, "The within treatement *a priori* analyses for each of the two exercise protocols will be the comparison of blood lipid concentrations from the pre-exercise time-points to all other time-points in the same protocol" (see page 72). Therefore, a repeated analysis of variance was conducted to find any across treatment blood concentration differences within each exercise protocol. If any ANOVA statistical significance was noted, a subsequent post-hoc t-test was performed to determine exact pre/post time-point significance.

Table 9

One Exercise Session Data

Variable (mg·dL^{-1})	IPreEx	24PEx	48PEx	72PEx	ANOVA Statistical Sign.
TCHL (U)	207.5±13.8	195.0±14.6†	197.0±12.7*	198.8±16.4	p=.02
TCHL (C)	207.5±13.8	198.1±15.2	197.0±13.2	196.5±16.4	p=.07
LDL-C (U)	134.7±10.3	117.7±7.1	118.9±9.1	126.5±12.4	p=.10
LDL-C (C)	134.7±10.3	119.7±7.7	118.9±9.3	124.9±12.3	p=.13
TG (U)	86.8±7.9	79.2±12.1	89.8±10.9	87.9±10.1	p=.52
TG (C)	86.8±7.9	80.3±12.2	90.0±11.2	86.7±10.1	p=.63
HDL-C (U)	55.9±5.3	53.9±5.1	52.3±5.2	54.7±5.1	p=.10
HDL-C (C)	55.9±5.3	54.8±5.3	52.3±5.2	54.2±5.1	p=.10

Values are averages±SEM
U – Uncorrected for plasma volume change; C – Corrected for plasma volume change; IPreEx – Immediately pre-exercise; 24PEx – 24 hours post-exercise; 48PEx – 48 hours post-exercise; 72PEx – 72 hours post-exercise; ANOVA – Analysis of variance
* Different from Immediately Pre-Exercise (p<.05).
† Different from Immediately Pre-Exercise (p<.01).

The uncorrected data showed a significant decrease in TG concentrations, but the plasma volume corrected TG data was not significant. Although not significant (p=.06), the corrected TG data showed a similar decreasing trend from exercise session #1 through 24 hours post-exercise, with a return toward baseline over the next two days. Also, there was a near significant (.06) decrease in TCHL from exercise session #1 through exercise session #3, which was due mostly to a drop in very low density lipoprotein concentrations. After plasma volume correction HDL-C concentrations were significantly elevated 24 and 48 hours post-exercise [moderate effect (.31)]. Final data for the three exercise sessions protocol are presented in Table 10.

Table 10

Three Exercise Sessions Data

Variable (mg·dL^{-1})	IPreEx-1	IPreEx-2	IPreEx-3	24PEx	48PEx	72PEx	ANOVA Statistical Sign.
TCHL (U)	200.7 ± 12.9	191.8 ± 14.7	185.5 ± 10.0	190.8 ± 12.6	189.9 ± 10.5	185.6 ± 10.9	p=.14
TCHL (C)	200.7 ± 12.9	195.6 ± 15.3	188.6 ± 10.1	202.8 ± 13.2	206.7 ± 12.2	202.8 ± 12.6	p=.06
LDL-C (U)	126.0 ± 8.4	122.5 ± 11.3	119.9 ± 8.2	123.9 ± 10.3	121.6 ± 8.3	118.0 ± 10.1	p=.57
LDL-C (C)	126.0 ± 8.4	124.9 ± 11.7	121.9 ± 8.3	131.6 ± 10.7	132.4 ± 9.6	129.1 ± 11.6	p=.28
TG (U)	90.0 ± 10.3	76.0 ± 10.2	66.5 ± 4.5*	60.4 ± 6.7*	68.8 ± 7.6	71.9 ± 8.6	p=.03
TG (C)	90.0 ±10.3	77.9 ± 10.9	67.6 ± 4.5	64.3 ± 7.1	75.1 ± 8.9	78.5 ± 9.4	p=.06
HDL-C (U)	55.5 ± 6.0	53.4 ± 5.1	52.3 ± 4.4	55.4 ± 5.3	54.5 ± 5.3	53.8 ± 5.2	p=.31
HDL-C (C)	55.5 ± 6.0	54.4 ± 5.2	53.2 ± 4.4	58.9 ± 5.7*	59.2 ± 5.7*	58.7 ± 5.6	p=.00

Values are averages ± SEM

U – Uncorrected; C – Corrected; IPreEx-1 – Immediately pre-exercise session #1; IPreEx-2 – Immediately pre-exercise session #2; IPreEx-3 – Immediately pre-exercise session #3; 24PEx – 24 hours post-exercise; 48PEx – 48 hours post-exercise; 72PEx – 72 hours post-exercise; ANOVA – Analysis of variance

* Different from Immediately Pre-Exercise Session #1 ($p<0.05$).

Control Session Data

The control session data (mgdL^{-1}) is presented in Table 11. No significant differences were noted between pre and post-control plasma concentrations for any blood variable.

One Exercise Session Versus Three Exercise Sessions Data

Specific aim #3 addresses the comparison of the effects of a single 90-minute treadmill exercise session performed at 60% VO$_{2max}$ versus three 90-minute exercise sessions performed at 60% VO$_{2max}$ on lipid and lipoprotein concentrations in young, sedentary obese adults. The two working hypotheses focus on how three consecutive days of accumulated exercise will produce greater improvements in plasma TG and HDL-C concentrations 24-72 hours after the last session of exercise, compared to the improvements observed 24-72 hours after a single session of exercise. Even though the two working hypotheses focus only on TG and HDL-C concentration changes, the results for TCHL and LDL-C are also presented. Data is presented after corrected for plasma volume change.

The Statistical Analysis section specifically states, "The across treatement *a priori* analyses will compare each subject's blood lipid concentrations across experimental protocols. Concentration changes between baseline and the 24-, 48-, and 72-hour post-exercise time-points will be compared across the two exercise protocols. This will indicate whether or not the two additional exercise sessions in protocol two resulted in greater blood lipid concentration changes." Therefore, a 2 X 4 repeated measures analysis of variance was

Table 11

Control Session Data

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Variable (mgdL^{-1})	Pre-Control	Post-Control	t-test Statistical Sign.
TCHL (U)	202.2±13.0	195.4±12.5	p=.12
TCHL (C)	202.2±13.0	196.4±14.0	p=.23
LDL-C (U)	127.7±10.2	118.4±11.7	p=.09
LDL-C (C)	127.7±10.2	119.4±12.9	p=.18
TG (U)	85.0±9.8	102.9±8.5	p=.11
TG (C)	85.0±9.8	103.2±8.7	p=.09
HDL-C (U)	57.7±4.7	55.7±4.2	p=.18
HDL-C (C)	57.7±4.7	55.7±4.2	p=.14

Values are average±SEM
U – Uncorrected for plasma volume change; C – Corrected for plasma volume change

conducted to assess blood concentration differences across the two exercise protocols. Final

data for the three exercise sessions protocol are presented in Figure 5.

A repeated measures analysis of variance indicated a significant treatment by time

difference for LDL-C [moderate effect (.24)]. However, the post-hoc t-test did not show any

specific timepoint differences. Also, there was a significant treatment x time difference for

HDL-C. The post hoc t-test indicated a difference between the two exercise sessions 48

hours post-exercise [moderate effect (.29)].

Average Post-Exercise Concentration Changes

The statistical analysis section (see page 72) indicates that peak post-exercise changes

in the two exercise protocols would be compared to the control protocol. For the two

exercising protocols the average of the three post-exercise concentrations were analyzed

Figure 5

One Versus Three Exercise Sessions Means±SEM
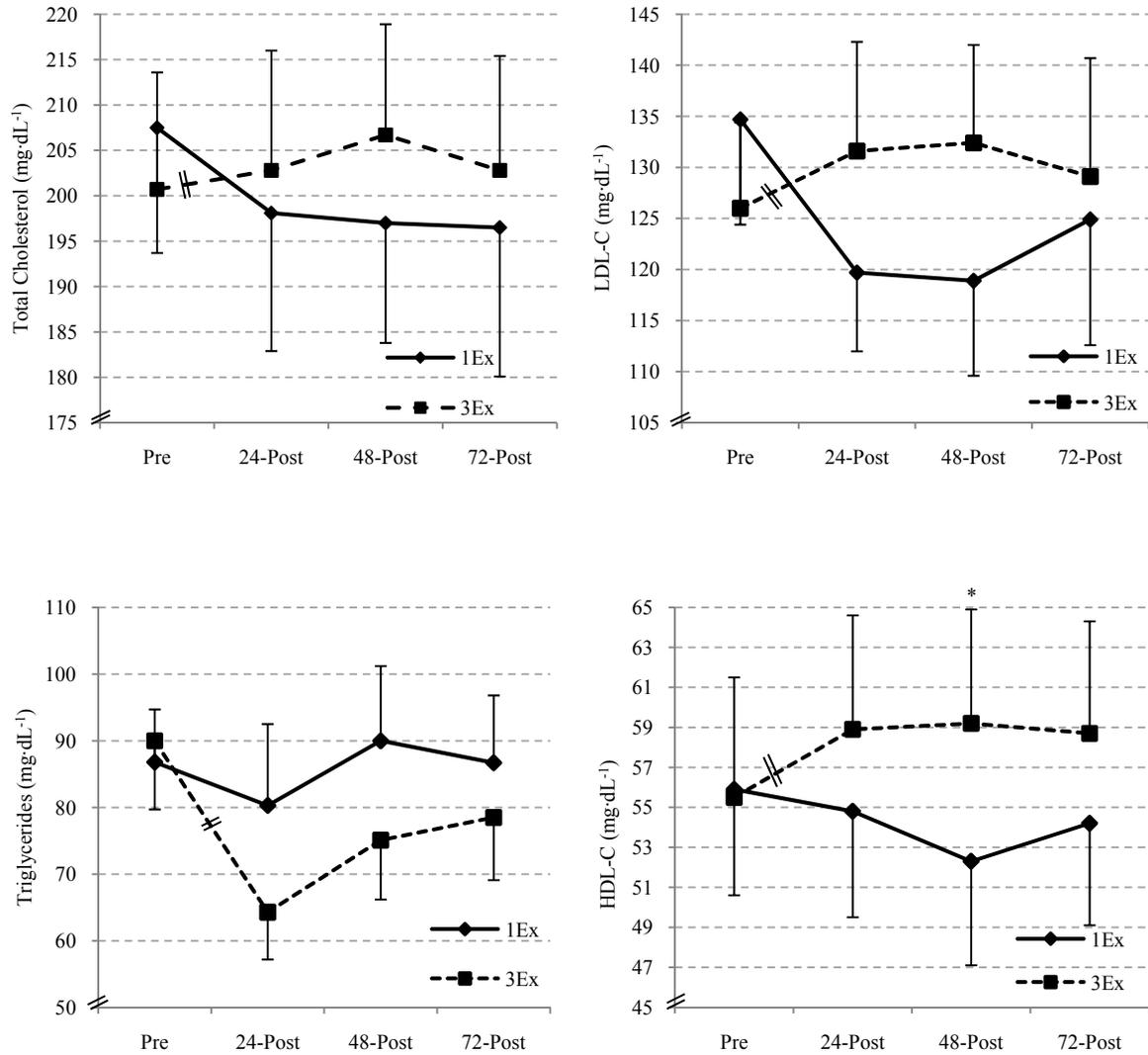
Values are mean±SEM
Pre – pre exercise session #1; 24-Post – 24 hours post-exercise; 48-Post – 48 hours post-exercise; 72-Post – 72 hours post-exercise
Only upper and lower confidence intervals are shown
* HDL-C = Different from 1Ex-48 hours post-exercise vs. 3Ex-48 hours post-exercise
    ($p<.05$)

