THESIS

A SINGLE SESSION OF MODERATE EXERCISE, WITHOUT ENERGY DEFICIT, MAY REDUCE sVCAM-1 CONCENTRATIONS IN YOUNG, SEDENTARY FEMALES

Submitted by

Molly Krause

Department of Food Science and Human Nutrition

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2010

Masters Committee:

Department Chair: Christopher Melby

Advisor: Christopher Melby

Matthew Hickey
Tracy Nelson

ABSTRACT

A SINGLE SESSION OF MODERATE EXERCISE, WITHOUT ENERGY DEFICIT, MAY REDUCE PLASMA SOLUBLE VASCULAR ADHESION MOLECULE-1 CONCENTRATIONS IN YOUNG, SEDENTARY FEMALES

Purpose: Cardiovascular disease remains the number one cause of death within the United States and globally [1, 2]. Postprandial lipemia and vascular adhesion molecules are becoming more widely recognized as biomedical makers associated with increased risk of developing CVD [3-5]. It has been well established that moderate exercise can improve some aspects of postprandial metabolism such as decreased triglycerides and improved insulin sensitivity [6]. However, there is limited data regarding the effect of prior moderate exercise on attenuating postprandial response specific to adhesion molecules [7, 8]. The purpose of this study was to determine the effects of a single bout of acute exercise, with energy replacement, on plasma soluble vascular adhesion molecule-1 (sVCAM-1), a marker of endothelial dysfunction, measured during fasting and in response to a high-fat, high-carbohydrate meal in young, non-obese, sedentary females.

Methods: Eight, non-obese (x body mass index=24.6 kg/m^2), habitually sedentary females (x age= 19.6 y) participated in this study. Following preliminary testing, each subject completed two trials in random order: 1) Exercise (Ex) 2) Non-exercise (Non-Ex). Each trial took place over 2 days. On the evening of day 1, subjects either rested (Non-Ex) or completed a cycle ergometer exercise bout at 65% peak heart rate, eliciting net exercise energy expenditure of ~285 kcalories. On the morning of day 2 of each trial a fasting venous blood sample was drawn for measurement of sVCAM-1, followed by the consumption of a high-fat, high-sugar meal by each

participant. Postprandial venous blood samples were then taken over at 2, 4, and 6 hours following meal ingestion for measurement plasma sVCAM-1 concentrations.

Results: There was no significant treatment by time interaction on sVCAM-1 concentrations, nor was there a significant main effect of time. There was a significant condition effect on circulating soluble VCAM-1 concentrations such that concentrations were lower before and following the high-fat, high-sugar meal challenge for the EX compare to the NonEx condition.

Conclusions: Results from the current study suggest that a single session of moderate exercise, without an energy deficit, may reduce sVCAM-1 concentrations in young, sedentary females. However, this finding must be viewed with caution owing to possible issues with the sVCAM-1 measurements, and the assay repeated prior to drawing any conclusions about the effect of acute exercise on circulating sVCAM-1 concentrations measured the morning after exercise.

Table of Contents

Introduction and Purpose Statement

Cardiovascular disease (CVD) encompasses a breadth of disorders including coronary artery disease (CAD), thrombotic stroke, and peripheral vascular disease, all of which are typically preceded by atherosclerosis. The role of inflammation in the initiation and subsequent progression of atherosclerosis is becoming recognized as a contributor to cardiovascular disease development, plaque rupture and possible death [1-3]. Cardiovascular diseases remain the number one cause of death globally, and approximately 433,000 females died in 2006 from CVD within the United states [4]. It is projected that by 2030 over 23 million people will die each year from CVD, mainly from coronary thrombosis and stroke, the prior a direct result of inflammation, plaque rupture or endothelial erosion [2, 5].

There are several clinical identifiers of cardiovascular disease prognosis and development. Traditional risk markers such as high plasma concentrations of low density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) and low plasma concentrations of high density lipoprotein-cholesterol (HDL-C) are typically assessed in a fasted state; however circulating lipids and lipoprotein concentrations during the postprandial period are becoming more widely recognized as risk markers for atherosclerosis. Postprandial lipemia has been strongly linked to increased risk of cardiovascular disease, specifically with progression of atherosclerosis [6, 7]. Moreover, the ingestion of a high-fat meal has been shown to acutely increase circulating levels of inflammatory markers such as tumor necrosis factor (TNF)-α and interleukin-6 (IL-6). Elevation of such molecules may induce endothelial expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), and predispose established

atherosclerotic plaques to rupture [8]. In otherwise healthy individuals, chronically elevated levels of these proinflammatory cytokines as well as VCAM-1 are significantly associated with an individual's risk of future myocardial infarctions [9, 10]. The acute postprandial lipemic response, as indicated by a substantial rise in plasma concentrations of triglyceride-rich lipoproteins, has now been generally accepted to play a causative role in atherogenesis. Although the perturbations of a single high fat feeding often normalize in the fasted state, the continual insult from frequent meals can lead to a chronic dislypidemic state and disease development [6, 11].

Although pharmaceutical advancements have been made in an effort to decrease CVD and associated outcomes, behavioral risk factors such as diet and physical activity remain responsible for 80% of coronary heart disease and cerebrovascular disease within the United States [5]. Furthermore, a single bout of exercise prior to a high-fat meal has been shown to significantly reduce the postprandial rise in TG in both healthy and diseased individuals [12, 13]. However, the effects of exercise on attenuating the postprandial rise in TG concentrations appear to be at least partially dependent upon the magnitude of the exercise-induced acute energy deficit. Recent efforts have focused on determining effects of exercise on the postprandial response when energy intake is increased to account for the energy cost of exercise; that is, replacement calories are provided to compensate for the net cost of exercise [14].

