

THESIS

THE EFFECTS OF A SINGLE BOUT OF EXERCISE ON PLASMA  
TRIGLYCERIDES, GLUCOSE, AND INSULIN FOLLOWING CONSUMPTION OF  
A HIGH-FAT MIXED MACRONUTRIENT MEAL

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## ABSTRACT

### THE EFFECTS OF A SINGLE BOUT OF EXERCISE ON PLASMA TRIGLYCERIDES, GLUCOSE, AND INSULIN FOLLOWING CONSUMPTION OF A HIGH-FAT MIXED MACRONUTRIENT MEAL

**Purpose:** The aim of the present study was to examine the effect of a single, acute bout of exercise with caloric replacement compared to a sedentary condition on plasma triglyceride, glucose, and insulin concentrations in response to a high-fat, mixed macronutrient (HFMM) meal challenge.

**Methods:** Eight non-obese, sedentary females aged  $19.6 \pm 1.3$  years participated in two trials: sedentary (SED) and exercise (EX). For the SED trial, subjects refrained from exercise the evening prior to the next morning's HFMM meal. The EX trial was designed to have subjects cycle at 65% of their  $VO_{2peak}$  to produce a net energy cost of 400 calories, with a snack provided shortly after to replace the extra calories expended with exercise. However, due to a methodological error, the net energy cost of exercise was less than the targeted value by approximately 100 kcal, which when accompanied by the replacement energy snack, likely resulted in a small acute positive energy balance. Thus, the unintended research question addressed was whether or not a low intensity bout of exercise could attenuate the postprandial lipemic response to a HFMM meal when

subjects slightly overcompensated for their exercise energy expenditure. During the trial day subjects reported to the laboratory following a 12-hour overnight fast. Blood samples were obtained by intravenous cannulation before ingestion of the HFMM meal challenge and at 30, 60, 90, 120, 150, 180, 210, 240, 300, and 360 minutes after ingestion. Plasma was analyzed for triglyceride, glucose, and insulin concentrations, with these variables compared across the SED and EX conditions using a repeated measures analysis of variance.

Results: There were no significant treatment by time interactions for any of the dependent variables. Low intensity exercise with modest energy overconsumption failed to attenuate the postprandial triglyceride, glucose, and insulin responses to a HFMM meal challenge in comparison to the SED condition.

Conclusion: A low intensity exercise bout accompanied by modest energy overconsumption failed to improve the postprandial response to a HFMM meal challenge compared to the HFMM meal challenge without exercise. Exercise alone may not be beneficial in attenuating postprandial lipemia unless it is accompanied by a resulting acute caloric deficit.

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# CHAPTER I

## LITERATURE REVIEW

### Introduction

The foundation of assessing clinical risk for the development of atherosclerosis and cardiovascular disease (CVD) is primarily based on **fasting** plasma lipid measurements. These risk indicators include total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, and triglycerides (TG), which are typically assayed from blood samples obtained 10-12 hours after consumption of the last meal. However, the postprandial period occupies a substantial portion of the day and there is increased research and clinical interest in the contribution of postprandial lipids to the development of atherosclerosis. The western diet typically provides 75-150 grams of fat per day, consumed during the course of 3-6 eating occasions (meals plus snacks) [1, 2]. Depending on the size and composition of a meal, the postprandial response can last up to eight hours [1]. With the exception of breakfast, each of these meals and snacks are most likely consumed before plasma TG levels have dropped back to baseline from their elevated postprandial concentrations [3]. Therefore during waking hours most humans are almost constantly in a postprandial state, with continuous fluctuations in the degree of lipemia throughout the day. Thus, research focused on attempts to attenuate the postprandial rise in plasma lipids has gained the interest of scientists.

Postprandial lipemia (PPL) describes the condition of elevated blood TG concentrations following ingestion of a meal. Zilversmit first proposed in 1973 that cholesterol enriched chylomicron remnants, which are remain elevated during episodes of extended postprandial lipemia, are as atherogenic as low-density lipoproteins (LDL) [3]. In 1996 a meta-analysis of 17 population-based prospective studies showed that an elevated plasma TG concentration was an independent risk factor for CVD [4]. PPL has since continued to be shown as an independent risk factor for CVD in numerous studies [1, 5-7]. Because coronary heart disease is the leading cause of death in the United States and industrialized countries [7, 8], interventions that have the potential to attenuate postprandial lipemia may help lower the risk of CVD. This literature review will provide an overview of normal metabolism of dietary lipids, further explore the metabolic aspects of PPL, explain why and how the postprandial state has been identified as a period when atherogenic events may occur, describe factors that affect the postprandial response, discuss the potential for exercise to exert favorable effects on the postprandial period, explore energy expenditure (EE) from physical activity in relations to energy intake (EI), and compare and contrast the potential for exercise to attenuate PPL with and without energy replacement (i.e. increasing energy intake to compensate for the calories expended during exercise).

## Overview of Dietary Lipid Metabolism

Lipoproteins serve to transport lipids and cholesterol via the bloodstream to the various tissues in the body. The lipoproteins include chylomicrons, chylomicron remnants, very low-density lipoproteins (VLDL), intermediate-density lipoproteins

(IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Chylomicrons are large TG rich particles packaged with apoB-48, a truncated form of apoB synthesized in the intestinal mucosal cells [9]. These chylomicrons transport dietary TG from the intestines to peripheral tissues. Chylomicron remnants transport dietary lipids to the liver and contain a structural component, apoE, which serves as a ligand for

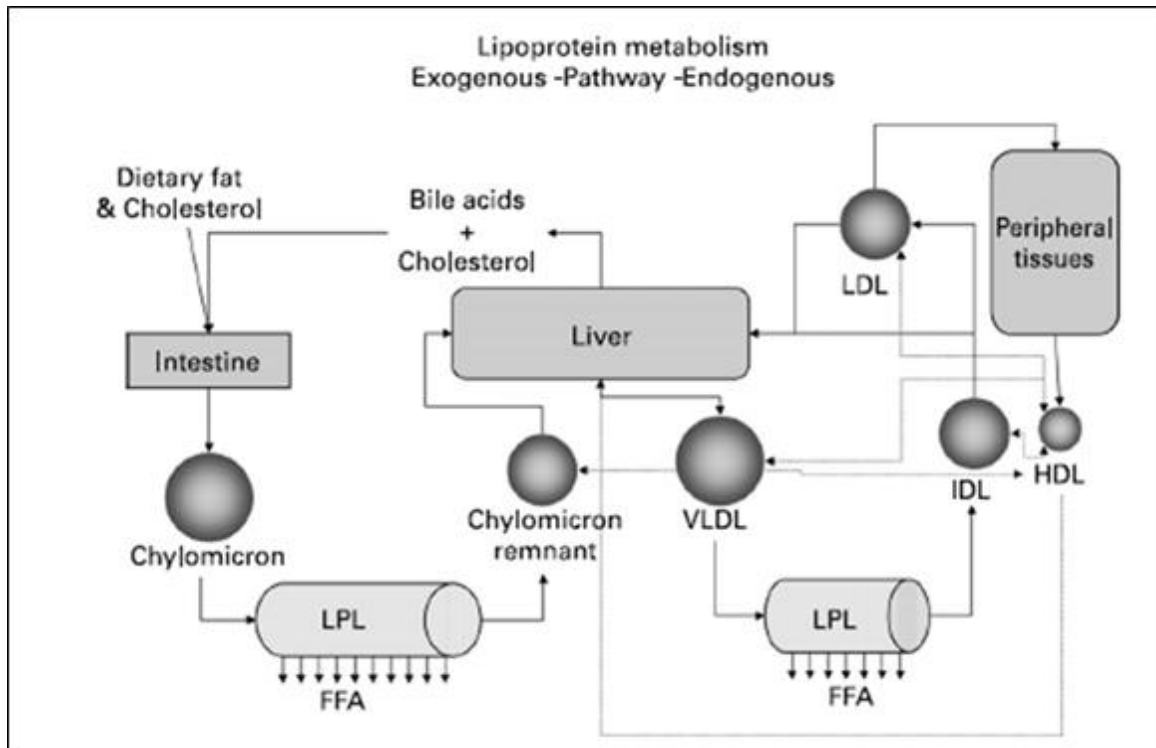


Figure 1: Dietary Lipid Metabolism (Taken from: Lopez-Miranda J, Williams C, & Larion D)[7]

the LDL receptor and the LDL receptor-related protein [5]. VLDLs are TG-rich particles that contain another structural apolipoprotein, apoB-100. VLDLs are synthesized by the liver and serve to transport endogenous TG from the liver to peripheral tissues. IDLs are putative VLDL remnants and contain apoB-100 and are transiently produced in the plasma from VLDL. LDLs contain apoB-100 and transport cholesterol throughout the body [7]. Small, dense LDLs are more atherogenic than the other lipoproteins because they readily enter the subendothelial space and become oxidized [6]. The atherogenic

nature of LDLs will be further discussed in a later section. HDL, the final lipoprotein, plays an important role in reverse cholesterol transport and its concentrations are inversely related to CHD risk [7].

These circulating lipoproteins are primarily derived from the liver and the intestine [10]. The liver secretes VLDLs in response to increased availability of lipids within hepatocytes in response to a caloric load from food consumption [10]. The intestine responds more directly to the presence of dietary fats by secreting chylomicrons [10].