because not all post-exercise time-points peaked in the same direction. Therefore, a 2 X 3 ANOVA was conducted to compare pre versus post-exercise average for the two experimental protocols and pre versus post-control for the control protocol.

The results for the average post-exercise concentration changes are presented in Figure 6. A significant decrease in LDL-C was observed from pre-exercise to post-exercise average for the single exercise session [moderate effect (.31)]. A significant elevation was observed for HDL-C from pre- to post-exercise average for the three exercise session protocol, and the post three exercise sessions' average change was significantly elevated compared to the post one exercise session average change [moderate effect (.39)].

The results observed in Figure 6 did not account for differing baseline lipid concentrations across treatments. Therefore, percent change scores were derived by subtracting the pre-exercise concentrations from the post-exercise average concentrations and then dividing the difference by the pre-exercise concentrations. A repeated measures ANOVA was conducted followed by paired samples t-tests. A significant percentage change between the two exercising protocols resulted with LDL-C [moderate effect (.29)]. Also, regarding the percentage changes in TG, there was a significant difference between the control protocol and the three exercise session protocol [moderate effect (.25)]. Finally, HDL-C showed significant percent change differences for the three exercise session protocol compared to the control and single exercise session protocols [large effect (.4)] (Figure 7).

The TG findings reported in Figure 7 were unexpected because, while the three exercise session protocol elicited a beneficial 17% decrease (i.e., pre- versus post-exercise), the control session resulted in an 18% increase in TG concentration. To further explore this

Figure 6

Average Post-Exercise Concentration Changes Means±SEM
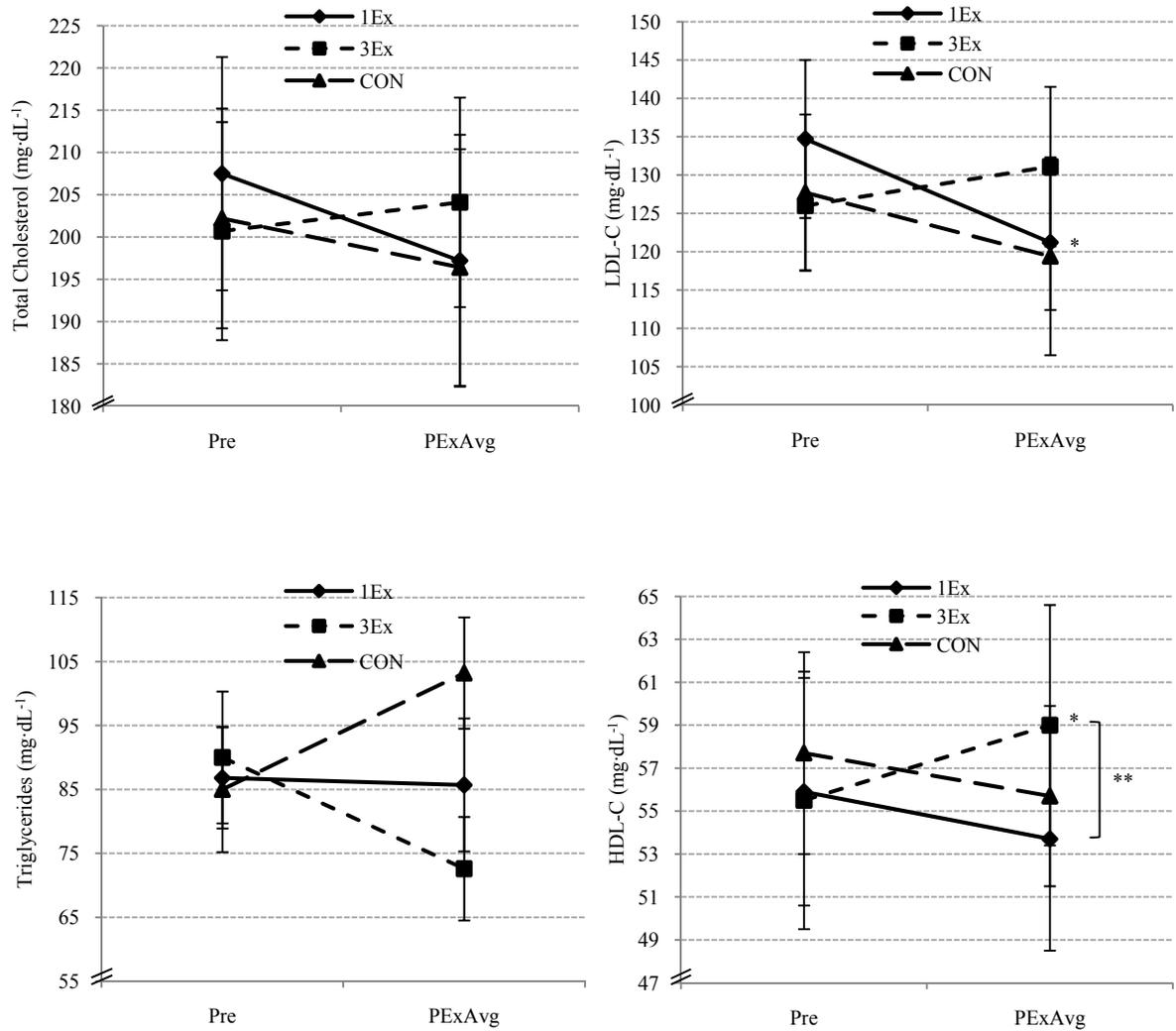
Pre – pre exercise session #1; PExAvg – post-exercise average
* LDL-C = Different from Pre1 vs. Post 1-Exercise Session Average (p<.05)
* HDL-C = Different from Pre3 vs. Post 3-Exercise Sessions Average (p<.05)
** HDL-C = Different from Post1 vs. Post 3-Exercise Sessions Average (p<.05)

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Figure 7

Pre Versus Peak Post-Exercise Percentage Changes Means±SEM
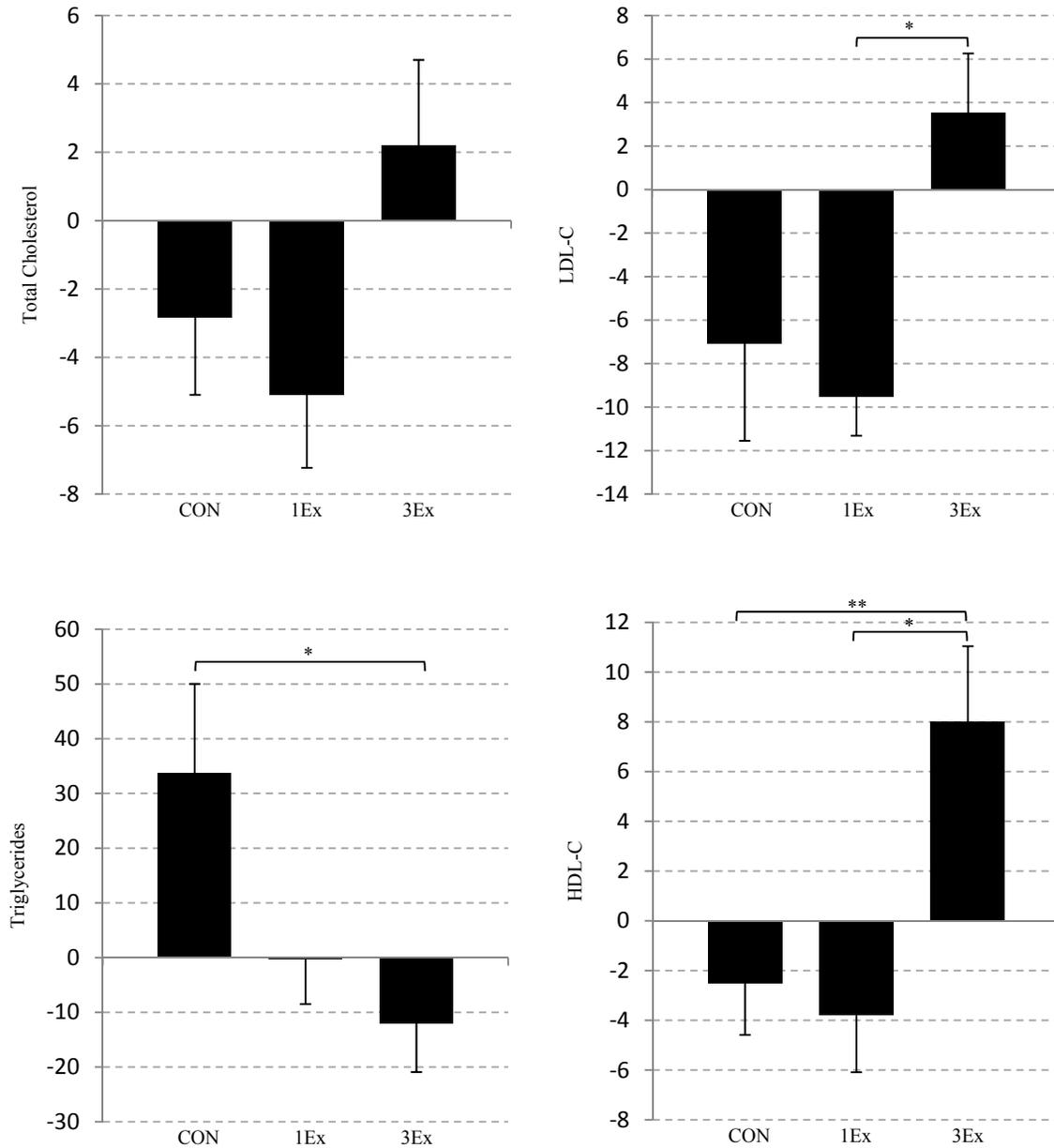
CON – control; 1Ex – 1 exercise session; 3Ex – 3 exercise sessions
* LDL-C – Different from 1Ex vs. 3Ex (p<.05)
* Triglycerides – Different from Control vs. 3Ex (p<.05)
* HDL-C – Different from 1Ex vs. 3Ex (p<.05)
** HDL-C – Different from Control vs. 3-Ex (p<.05)