The effect of exercise on inflammation is complex. Initial inflammatory responses during and following acute exercise bouts may appear to be detrimental. Various markers of inflammation as well as soluble forms of circulating adhesion molecules are often elevated during exercise sessions [15, 16]. However, endurance exercise training has been shown to be beneficial

to immune function and overall health, with subsequent decreases in basal circulating levels of sVCAM-1 [16, 17]. Few studies have investigated the effect of a single bout of moderate exercise with energy replacement on the postprandial response and endothelial adhesion molecules in females.

Purpose Statement

The purpose of this study was to determine the effects of a single bout of acute exercise with energy replacement on plasma sVCAM-1, a marker of endothelial dysfunction, measured during fasting and in response to a high-fat, high-carbohydrate meal in non-obese, sedentary females.

Hypothesis

Aim: To determine the effects of a single bout of moderate, aerobic exercise with energy replacement versus a no-exercise condition on fasting plasma concentrations of soluble-vascular circulating adhesion molecule-1 (sVCAM-1) measured 12 hours following the exercise as well as 6-hour postprandial sVCAM-1 concentrations following consumption of a high-fat, high-carbohydrate meal in healthy, non-exercise trained females.

Hypothesis: A single bout of moderate, aerobic exercise will not decrease baseline plasma sVCAM-1 concentrations, but will decrease 6-hour postprandial sVCAM-1 concentrations relative to the non-exercise condition in healthy, non-exercise trained females.

LITERATURE REVIEW

Cardiovascular Disease

Cardiovascular disease (CVD) remains the number one cause of death globally with an estimated 17.1 million deaths in 2004, of which the majority are related to heart attack and stroke [3]. Since 1985 women have averaged higher mortality rates than men and currently comprise 52.1% of all deaths from CVD [4]. Cardiovascular diseases encompass a group of disorders involving the heart and blood vessels including coronary heart disease (CHD), also known as coronary artery disease (CAD), and arteriosclerosis. CHD is a condition in which plaque from fat, cholesterol, calcium and other substances builds up inside the coronary arteries eventually decreasing blood flow to the heart muscle[5]. The build up of plaque occurs through the process of atherogenesis. Progressive plaque accumulation leading to narrowing of the arteries and cardiac ischemia, combined with inflammation and plaque rupture can lead to heart attack, stroke and death. Atherosclerosis is often asymptomatic and considered a silent killer, such that a heart attack or stroke may be the first warning of any underlying disease. Although new knowledge regarding progression, causes and treatment is continually forthcoming, it remains the number one cause of death in the U.S. and warrants further attention.

Cardiovascular Disease Risk Factors and Endothelial Function

The specific mechanistic causes of atherosclerosis remain unclear; however, certain behavioral risk factors can increase ones risk of disease development [5]. Behavioral risk factors such as an unhealthy diet, physical inactivity, and tobacco use are responsible for approximately

80% of coronary heart disease and cerebrovascular disease[3]. Additionally, traditional risk factors such as elevated low density lipoprotein (LDL), decreased high density lipoprotein (HDL), high blood pressure, central adiposity and family history are also considered major risk factors for disease development. Furthermore, research has implicated the roles of chronic inflammation and an abnormal postprandial response in the development of coronary heart disease. Postprandial lipemia (PPL) is characterized by a rise in plasma concentration of triglyceride-rich lipoproteins following the consumption of a high-fat meal and has become a novel risk factor for cardiovascular disease [12]. Increased circulation of triglyceride-rich lipoproteins is often associated with high-fat diets and has been suggested to be atherogenic, potentially through induction of endothelial dysfunction, a contributor to atherosclerosis [18, 19]. Endothelial dysfunction, such as decreased permeability and abnormal vasoactivity, is induced by a variety of mediators, one of which is dyslipidemia. As endothelial dysfunction develops, a shift away from anti-atherogenic factors and toward proatherogenic factors ensues. Inflammatory mechanisms coupled with dyslipidemia appear to play a key role in atherosclerotic plaque formation, in part by inducing endothelium expression of adhesion molecules such as vascular adhesion molecule-1 (VCAM-1) [11].

Early stages of atherosclerosis are typically asymptomatic and can lead to fatty streak development. Fatty streaks, made primarily of macrophages accumulate as lipid-laden cells beneath the endothelium, may progress to atheromata, or atherosclerotic lesions [2]. These lesions are comprised of a core of foam cells and extracellular lipid droplets surrounded by a cap of smooth-muscle cells and a collagen-rich matrix. T-cells, macrophages and mast cells can

infiltrate the atheromata leading to growth of the lesion. Such activation of plaque has been shown to precipitate ischemia and infarction[2]. Moreover, most cases of infarction are due to the formation of an occluding thrombus on the surface of plaque due to either plaque rupture or endothelial erosion. This process differs from the previously held view that the thrombus was solely caused from marked stenosis. It is now recognized that endothelial dysfunction can initiate the entire process of atherosclerotic development [20]. A schematic cross-section of an artery is provided in Figure1, which shows some of the key contributors to plaque formation [2]. Endothelial dysfunction is a term describing maladaptive changes in endothelial functions induced by stimuli resulting in localized functional alterations of vascular tone. Endothelial dysfunction encompasses a breadth of maladaptive changes such as altered permeability to plasma lipoproteins, hyper-adhesiveness to blood leukocytes, and increased cytokine and growth factor production. Endothelial activation specifically describes the functional changes that endothelia may undergo and the acquisition of new functional properties mainly influencing interactions with blood leukocytes. Endothelial activation is considered to play a key role in the initiation, progression and clinical emergence of atherosclerosis. Furthermore, it is a pivotal process in monocyte adhesion, a prominent feature of several inflammatory disorders [20]. The role of endothelial activation and adhesion molecules is discussed in the following sub-section.