A variety of different types of lipids are consumed in the human diet. The greatest quantities of dietary lipids are in the form of TGs. The digestion of TGs is a complex process that occurs in the lumen of the gastrointestinal tract plus additional processing of the absorbed lipids that occurs once digestion products have been taken up into the enterocytes of the small intestinal mucosa [11]. Most of the digestion of dietary TG is brought about by pancreatic lipase in the lumen of the small intestine [11]. This enzyme catalyzes the hydrolysis of TG to produce monoacylglycerol and free fatty acids. These two products are absorbed into the enterocyte where they are re-esterified into TG [1]. The absorbed dietary TG then combines with apolipoproteins in a reaction catalyzed by microsomal triglyceride transfer protein to produce chylomicrons [1].

Chylomicrons are characterized by their low density and the presence of apoB-48. Chylomicrons are synthesized exclusively in the intestine. In the postprandial period, they move from the intestine into lymphatic circulation carrying the dietary lipids, which are primarily TGs. Over the course of several hours, they migrate into the subclavian vein and enter general circulation. The chylomicrons primarily function to transport

exogenous dietary TG within the body's circulation to the liver, skeletal muscle, and adipose tissue. Lipoprotein lipase (LPL) is the enzyme responsible for the hydrolysis of core TGs in chylomicrons [12]. This reaction produces non-esterified fatty acids and glycerol, with the former being taken up by muscle cells for oxidation or stored in adipocytes. The remaining particles, known as chylomicron remnants are removed from the circulation by the liver through binding of apoE found on the surface of chylomicron remnants to LDL receptors or LDL receptor-related proteins located on the hepatocyte membrane [6].

With the lipids available from the chylomicron remnants the liver is then able to synthesize TG-rich apoB-100 containing VLDL particles. Like chylomicrons, VLDLs are hydrolyzed by LPL. VLDL remnants, also known as IDLs, are taken up by liver receptors via apoE on the surface of IDL or they are converted to a cholesterol rich lipoprotein, LDL, by the action of hepatic triglyceride lipase [6].

#### Metabolic Aspects of Postprandial Lipemia (PPL)

The postprandial period is a dynamic condition during which rapid remodeling of lipoproteins takes place compared to the relatively stable fasting condition [6]. To understand the suspected role of PPL in the development of atherosclerosis it is important to understand that the postprandial response represents both the influx of dietary TG into the circulation and the significant period during which the composition of plasma lipoproteins is significantly changed [1].

When dietary fat is ingested, chylomicrons are secreted into the mesenteric lymph and are transported to the thoracic duct which allows them to enter the circulation via the

subclavian vein. This results in a significant increase in plasma TG and apoB-48 concentrations during the postprandial response [1]. Once in the circulation the nascent chylomicrons acquire a multitude of apolipoproteins from HDL including variants of apoE, which facilitate the removal of chylomicron remnants, and variants of apoC, which modulates LPL-mediated chylomicron catabolism [1]. LPL is localized on the luminal face of the endothelial cells that form the “walls” of capillaries [13]. This enzyme is primarily synthesized by adipocytes and monocytes and is secreted into the interstitial space [13]. LPL is then taken up at the interstitial side of the endothelial cells, is transported across these cells, is secreted, and then bound to the plasma side of the luminal surface [13]. Adipose tissue LPL catalyzes the removal of chylomicron TGs, which have a relatively short (5-10 minute) residence time within the circulation. Once LPL hydrolyzes the TG the fatty acids can be transported into nearby cells such as adipocytes and myocytes. In adipose cells the absorbed fatty acids are used to rebuild TG for storage. LPL is also present in skeletal muscle in which the individual myocytes generally use the absorbed fatty acids for energy but can use these fatty acids for TG synthesis and storage. Cardiac muscle and the mammary gland also require high concentrations of fatty acids and therefore contain LPL for TG breakdown so that the fatty acids can be utilized [1].

During the postprandial period insulin appears to be the exclusive activator of adipose tissue LPL and inactivates LPL in other tissues [1]. When adipose tissue LPL is active the free fatty acids are being primarily used for storage in the fat cells since LPL is less active in other tissues. LPL is therefore the rate-limiting enzyme that controls the removal of triglyceride rich lipoproteins from the circulation. Thus, the activity of

adipose tissue LPL is a major determinant of the magnitude and duration of postprandial lipemia based on how long it takes for plasma TG levels to return to baseline. Once LPL removes the triglyceride-rich lipid from circulation the resulting apoE containing chylomicron remnant returns to the liver and is catabolized to provide lipids for VLDL synthesis.

The resulting apoB-100 containing VLDL particles also play an important role in postprandial lipid metabolism. It has been shown by several studies that VLDL accounts for a significant proportion of the increased plasma TG concentration during postprandial lipemia [14-16]. The rise of VLDL TG seen in the 4-6 hours following the ingestion of a meal has been attributed to both a reduced rate of VLDL clearance and an increased hepatic VLDL synthesis and secretion [1]. Both chylomicrons and VLDLs compete for LPL-mediated triglyceride rich lipoprotein hydrolysis, however, the TG in chylomicrons are the preferred substrate, which leads to a delay in VLDL removal and a postprandial accumulation of VLDL [1]. In addition, up to 50% of chylomicron TG escapes into circulation as non-esterified fatty acids (NEFAs) during the lipolysis of chylomicrons. The NEFAs that are released into the circulation provides the substrate for hepatic TG synthesis which thereby promotes the secretion of VLDL [1]. Although VLDL transports exogenous TG, its presence plays an important role in determining the duration and magnitude of the postprandial response to exogenous (dietary) lipid.

Both chylomicrons and VLDLs are present during postprandial lipemia. ApoC-II and apoC-III are two surface proteins on chylomicrons and VLDLs that are involved with LPL action. ApoC-II is a required cofactor for LPL activation and the resulting hydrolysis of TG by LPL and apoC-III additionally regulates this hydrolysis by impeding

the binding to LPL [17]. When in circulation, VLDL is converted into IDL, via the action of LPL that is attached to endothelial cells [17]. The resulting IDLs are enriched with apoE in the circulation. IDLs are taken up by liver receptors via apoE or are converted to LDLs [6]. These LDLs return to circulation for further cholesterol transport. TG enrichment of LDL particles render them better substrates for hepatic lipase, which hydrolyzes TG from the core of LDL and turns them into smaller and denser particles [6].

Now that the general pathway leading to the production of small, dense, LDL particles has been reviewed, postprandial glucose and insulin action must be considered to further understand the metabolic aspects of PPL.

#### Postprandial Glucose in Relation to Postprandial Lipemia

Epidemiological data have indicated that a postprandial state, characterized by abnormally increased levels of both lipids and glucose, is an independent predictor of future cardiovascular events [15]. Oxidant stress appears to mediate at least some of the increased atherogenic risk associated with increased levels of circulating glucose and lipids in the postprandial state [15]. This temporary rise in glucose and lipids generates excess reactive oxygen species (free radicals) that can trigger a biochemical cascade that acutely triggers inflammation, endothelial changes, hypercoagulability, and sympathetic hyperactivity [15, 16]. Repeated multiple times throughout the day these postprandial changes in both lipids and glucose can eventually lead to atherosclerotic risk factors and CVD [18].

Challenges to glucose homeostasis that provide relevant information on disease risk are often determined by the use of an oral glucose tolerance test (OGTT). This test is

administered after a 10-12 hour fast and provides the individual with a 75 g oral glucose load followed by periodic blood sampling during the 2 hours following ingestion of the glucose. Impaired glucose tolerance, or prediabetes, is then defined as a glucose concentration at this 2 hour timepoint that is between 140-200 mg/dl [15]. Emerging data have indicated that even the earliest abnormality of glucose homeostasis, borderline high (100-140 mg/dl) postprandial glycemia levels, may predispose to atherosclerosis progression and CVD [17]. It is therefore necessary to measure not just postprandial triglycerides but also glucose concentrations when considering the relation between the postprandial state and CVD.

In regard to glucose tolerance, it should also be noted that in small doses, dietary fructose appears to be beneficial in enhancing glucose tolerance [19]. When consumed in large doses characteristic of the American diet, however, fructose may lead to hypertriacylglycerolemia [19]. Fructose may increase plasma TG concentrations by altering the rate of hepatic fatty acid synthesis. Both acute and chronic fructose feeding has been shown to stimulate hepatic de novo lipogenesis [19]. The extent to which de novo lipogenesis contributes to the hypertriacylglycerolemic effects of fructose ingestion in an acute setting however is currently unclear.

#### Postprandial Insulin in Relation to Postprandial Lipemia

Insulin is an anabolic hormone that promotes cellular uptake of energy substrates in times of excess nutrient bioavailability. This hormone has a multitude of biological effects including, but not limited to, promoting an increase of lipid and glucose uptake while decreasing lipolysis and gluconeogenesis, increasing protein synthesis while

inhibiting protein degradation, and enhancing vasodilation and cell proliferation. The biological response of insulin that is most studied is insulin-mediated glucose disposal [18]. For the sake of this study a brief overview of insulin action and insulin stimulated glucose transport is provided to highlight insulin's role in postprandial glucose levels. The relation to insulin and LPL in postprandial lipemia is also presented.