finding, a Pearson Product Moment correlation analysis was conducted (pre-exercise

concentration versus percent change) to determine if TG change was affected by baseline

concentrations. Regarding the three exercise session protocol, subjects with the highest

baseline concentrations experienced the greatest post-exercise TG reductions ($r = -0.655$, $p = $

$.021$) (Figure 8). Secondly, a separate correlational analysis indicated that subjects with

lower pre-control concentrations experienced the highest increase in TG across the control

treatment ($r = -0.593$, $p = .042$) (Figure 9).

Figure 8

Triglycerides Percentage Change Post-hoc Analysis *(3 Exercise Sessions)*
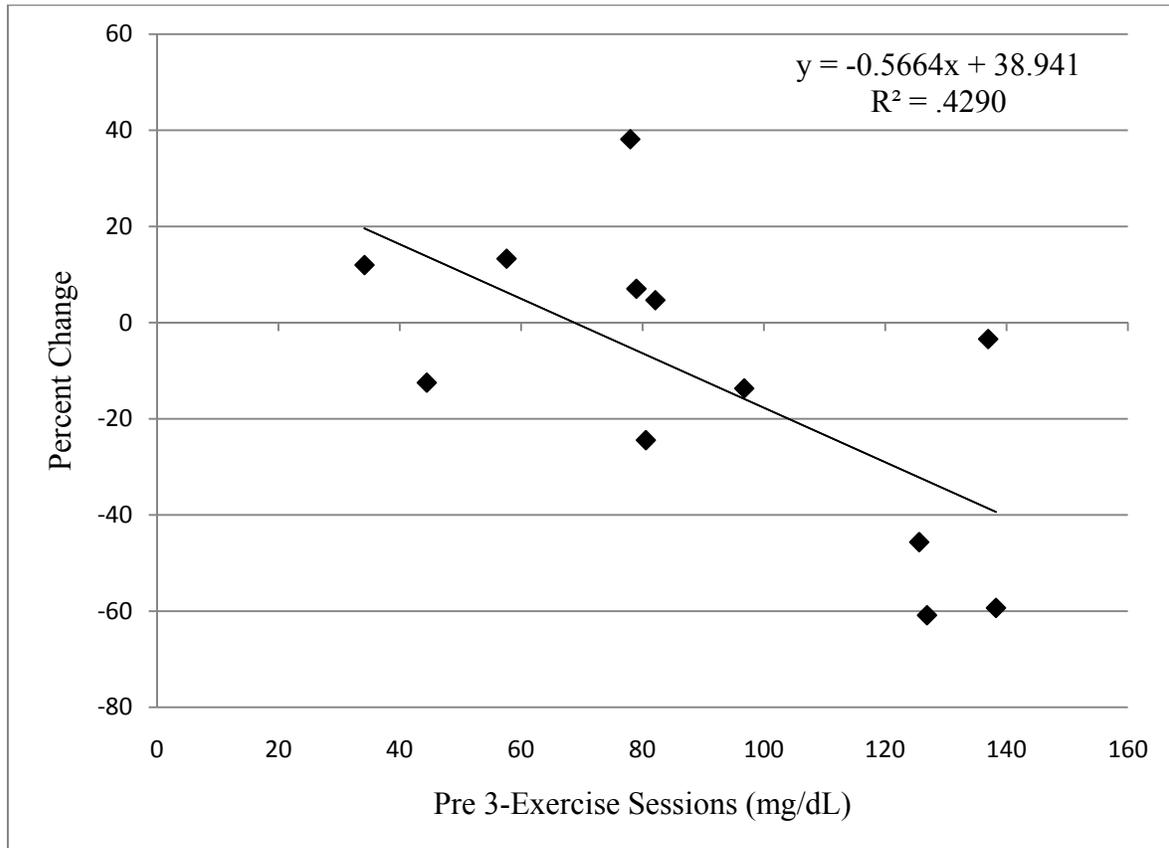
Pre 3-Exercise Sessions – pre three sessions of exercise
$p < .05$

Figure 9

Triglycerides Percentage Change Post-hoc Analysis (*Control Session*)

$$y = -0.9889x + 117.7$$
$$R^2 = .3516$$
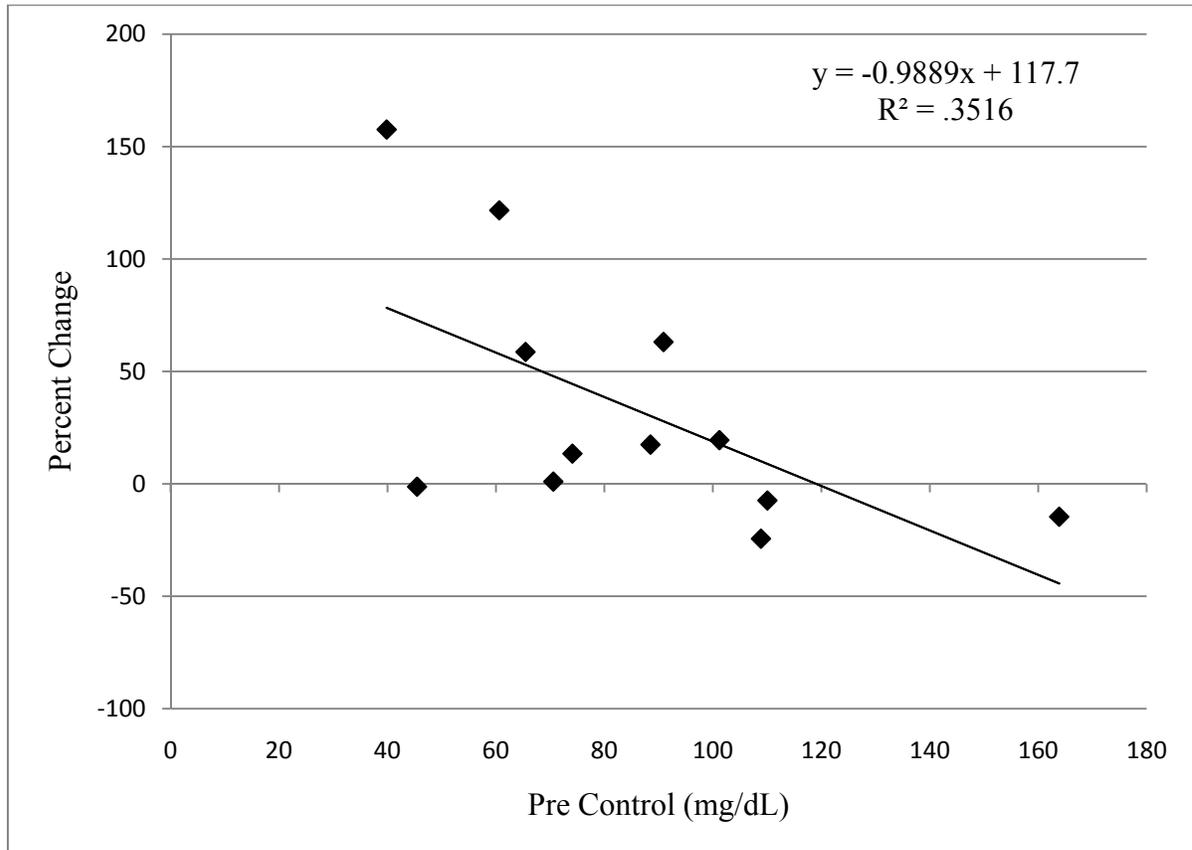
Pre Control – pre control session
$p<.05$

CHAPTER V

DISCUSSION AND CONCLUSIONS

Two factors associated with cardiometabolic risk are decreased HDL-C and increased TG plasma concentrations. Exercise has been shown to concomitantly increase HDL-C and decrease TG (Durstine & Haskell, 1994). While past research indicates that these beneficial changes can occur due to exercise *training*, studies employing single prolonged sessions of exercise have produced similar results that last one to three days after exercise. However, current research has not defined the potential interaction between the acute and training responses (Haskell, 1994). Specifically, accumulating the effects of single sessions of exercise may produce an additive post-exercise effect on plasma lipid concentrations beyond those observed after a single session of exercise. Therefore, the purpose of the present study was to compare three experimental protocols: 1) a single exercise session, 2) identical prolonged exercise sessions performed on three consecutive days, and 3) a control period with no exercise. The duration of each moderate-intensity exercise session (90 minutes) reflects a recent physical activity recommendation for obese individuals attempting to lose weight (Brooks et al., 2004).