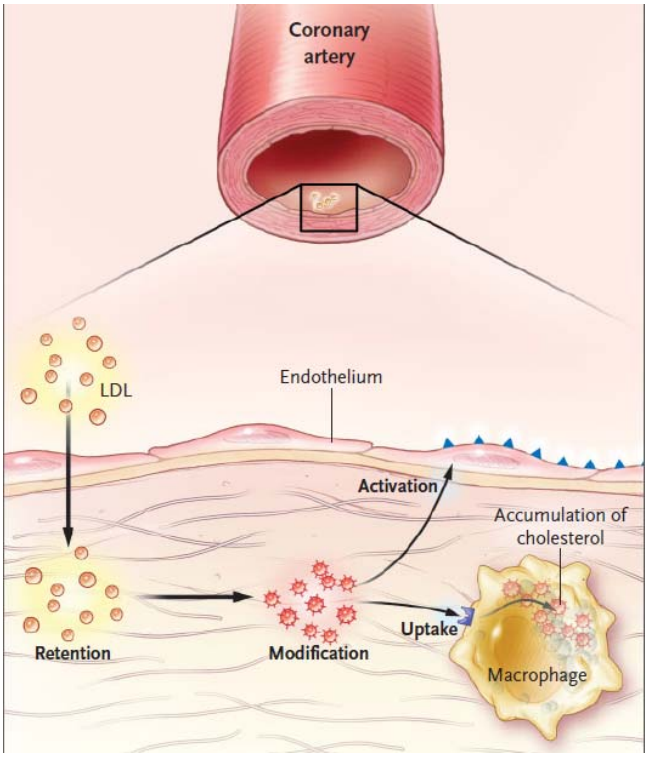

Figure 1 *[2]* **.** Illustrating the infiltration and retention of LDL within the intima of the artery. Oxidative modification of LDL leads to the release of inflammatory lipids, uptake into macrophages which can evolve into foam cells and plaque formation. Induction of endothelial cells to express leukocyte adhesion molecules is also upregulated.

Endothelial Adhesion Molecules

Adhesion of circulating leukocytes to cultured endothelial cells in vitro has been studied in an attempt to better understand mechanisms mediating cell-to-cell adhesion [21], and has led to the identification of several protein families which provide "traffic signals" for leukocytes[20]. The initiation of atherosclerosis, in part due to the adhesion of circulating leukocytes to the endothelial cell and subsequent transendothelial migration, is mediated by members of the immunoglobulin superfamily on the endothelium surface [22]. Increased expression of adhesion

molecules and inflammation has been shown to be upregulated by hypercholesterolemia due to activation of the endothelium with subsequent infiltration, retention and alteration of LDL in the arterial intima. Once activated, the endothelial cells express adhesion molecules such as VCAM-1, upregulating adhesion and chemokine migration through the interendothelial space. In the presence of a hyperadhesive state, cytokines produced in the inflamed intima induce monocyte entry into the plaque, differentiating into macrophages, a step critical for the development of atherosclerosis [2, 20]. This process is represented in Figure 2 taken from reference 2.

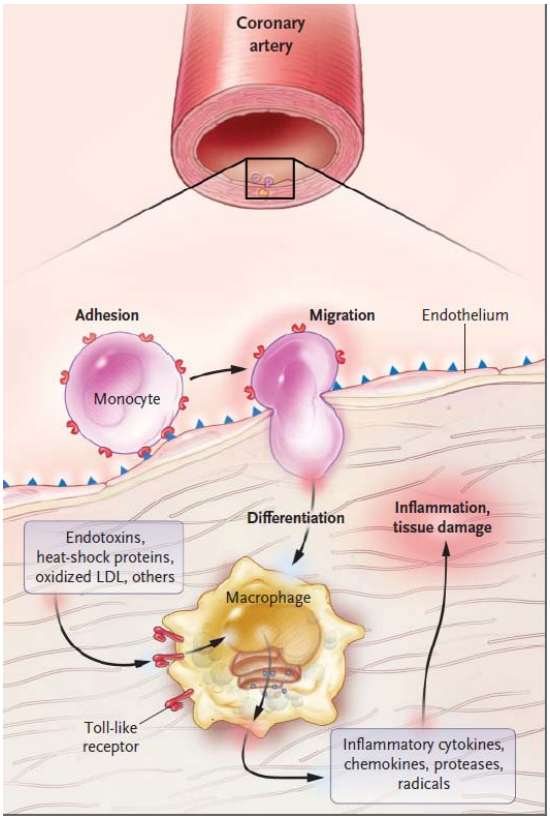

Figure 2. *Taken from Hansson et al [2]*

During the past decade an increase in knowledge regarding atherosclerosis development and classification as an inflammatory disease has resulted in greater understanding of the complex pathophysiology of this disease. Traditionally, individuals were assessed based on disease risk factors including anthropometric measures, lifestyle behaviors, biomarkers and family history. Over the past few decades the use of adhesion molecules and circulating leucocytes as markers of disease state and risk of coronary events has expanded. Within the literature, adhesion structures are often divided into three main families: the selectins, the chemoattractants and the immunoglobulin superfamily (IGSF) [23]. Specific

members of these families are expressed by endothelial cells and soluble isoforms can be found in plasma as a result of shedding from the cells surfaces during times of inflammation [16, 24].