Overview of Insulin Action. Normal insulin action results from the hormone's effects on specific insulin signaling cascades that involve a variety of signaling molecules. Insulin action in insulin-sensitive peripheral tissues, such as muscle and fat, begins with specific binding to high-affinity receptors on the plasma membrane of the target tissue [18]. The insulin receptor is a large, transmembrane protein that consists of 2  $\alpha$ - and 2  $\beta$ -subunits. The insulin signaling cascade is initiated when insulin binds to the  $\alpha$ -subunit of the insulin receptor which leads to the autophosphorylation of specific tyrosine residues of the  $\beta$ -subunit [19]. Tyrosine kinase activity is present within the  $\beta$ -subunit. This process enhances the tyrosine kinase activity toward other protein substrates. When the insulin receptor tyrosine kinase is activated, tyrosine phosphorylation of insulin receptor substrates (IRS) results. These cytoplasmic proteins have multiple phosphorylation docking sites for cytosolic substrates that contain specific recognition domains which are termed SH2 domains [18]. These SH2 domains provide an extensive potential for interaction with downstream signaling molecules via multiple phosphorylation motifs [18, 19]. Therefore, the phosphorylated form of IRS proteins plays a role in regulating insulin signaling because it serves as the docking site which binds to and regulates intracellular enzymes containing SH2 domains that allow insulin signal transduction throughout the target cell to occur.

*Insulin-Stimulated Glucose Transport.* Glucose is a water soluble molecule and as such readily diffuses through the lipid rich plasma membrane of cells. Tissue specific glucose transport proteins are required for glucose to traverse across the cell membrane. An important insulin signaling pathway that leads to the translocation of cytosolic sequestered glucose transporters to the cell surface in skeletal muscle, cardiac muscle, and adipose tissue involves the enzyme phosphatidylinositide-3 kinase (PI-3 kinase) [18]. Insulin binding increases the amount of PI-3 kinase associated with IRS, which activates the enzyme. This pathway ultimately results in the translocation of an intracellular pool of Glut-4 molecules to the plasma membrane for glucose transport from the interstitium into the cell. The use of Glut-4 for glucose transport differs from other transporters because without insulin stimulation, 90% of the proteins are sequestered intracellularly, allowing Glut-4 to be recycled between the plasma membrane and intracellular storage pools [20]. This favors low uptake of glucose in skeletal muscle and adipocytes during a fasting state, and enhanced glucose clearance under post-prandial conditions. When insulin is present the equilibrium of this Glut-4 recycling is altered to favor translocation of the transporter to the plasma membrane and transverse tubules in skeletal and cardiac muscle which results in an increase in the maximal velocity of glucose uptake into the cell [20]. Without the proper functioning of this insulin signaling cascade, most of the Glut-4 remains sequestered in the cytosolic compartment, and glucose uptake is low in these tissues. This phenomenon is one characteristic of insulin resistance.

*Insulin in the Postprandial State.* Insulin is involved in promoting fat uptake, increasing TG synthesis, enhancing the proliferation of fat cells, and decreasing lipolysis.

During the postprandial state, insulin is the major activator of adipose tissue LPL, but inactivates LPL in other tissues [2]. As previously discussed, LPL is the rate-limiting enzyme that controls the removal of triglyceride rich lipoproteins from the circulation, thereby determining the duration and magnitude of PPL [2]. Insulin resistance is a state in which physiologic concentrations of insulin produce a subnormal response and it underlies glucose and lipid abnormalities [21]. Therefore triglycerides, glucose, and insulin all have major roles in the postprandial state and were selected as the dependent variables in this study.

#### The Postprandial State as a Period when Atherogenic Mechanisms can Occur

As previously mentioned, in 1979 a widely referenced paper by Zilversmit [3] proposed that the hydrolysis of chylomicrons by LPL resulted in the subsequent internalization of cholesterol ester-enriched chylomicron remnants by arterial smooth muscle cells. After this paper, more attention has been directed towards the potential atherogenicity of postprandial TG and TG-rich lipoprotein levels.

It is now widely recognized that atherogenesis is an inflammatory disease process. Elevated postprandial plasma TG levels lead to the production of excessive chylomicrons and VLDL remnants, which are both proinflammatory and can enhance the progression of atherosclerosis [6]. When LDLs are produced and accumulate in circulation they can readily enter the artery wall where they are able to become modified. Modified LDL particles are also proinflammatory and are a determinant of the inflammation that can lead to CVD.

## Factors Affecting the Postprandial Response

As previously mentioned, most of the waking hours for humans are spent in a postprandial state due to successive meal intake throughout the day. The duration and kinetics of the postprandial changes are highly variable and are modulated by many factors. Postprandial lipoprotein metabolism is modulated by meal size and composition, several lifestyle conditions, and physiological factors.

*Meal Size and Composition.* The length of the postprandial response depends on the size and the composition of the ingested meal and can last up to eight hours [1]. Most Americans consume 3-6 meals/snacks per day and therefore during waking hours are in an almost constant postprandial state. Therefore the postprandial state is not only determined by the most recent feeding but also from previous food consumption resulting in elevated TGs which have not yet returned to baseline. Meal composition is also a key determinant of PPL. As discussed below, the amount and type of dietary fat and carbohydrate present in a meal, as well as the presence of any protein or alcohol, influence PPL response.

*Dietary Fat.* The amount and type of dietary fat consumed in a meal modulate postprandial lipemia. Thirty to fifty grams of dietary fat result in significant elevations in plasma TG concentration [6, 20]. Most meals contain 20-40 g of dietary fat and consecutive meals containing dietary fat appear to increase the magnitude of postprandial lipemia [21]. Therefore if three meals along with snacks are consumed it can be expected that circulating TG levels will be maintained above fasting concentrations for the majority of the day.

The type of dietary fat consumed modulates postprandial lipemia. Short or medium chain dietary fatty acids have a limited effect of postprandial plasma TG response because they enter the portal route rather than chylomicron secretion initially into the lymphatic system [6, 20]. It has also been shown that the intake of long-chain omega-3 polyunsaturated fatty acids, predominately found in fish oils, results in lower circulating TG levels and attenuates postprandial lipemia [22].

Dietary Carbohydrate. It has been shown in clinical studies that highly digestible carbohydrate can lead to increases in fasting plasma TG as a result of hepatic VLDL chylomicron remnant accumulation secondary to altered lipoprotein secretion and/or clearance [23, 24]. It is also known that the insulin response to dietary carbohydrate can stimulate glucose disposal, inhibit VLDL release and formation, increase LPL activity, and decrease lipolysis [25]. Therefore, insulin release following a high-fat test meal that also contains significant metabolizable carbohydrate may attenuate the actual postprandial triglyceride response of a test meal that solely contains fat. Meals and snacks in the American diet however rarely contain a single macronutrient and it is necessary to consider the effects of the carbohydrate present in a high-fat mixed macronutrient test meal when evaluating PPL.

The amount and nature of dietary carbohydrate in a meal can alter postprandial metabolism. The addition of glucose (50 g - 100 g) to an oral fat tolerance test (OFTT) meal has not produced consistent findings in healthy individuals [6]. However, the addition of fructose to an OFTT, either alone or in equimolar concentrations with glucose (i.e. sucrose), increases the accumulation of chylomicrons in the postprandial state [6, 20]. The presence of dietary fiber has also been shown to affect PPL. When dietary fiber

is added to a mixed meal at the level of 4-10 g it has been shown to moderately reduce PPL or chylomicron lipids as generated by the mixed meal [6, 20].

*Lifestyle Conditions.* Smoking habits, alcohol consumption, and physical activity are three lifestyle conditions that affect PPL. In regards to smoking habits, Axelson *et al.* [26] showed a 50% greater TG postprandial increase in habitual smokers without changes in fasting TG. Smoking habits must therefore be considered when evaluating PPL. Alcohol also affects PPL because when ethanol is consumed with a meal both total plasma and VLDL-TG are elevated due to alcohol metabolism.

There is clear evidence from a variety of cross-sectional studies comparing endurance trained men with untrained control subjects that regular exercisers exhibit low levels of postprandial lipemia [27]. It has been shown that physical activity within the 24-h period preceding a high-fat meal improves the rate at which lipids are removed from the circulation [6]. The potential favorable effects of acute exercise will be discussed in further detail below.

#### Favorable Effects of Acute Exercise

There is a large body of evidence that supports the ability of a prior session of exercise to attenuate PPL [27]. The decreased postprandial TG concentrations observed following an acute bout of exercise could theoretically be due to a number of possibilities: 1.) an increased clearance rate of TG from the circulation; 2.) a reduced rate of appearance of either chylomicrons into the circulation and/or to a reduced hepatic secretion of VLDL into the circulation; and 3.) both a decreased rate of TG appearance and an increased rate of clearance. An increased clearance rate of TG is likely mediated

by an upregulation of LPL activity. This phenomenon is supported by numerous observations of low-levels of PPL in endurance trained athletes, and by the attenuation of circulating TG following prolonged exercise [27]. However, there has been an emerging body of evidence suggesting that increased TG clearance cannot entirely account for exercise-induced TG reductions seen with exercise bouts of moderate duration and intensity.

It seems unlikely that a reduced rate of chylomicron appearance into the circulation from the gut would be an important reason for the exercise-induced reduction in the lipemic response to a meal. It has been shown that prior exercise does not delay the time to reach peak postprandial chylomicron concentrations [28]. Additionally, studies that administered paracetamol during an OFFT as a marker of gastric emptying suggest that macronutrient absorption is not delayed by participation in an exercise bout the day before [28, 29].

If an increased clearance rate of TG from the circulation and a reduced rate of appearance of chylomicrons into the circulation cannot entirely account for the reduction in TG following a bout of moderate exercise, it is plausible that reduced hepatic VLDL secretion could also contribute. Therefore, it is possible that an acute bout of exercise could reduce PPL by two complementary mechanisms involving an increase in TG clearance and a reduction in VLDL secretion from the liver [27].