In order to assess blood cholesterol transport changes, HDL-C, TG, TCHL, and LDL-C were measured. Based upon previous literature, HDL-C and TG concentrations were the two variables most expected to change following exercise (Durstine et al., 1994; Thompson et al., 2001). For this reason, the working hypotheses of this dissertation focused on these

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two variables. Since the results of HDL-C and TG differed, each working hypothesis of this dissertation will be addressed in relation to HDL-C first, and then TG. Although TCHL and LDL-C were not included in the hypotheses, they will still be discussed. To our knowledge, this is the first investigation to compare single versus accumulated exercise sessions on post-exercise lipid and lipoprotein changes utilizing both male and female subjects.

<center>HDL-C Concentrations</center>

A single 90-minute session of moderate intensity exercise, resulting in ~850 kilocalories of energy expenditure, did not induce a significant elevation of HDL-C concentration. *Therefore, the hypothesis indicating that a single session of exercise would produce improved HDL-C concentrations compared to the control group is rejected.*

While most of the existing single session exercise literature supports a post-exercise increase in HDL-C (Bounds et al., 2000; Crouse et al., 1995; Crouse et al., 2001; Ferguson et al., 1998; Grandjean et al., 2000; Kantor et al., 1984), some studies report no change or even reduced concentrations. Swank et al. (1987) recruited young, trained, normal weight females to perform a 40-minute treadmill run at 70% VO_{2max}. No significant HDL-C concentration changes were reported 24, 48, or 96 hours post-exercise. Davis et al. (1992) required young, trained, lean males to perform a 90-minute treadmill run at 50% VO_{2max}. No significant HDL-C concentration changes were found 24 through 72 hours post-exercise. Frey et al. (1993), who required young, lean, well-trained males to perform a 30 kilometer run (~130 minutes) at an average of 78% VO_{2max}, reported no significant HDL-C concentration changes

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20 hours post-exercise. While these studies found no HDL-C concentration changes utilizing subjects who were leaner and more trained, they were similar in age to the current study.

In addition to research conducted with trained individuals, studies conducted on young, *sedentary* individuals also support the current study's findings. Angelopoulos and colleagues (1993) assessed young, overweight, sedentary males and reported no HDL-C change 24 or 48 hours after a 30-minute treadmill run at 65% VO_{2max}. Imamura et al. (2000) noted no significant post-exercise HDL-C concentration changes 24 hours after 60 minutes (~322 kilocalorie average) of cycle ergometry work at 60% VO_{2max} in sedentary, lean young women. It is possible that these studies did not yield significant changes due to their lower exercise volume. However, a study utilizing a single exercise session protocol similar to that of the current study was conducted by Tsetsonis & Hardman (1995) who reported no post-exercise (24 hours) HDL-C concentration changes after requiring young, untrained, normal weight men and women to perform 90 minutes of treadmill exercise at 60% VO_{2max}. More recently, Wooten et al. (2008) showed a significantly decreased HDL-C concentration 48 hours after sedentary, overweight, young men performed a 500 kilocalorie treadmill session at 65% VO_{2max}.

Although the above studies support the current study's findings that HDL-C was not increased after young, sedentary individuals performed a single moderate intensity exercise session lasting up to 90 minutes, other studies utilizing similar subjects and exercise protocols have yielded significantly elevated HDL-C concentrations (Kantor et al., 1987; Park & Ransone, 2003). As such, the 90-minute exercise duration of the current study was developed based upon this previous research showing HDL-C elevations and upon a current

exercise recommendation advocated for weight management (Brooks et al., 2004). Specifically, past research has elicited HDL-C elevations after an acute exercise session requiring 350 to 500 kilocalories (Durstine et al., 2001; Thompson et al., 2001). These findings suggest that a minimum energy expenditure threshold must be met in order to stimulate elevated HDL-C concentrations. However, this suggested minimal threshold may vary based upon subject characteristics (i.e., age, training status, baseline cholesterol concentrations, body composition, etc.).

The following studies conducted on sedentary individuals support the potential to elevate HDL-C concentrations after an acute exercise session requiring between 350 to 500 kilocalories. Park & Ransone (2003) noted significant HDL-C elevations 24 hours after requiring young, sedentary, normal weight (normocholesterolemic) males to perform a 350 kilocalorie treadmill session at lactate threshold. Crouse et al. (1995) required middle-aged, hypercholesterolemic, sedentary, overweight males to perform two separate 350 kilocalorie cycle ergometer exercise sessions (50% VO_{2max} or 80% VO_{2max}). HDL-C was significantly elevated 24 through 48 hours after each exercise session. Another study by Crouse et al. (1997) reported significant HDL-C elevations 24 through 48 hours after requiring middle-aged, overweight, hypercholesterolemic males to perform a 350 kilocalorie cycle ergometer session at 50% VO_{2max}. These studies suggest that sedentary, overweight males can elicit significant post-exercise HDL-C concentration elevations after performing a relatively low-volume exercise session. It does not suggest that only men can produce changes, because past research indicates that no gender differences exist (Tsetsonis & Hardman, 1995).

A study directly comparing normocholesterolemic to hypercholesterolemic individuals provides further support that a low volume exercise duration can elicit HDL-C elevations. Grandjean and colleagues (2000) required middle-aged, sedentary, overweight, normocholesterolemic and hypercholesterolemic men to perform a 500 kilocalorie treadmill walk at 70% VO_{2max}. HDL-C was elevated 24 and 48 hours post-exercise for both normocholesterolemic (14%) and hypercholesterolemic (11%) men. Compared to the current study, this study elicited HDL-C elevations utilizing a shorter exercise duration on sedentary subjects exhibiting similar baseline HDL-C concentrations (for normocholesterolemic subjects) and body composition.

A study conducted by Crouse et al. (2001-abstract) required sedentary middle-aged men (body weight was not reported) to perform a 500 kilocalorie treadmill session at 60-70% VO_{2max}. Prior to initiation of this study, subjects were screened into moderate (~50 mg/dL) or low (~35 mg/dL) baseline HDL-C concentrations (TCHL concentrations were not reported). Both groups noted a significant HDL-C increase (9%) 24 and 48 hours post-exercise. The moderate baseline values reported were comparable to the current study's subjects (~56 mg/dL). Compared to the current study, this study reported elevated HDL-C concentrations utilizing a shorter exercise duration on sedentary individuals exhibiting similar baseline HDL-C concentrations.

The above studies suggest that some individuals, independent of baseline TCHL or HDL-C, can elicit HDL-C concentration elevations with an exercise duration requiring 350 to 500 kilocalories. However, these findings may only be applicable to middle-aged or older individuals. Therefore, while researchers have suggested that an exercise duration requiring

350 to 500 kilocalories may be sufficient to induce significant post-exercise plasma volume corrected HDL-C elevations (Thompson et al., 2001), the current study, which required ~850 kilocalorie energy expenditure, does not support this notion.

Beyond the effects of a single session of exercise, a very limited amount of research has attempted to assess a potential accumulated effect of repeated sessions of exercise on plasma lipid and lipoprotein concentrations (Grandjean et al., 2001; Thompson et al., 2001). The results of the current study provide support for an additive effect of three consecutive daily sessions of exercise. HDL-C concentrations were statistically elevated 24 and 48 hours after performing three sessions of exercise when compared to the change across time in the control session. *These findings lead to the acceptance of the hypothesis that three sessions of exercise will produce improved HDL-C concentrations post-exercise compared to the control group.* Also, when comparing post-exercise concentration changes between the two exercise protocols (one day of exercise versus three consecutive days of exercise), a significant time by trial interaction was observed with HDL-C elevated at the 48 hour timepoint following the three-day trial (Figure 5). *This finding leads to the acceptance of the hypothesis that three consecutive sessions of exercise will produce greater post-exercise improvements in HDL-C compared to one session of exercise.*

Furthermore, the *a priori* analysis was to compare the peak changes across the two exercise protocols and control session. This was conducted by assessing baseline concentrations compared to the average post treatment change [i.e., average of 24, 48, and 72 hour timepoint concentrations ("peak" concentrations could not be assessed because timepoints changed in differing directions for some variables)]. The treatment by time

ANOVA indicated significant concentration elevations from 1) pre-three sessions of exercise versus post-three sessions of exercise, and 2) post-three sessions of exercise versus post-one session of exercise (Figure 6). Lastly, a repeated measures ANOVA indicated a percentage change (i.e., pre versus post-average) for HDL-C concentrations when comparing the three sessions of exercise to both the control and one session of exercise (Figure 7). Taken together, these results indicate that performing prolonged moderate intensity exercise for three consecutive days is more effective in elevating HDL-C concentrations than one session of exercise or no exercise.

Currently there are only three studies that specifically assess the accumulated effects of exercise on HDL-C concentrations. Angelopoulos et al. (1993) recruited young, sedentary, overweight males to perform three separate exercise protocols: 1) one session, 2) two sessions, with 48 hours separation, and 3) three sessions, each with 48 hours separation. All treadmill exercise sessions were 30 minutes at 65% VO_{2max}. HDL-C concentrations were significantly higher 24 hours post-exercise, before returning to baseline by 48 hours post-exercise, after all three sessions. As previous research has suggested, the quick return to baseline concentrations 48 hours post-exercise, for all three exercise sessions, may be related to the short exercise duration (Durstine et al., 1994; Thompson et al., 2001). By implementing an ~850 kilocalorie exercise output, the current study observed longer post-exercise HDL-C concentration elevations.