Within the IGSF is a subset of cell-to-cell adhesion molecules, including intercellular adhesion molecules-1,-2,-3 (ICAM-1,-2,-3), vascular cell adhesion molecule-1 (VCAM-1) and platelet-endothelial cell adhesion molecule (PECAM). ICAM-1,-2,-3 and VCAM-1 are expressed on endothelial cells and function as ligands for integrins allowing for the firm adhesion of monocytes to the endothelium [1]. Adhesion of leukocytes and platelets to the adhesion molecules enables signal transduction in endothelial cells that trigger changes in shape and allow for leukocyte emigration [1]. Furthermore, this process has been implicated early on in atherogenesis development[1, 2]. Moreover, these transmembrane glycoproteins allow for cell to cell interaction with an upregulation of cell surface expression subsequent to activation due to inflammatory and immune responses, mediating both cell migration and activation [24, 25]. Specifically, the upregulation of VCAM-1 expression is induced by cytokines such as IL-1 and TNF-alpha. The basic structure of cellular adhesion molecules (CAMs) are composed of an extracellular component, a hydrophobic transmembrane domain and an intracytoplasmic component [23]. Activation and adhesion are dependent upon interactions between CAMs and activated integrins on leukocytes[25].

The process of leukocyte adhesion and transmigration across the endothelial monolayer is a complex process requiring overlap and interaction of all classes of adhesion molecules. Briefly, the first steps in recruitment, termed tethering and rolling, involve interaction of selectins with carbohydrate ligands on the leucocytes. The process of rolling causes a change in conformation of α and β subunits of integrins, supporting high-affinity binding with their respective ligands.

Firm adhesion occurs as leukocytes encounter activating signals while rolling along the endothelium. Specifically, interaction between very late activation-4 (VLA-4) /VCAM-1 and leukocyte function association-1 (LFA-1)/ICAM-1 pathways allow for activation-dependent attachment of monocytes and lymphocytes as well as neutrophils to the vessel wall, respectively [23]. Transmigration of adherent cells across the endothelial monolayer occurs when a favorable chemotactic gradient exists requiring the involvement of platelet/endothelial cell adhesion molecule-1 (PECAM-1). A schematic of these steps is represented in Figure 3 [23, 25].

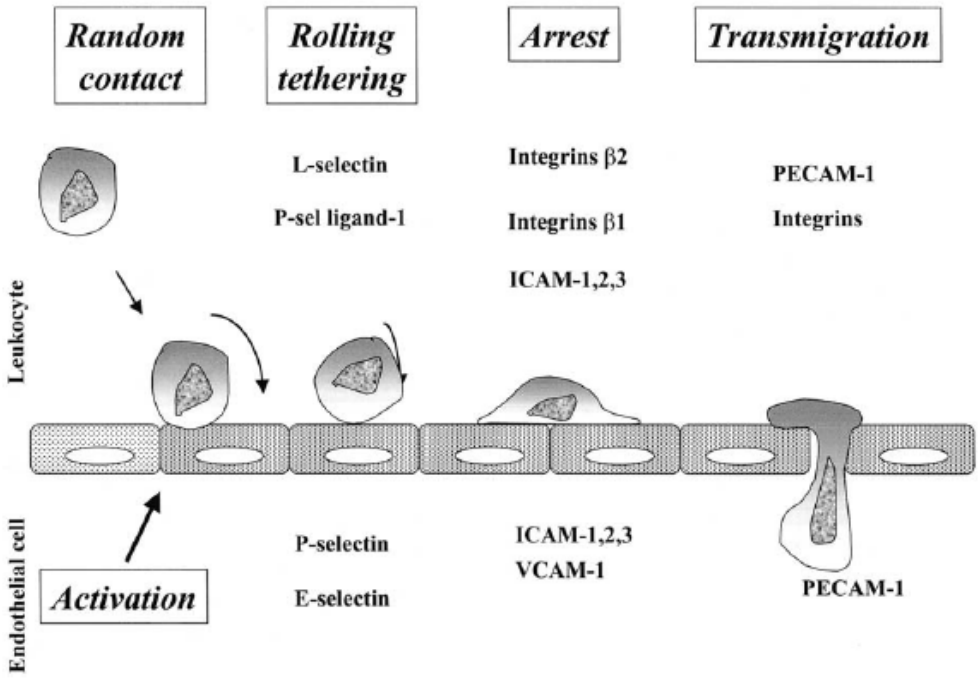

Figure 3. Illustration of the initial steps of atherosclerosis, with representation of leukocyte-endothelial cell interactions and the role of various adhesion molecules [1]

In response to inflammation and cytokine release due to injury to the endothelium, increased expression of VCAM-1 occurs. VCAM-1 expression is also induced by lysophosphatidylcholine, TNF-α and IL-6 secretion by endothelial cells, macrophages and myoblasts. The initiation of VCAM-1 signals from endothelial cells modulates the shape of

leukocytes, allowing migration into subendothelial space across the cell barrier. Soluble VCAM-1 is released into circulation following shedding from the endothelial cell. Although an increase in shedding is associated with greater substrate availability and activation of proteolysis, the full mechanism of this process has not been elucidated [26].

The induction of cellular adhesion molecules to the endothelial surface have been noted as early indicators in atherogenesis as well as predictors of future untoward events such as acute myocardial infarction. As described earlier, the recruitment of leukocytes during inflammation, specifically in atherosclerosis, is predominantly mediated by adhesion molecules. Focal expression of these molecules has been observed repeatedly in atherosclerotic plaques[27]. Furthermore, the use of soluble adhesion molecules, specifically sICAM-1 and sVCAM-1, have been proposed as useful tools in establishing risk of CVD in healthy and diseased populations [1]. Levels of soluble adhesion molecules have been shown to positively correlate with CVD risk factors such as smoking, HTN, low HDL, diabetes, and are higher in individuals with insulin resistance[1]. Furthermore, the use of sVCAM-1 has been found to strongly correlate with carotid intima-media thickness, an index often used in detection of early atherosclerosis [28]. The upregulation of sVCAM-1 appears to occur very early on in endothelial dysfunction and has been hypothesized to be a predisposing factor for atherosclerosis formation [29].