Most published studies examining the effect of prior acute exercise on postprandial metabolism show an exercise-induced decrease in PPL. In a meta-analysis by Pettit and Cureton [30] it was suggested that exercise has a moderate effect on PPL and that the energy expenditure of prior exercise appears to be the major factor

influencing the magnitude and duration of the postprandial lipemic response. As discussed below, the type of OFTT administered, gender, body composition, exercise history, control of menstruation, the mode and duration of exercise, and caloric replacement also affect the postprandial lipemic response.

In a study by Aldred *et al.* [31] one bout of low intensity walking on a treadmill for 2 hours at  $30.9\% \pm 1.6\%$  of  $VO_2\text{max}$  was able to reduce postprandial lipemia following a high-fat meal the following day. The study included 6 normolipidemic females and 6 normolipidemic males. All study participants took part in regular physical activity. Since the study participants were regularly active individuals the results in this study may not be applied to sedentary individuals, whose responses could differ from regularly active individuals. The possibility of such differences was addressed in a comparative study of trained and untrained individuals by Tsesonis *et al.* [32]. They examined the PPL differences between trained and untrained middle-aged women. The acute bout of exercise involved treadmill walking for 90 minutes at 60% of  $VO_2\text{max}$ . A high-fat test meal was consumed the day following exercise after a 12-h fast. When compared to the control trial, total oxidation of fat over the 6-h postprandial period was enhanced by exercise to a similar degree in both groups of women. MacEneaney *et al.* [33] studied normal weight and overweight adolescent boys in a similar study design where the subjects underwent two 6-h OFTTs after a sedentary trial and an exercise trial that varied in duration. Subjects exercised on a treadmill for a calculated amount of time that would expend 600 calories while working out at 65% of  $VO_2\text{max}$ . The study concluded that a moderate exercise bout prior to the OFTT effectively reduced PPL to a similar degree in both groups.

Each of these three studies did not replace the calories expended during the exercise trial so the results were influenced by the exercise-induced caloric deficit. These previous studies of acute exercise and postprandial lipemia may not be representative of real life conditions since these studies often fail to address the potential of increased energy intake following moderate exercise. There is a belief that physical activity drives up hunger and increases food intake, however, supporting evidence is lacking for such an immediate or automatic effect [34]. It is therefore necessary to consider the relationship between energy expenditure (EE) from physical activity and energy intake (EI). Further the favorable effects of exercise on PPL may not be seen if the exercise-induced energy deficit is corrected with dietary caloric replacement.

#### Energy Expenditure and Energy Intake

For over 50 years the relationship between EE from physical activity and EI has been a central research theme when studying energy balance (EB) [34]. A study by Stubbs et al. [35] aimed to assess the effect of graded increases in exercise-induced EE on EI. They used a within-subject, repeated measures design where six men were studied three times during a nine day protocol, corresponding to three conditions; no exercise (0kcal expended), medium exercise level (382kcal expended during exercise), and high exercise level (764kcal expended during exercise). On days 1-2 the subjects were provided a medium fat maintenance diet and on days 3-9 subjects were allowed to eat ad lib while they kept food diaries that included self-weighted intakes. The study showed that EE amounted to 2,795, 3,081 and 4,013kcal/day on the no exercise, medium exercise, and high exercise conditions, respectively. Corresponding values for EI were 2,771,

2,818, and 2,818 kcals/day. The study concluded that increasing EE did not lead to compensation of EI over 7 days.

In a different study by Stubbs et al. [36] using the same study design six women participated in 7 day treatments, three times. The three conditions were no exercise (0kcals/expended), medium exercise level (454kcals expended during exercise), and high exercise level (812kcals expended during exercise). In this study EE amounted to 2,197, 2,627, and 2,890 kcals/day on the no exercise, medium exercise, and high exercise conditions. Corresponding values for EI were 2,126, 2,197, and 2,388 kcals/day. These results showed that markedly increasing EE through exercise was able to produce partial compensations in EI, about 33% of EE due to exercise.

In these studies an acute increase in EI was not seen at the level where individuals were consuming more kcals than that expended during acute bouts of exercise. As a result accurate adjustments of EI to acute increases in EE are likely to take weeks rather than days. Therefore it is not likely to see an overcompensation of EI following a single acute bout of exercise. However, for the sake of the present study it is important to distinguish if an acute bout of exercise without caloric replacement is enough to produce positive effects on postprandial lipemia.

#### Exercise-Induced Calorie Deficit vs. Calorie Replacement

Both positive and negative energy status can have profound effects on carbohydrate and lipid metabolism. Therefore the internal validity of studies addressing the effects of acute exercise on PPL may be compromised if energy status is not strictly controlled. If caloric replacement is not included to match the increased energy

expenditure of the exercise bout, the effects of exercise are likely to be confounded by the resulting energy deficit. A recent study by Harrison *et al.* [37] examined the influence of acute exercise with and without carbohydrate replacement on postprandial lipid metabolism. This study included eight recreationally active young men who underwent an OFTT on the morning after three experimental conditions: no exercise, prolonged exercise without carbohydrate replacement, and prolonged exercise with carbohydrate replacement to restore carbohydrate and energy balance. The exercise bout consisted of cycling at 70%  $\text{VO}_2\text{peak}$  for 90 minutes followed by 10 maximal one minute sprints producing an average energy expenditure of  $\sim 17.5$  kcal/kg body mass. This study concluded that the influence of acute exhaustive exercise on postprandial lipid metabolism is largely dependent on the associated carbohydrate and energy deficit.

Burton *et al.* [38] recently shed light on the effects of exercise, with and without energy replacement, on postprandial lipemia. In their study subjects underwent three 2-day trials. On day 1 of each trial subjects either rested (control), walked at 50% maximal oxygen uptake for 75-120 minutes to create a net energy expenditure of 6.5kcal/kg body mass (energy-deficit), or completed the same walk with the net energy expended replaced (energy-replacement). On day 2 subjects underwent an 8.5-h metabolic assessment during which they received a high-carbohydrate test breakfast and lunch. The study found that exercise with energy replacement lowered postprandial insulinemia and increased fat oxidation. Importantly, however, an exercise-induced energy deficit augmented these effects and was necessary to lower postprandial lipemia.

## Summary

In summary, significant and prolonged elevations of postprandial TG-rich lipoproteins are now recognized to contribute to CVD risk. Owing to the frequency of meal consumption during the waking hours, the postprandial state is clearly an important period to consider when examining the risk for atherogenesis in humans. Exercise, both chronic and acute, has the potential to attenuate the postprandial rise in circulating TG in response to a high-fat meal. However, fat tolerance tests have limited external validity, as most often significant amounts of dietary fat are not consumed in isolation, but rather in association with dietary carbohydrate. Also, there is a paucity of data examining the effect of acute exercise with energy replacement on the postprandial lipemic response to a high-fat mixed macronutrient meal in sedentary women. Given these limitations of previous studies, the aim of the present study was to determine the effect of a single, acute bout of exercise with caloric replacement on plasma triglyceride, glucose, and insulin concentrations following a test meal containing significant amounts of both fat and carbohydrate. We hypothesized that the exercise bout with caloric replacement would result in an attenuated rise in plasma TG and glucose concentrations along with an attenuated postprandial plasma insulin response when compared to the sedentary trial. However, this attenuation would be to a lesser degree than that reported by previous OFTTs that have followed an acute exercise bout that produced a caloric deficit.

## CHAPTER II

### METHODS

#### Subjects

A total of eight sedentary (exercise  $\leq 2$  times/week), non-obese [body mass  $68.2 \pm 8.4$  kg, body mass index (BMI)  $24.6 \pm 1.8$  kg/m, % body fat  $29.8 \pm 3.2\%$ ], young adult females ( $20 \pm 1.3$  years of age) participated in this study. Aerobic fitness [maximal O<sub>2</sub> uptake (VO<sub>2peak</sub>)  $31.3 \pm 3.0$  ml/kg/min] was measured by a graded exercise test. Subjects were apparently healthy individuals with no overt signs or symptoms of disease as determined by a health history questionnaire. Subjects were excluded from the study if they had diabetes ( $>126$  mg/dl), hypertension ( $>140/90$  mmHg), exercised more than twice per week, had a VO<sub>2peak</sub>  $\geq 40$  ml/kg/min, or if they had a past or present history of endocrine disorders or cardiovascular disease. Individuals were also excluded from the study if they were pregnant, used tobacco, were vegetarian or lactose intolerant, if they had a history of an eating disorder, or if they were taking any medications known to influence carbohydrate or lipid metabolism. All participation was voluntary and both oral and informed written consent was obtained from each subject prior to enrollment. The study protocol was approved by the Colorado State University (CSU) Institutional Review Board.

## Experimental Design

Each subject underwent two trials in random order, one trial with exercise (EX) and one sedentary trial without exercise (SED). It has been shown previously that the effects of exercise training on postprandial lipemia diminish within sixty hours of the last training session [39], therefore, a washout period of a minimum of seven days was scheduled between trials for each participant. The day before each of the trials a standardized isocaloric diet (57% CHO, 24% fat, 19% protein) was provided for the subjects to consume. On the evening before the EX trial subjects reported to the laboratory to complete an exercise bout on a stationary bicycle, which was designed to achieve a net caloric cost of ~ 400 kcal. Subjects were monitored throughout the exercise bout and heart rate was recorded at each minute of exercise. Water was provided as needed throughout the exercise. After the exercise was completed subjects were provided with an isocaloric replacement snack (Yoplait® yogurt, Kashi® trail mix bar, and string cheese; 380 calories, 57% CHO, 25% fat, 20% protein) to restore energy balance. On the evening before the SED trial subjects were instructed to refrain from physical activity. For each trial all food and the snack provided after the EX trial were consumed at least 12-h prior to administering the test shake the next morning.