More recently, Biggerstaff et al. (2005 - abstract) described a three exercise session protocol, each session separated by 48 hours. Young, moderately active, overweight women performed treadmill exercise for 500 kilocalories at 65% VO_{2max}. HDL-C concentrations

statistically increased prior to the third session of exercise, but had returned to baseline concentrations 48 hours post-exercise (compared to a resting protocol). The elevated timepoint prior to the third session of exercise did not exactly match the current study's significant elevation (timepoint 24 and 48 hours after the third session of exercise). This different response pattern may be due to differing periods of time between the three sessions of exercise (48 versus 24 hours, respectively).

Also, Grandjean et al. (2001 - abstract) utilized hyperlipidemic, middle-aged, obese men for assessment of a 350 kilocalorie treadmill protocol at 70% VO_{2max}. Similar to the findings of the current study, one session of exercise did not elicit a change in HDL-C concentration. However, exercising on four consecutive days induced an additive effect on raising HDL-C concentrations. Two days of exercise were required before HDL-C concentrations were significantly elevated. Then concentrations held steady through the fourth day of exercise and continued to increase 24 and 72 hours post-exercise (a 48 hour post-exercise time point was not collected). The current study found similar HDL-C elevations after three consecutive sessions of exercise, but utilized younger participants (including females) who exhibited lower TCHL and higher HDL-C baseline concentrations. Taken together, these findings suggest that neither age nor baseline concentrations prevent an individual from improving HDL-C after performing three or four repeated sessions of exercise.

In summary, the results of this study and those previously investigating the accumulated effects of several days of exercise indicate that post-exercise HDL-C elevations

are affected by the amount of exercise performed and/or manner in which the exercise sessions are repeated (i.e., every day versus every other day).

TG Concentrations

A single 90-minute session of moderate intensity exercise, resulting in ~850 kilocalories of energy expenditure, did not induce statistically significant post-exercise TG concentration changes. *Therefore, the hypothesis stating that a single session of exercise will improve post-exercise TG concentrations compared to the control session, is rejected.*

Much of the existing acute exercise TG literature reports significant post-exercise reductions (Durstine et al., 2001; Thompson et al., 2001). In particular, individuals with elevated baseline TG concentrations typically show the greatest, and most consistent, post-exercise decreases, while individuals with lower baseline TG concentrations do not show reductions as often. Studies with participants having baseline concentrations >125 mg/dL have consistently reported decreases in TG concentrations (Crouse et al., 1995; Crouse et al., 1997; Crouse et al., 2001; Grandjean et al., 2000).

While some acute studies have failed to yield reduced TG concentrations on the days following exercise, most of these have involved trained participants (Davis et al., 1992; Gordon et al., 1998; Hughes et al., 1990) or have employed relatively low volumes of exercise ($\leq$60 minutes, $\leq$70% VO_{2max}) (Angelopoulos et al., 1993; Imamura et al., 2000; Lennon et al., 1993; Swank et al., 1987; Tsetsonis & Hardman, 1995). On the other hand, some studies have reported post-exercise reductions in sedentary individuals with baseline TG concentrations comparable to those of the current study (90 mg/dL). Wooten et al.

(2008) showed significantly reduced TG 48 hours after young, sedentary, overweight (baseline TG=103 mg/dL) men performed a 500 kilocalorie treadmill session at 65% VO_{2max}. Another study by Crouse et al. (2001-abstract) required sedentary middle-aged men to perform a 500 kilocalorie treadmill session at 60-70% VO_{2max}. Prior to initiation of this study, subjects were stratified into moderate (~103 mg/dL) or elevated (~190 mg/dL) baseline TG. Significant TG reductions (-13%) were noted in both groups 24 and 48 hours post-exercise. Therefore, individuals exhibiting normal TG concentrations also have the ability to experience significant reductions after a single session of exercise. As such, it would have been reasonable to expect the 90-minute exercise session employed in the current study to reduce TG concentrations in young, sedentary, overweight individuals.

When considering an accumulated effect of exercise, TG concentrations following three consecutive days of 90-minute exercise sessions resulted in a significant reduction when compared to the control period (Figure 7). *Therefore, the hypothesis stating that accumulating three sessions of exercise will produce improved TG concentrations compared to the control group is accepted.* However, it is important to note that the inter-treatment difference was due to a divergent response (i.e., TG *decreased* following the three-day exercise treatment, but *increased* over the control period). Although a treatment-by-time effect was present, neither treatment by itself produced a statistically significant difference over time. In addition, since no difference existed between the TG responses to one day versus three days of exercise (Figure 6), *the hypothesis indicating that three consecutive days of exercise will produce greater improvements in TG concentrations compared to a single session of exercise is rejected.*

While the current study observed a significant percent change difference between the control and three exercise sessions, this difference is driven as much by the increase in TG during the control session than by the decrease following exercise. Specifically, the control protocol showed an 18% increase while the three exercise sessions protocol elicited an average decrease of 17%. While previous literature supports a 17% decrease in TG concentrations after an accumulated exercise protocol (Thompson et al., 2001; Durstine et al., 2001), the increase in the control group was unexpected. Since dietary variables were not significantly altered throughout the study it is possible that the increase in TG concentrations in the control group may reflect day-to-day variations. While not statistically significant on its own (p=.06; Table 10), the decrease in TG concentrations after three exercise sessions indicates that TG was altered substantially by the accumulated sessions of exercise in some subjects. However, the large standard error indicates a considerable variation of responses across subjects. It is important to note that while the standard error was large, the average intra-assay coefficient of variation for this variable was small (2.4%).

Only a limited amount of literature exists regarding the accumulated effect of exercise on TG concentrations. Angelopoulos et al. (1993) recruited young, sedentary, overweight males to perform three different exercise protocols; 1) a single session, 2) two sessions, with 48 hours separation, and 3) three sessions, each with 48 hours separation. All treadmill sessions were 30 minutes at 65% VO_{2max}. TG concentrations were not significantly altered during any exercise protocol 24 or 48 hours post-exercise. Potential reasons for the non-significant findings may be the shorter exercise duration or the longer amount of time between exercise sessions. However, the current study was also unable to elicit statistically

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significant TG changes when implementing a longer exercise duration and decreasing the time between sessions.

Two abstracts have reported significant post-exercise TG reductions following multiple sessions of exercise. Four consecutive days of exercise at 70% VO_{2max} (350 kcal, each separated by 24 hours) induced an additive effect of reduced TG concentrations in hyperlipidemic, middle-aged, obese men (Grandjean et al., 2001). Baseline TG concentrations (266 mg/dL) were significantly reduced after the first exercise session and remained significantly lower after each successive session (i.e., separated by 24 hours), before reaching their nadir 24 (-80 mg/dL) and 72 hours (-75 mg/dL) after the fourth exercise session. These findings suggest that accumulating successive sessions of exercise may elicit an additive effect beyond those observed after a single session of exercise in subjects with elevated TG concentrations. In addition, the timing of these significant post-exercise reductions support the current study's decreasing trend in TG concentrations through 24 hours after the third exercise session (Table 10).

Another abstract reported the results from a three exercise session protocol, each session separated by 48 hours (Biggerstaff et al., 2005). Young, sedentary, overweight women performed treadmill exercise for 500 kilocalories per session at 65% VO_{2max}. TG concentrations were significantly reduced 48 hours after the the last session of exercise. Specifically, these significant TG reductions (i.e., 20 mg/dL) were from a baseline of 103 mg/dL. The current study noted a 90 mg/dL baseline, with a 26 mg/dL reduction 24 hours post-exercise and 15 mg/dL reduction 48 hours post-exercise. Therefore, while the current

study's findings are not significantly different from baseline at p<0.05, the absolute TG reductions are comparable to the study above.

The decreasing TG concentration trend observed in the present study would not be possible if it were not for some individuals experiencing large absolute decreases. Although past research shows TG concentrations to decrease independent of baseline concentrations, the current study showed subjects with elevated baseline concentrations to have the largest decreases following three consecutive days of 90 minutes of moderate-intensity exercise (Figure 8). Interestingly, exercise *training* studies employing hypertriglyceridemic participants have consistently resulted in decreased TG concentrations, while results from studies employing normotriglyceridemic participants are inconsistent (Durstine & Haskell, 1994). Oddly, a non-significant increase was observed over the control protocol, but this trend, too, was dependent upon baseline concentrations (i.e., subjects with the lowest concentrations increased the most).

TCHL and LDL-C Concentrations

Consistent with previous single-session (Angelopoulos et al., 1993; Davis et al., 1992; Gordon et al., 1998; Grandjean et al., 2000; Hughes et al., 1990; Imamura et al., 2000; Park & Ransone, 2003; Tsetsonis & Hardman, 1995; Wooten et al., 2008) and acute accumulated-session investigations (Angelopoulos et al., 1993; Grandjean et al., 2001), the current study did not result in a significant post-exercise TCHL concentration change during any protocol. LDL-C concentrations were significantly decreased following the single exercise session (Figure 6) and the pre- versus post-exercise percentage change was

significantly different between the single exercise session and the three sessions of exercise protocol (Figure 7).