Soluble adhesion molecules such as VCAM-1 and ICAM-1 have been shown to correlate with CAD risk factors as well as predict CAD events[1]. The Atherosclerosis Risk In Communities (ARIC) study was the first to identify sICAM-1 as a significant predictor of future CAD events [30]. Additionally, the Physician's Health Study observed and reported a significant association between increasing concentrations of sICAM-1 and risk of future CAD events in

healthy individuals [10]. Furthermore, in individuals previously diagnosed with CAD, sVCAM-1 has been reported as a strong predictor of risk with an over two-fold increased risk of death for individuals in the highest versus the lowest quartiles [27]. Although the use of sVCAM-1 and sICAM-1 as markers of inflammation and cardiovascular risk often are measured in conjunction to one another, there are underlying differences between the two. Moreover, sICAM-1 appears to be expressed by a host of cells such as hematopoietic lineage and fibroblasts where as sVCAM-1 is mainly expressed on atherosclerotic plaques by activated endothelial cells and smooth muscle cells [9]. Although sVCAM-1 may be a more specific predictor of CVD risk and risk of death it has been suggested that it may be limited to those with more advanced atherosclerosis[9]. However, others argue that the use of sVCAM-1 may be a more accurate tool for early detection and a predisposing factor for atherosclerosis formation as it may play a more dominant role in foam cell development compared to ICAM-1[29].

Postprandial Response

Following ingestion of exogenous lipids, digestion essentially begins in the stomach and is completed in the lumen of the small intestine, allowing for migration of micelles into enterocytes and into circulation via the lymphatic system. Briefly, lipases hydrolyze TG with emulsification aided by bile acids and salts in the small intestine through the formation of micelles. The hydrophilic exterior of the micelles allows for transportation to the intestinal brush border of enterocytes. At the surface of the brush-border micelles enter an acidic microenvironment, allowing for protonation of fatty acids, lysophospholipids and cholesterol that then enter into enterocytes, which allows for the lipid components to diffuse inward with subsequent reassembly in the endoplasmic reticulum. Upon reassembly a layer of surface protein is added forming lipoproteins. The primary lipoproteins derived from dietary fat are

chylomicrons. The lipoprotein particles are then released into lymphatic circulation and ultimately into the subclavian vein via the thoracic duct. Following a mixed meal ingestion, an increase in insulin secretion stimulates lipoprotein lipase (LPL) activity and decreases lipolysis and β-oxidation of fatty acids, with subsequent favoring of triacylglycerol storage within adipocytes [31]. Hepatocytes play a key role in clearing chylomicron remnants, the smaller particle of the chylomicron left remaining following lipolytic action of LPL. The reuptake of lipids from the bloodstream into the liver allows for further catabolism of exogenous lipids ultimately resulting in formation of cholesterol rich very-low density lipoprotein (VLDL) and low-density lipoprotein (LDL) from endogenous sources [31].

When energy intake is greater than energy demand, especially following a high-fat, high-carbohydrate meal, accumulation of lipids in the form of triacylglycerol occurs primarily within adipocytes. Although fatty acids serve as a source of energy, an excess of dietary lipids has been implicated in hypercholesterolemia and atherogenesis development. The atherogenic process is believed to start in the endothelium, the single layer of cells lining the wall of the arteries. When the monolayer of endothelial cells are insulted, such as from elevated levels of cholesterol and triglycerides in the blood, particles such as LDL can penetrate the arterial intima and become oxidized. Oxidized LDL is a known toxin to endothelial cells. As injury occurs, an increase in immune response follows and subsequent signaling for platelet aggregation follows. As described earlier, leukocytes and immune responses transmigrate through the endothelial layer, with subsequent differentiation into macrophages leading to the formation of foam cells as well as the proliferation of smooth muscle cells [1, 2, 31, 32]. This process cyclically induces further injury, plaque formation and narrowing of the arterial lumen. Additionally, decreased blood flow and subsequent endothelial dysfunction can decrease the production of nitric oxide (NO) a potent vasodilator, inhibitor of growth and inflammation, and has platelet aggregate effects. Low NO

bioavailability has also been implicated in the increased expression of VCAM-1, contributing to the progression of atherosclerosis [31, 32]. A schematic of this process is represented in figure 4 below.

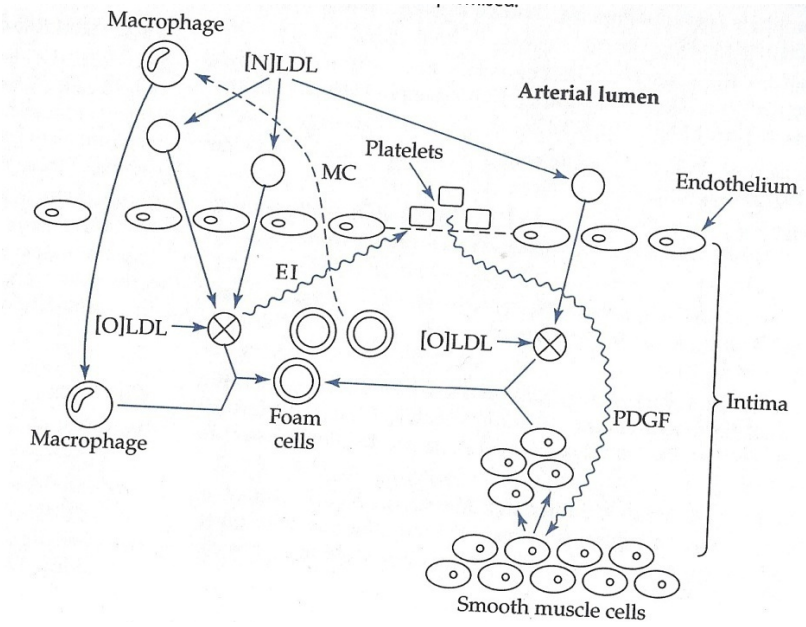

Figure 4. Schematic representation of events leading to atherogenesis. Diagram taken from *Advanced Nutrition and Human Metabolism* [31].