*High-Fat Mixed Macronutrient (HFMM) Test Meal.* Subjects arrived at the laboratory on each trial day at approximately 0700 hours having refrained from any food or beverage intake (with the exception of water) for the previous 12-h. Weight was obtained upon arrival at the lab and then the subjects rested in bed while a catheter was placed in the antecubital vein of the forearm. Two baseline blood samples were drawn and placed on wet ice. Subjects were then provided with a liquid test meal that consisted

of whole milk, heavy whipped cream, sugar, chocolate syrup and whey protein powder. . Each study participant received a relative dose of the test meal based on their body weight (12.5 kcal/kg body weight), with a macronutrient intake of 0.75 g fat per kilogram body weight, 1.1 g CHO/kg body weight, and 0.35 g protein/kg body weight. The macronutrient distribution as a percentage of calories in the liquid meal was 53% fat, 34% CHO, and 11% protein. Subjects were given 15 minutes to consume the meal. When the meal was finished a timer was started and venous blood samples were obtained at 30, 60, 90, 120, 150, 180, 210, 240, 300, 360 minutes after completion of the test shake. All blood samples were drawn into EDTA tubes and initially stored on wet ice until they were centrifuged at 2500 rpm at 4 degrees Celsius within 2 hours. The plasma was then removed and placed in bullet tubes and stored at -80 Celsius for later analysis. Throughout the 6-h trials subjects rested quietly in bed.

### Specific Procedures

*Preliminary Measurements.* During the initial meeting, after participants provided informed written consent they completed the following surveys: a CSU Confidential Health History Questionnaire that included a section on exercise history, a Food Preferences and Acceptability questionnaire, and an Eating Attitude Test (EATS-26) to rule out eating disorders [40]. Pregnancy tests were available if subjects had a possibility of being pregnant. Subjects were then instructed to keep a 3-day food record prior to the trial days and set up a meeting time to complete the remainder of the preliminary testing.

On the preliminary testing day all subjects arrived at the laboratory after an overnight fast. Body height was measured without shoes on a standard stadiometer to the

nearest 0.1 centimeter. Body weight was measured without shoes on a balance scale to the nearest 0.5 kg and BMI was calculated. Blood pressure was measured with a sphygmomanometer (Dynamap, XL vital signs monitor and a Dura-Cuf, Johnson and Johnson Medical Inc. Tampa, FL). Resting energy expenditure was performed prior to obtaining fasting blood glucose which was determined via finger stick and glucometer (Precision Xtra, MediSense, Abbott, Alameda CA).

Resting Energy Expenditure (REE). REE was measured on the preliminary testing day using indirect calorimetry (Parvo medics, Model No. MMS 2400, True One 2400 Metabolic Measurement System, Sandy, UT). Subjects were instructed to lie quietly in a recumbent position on a comfortable bed for 20 minutes with a plastic canopy over their head while respiratory gas exchange measures were obtained. The REE was calculated using the  $VCO_2$  and  $VO_2$  gas exchange measures, which were converted to kilocalories via the Weir equation [41]. The last 10 minutes of gas analysis data was averaged to determine REE which was extrapolated to 24-h and used in subsequent design of the subjects' control diets.

Body Composition. Percentage of body fat, absolute fat mass, and fat-free mass were measured using a dual energy X-ray absorptiometer (DEXA) (Hologic, Discovery, QRD series). Testing was completed at Colorado State University (Fort Collins, CO) in the Human Performance & Clinical Research Laboratory. Subjects were instructed to remove all metal from their body and lie supine on the DEXA platform where they were positioned according to the manufacturer instructions. Body composition was analyzed and printed using the Hologic computer software.

Cardiorespiratory Fitness. Subjects were recruited based on self report of a sedentary lifestyle (exercise  $\leq 2$  times per week). Subjects were excluded if  $VO_2$ peak exceeded 40 ml/kg/min.  $VO_2$ peak was measured during a graded cycling exercise to volitional exhaustion using a Monark, Ergomedic bicycle and a progressive maximal workload protocol (Medgraphics, Monark, Ergomedic, Model No. 8082E, St Paul, MN). Each subject wore a heart rate monitor (Polar Target, Hong Kong) and heart rate was continuously recorded every 30 seconds during testing. Subjects were fitted to the bike and familiarized with the testing procedure prior to starting the bout of exercise. Following a 2-5 minute warm-up period subjects were instructed to pedal at a cadence between 70-100 reps per minute (rpm) and an initial work-load of 1 kp was set for 2 minutes. Workload was then increased by 0.5 kp every 2 minutes until the test was terminated when subjects could not maintain a cadence of 70 rpms or when an increase in oxygen uptake was not seen with an increase in workload. Throughout the cycling exercise bout measurements of carbon dioxide production, oxygen consumption, pulmonary ventilation, and respiratory exchange ratio were determined by computer-assisted open-circuit spirometry (Parvo Medics, Model No. MMS 2400, True One 2400 Metabolic Measurement System, Sandy, UT). Peak  $VO_2$  was identified as the highest oxygen uptake values maintained for a full minute.

Dietary Control. It was necessary to control dietary intake the day preceding the EX and SED test days. This was accomplished in the following manner. Using the completed Food Preferences and Acceptability Questionnaire a registered dietitian prepared all food in the Department of Food Science and Human Nutrition metabolic kitchen. For the standardized pre-test diet, total 24-h energy intake for each subject was

determined by multiplying their measured REE by an activity factor of 1.4, corresponding to the physical activity level of a non-active adult [42]. The macronutrient breakdown of the meals provided the day preceding the trial was 57% of energy from carbohydrate, 24% of energy from fat, and 19% of energy from protein. Subjects reported to the laboratory two days prior to each trial to pick up the food and receive dietary instruction to consume all of the provided food and return any remaining items to the lab for collection and recording. Subjects were allowed to consume water *ad libitum* and were instructed to finish eating 12-h prior to reporting to the laboratory the following morning. Any questions about the standardized diet were answered at this time by a member of the research team.

Exercise Trial. Between 1700 and 1900 the evening before the EX trial, subjects completed the acute bout of cycling exercise designed with the goal to achieve a net energy cost of 400 *kcalories*. Each subject cycled for a set amount of time determined by the following equations:

$\underline{\hspace{2cm}} \text{ L/min (65\% } VO_2\text{peak)} \times 4.95 \text{ kcal/L} = \underline{\hspace{2cm}} \text{ kcal/min of exercise}$ <p><b><i>Calories from RMR accounted for during exercise:</i></b></p> $\underline{\hspace{2cm}} \text{ kcal/day} \div 1,440 \text{ min/day} = \underline{\hspace{2cm}} \text{ kcal/min resting}$ $\underline{\hspace{2cm}} \text{ kcal/min of exercise} - \underline{\hspace{2cm}} \text{ kcal/min resting} = \underline{\hspace{2cm}} \text{ kcal/min}$ $400\text{kcal} \div \underline{\hspace{2cm}} \text{ kcal/min} = \underline{\hspace{2cm}} \text{ total minutes of exercise}$
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Unfortunately, after determining the duration of the exercise, the exercise intensity was erroneously based on *65% of peak heart rate* rather than on *65% of peak VO<sub>2</sub>*. Subjects were instructed to adjust their cadence in accordance to their heart rate to maintain 65% of their peak heart rate. In attempt to offset the acute energy deficit subjects were provided with an energy replacement snack that contained 380 kcals (57% CHO, 25% fat, 20% protein). This mixed meal snack was consumed shortly after the exercise session to ensure a 12-h fast prior to starting the trial the following morning.

Due to the erroneous exercise design the net energy cost of the exercise was determined to approximate 285 kcals (~4.2kcal/kg). The actual energy cost was determined post data collection and was based on the VO<sub>2</sub> corresponding to 65% of peak heart rate during the graded exercise test. The first equation presented above was then used to multiply the L/min at 65% VO<sub>2peak</sub> by 4.9 kcal/L O<sub>2</sub> to convert the oxygen consumption values to kcals/min. These values were then multiplied by the duration of the subjects exercise bout. The duration of exercise for the subjects averaged 70 ± 14.9 (SD) minutes.

#### Blood Analysis

Plasma glucose and insulin concentrations were determined at the Colorado Clinical and Translational Sciences Laboratory. Glucose concentrations were measured by the standard glucose oxidase method using a glucose analyzer (YSI 2300, YSI Inc. Yellow Springs, OH). Plasma insulin concentrations were measured by an immunoenzymatic (“sandwich”) assay for use with the Beckman-Coulter Access

Immunoassay System (Beckman Coulter, Inc. Fullerton, CA). Plasma TG concentrations were measured by standard spectrophotometric procedures.

### Statistical Analysis

The dependent variables, pre- and post-prandial TG, glucose, and insulin were analyzed across the EX and SED conditions using a repeated measures ANOVA to examine the main effects of condition, time, and their possible interactions. The probability of a type 1 error was established as  $p < 0.05$ .

## CHAPTER III

### RESULTS

#### Characteristics of the Study Participants

Table 1 provides information regarding the physical characteristics of the study participants. The data indicate that the eight volunteers were non-obese young women with normal fasting blood glucose concentrations, normal blood pressure, and relatively low levels of physical fitness as would be expected owing to their sedentary nature.