Most acute studies have not shown significant changes in LDL-C concentrations when controlling for various confounders (i.e., gender, dietary intake, baseline concentrations, plasma volume, body composition, etc.) (Angelopoulos et al., Bounds et al., 2000; 1993; Davis et al., 1992; Gordon et al., 1998; Grandjean et al., 2000; Hughes et al., 1990; Imamura et al., 2000; Kantor et al., 1987; Park & Ransone, 2003; Tsetsonis & Hardman, 1995; Wooten et al., 2008). Moreover, studies utilizing subjects and an exercise duration comparable to the current study have not reported significant post-exercise LDL-C reductions (Bounds et al., 2000; Tsetsonis & Hardman, 1995). Therefore, these studies suggest that a single extended session of exercise may not be sufficient to elicit significant LDL-C changes for young, sedentary individuals. However, in support of the current study's findings, a few studies have shown significant LDL-C reductions after a single session of exercise (Ferguson et al., 1998; Frey et al., 1993; Kantor et al., 1984). These studies observed LDL-C reductions after well-trained males performed a moderate to high intensity extended session of exercise (i.e., 130 to 244 minutes or 1,500 kilocalories). Although the exercise volume in these studies was abnormally high, it should be understood that the exercise volume employed in the current study was considerably high for untrained subjects. Therefore, while most acute exercise studies report no changes, LDL-C reductions may be observed after performing an extended session of exercise. The exercise volume required to reduce LDL-C may depend upon baseline activity level.

While one session of exercise produced a significant reduction in LDL-C, three consecutive sessions of exercise did not significantly alter LDL-C concentrations. Most endurance exercise training studies have not reported LDL-C changes (Altena et al., 2006; Couillard et al., 2001; Halverstadt et al., 2003; Kraus et al., 2002; Leon et al., 2002; Leon et al., 2000; Leon et al., 1996; Nieman et al., 2002; Vislocky et al., 2009), however, nearly all studies estimate LDL-C concentrations (Friedwald et al., 1972) rather than measuring it directly. Therefore, any error in measuring TCHL, HDL-C or TG concentrations will alter LDL-C estimation.

Summary

The results of this study suggest that a single 90-minute session of moderate intensity aerobic exercise performed by young, sedentary individuals was not sufficient to elicit significant HDL-C or TG concentration changes. However, three consecutive exercise sessions elicited a: 1) significant increase in post-exercise HDL-C concentrations, and 2) downward trend in post-exercise TG concentrations, mainly in those individuals with initially elevated concentrations. Also, as previously demonstrated, the reponses for all measured variables were similar between men and women (Tsetsonis & Hardman, 1995) and black and white (Alhassan et al., 2001) participants.

A few underlying biochemical mechanisms for these aerobic exercise induced lipid and lipoprotein changes have been proposed. It is hypothesized that aerobic exercise acutely depletes intramuscular triglycerides, stimulating the synthesis of lipoprotein lipase (LPL), facilitating the catabolism of triglyceride rich lipoproteins (i.e., chylomicrons and very low density lipoproteins) which leads to a reduction in plasma TG concentrations (Durstine &

Haskell, 1994). Also, the catabolism of triglyceride-rich lipoproteins can result in the production of more remnants to be converted into HDL. Since intramuscular triglyceride depletion can stimulate LPL activity, a greater depletion over the course of three days may have been a key factor in yielding larger HDL-C and TG changes. Secondary to LPL activity, hepatic lipase (HL) activity has been shown to reduce or not change after aerobic activity, resulting in a prolonged survival time for HDL-C and, therefore, increased circulating HDL-C concentrations (Thompson et al., 2001). While the primary exercise-induced increases in HDL-C are attributed to changes in plasma lipase activities, a decrease in cholesteryl ester transport protein (CETP) levels after aerobic exercise are viewed as a secondary factor for the increased HDL-C concentrations (Seip et al., 1993). Rather than being transferred to VLDL and LDL, this would allow for more cholesterol to remain within HDL-C. While the current study did not assess these enzymatic processes, it appears that three sessions of exercise was sufficient to beneficially augment LPL, HL, and/or CETP activity.

Past discussion has suggested that blood lipid and lipoprotein concentrations can improve once a minimal exercise dose has been achieved (Haskell, 1994). While this exact exercise dose has not been established, it is thought to be linked to the interaction between exercise intensity, frequency, and duration of each exercise session along with the length of the exercise training period. The findings of this dissertation suggest that a threshold number of accumulated exercise sessions may exist for stimulating blood lipid and lipoprotein improvements. In addition, this threshold may differ across various populations. Specific to the current study, young, sedentary, overweight individuals performing three consecutive

prolonged sessions of moderate intensity exercise may experience HDL-C and TG improvements, beyond those observed after a single session of exercise.

In conclusion, in addition to two published abstracts (Grandjean et al., 2001; Biggerstaff et al., 2005), this study suggests that a beneficial additive exercise effect exists regarding HDL-C and, when elevated at baseline, TG concentrations. While most exercise training studies have focused on the effects of intensity or overall volume of exercise, this "accumulated session" research provides an important beginning step in studying the effects of exercise frequency on plasma lipid and lipoprotein concentrations. These results provide the justification for future exercise training studies to investigate a more exact manner in which exercise training frequency (e.g., every day, every other day, etc.) will enhance an individual's lipid and lipoprotein profile. While more research is necessary, the findings of this dissertation suggest that exercise on a *daily* basis may be most effective in improving HDL-C and TG concentrations.

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APPENDIX A

RECRUITMENT FLYER

SUBJECTS NEEDED FOR RESEARCH STUDY

THE UNIVERSITY OF NORTH CAROLINA @ GREENSBORO
DEPARTMENT OF EXERCISE AND SPORT SCIENCE

The study protocol is investigating the effect of a single session of exercise versus accumulated sessions of exercise on blood lipid and lipoprotein concentrations.

UNTRAINED/SEDENTARY SUBJECTS AGE 18-30 ARE NEEDED:

You are eligible for participation if:
- Over the past year you have exercised less than one day per week.
- You are between 18 and 30 years of age.

You are **not** eligible for participation if you:
- Have a resting systolic blood pressure of 140 mmHg or greater.
- Have a resting diastolic blood pressure of 90 mmHg or greater.
- Use tobacco products.
- Use illegal drugs.
- Drink more than an average of 2 alcoholic beverages per day.
- Have diabetes.
- Are, or think you might be, pregnant.
- Have been pregnant or have breastfed within the past six months.
- Have a disease affecting the heart, kidneys, or any other major organ.
- Have a muscle or bone condition that would affect your ability to walk/run on a treadmill.

Benefits include:
- **FREE-** Assessment of maximal aerobic capacity (VO_{2max}).
- **FREE-** blood lipid profile (i.e., measurement of total cholesterol, "good" and "bad" cholesterol, and triglyceride levels). (Generally a $75 fee at a doctor's office)
- **FREE-** Body composition analysis (Generally a $100 fee at a health club).
- **FREE-** Dietary analysis (Generally a $75 fee at a registered dietician).
- Information regarding your current level of physical fitness.

For more information, please contact:
Jason Wagganer
(336)272-7102 ex.468
jwagganer@greensborocollege.edu

APPENDIX B

DETERMINATION OF SAMPLE SIZE

This study will utilize a repeated measures (12 levels) design. It is expected that 90 minutes of physical activity will elicit HDL-C and TG concentration changes from pre- to post-exercise. These concentration changes will only be observed at certain time points (i.e., repeated measurements) during testing. Therefore, in order to determine proper sample size a correlation between the time points of emphasis is required. Since this correlation can only be determined from final data, an estimated correlation must be utilized for pre-study sample size determination. Therefore, the following effect size calculations are based upon a moderate correlation of 0.70:

$$\text{Small effect size} = \frac{f^2 \times m}{1-p} = \frac{0.01 \times 12}{1-0.70} = \frac{0.12}{0.3} = 0.4$$

$$\text{Moderate effect size} = \frac{f^2 \times m}{1-p} = \frac{0.0625 \times 12}{1-0.70} = \frac{0.75}{0.3} = 2.5$$

$$\text{Large effect size} = \frac{f^2 \times m}{1-p} = \frac{0.16 \times 12}{1-0.70} = \frac{0.1.92}{0.3} = 6.4$$

Calculations of degrees of freedom are based upon the following equations:

$$df_{effect} = 2 \times (m-1)$$

$$df_{error} = n \times (m-1)$$

Power calculations using G-power (statistical software) are as follows:

n	m (# rep. meas.)	rho (corr.)	f-squared	adj. f-squared effect size	df effect	df error	Power
9	12	0.7	0.0625	2.5	22	99	0.7317
10	12	0.7	0.0625	2.5	22	110	0.7977
11	12	0.7	0.0625	2.5	22	121	0.8512
12	**12**	**0.7**	**0.0625**	**2.5**	**22**	**132**	**0.8929**
13	12	0.7	0.0625	2.5	22	143	0.9246
14	12	0.7	0.0625	2.5	22	154	0.9479

APPENDIX C

HEALTH HISTORY QUESTIONNAIRE

Health History Questionnaire
All of the information below is necessary to 1) insure your safety as a participant in this study, 2) to determine if you meet the qualification guidelines of this study, and/or 3) for the reporting of the demographics of this study. Please answer all questions accurately. If you are unsure as to how to answer any question, please notify the investigator. All information on this form will be kept confidential.

Name _____

Age_____   Birth Date_____

Emergency contact: Name_____

Phone # (_____)_____

Please check the appropriate responses:
1. What is your race?
    _____African American   _____Caucasian   _____Asian   _____Hispanic
    _____Other (please list)_____

2. Do you currently use tobacco products:
    _____Yes   _____No

3. If you answered "no" to Question #2, have you ever used tobacco products on a regular basis in the past?
    _____Yes   _____No

4. If you answered "yes" to Question #3, how long ago did you quit?
    _____

5. Have you ever been told that you have high blood pressure?
    _____Yes   _____No

    To the best of your knowledge, what is your blood pressure?_____

6. Have you ever been told by a doctor that you should not exercise or that you should only exercise under medical supervision?
    _____Yes   _____No

7. Have you ever had problems with chest discomfort or dizziness?
    _____Yes   _____No

8. Have you ever had a stroke or heart attack?
    _____Yes   _____No

9. Are you diabetic?
    _____Yes   _____No

10. Are you, or do you think you might be, pregnant?
    _____Yes   _____No

11. Do you have any bleeding disorders?
    _____Yes   _____No

12. How many days per week do you exercise?

13. In what type(s) of exercise do you participate?

14. What is the average amount of time you spend participating in each activity?

15. Do your answers to Questions 12-14 represent your exercise habits for at least the past six months?
    _____Yes   _____No

16. Please list *all* prescribed and/or over-the-counter medications (including birth control), nutritional supplements, and vitamins that you take on a regular basis.