The importance of the postprandial response regarding the development of metabolic disorders and heart disease has gained attention and recognition over the past decade. Abnormal postprandial hypertriglyceridemia, hyperinsulinemia and hyperglycemia have been associated with increased risk of diabetes, atherosclerosis and the metabolic syndrome [6, 8]. Additionally, given the increased awareness that adhesion molecules play as an important role in the initiation of atherosclerosis, investigations into the postprandial state of this biomarker have also increased [1, 27, 28, 30]. Ceriello and colleagues examined the effect of a high fat meal and a high-fat meal plus oral glucose tolerance test (OGTT) in both diabetic and healthy individuals. Results from this study indicate that a high-fat load alone produced an increase in sICAM-1 ands VCAM-1 and the combination of high-fat plus OGTT further exacerbated this response [33]. The high-fat meal alone produced an increase from 1 to 3 hours post-meal in normal weight individuals and from 1

to 4 hours post-meal in type 2 diabetic individuals for both ICAM and VCAM levels. Additionally, high-fat plus OGTT induced a more pronounced increase than high-fat alone with maximal elevation occurring at hours 1 and 2 in healthy and type 2 diabetic subjects, respectively [33]. A similar study by Nappo *et al* investigating the effects of both high fat and high carbohydrate meals in both healthy and diabetic patients on plasma concentrations of CAMs produced similar results. sICAM-1 and sVCAM-1 rose significantly after high fat meal in both groups; however, sCAMs did not significantly increase following the high-carbohydrate meal in healthy individuals [8]. Results from these studies indicate a strong association between postprandial response of a high fat meal and increased CAM expression in lean, healthy and diabetic individuals. However, the effects of high-fat and high-carbohydrate levels within the same meal warrants further investigation.

Implications of Prior Exercise

It is well established that moderate exercise can improve some aspects of postprandial metabolism such as decreased TG and insulin concentrations [34]. These improvements are most likely due to improvements in both insulin sensitivity and TG clearance. Moreover, exercise may reduce both fasting values of insulin as well as the postprandial insulin response up to 24-hours following exercise [35, 36]. During a single session of moderate, aerobic exercise, at intensities between 35-55% VO_2max, muscle derives similar relative amounts of energy from circulating free fatty acids (FFAs), glycogen stores and intra-muscular TG stores [37]. During recovery periods, TG stores within skeletal muscle are restored by an increase in LPL activity with increased TG uptake and storage. Acute reductions of circulating TG and LDL levels are likely due to this accelerated clearance and storage. Additionally, depletion of both hepatic and skeletal muscle glycogen content occurs. However, due to the homeostatic need to maintain blood glucose levels, circulating levels of glucose following exercise without energy replacement will remain stable while a reduction in TG occurs. The decrease in circulating TG levels may be due to increased clearance into skeletal muscle and/or by directing hepatic fatty acid flux toward oxidation and away from esterification[14, 31]. Thus, the magnitude of postprandial changes following exercise may be a consequence of tissue specific energy deficits. However, it has been demonstrated that deficits created by dietary restriction do not yield the same metabolic improvements as the energy deficits following exercise [38]. A recent study by Gill *et al.* investigating the effects of exercise with and without energy replacement demonstrated that exercise without energy deficit can reduce postprandial insulin concentrations, as well as attenuate the decline in postprandial lipid oxidation[14]. However, an energy deficit was required to reduce fasting and post-prandial TG levels [14]. Results from this study suggest that the TG-lowering effect of exercise is likely to be at least partially explained by the temporary exercise-induced energy deficit.

Regular participation in exercise is known to reduce cardiovascular disease risk in healthy individuals [39-41]. These benefits induce protection despite acute physiological stress induction such as inflammatory responses, reactive oxygen species production, and increased shear stress. Of these responses, shear stress is arguably the most closely related with endothelium and cardiac involvement. Shear stress, defined as frictional force in plane with the endothelial cell as a result of blood flow, is increased during exercise, resulting from an increase in oxygen demand eliciting a subsequent increase in cardiac output [42]. Shear stress is also a key player in the development of atherogenesis within specific sites of the vasculature. Specifically, in areas of low shear stress, monocyte adhesiveness is increased and can lead to increased vulnerability of plaque formation and rupture. Furthermore, low shear stress can also lead to increased VCAM-1 expression, also increasing risk of plaque formation[23]. Potential benefits from exercise in regards to a decrease in plaque formation could be linked to an increase in shear stress with subsequent decrease in VCAM-1 expression. However, evidence suggests that increased shedding of adhesion molecules and subsequent increases in soluble isoforms occurs in part from inflammation or increased shear stress, as seen in disease states and exercise [24]. As such, following bouts of strenuous exercise, soluble isoforms have been shown to increase [15, 16]. However, training programs amongst patients with coronary heart failure have indicated a significant decrease in VCAM-1 and ICAM-1 levels, an effect likely resulting from attenuation of inflammation with increased exercise capacity [43]. Although an acute increase in adhesion molecules within healthy subjects has been demonstrated, the impact on cardiovascular health remains unclear.

Several studies have focused on the impact a single bout of exercise on fasting and postprandial lipids. A single bout of exercise performed 4-24 hours prior to a high-fat meal has been shown to reduce postprandial plasma TG excursions in several populations [14, 34].

However, there are limited data regarding the effect of prior moderate exercise on attenuating postprandial lipemia and specific adhesion molecules. Following an extensive search of the literature, only two such studies have been published in which the effects of a prior bout of exercise on the postprandial adhesion molecule response have been investigated. Both studies indicate no change in postprandial or baseline values of VCAM-1 following an acute exercise bout [12, 44]. However, it should be noted that both studies solely investigated male subjects[12, 44]. To date no studies have been conducted on the effect of prior moderate exercise on attenuating the postprandial response of markers of endothelial activation in young, non-exercise trained females. Therefore, the purpose of this study was to investigate the effects of a single bout of moderate exercise with energy replacement versus a no-exercise condition on sVCAM-1 following ingestion of a high-fat, high-carbohydrate meal in non-obese, untrained women.