Table 1: Subject Baseline Characteristics. *n*=8

<b>Variable</b>	<b>Mean</b>	<b>St. Dev.</b>
Age (yrs)	19.6	1.3
Weight (Kg, baseline)	68.2	8.5
Weight (Kg, ex)	67.1	9.4
Weight (Kg, no ex)	65.2	9.3
Height (cm)	166.4	7.1
BMI (baseline)	24.6	1.8
Waist (cm)	92.0	8.0
Hip (cm)	77.2	7.0
WHR	0.84	0.06
Body Fat %	29.8	3.2
Blood Glucose (mg/dl)	79.6	15.4
Systolic BP (mm Hg)	108	9.2
Diastolic (mm Hg)	74	13.3
Pulse (bpm)	76	11.7
VO <sub>2</sub> peak (ml/kg/min)	31.3	3.0
RMR (Kcal)	1501.8	168.9

Furthermore, the subjects' body weight did not vary significantly prior to each of the two trials.

## Dietary Control

The test meal for the subjects averaged  $871.7 \pm 108.9$  calories. The equations and test shake composition were described previously in the Methods section. The standardized diet provided the day preceding the EX trial provided an average of  $2,425 \pm 233.1$  kcals, 57% CHO, 24% fat, and 19% protein. The standardized diet provided the day preceding the SED trial provided an average of  $2,132 \pm 256.4$  kcals, 57% CHO, 24% fat, and 19% protein.

## Exercise

On average subjects exercised for  $70 \pm 14.9$  (SD) minutes and maintained an average heart rate of  $127 \pm 15.1$  beats per minute.

## Serum glucose concentrations during the two trials

Figure 2 shows the mean ( $\pm$  SEM) baseline and postprandial serum glucose concentrations over the 6-h trial for the EX and SED conditions. The baseline fasting plasma glucose concentrations prior to the test meal were not significantly different between the EX and SED trial. Despite the ingestion of the test meal there was no marked changes during the 6-h trial and therefore no significant time effect. Additionally, there were no significant main effect for condition ( $p=0.53$ ), nor was there a condition versus time interaction,  $p = 0.20$ .

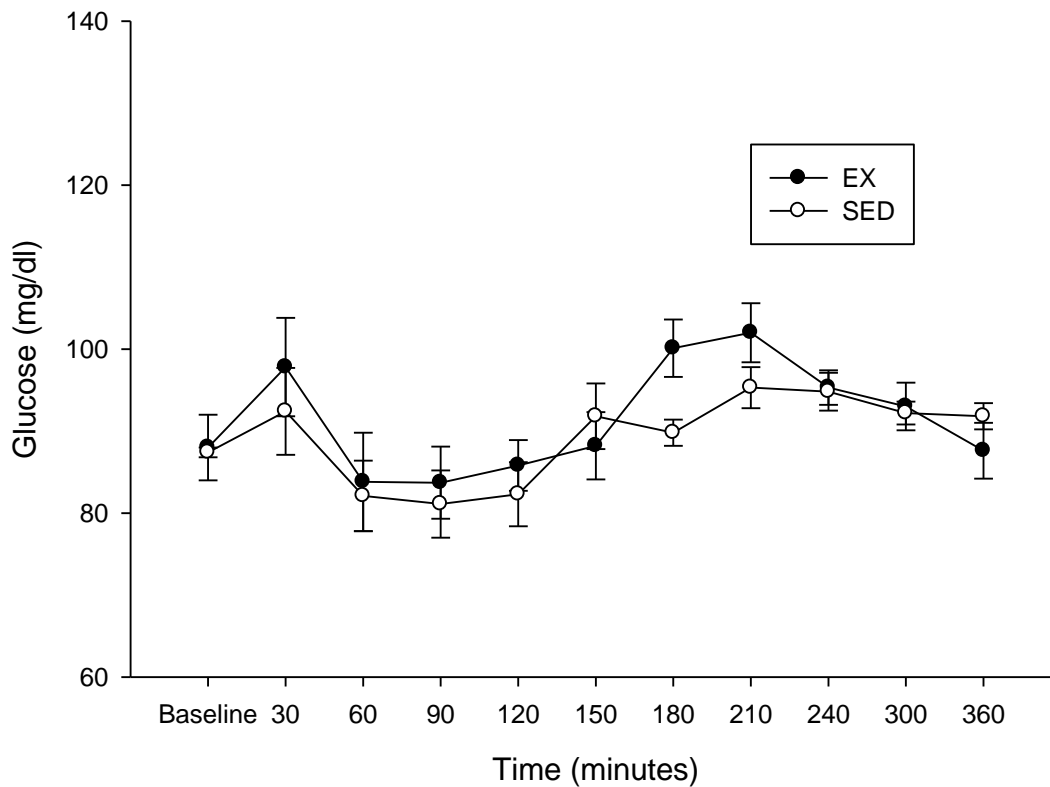


Figure 2. Plasma glucose concentrations for the EX and SED conditions at baseline and for the 6-h following the HFMM meal challenge in 8 young adult women.

#### Serum insulin concentrations during the two trials

Figure 2 depicts the mean ( $\pm$  SEM) baseline and postprandial plasma insulin concentrations over the 6-h trial for the EX and SED conditions. The baseline fasting plasma insulin concentrations prior to the test meal were not significantly different between the EX and SED trials. There was not a main effect of condition and there was not a significant condition versus time interaction. There was a significant time effect for insulin, with mean concentrations peaking at 30 minutes and returning to baseline values within the 360 minutes of test meal ingestion.

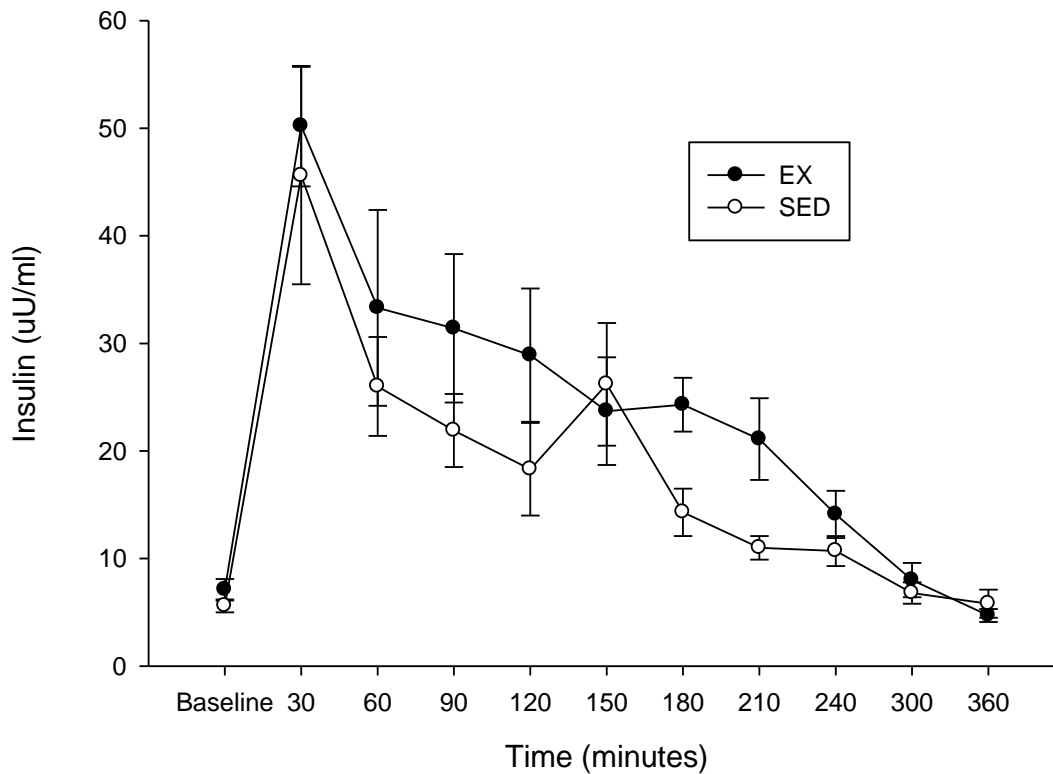


Figure 3. Plasma insulin concentrations for the EX and SED conditions at baseline and for the 6-h following the HFMM meal challenge in 8 young adult women.

#### Serum triglyceride concentrations during the two trials

In Figure 4 the mean ( $\pm$  SEM) baseline and postprandial serum triglyceride concentrations are shown at baseline and for the measured 6-h postprandial period. There was no condition by time interaction, nor was there a significant main effect of treatment. There was a significant time effect, with plasma TG concentrations peaking at about 180 minutes after test meal ingestion and returning to baseline in both groups within the 360 minute timeframe.