I have answered all the above questions truthfully to the best of my ability.

Signature of Participant_____

Date_____

APPENDIX D

PHYSICAL ACTIVITY QUESTIONNAIRE

1. For the last twelve months, which of the following moderate or vigorous activities have you performed **regularly**? (*Please circle YES for all that apply and NO if you do not perform the activity; provide an estimate of the amount of activity for all marked YES. Be as complete as possible*).

Walking
NO   YES   →   How many sessions per week?   _____
                  How many miles (or fractions) per session?   _____
                  Average duration per session?   _____ (minutes)

        What is your usual pace of walking? (*Please circle one*)

        CASUAL or   AVERAGE or   FAIRLY   BRISK or
        STROLLING   NORMAL   BRISK   STRIDING
        (< 2 mph)   (2 to 3 mph)   (3 to 4 mph)   (4 mph or faster)

Stair Climbing
NO   YES   →   How many flights of stairs do you climb UP each day? _____
                  (1 flight = 10 steps)

Jogging or Running
NO   YES   →   How many sessions per week?   _____
                  How many miles (or fractions) per session?   _____
                  Average duration per session?   _____ (minutes)

Treadmill
NO   YES   →   How many sessions per week?   _____
                  How many miles (or fractions) per session? _____ (minutes)
                  Speed? _____ (mph) Grade? _____ (%)

Bicycling
NO   YES   →   How many sessions per week?   _____
                  How many miles per session?   _____
                  Average duration per session?   _____ (minutes)

Swimming Laps → How many sessions per week?   _____
NO   YES   How many miles per session?
                    (880 yds = 0.5 miles)   _____
                  Average duration per session?   _____ (minutes)

Aerobic Dance/Calisthenics/Floor Exercise
NO   YES   →   How many sessions per week?   _____
                   Average duration per session?   _____ (minutes)

Moderate Sports
(e.g. Leisure volleyball, golf (not riding), social dancing, doubles tennis)
NO   YES   →   How many sessions per week?   _____
                   Average duration per session?   _____ (minutes)

Vigorous Racquet Sports
(e.g. Racquetball, singles tennis)
NO   YES   →   How many sessions per week?   _____
                   Average duration per session?   _____ (minutes)

Other Vigorous Sports or Exercise Involving Running
(e.g., Basketball, soccer)
NO   YES   →   Please specify:_____
                   How many sessions per week?   _____
                   Average duration per session?   _____ (minutes)

Other Activities
NO   YES   →   Please specify:_____
                   How many sessions per week?   _____
                   Average duration per session?   _____ (minutes)

Weight Training
(Machines, free weights)
NO   YES   →   How many sessions per week?   _____
                   Average duration per session?   _____ (minutes)

Household Activities (i.e., Sweeping, vacuuming, washing clothes, scrubbing floors)
NO   YES   →   How many hours per week?   _____

Lawn Work and Gardening
NO   YES   →   How many hours per week?   _____

2. How many times a week do you engage in vigorous physical activity long enough to
    work up a sweat? _____ (*times per week*)

APPENDIX E

INFORMED CONSENT FORM

The University of North Carolina at Greensboro
CONSENT TO ACT AS A HUMAN SUBJECT: LONG FORM

Project Title: Effect of Exercise Accumulation on Plasma Lipids

Project Director: Jason D. Wagganer

Participant's Name: _____

Date of Consent: _____

Description and Explanation of Procedures:
This study is designed to compare single session to accumulated sessions of exercise on blood cholesterol levels. As a participant, you will need to report to UNCG's Exercise and Sport Science Department's Exercise Physiology Laboratory for all testing procedures: Protocol One: four visits, Protocol Two: six visits, Protocol Three: two visits; each protocol separated by at least one week and each visit separated by 24 hours.

- Blood collection: Blood (28mL, approximately 2 tablespoons) will be collected at the beginning of each laboratory visit by a blood collection specialist (e.g., phlebotomist). Blood will be analyzed for concentrations of total cholesterol, HDL- and LDL-cholesterol, and triglyceride. Experimental exercise protocols will be timed based upon initiation of menstrual cycle for female participants. Blood collection for all female participants will occur between the first and tenth day of your menstrual cycle. Male participants will have blood collected prior to laboratory visits involving exercise.

- Graded exercise test visit: Prior to exercise testing, resting blood pressure, heart rate, and body fat percentage will be conducted. Blood pressure and heart rate readings will be conducted after a 10-minute rest period in a quiet environment. Body fat percentage will be estimated from a three-site skinfold technique. Waist circumference will also be measured. Maximal oxygen consumption (VO_{2max}), a measure of physical fitness, will then be measured during a graded exercise test on a motorized treadmill. For your safety the electrical activity of your heart will be monitored during the VO_{2max} test. The exercise protocol will start at a moderate walk/run pace, with intensity increasing every two minutes until you can no longer continue. It is important for you to give a maximal effort during this test. However, if you feel anything unusual at any time, you should inform the researcher immediately and, if necessary, the test will be terminated. You also have the right to terminate any procedure of this experiment at any time. This session will last approximately one hour.

- Experimental Exercise Protocols: *Protocol One* will require you to complete a 90-minute treadmill exercise session at a moderate (60% VO_{2max}) pace, followed by three visits (each separated by 24 hours) for fasted blood draws. *Protocol Two* will require you to complete three 90-minute treadmill exercise sessions at a moderate (60% VO_{2max}) pace. After completion of the third exercise session, three more visits will be required for fasted blood sampling (each separated by 24 hours). *Protocol Three* will require two laboratory visits over a six day period. You will report to the lab on day one and day six for fasted blood draws.

To participate in this study, you <u>must</u>:
- Be 18-30 years of age
- Be obese based upon waist circumference and body weight. The criteria for waist circumference and weight depend on sex and height, respectively.
- Exercise less than one time per week over the past six months

You are not eligible for participation if you:
- Have a resting systolic blood pressure of 140mm Hg or greater
- Have a resting diastolic blood pressure of 90mm Hg or greater
- Use tobacco products
- Use illegal drugs
- Drink more than 2 alcoholic beverages per day
- Have diabetes
- Are, or think you might be, pregnant
- Have a disease affecting the heart, kidneys, or any other major organ
- Have a muscle or bone condition that would affect your ability to walk/run on a treadmill

Risks and Discomforts:
A very slight chance of heart attack and even death exists during exercise. The chance of such an incident will be minimized by pre-screening and maintaining a proper emergency protocol to include, if needed, cardiopulmonary resuscitation (CPR) and notification of the Emergency Medical Service (EMS). In addition, there is a slight possibility that you will feel weak or faint from fasting. You should tell the researcher if you experience any unusual sensations before, during, or after any exercise tests. Since you do not have known heart, lung, or metabolic disease, or smoke, and do not have severely high blood pressure, the potential benefit of participation most likely outweighs the risks. In addition, a chance of bruising and a very slight chance of infection exists with all blood collection procedures. This will be minimized through use of a trained phlebotomist and sterile materials.

Potential Benefits:
For no charge, each participant will receive information concerning cardiorespiratory fitness, body composition, blood pressure, and blood cholesterol levels relating to risk of cardiovascular disease (CVD). Data from this study will be used to define optimal exercise

frequency for individuals at risk for CVD. You will be paid $100.^{00} upon completion of **all** required laboratory visits. The entire experiment must be completed in order to receive this payment.

Consent:

By signing this consent form, you agree that you understand the procedure and any risks and benefits involved in this research. You are free to refuse to participate or to withdraw your consent to participate in this research at any time without penalty or prejudice; your participation is entirely voluntary. Your privacy will be protected because you will not be identified by name as a participant in this project. Only members of the research team will have access to your records unless you give written permission for your information to be given to someone else. Data from this experiment will be kept in a locked filing cabinet for at least five years after the study's completion and will then be disposed of by paper shredding. Your blood may need to be tested for evidence of hepatitis, HIV, or other infections in the event anyone involved in the study is exposed to your blood.

The research and this consent from have been approved by the University of North Carolina at Greensboro Institutional Review Board, which insures that research involving people follows federal regulations. Questions regarding your rights as a participant in this project can be answered by calling Mr. Eric Allen at (336) 256-1482. Questions regarding the research itself will be answered by Jason D. Wagganer by calling (336) 686-7825. Any new information that develops during this project will be provided to you if the information might affect your willingness to continue participation in the project.

By signing this form, you are agreeing to participate in the project described to you by

_____ (investigator).

_____
Participant's Signature

_____
Witness to Signature

APPENDIX F

MEDICAL RELEASE FORM

To the attending physician of _____

Your patient has expressed interest in participating in our study entitled, "Effects of Exercise Accumulation on Plasma Lipids and Lipoproteins". This study is designed to compare the effects a single session of exercise to the accumulation of exercise sessions in young sedentary and obese individuals. Factors to be measured include maximal oxygen consumption (VO_{2max}), blood pressure, body composition, and plasma concentrations of total cholesterol, LDL, HDL, and triglycerides. Because many subjects may possess cardiovascular risk factors, as well as being sedentary and obese, we are requiring all study participants to obtain their physician's approval before participation. Please read the study description below and sign the release form if, in your medical judgment, this individual shows no contraindication to study participation.

Inclusion Criteria:
*   Be 18-30 years of age
*   Be obese based upon waist circumference and body mass index:
    -   Waist circumference >102 cm for men, >88 cm for women.
    -   Body mass index of 25-35 kg/m^2
*   Have performed less than one day per week of physical activity during the previous six months.