METHODS

Subjects

It should be noted that a portion of this data has been presented as part of a previous thesis. Participants for this study were healthy, eumenorrheic females between the ages of 18-40 years old, who were recruited using flyers posted on the campus of Colorado State University, by online on-campus advertising, and by word of mouth. A total of eight subjects completed this study. All were non-smokers, and none had a personal history of diabetes or hypertension as evidenced by self-report and measured fasting blood glucose concentrations ≤ 126 mg/dl and resting blood pressure < 140/90 mmHg. To be eligible for participation, subjects could not be exercise trained (exercise ≤2times per week), exhibited body mass indexes (BMI) between 21-29 kg/m^2, and were weight-stable during the previous 6-months. Individuals who were vegetarians, lactose-intolerant, had extreme dietary patterns such as eating disorders, or who were taking medications that could interfere with study results were not allowed to participate in this study. The study was approved by the Institutional Review Board at Colorado State University, and all subjects provided voluntary oral and written consent prior to enrollment.

Experimental Design

Following preliminary testing, each subject completed two trials in random order: 1) Exercise (Ex) 2) Non-exercise (Non-Ex). Each trial took place over 2 days. On the evening of day 1, subjects either rested (Non-Ex) or completed a cycle ergometer exercise bout at 65% peak heart rate achieved in a prior VO_{2max} test, with the goal of eliciting a net exercise energy expenditure of approximately 400 kcalories. For energy replacement, a mixed macronutrient snack consisting of 380 calories was consumed directly following exercise intervention. However, as will be discussed later, due calculation errors, the estimated net cost of the exercise

bout was only approximately 285 kcal, which coupled with the 380 kcal snack resulted in an acute positive energy balance, rather than zero energy balance. On day 2 of each trial subjects arrived at the Nutrition and Fitness Laboratory at Colorado State University between 0800 and 1000 h following a 12-hr fast. An antecubital vein catheter was placed and following 5-min of rest two baseline venous blood samples were taken 5 minutes apart. Subjects then consumed, under supervision, a high-fat, high-sugar liquid meal. The test meal consisted of whole milk, heavy whipping cream, whey protein powder, chocolate syrup and sugar. Detailed macronutrient assessment of the liquid meal is described within the Metabolic Assessment portion of this chapter. Subjects consumed each test shake within a 15 minute time period. Postprandial venous blood samples were then taken at specific times over a six hour period for measurement of glucose, insulin, and soluble vascular adhesion molecule-1 (VCAM-1). Subjects rested quietly during this time period and were allowed to consume water ad libitum. Trials were completed in random order and separated by a wash-out period of at least 7-days.

Specific Procedures

Preliminary Screening- Prior to the beginning of the study, baseline screening was completed to determine eligibility. Subjects completed a health history questionnaire, the Eating Attitudes Test (EAT-26) to rule out eating disorders, a food preferences questionnaire and an ethnicity questionnaire [45]. Baseline body weight was measured using a balance beam scale (Detecto, Webb City, MO); height without shoes was measured to the nearest 0.1 cm using a wall-mounted stadiometer, resting blood pressure was obtained using an automated sphygmomanometer (Dynamap, Denver, CO); and fasting blood glucose was measured by fingerstick using a glucometer (Precision Xtra, MediSense, Abbott, Alameda CA). None of the

female subjects were pregnant and testing was completed during the follicular phase of their menstrual cycles, or between days 6-14 after starting menstruation.

Resting Metabolic Rate- Resting Metabolic Rate (RMR) was measured following a 12-hour fast by indirect calorimetry using True One 2400 Metabolic Measurement System (Parvo Medics, Model No MMS 2400, Sandy, UT). Prior to testing, the metabolic cart was calibrated using known gas concentrations. Subjects were familiarized with the procedure and were instructed to lie quite in the supine position for 30-minutes, during which time their head was enclosed in a canopy and respiratory gas exchanged measures were obtained. The respiratory exchange ratio (RER) was calculated as VCO_2/VO_2 and the Weir equation was used to convert the gas exchange measures to kilocalories [46]. Gas analysis was averaged over the last 10-minutes to determine RMR, with the resting energy expenditure values extrapolated to 24 hours and used in subsequent design of control diets.

Peak Oxygen Consumption-To determine each subject's sub-maximal exercise intensity required for the subsequent EX-trial, peak oxygen consumption was measured using a Monark, Ergomedic bicycle and a progressive maximal workload protocol (Medgraphics, Monark, Ergomedic, Model No. 8082E, St Paul, MN). Gas analysis was completed using Parvo Medics metabolic system to determine VO_{2peak} (Parvo Medics, True One 2400 Metabolic Measurement System, Sandy, UT). Prior to testing, calibration of the metabolic system was complete using known gas concentrations. Each subject was fitted to the bike and familiarized with testing procedures. Following a 2-5 minute warm-up period subjects were instructed to pedal at a cadence of 70-100 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 and testing was ceased when subjects could not

maintain maximum cadence or oxygen uptake was not increased with an increase in workload. Heart rate (Polar Target, Hong Kong) was continuously measured every 30 seconds during testing. The exercise test was stopped based on volitional fatigue and peak oxygen consumption was determined as the highest VO_2 value for 30 seconds.

Body Composition- Dual X-ray Absorptiometry (DEXA) (Hologic, Discovery, QRD series) was used to determine subjects' body composition. Testing was completed at the Human Performance/ Clinical Research laboratory at Colorado State University. Subjects were informed to lie supine and were positioned according to manufacturer instructions. Body composition was then analyzed using computer software.