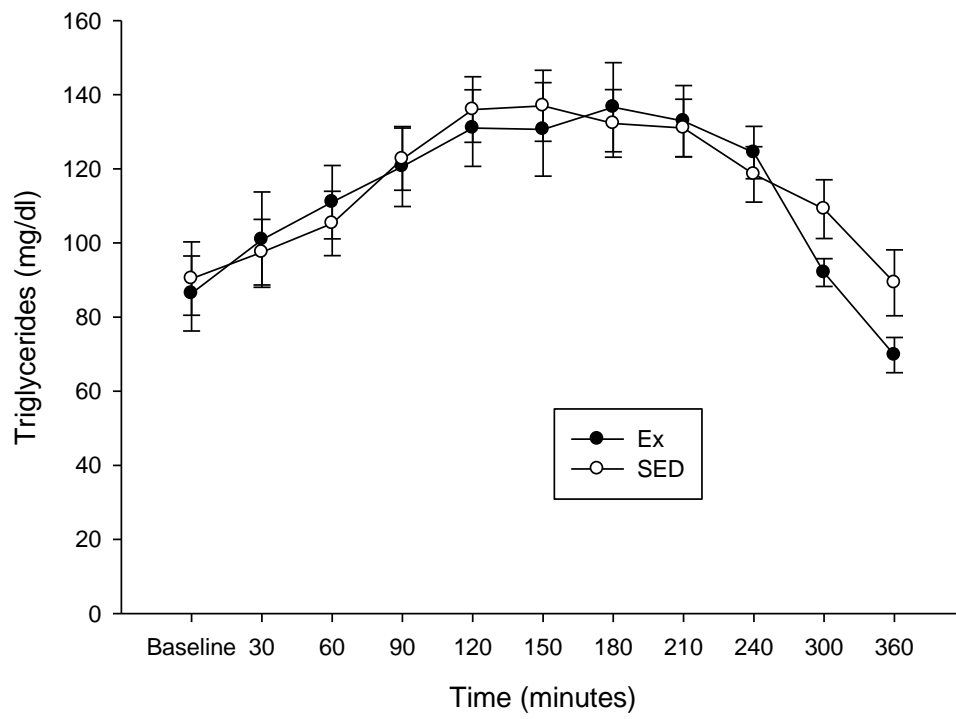


Figure 4. Plasma triglyceride concentrations for the EX and SED conditions at baseline and for the 6-h following the HFMM meal challenge in 8 young adult women.

## CHAPTER IV

### DISCUSSION

The major finding for our study is that exercise of low-to-moderate intensity (65% of peak heart rate) and modest net energy expenditure (estimated at 285 kcal) failed to improve postprandial metabolic markers in comparison to a nonexercise condition. This finding is at odds with a considerable body of evidence indicating that moderate exercise improves a number of aspects of postprandial metabolism [43]. For example, Gill *et al.* [44] previously showed that postprandial TG and insulin concentrations are lower on the day following an acute exercise bout of 90 minutes at 60% of  $\text{VO}_2\text{max}$ . A meta analysis by Petitt and Cureton [30] also showed a moderate effect of prior exercise on the attenuation of PPL and further concluded that the energy expenditure of the exercise appeared to be the major factor in influencing the magnitude of the effect. Why the disparate results between our study and these others?

These and additional earlier studies that showed reduced PPL following a high-fat meal challenge the day after an acute bout of exercise often failed to control for the caloric deficit that resulted from the exercise bout. More recent work by Burton *et al.* [38] aimed to determine the effects of exercise, with and without energy replacement, on postprandial metabolism in overweight/obese men. Each subject completed three 2 day trials that included one of the following three interventions on day 1; control, energy-

deficit, and energy-replacement. An 8.5-h metabolic assessment following a high-carbohydrate test meal was then completed on day 2. The exercise bout for the energy-deficit and energy-replacement trials involved a treadmill walk at 50% of  $\text{VO}_2\text{max}$  with an aim to induce a net energy expenditure of 6.5 kcals/kg of subject's body weight. For the energy-replacement trial a meal-replacement drink was consumed in a calculated amount to ensure that the entire exercise-induced energy expenditure was replaced. The results of the study demonstrated that a single session of exercise, without an energy deficit was able to decrease postprandial insulin concentrations and increase postprandial fat oxidation. However, the exercise-induced deficit was required to further lower postprandial concentrations and to enhance increased postprandial fat oxidation when compared to the exercise with energy-replacement. In addition, the exercise-induced deficit was necessary to reduce fasting and postprandial TG concentrations.

Subjects in a study by Harrison *et al.* [40] underwent three 6-h OFTTs that included the same three interventions; control (Con), exercise with energy balance (Ex-Bal), and exercise with exercise deficit (Ex-Def). For the exercise trials in this study subjects completed a more vigorous exercise bout that consisted of cycling at 70%  $\text{VO}_2$  peak for 90 minutes followed by ten 1-min full-effort sprints interspersed with 1-min of resting recovery. For the exercise with energy balance trial subjects consumed 105% of the carbohydrate oxidized during exercise to restore both carbohydrate and energy balance. The carbohydrate was consumed as an 18% carbohydrate drink and 85% glucose confectionary. The results of this study showed lower postprandial TG in the Ex-Def group compared to Con and Ex-Bal. Postprandial glucose, insulin, and homeostatic model of assessment of insulin resistance did not vary significantly across the three trials.

The study concluded that the influence of acute, exhaustive exercise on PPL is largely dependent on the associated carbohydrate and energy deficit.

Unlike the study by Burton *et al.* our study focused on the lipemic response following a higher-fat test meal (53% fat, 34% CHO, and 11% protein) rather than a high-carbohydrate test meal (49% CHO, 37% fat, 14% protein). We were also interested in studying sedentary young females, a group largely overlooked in research studies to date. Unlike Harrison *et al.*, our study examined the lipemic responses in a sedentary population and therefore a lower absolute exercise intensity was used.

The study at hand was originally designed to address the question as to whether or not a moderate exercise bout with energy replacement completed the evening prior to consuming a HFMM meal the following morning would attenuate the lipemic response to said meal. Unfortunately, the exercise bout produced a lower net energy cost than our original intent, and thus with the energy replacement, the study participants were actually overfed by approximately 80-100 kcals. Thus, the unintended research question we addressed was whether or not a low intensity bout of exercise could attenuate the postprandial lipemic response to a HFMM test meal when subjects are in a modest state of positive energy balance. This is actually an important question, because there is evidence of increased appetite during the later stages of recovery following acute exercise [45]. It is conceivable that in the current obesogenic environment, many individuals overcompensate for the energy expended in an exercise bout of low to moderate intensity by increasing their energy consumption following exercise. Our data clearly demonstrate that in the face of modest energy overcompensation, a low intensity exercise bout fails to attenuate the postprandial lipemic response in comparison to a non-exercise condition.

Without an exercise-induced energy deficit or at minimum a maintenance of energy balance, the postprandial response to a HFMM meal is no different than the response seen without any prior exercise. Thus, it appears that in regard to postprandial TG, glucose, and insulin responses, a positive energy balance negates the benefits of a modest exercise bout.

Given the confounding effect of the state of energy balance on the exercise-induced postprandial insulinemic and lipemic responses, it is important to explore how energy balance and imbalance could theoretically affect these responses. Previous data by Gill & Hardman [46] reported that the TG-lowering effect of an exercise-induced energy deficit was greater than the TG-lowering effect of an equivalent dietary-induced energy-deficit; they further suggested that either the effects of exercise on postprandial metabolism were independent of an energy deficit, or that dietary-induced and exercise-induced energy deficits elicited different effects on postprandial metabolism. Data from Burton *et al.* [38, 43] and Harrison *et al.* [37] described above suggest that the later interpretation is the correct one. The contrasting effect of TG metabolism of a dietary- or exercise-induced energy deficit may be related to specific body tissues in which the energy deficit or macronutrient decrement occurs. During exercise the utilization of skeletal muscle glycogen increases and following exercise muscle TG utilization is elevated during the recovery period—thus muscle glycogen and TG are at least partially depleted following exercise [38]. In contrast, a diet-induced energy deficit would result in a proportionally greater utilization of adipose tissue TG in the short term [38]. Hepatic fuel utilization may also differ in response to dietary- and exercise-induced energy deficits. The level of hepatic glycogen content is reduced by about two-thirds following a

24-h fast which is equivalent to hepatic glycogen depletion seen following about 80-90 minutes of exercise at 70%  $\text{VO}_2\text{max}$  [47]. Therefore, exercise induces quantitatively larger muscle and hepatic substrate deficits than dietary energy restriction [38]. These substrate deficits in muscle and/or the liver may mediate the TG-lowering effects of exercise by stimulating muscle LPL activity and enhancing TG clearance and/or reducing VLDL production by directing the hepatic fatty acid flux towards oxidation and away from re-esterification [27]. Thus, the larger effect of exercise-induced deficits on TG metabolism when compared to dietary-induced deficits could be mediated by greater energy substrate deficits in both muscle and liver tissue.

The above reasoning may also help explain why replacement of the exercise-induced energy deficit does not produce the TG-lowering effect seen with an exercise-induced energy deficit. Increased carbohydrate ingestion following exercise markedly increases both the rate of hepatic and muscle glycogen re-synthesis [47] and therefore muscle and hepatic substrate would be less following energy replacement, or in the sake of this study negated with overfeeding, when compared to exercise with an energy deficit. Additionally, increased carbohydrate intake *per se* has been shown to increase both fasting and postprandial TG concentrations, most likely by increasing hepatic VLDL production [48]. Thus the overfeeding in our exercise trial would have acted to oppose a TG-lowering effect of the exercise.

After the ingestion of a mixed meal containing carbohydrate, glucose is generated and the resulting postprandial hyperglycemia is accompanied by dose-response hyperinsulinemia [49]. It has been shown that a short-term energy surplus of 3-7 days during which dietary intake exceeds expenditure, usually causes a relative insulin

resistance, even in the absence of significant weight gain [50]. Short-term exercise can enhance insulin action, but the effect may be negated by the opposing action of energy surplus [50].