Exclusion Criteria:
*   Resting systolic blood pressure of 140 mm Hg or greater
*   Resting diastolic blood pressure of 90 mm Hg or greater
*   Use of tobacco products
*   Use of illegal drugs
*   Average consumption of more than 2 alcoholic beverages per day
*   Diabetes
*   Known or suspected pregnancy
*   Have been pregnant or have breastfed within the past six months
*   Disease affecting the heart, kidneys, or any other major organ
*   Any muscle or bone condition that would affect the ability to walk/run on a treadmill

Study Protocol:
This study is designed to compare single session to accumulated sessions of exercise on post-exercise plasma lipid and lipoprotein concentrations. Participants will report to UNCG's Exercise and Sport Science Department's Exercise Physiology Laboratory for all testing procedures: **Protocol One**: four visits, **Protocol Two**: six visits, **Protocol Three**: two visits; each protocol separated by one month and each visit separated by 24 hours.

- Blood collection: Blood (28mL, approximately 2 tablespoons) will be collected at the beginning of each laboratory visit by a trained phlebotomist. Experimental **Protocol 1** will require 112mL, **Protocol 2** will require 168mL, and **Protocol 3** will require 56mL, totaling 336mL of blood for all three experimental protocols (e.g., spanning a three month time period). Experimental exercise protocols will be timed based upon initiation of menstrual cycle for female participants. Blood collection for all female participants will occur between the first and tenth day of your menstrual cycle. Male participants will have blood collected in the same pattern as female participants, with one month in between each exercise protocol.

- Graded exercise test visit: Prior to exercise testing, participants will undergo testing for resting blood pressure, heart rate, and body fat percentage. Blood pressure and heart rate readings will be conducted after a 15-minute rest period in a quiet environment. Body fat percentage will be estimated from skinfold measurements. Waist circumference will also be measured. Maximal oxygen consumption (VO_{2max}) will then be measured while exercising to fatigue during a graded exercise test on a motorized treadmill. During this test, an ECG will be used for measurement of heart rate. The exercise protocol will start at a moderate walk/run pace, with intensity increasing by 2.5% (primarily by grade increases) every two minutes until the participant can no longer perform the exercise. It is important for each participant to give a maximal effort during this test. In addition to these requirements, criteria for the attainment of VO_{2max} will include heart rate within 10 beats/minute of age predicted maximum (220-age), respiratory exchange ratio >1.1, and oxygen consumption plateau despite increased work rate.

- Experimental Exercise Protocols: Protocol One will require four visits to the exercise physiology laboratory, each visit separated by 24 hours. The first visit will require participants to complete a 90-minute treadmill exercise session at a moderate (60% VO_{2max}) pace, followed by three visits (each separated by 24 hours) for fasted blood sampling. Protocol Two will require six visits to the exercise physiology laboratory, each visit separated by 24 hours. The first three visits to the lab will require blood samples prior to a 90-minute treadmill exercise session at a moderate pace (60%VO_{2max}). After completion of the third exercise session, three more visits will be required for fasted blood sampling. Protocol Three will require two laboratory visits over a six day period. Participants will report to the lab on day one and day six for fasted blood sampling.

Data from this study will be used to identify the optimal exercise frequency related research in young overweight and obese individuals at risk for CVD. These studies will also provide baseline information for future research involving exercise frequency in different age groups and CVD risk profile. If further information is needed, or there are further questions regarding the experimental protocol, please feel free to contact the principal investigator/co-investigator:

Jason D. Wagganer, MS   Paul G. Davis, PhD
Dept. of Kinesiology   Dept. of Exercise and Sport Science
Greensboro College   UNCG
(336) 272-7102 ex.468   (336) 334-3030
jwagganer@greeensborocollege.edu   pgdavis@uncg.edu

Thank you for taking the time to consider your patient's participation in this study.

Release

I believe my patient to be capable of safely participating in the investigation, "Effects of Exercise Accumulation of Plasma Lipids and Lipoproteins", as described above.

Signature_____ Print Name_____
Date_____
Adress_____
_____

SUBJECT DATA FORM

Subject's Name _____ Age: _____ Date:_____

Date menstrual cycle began_____ Fasted? (check if "yes") _____

Average

Waist Circumference _____ _____ _____ _____

Resting BP:

Left Arm ____/___ ____/___ ____/___

Right Arm ____/___ ____/___ ____/___

Resting HR:

Left Arm _____ _____ _____

Right Arm _____ _____ _____

Male Skinfolds:

Chest _____ _____ _____ _____

Abdominal _____ _____ _____ _____

Thigh _____ _____ _____ _____

Female Skinfolds:

Tricep _____ _____ _____ _____

Suprailiac _____ _____ _____ _____

Thigh _____ _____ _____ _____

Total: _____

% Body Fat: _____

APPENDIX H

RATING OF PERCEIVED EXERTION SCALE

Category Scale
6
7 Very, Very Light
8
9 Very Light
10
11 Fairly Light
12
13 Somewhat Hard
14
15 Hard
16
17 Very Hard
18
19 Very, Very Hard
20

APPENDIX I

STUDY PROCEDURES (PRE-STUDY SCREENING)

1. Assess waist circumference (men >102cm; women > 88cm) and verify previous sedentary lifestyle (i.e., less than one exercise session per week for the past six months).

2. During a 10-minute resting period have subject complete physical activity questionnaire, health history questionnaire, and informed consent.

3. Conduct BP and HR measurements.

4. Conduct resting 12-lead EKG for 5 minutes.

5. Schedule physical with health center.

6. Fully describe future scheduling dates and exercise protocols.

APPENDIX J

STUDY PROCEDURES (MAXIMAL EXERCISE VISIT)

1. Have subject rest in a seated position for 10 minutes, then obtain HR and BP.

2. Obtain height, weight, and skinfolds (% fat, fat mass and LBM with program on lab computer).

3. VO_{2max} test on treadmill (have metabolic cart calibrated and ECG prep materials ready). While prepping subject for ECG, explain RPE scale and test procedures. Allow subject to drink prior to fitting mouthpiece and conducting warm-up.

4. Get treadmill speed to subject selected RPE between 10-12. Begin test (2-min stages). Increase speed 0.5 mph until 75% predicted HRmax is reached. Increase grade 2.5% every stage thereafter until volitional exhaustion.

5. After completion of max test, transfer vital data to the 60% VO_{2max} data form.

6. Give subject instructions – maintain regular sedentary lifestyle throughout the entire study, complete food diary records the three days prior to the first visit of each experimental protocol and then daily records for each experimental session.

7. Schedule subjects for all experimental sessions (give randomization). Have full copies of calendars indicating exact dates and times of future sessions.

8. Call each subject prior to each test day (starting with the three days preceding each test day for dietary compliance) to remind subject of proper participation.

APPENDIX K

VO$_{2max}$ TEST DATA FORM

NAME:_____   AGE:_____   DATE:_____

Minute	Speed	Grade	Heart Rate	BP	RPE	VO$_2$	Comments
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

Termination time:_____   Max HR:_____   Max VO$_2$:_____

Reason for Termination:

Session:_____

60% VO$_{2max}$ DATA FORM

Trial:_____

NAME:_____   AGE:_____   DATE:_____

VO$_{2max}$:_____   60% VO$_{2max}$:_____   HR$_{max}$:_____   60% HR$_{max}$:_____

Min.	Speed	Grade	Heart Rate	Kcal Output	RPE	VO$_2$	mL/min	Mouthpiece
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
20								
25								
30								
35								
40								
45								
50								
55								
60								
65								
70								
75								
80								
85								
90								

Total kcal expended:_____   Avg. HR:_____   Avg. % HRmax:_____

Avg. VO$_2$:_____   Avg. % VO$_2$ max:_____

APPENDIX M

STUDY PROCEDURES (EXPERIMENTAL EXERCISE SESSIONS)

1. Know at what workload and heart rate subject achieved 60% VO_{2max}; prep cart.

2. Obtain subject's current weight, then have them sit for 10 minutes; obtain RBP/RHR. Take blood sample and perform plasma volume test, spin down blood (1500 rpm x 20 minutes). Have subject drink water and loosen up during this time.

3. Put on heart rate monitor, fit mouthpiece and noseclip, conduct a brief warm-up.

4. Start 60%VO_{2max} treadmill exercise (put mask on at 5:00 and begin acquiring gas exchange data at 7:00).

5. Acquire gas exchange until 23:00 minutes.

6. After the first gas exchange reading, remove mouthpiece; monitor HR during next 15 minutes (36:00 minute). If necessary, allow subject to rest in a seated position for five minutes. Allow subject to drink water (i.e., 4 mL/kg) during this rest period.

7. If no rest is required, replace mouthpiece and begin calculating caloric expenditure at 38:00 minutes.

8. At 55:00 minute mark remove mask and continue exercising until 75:00 minute mark, at which time another five minute rest period (if necessary) will be conducted (same as above).

9. At the 75:00 minute mark, begin exercise; replace mouthpiece and begin calculating caloric expenditure based upon VO_2, until 90:00 minute mark.

10. End test and print-out VO_2 data sheet (multiply all VO_2 numbers by 1.13) (Cool-down)

FOOD DIARY DATA FORM

Please list ALL food and drink consumed during the three days before the first day of each experimental session, in addition to each experimental day. Use a separate sheet for each day and refer to the following examples for ideas on how specific you should be.

7:00am	Eggs (fried)	2 whole (jumbo)	180
		4 whites (jumbo)	75
	wheat bread	3 slices	200
	jelly (grape)	3 tsp.	?
	orange juice (conc.)	2 cups	200
11:00am	Rice (instant/white)	1 cup (dry)	300
	baked chicken	8 oz.	300
	(boneless/white meat)		
	olive oil	2 tbs.	240

Time	Food Source	Quantity (oz./cups)	Calories (if known)

Name:_____ Day:_____ Date:_____