*Limitations.* The major limitation of this study resulted from how the net energy cost of exercise was determined. Although subjects initially underwent  $VO_2$ max testing with recorded heart rate values, appropriate response curves for each subject were not developed and utilized. Rather, in attempts to create a net cost of 400 kcal the exercise trial was erroneously based on 65% of peak heart rate rather than on 65% of peak  $VO_2$ . As a result the net cost of the exercise bout was estimated to be approximately 285 kcal. Because a 380 kcal mixed meal snack was consumed following the exercise bout in order to compensate for the exercise energy expenditure, an approximate 100 kcal surplus after the exercise was unintentionally created. The HFMM meal administered the day after the EX trial therefore followed a kcal surplus rather than following an exercise bout with caloric replacement. This limitation, however, allowed for the evaluation of the unintended research question discussed above which addresses the issue of compensatory increases in energy intake following acute exercise.

There may be a further limitation with the estimated mean 80-100 kcal surplus present following the exercise bout. It is difficult to accurately quantify energy balance under the conditions tested, and is possible that the true energy balance across all subjects was not the same. Also, it is unclear what the effect of overfeeding such a small amount of energy would have on the metabolic variables measured. A single day of ingesting 100 kcal in excess of energy needs may not be much different than maintaining energy balance for the day. If individuals were faced with repeated 100 kcal surplus' on a daily

basis this small increase could result in more significant changes over time. However, a single 100 kcal surplus may be meaningless in regards to long term changes given the immense difficulty with which precise and accurate estimates of daily changes in EE and EI can be made.

The lack of a third trial, wherein subjects completed the exercise bout without receiving caloric replacement, is another limitation to our study. A third trial with the subjects completing the same exercise bout but not receiving the post-exercise mixed meal snack would have allowed us to determine if the low intensity exercise, resulting in a 285 caloric deficit could have resulted in a change in postprandial TG, glucose, and insulin concentrations. This would have allowed us to determine if a low intensity exercise bout, reasonable for a sedentary individual to undertake, can have positive effects on the postprandial response following a HFMM meal.

Additionally the small sample size of this study does not allow conclusions to be extrapolated to individuals other than sedentary, healthy weight, young females who had overcompensated energy intake related to a low intensity bout of exercise.

## CHAPTER V

### CONCLUSION

In summary, the original intent of this study was to determine if an exercise bout with energy replacement in comparison to a nonexercise condition could attenuate the lipemic response to a HFMM consumed the following morning. Due to an error in calculating the targeted exercise intensity used for this study, the unintended research question we addressed was whether or not a low intensity bout of exercise could attenuate the postprandial lipemic response to a HFMM meal when subjects are in a modest state of positive energy balance. Our results indicated that in absence of an exercise-induced energy deficit, or at minimum a maintenance of energy balance, a low intensity exercise bout compared to no-exercise fails to improve postprandial TG, glucose, and insulin responses to a test meal in non-obese sedentary young women. Therefore, exercise without a resulting energy deficit may not be beneficial in attenuating postprandial lipemia unless it is accompanied by a resulting caloric deficit.

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## APPENDIX I

### ACSM Risk Factor Thresholds for CAD

If you fall into 2 or more of the following categories you are considered "moderate to high" risk:

Hypertension: SBP above 140 or DBP above 90

Dyslipidemia: LDL above 130mg/dl or HDL below 40mg/dl or Total Cholesterol above 200mg/dl

Impaired Fasting Glucose: above 100mg/dl

Obesity: BMI above 30 or W/H ratio above 102cm for men and above 88cm for women

Cigarette Smoking: Current or those who just quit within previous 6 months

Family History: Myocardial Infarct or Sudden Death before 55 years of age in father (or brother), or before 65 years of age in mother (or sister)

Negative Risk Factor: HDL above 60mg/dl

### ACSM/AHA Risk Factors:

1. Family History
2. Smoking: current or quit within past 6 mo
3. HTN: >140.90 or medicated for htn
4. Hypercholesterolemia: TC >200, HDL< 35, LDL> 130
5. IFG: fasting glucose > 110
6. Obesity: BMI>30
7. Sedentary: no regular exercise

Risk stratification:

Low risk: Males < 45, females <55, asymptomatic and no more than 1 risk factor present

Moderate risk: Males > 45, females > 55, 2+ risk factors

High risk: individuals with one or more signs or symptoms or with known disease

Physician supervision for maximal stress test:

Low risk: not necessary

Moderate risk: recommended

High risk: recommended

## APPENDIX II

### EATS-26

Please place an (X) under the column which applies best to each of the numbered statements. All of the results will be strictly confidential. Most of the questions directly relate to food or eating, although other types of questions have been included. Please answer each question carefully. Thank you.

Always	Very Often	Often	Sometimes	Rarely	Never
--------	------------	-------	-----------	--------	-------

- |     |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|--|
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 1. Engage in dieting behavior.   |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 2. Have the impulse to vomit after meals.                              |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 3. Cut my food into small pieces.                                      |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 4. Eat diet foods.   |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 5. Feel uncomfortable after eating sweets.                             |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 6. Vomit after I have eaten.   |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 7. Take longer than others to eat meals.                               |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 8. Enjoy trying new rich foods.  |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 9. Have gone on eating binges and feel that I may not be able to stop. |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 10. Other people think I am too thin.                                  |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 11. Avoid foods with sugar in them.                                    |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 12. Particularly avoid foods with high carbohydrate content.           |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 13. Give too much time and thought to food.                            |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 14. Feel that others would prefer if I ate more.                       |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 15. Am preoccupied with a desire to be thinner.                        |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 16. Like my stomach to be empty.                                       |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 17. Am preoccupied with the thought of having fat on my body.          |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 18. Find myself preoccupied with food.                                 |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 19. Feel that others pressure me to eat.                               |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 20. Am terrified about being overweight.                               |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 21. Avoid eating when I am hungry.                                     |
| ( ) | ( ) | ( ) | ( ) | ( ) | ( ) | 22. Think about burning up calories when I exercise.                   |

23. Feel extremely guilty after eating.
24. Feel that food controls my life.
25. Display self-control around food.
26. Aware of the calorie content of foods.

APPENDIX III

		Food Preferences and Acceptability Questionnaire									
		<i>Please circle the number that corresponds to your response.</i>									
Have not tried item (check box)	Food Item	Like	Like	Like	Like	Neither	Dislike	Dislike	Dislike	Dislike	
		Extremely	Very	Moderately	Slightly	Like Nor	Slightly	Moderately	Very	Extremely	
			Much			Dislike			Much		
<input type="checkbox"/>	Apple	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Banana	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Bell Peppers	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Broccoli	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Carrots	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Cheerios	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Chicken	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Chocolate Chip Cookies	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Cucumber	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	French Bread	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Granola Bar	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Honey Nut Cheerios	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Italian Dressing	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Lettuce	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Mayonaise	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Mozzarella Cheese	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Mustard	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Oatmeal	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Oreo's	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Parmesan Cheese	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Parsley	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Peaches	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Peanut Butter	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Pears	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Pepperoni	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Pizza	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Provolone Cheese	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Pudding	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Red Onion	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Romano Cheese	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Sausage	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Skim Milk	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	String Cheese	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Tomato	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Tomato Sauce	1	2	3	4	5	6	7	8	9	
<input type="checkbox"/>	Triscuit's	1	2	3	4	5	6	7	8	9	

<input type="checkbox"/>	Turkey	1	2	3	4	5	6	7	8	9
<input type="checkbox"/>	Wheat Thins	1	2	3	4	5	6	7	8	9
<input type="checkbox"/>	Whole Wheat Bread	1	2	3	4	5	6	7	8	9
<input type="checkbox"/>	Whole Wheat Pasta	1	2	3	4	5	6	7	8	9
<input type="checkbox"/>	Whole Wheat Tortilla	1	2	3	4	5	6	7	8	9
<input type="checkbox"/>	Yogurt	1	2	3	4	5	6	7	8	9

Please list any food allergies you have:

APPENDIX IV

Date: \_\_\_\_\_

Calorie and Exercise Requirements

RMR: \_\_\_\_\_ kcal/day

Daily energy requirement without exercise:

\_\_\_\_\_ kcal/day x 1.4 Activity Factor = \_\_\_\_\_ kcal/day

VO2 Max: \_\_\_\_\_ L/min

65% VO2 Max: \_\_\_\_\_ L/min

\_\_\_\_\_ L/min x 4.95 kcal/L = \_\_\_\_\_ kcal/min of exercise

Calories from RMR accounted for during exercise:

\_\_\_\_\_ kcal/day ÷ 1,440 min/day = \_\_\_\_\_ kcal/min resting

\_\_\_\_\_ kcal/min of exercise - \_\_\_\_\_ kcal/min resting = \_\_\_\_\_ kcal/min

400kcal ÷ \_\_\_\_\_ kcal/min = \_\_\_\_\_ total minutes of exercise

APPENDIX V

FAT TOLERANCE TEST

Subject: \_\_\_\_\_

Blood Pressure: \_\_\_\_\_

Pulse: \_\_\_\_\_

Hours of Sleep: \_\_\_\_\_

Hours since last exercise : \_\_\_\_\_

TIME	PROCEDURE	COMMENTS
-2:00	Baseline blood draw	
-1:00	Baseline blood draw	
0:00	Shake	
30:00	Blood draw	
1:00:00	Blood draw	
1:30:00	Blood draw	
2:00:00	Blood draw	
2:30:00	Blood draw	
3:00:00	Blood draw	
3:30:00	Blood draw	
4:00:00	Blood draw	
5:00:00	Blood draw	
6:00:00	Blood draw	