Dietary Control- In an effort to control energy balance for subjects between trials all meals were provided for the entire day prior to each high-fat, high-carbohydrate test meal. Energy intake was individualized for each subject's RMR multiplied by a physical activity level of 1.4, corresponding to the physical activity level of a non-active adult [47]. To ensure consistency of macronutrient intake total daily intake provided 57% carbohydrate, 24% fat and 20% protein. All meals were prepared in the Department of Food Science and Human Nutrition metabolic kitchen. Individuals were instructed to consume all foods provided and return all uneaten food items to the lab for collection and recording. Ad libitum water was allowed and subjects were instructed to stop eating 12-hours prior to the test meal to ensure fasting conditions the following morning.

Exercise Intervention- On the evening of the EX trial subjects arrived at the laboratory 14-16 hours prior to the consuming the test meal the following morning. They were instructed to cycle at a given intensity and duration, with the goal of achieving an estimated net energy cost of 400 kcal. However, because the exercise intensity was erroneously based on 65% of peak heart rate, rather than 65% of peak VO_2, the net energy cost of the exercise was determined to approximate 285 kcal based on the following assumptions. The energy cost of the exercise was determined based on the VO_2 corresponding to 65% of peak heart rate during the graded exercise test. The minute VO_2 was then multiplied x 4.9 kcal/L O_2 to convert the oxygen consumption values to Calories per minute. This value was then multiplied by the duration of the exercise bout, and the resting energy expenditure for the time spent exercising was subtracted. The duration of exercise averaged 70 ±15 (SD) minutes. Heart rate was recorded continuously at each minute throughout the exercise bout and subjects were instructed to adjust their cadence accordingly to maintain exercise intensity. Directly following completion of the exercise bout, the acute energy deficit was offset with a 380 kcal mixed meal snack consisting of a 58% carbohydrate, 28% fat and 21% protein.

Metabolic Assessment- On day 2 of each trial subjects reported to the lab after an overnight fast of at least 12-hours. Individuals either rested (Non-EX) or completed the exercise bout (EX) the night prior to the high-fat, high-carbohydrate test meal. An antecubital vein catheter was placed and following a 5-minute rest period 2-baseline blood samples were taken. Subjects then consumed a high-carbohydrate, high-fat test meal within a 15-minute period. The test meal was comprised of whole milk, heavy whipping cream, sugar, chocolate syrup and whey protein. The macronutrient breakdown averaged 850.3±134.2 kcal of energy, 51 ±6 grams of fat, 75 ±9 grams of carbohydrate and 23 ±3 grams of protein at 53%, 34% and 11% fat, carbohydrate, protein, respectively. Following consumption of the test meal, blood samples were taken at 30,

60, 90, 120, 180, 240, 300 and 360 minutes later. During the observation period subjects lay quietly in bed and were provided water ad libitum.

Blood Analysis- Blood samples were collected in EDTA-coated tubes and immediately placed on wet ice. All samples were separated by centrifugation at 2500 rpm for 10 minutes. Plasma was then drawn off, placed in plastic eppendorf tubes and stored at -80 °C until further analysis. Plasma glucose and insulin assays were performed at the University of Colorado Clinical and Translational Sciences Laboratory. Glucose concentrations were measured using the glucose oxidase method on an automated glucose analyzer (YSI 2300, YSI Inc. Yellow Springs, OH). Plasma insulin concentrations were measured using a simultaneous one-step immunoenzymatic ("sandwich") assay for use with the Beckman-Coulter Access Immunoassay System (Beckman Coulter, Inc. Fullerton, CA). Plasma sVCAM concentrations were determined using an Enzyme-Linked Immunosorbent Assay (ELISA) procedure (R&D Systems, Minneapolis).

Statistical Analysis- The dependent variables, post-prandial glucose, insulin, and sVCAM were analyzed across the two 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 at $p<0.05$.

RESULTS

Subject characteristics

A total of eight, female volunteers participated in this study. Their physical characteristics presented in Table 1 below indicate that they were young, non-obese individuals with relatively low cardiorespiratory fitness, and with normal resting blood pressure and fasting blood glucose concentrations.

Table 1 Subject baseline characteristics. *n=8*

Variable	**Mean**	**Standard Deviation**
Age (yrs)	19.6	1.3
Weight (Kg)	68.2	8.4
Ht (cm)	166.4	7.1
BMI (kg/m^2)	24.6	1.8
Hip circumference(cm)	92.0	7.9
Waist circumference(cm)	77.2	7.0
Body Fat %	29.8	3.2
Resting Systolic Blood Pressure (mmHg)	108.0	9.3
Resting Diastolic Blood Pressure (mmHg)	74.0	13.3
Fasting Blood Glucose (m g/dl)	80.0	15.4
Resting Pulse (Beats Per Minute)	75	11.5
VO_2max (ml/kg/min)	31.3	2.9

Exercise

The mean exercise duration for the study participants was 70 ±14.9 (SD) minutes. Furthermore, subjects maintained an average heart rate of 127 ± 15.1(SD) beats per minute, which corresponded to approximately 65% of their peak heart rates.

Fasting and Postprandial Plasma Glucose Concentrations

The mean (± SEM) pre- and postprandial plasma glucose concentrations for the EX and NonEX conditions are provided in Figure 1. The baseline fasting plasma glucose concentrations prior to the meal challenge were not significantly different between EX and NonEX trials. There was not a significant time effect, indicating no marked changes during the 6 hour measurement period, despite the ingestion of the meal. Additionally, there was not a main effect of condition, indicating no difference in the postprandial glucose response between the EX and NonEx conditions ($p=0.53$), nor was there a condition by time interaction, $p= 0.20$.

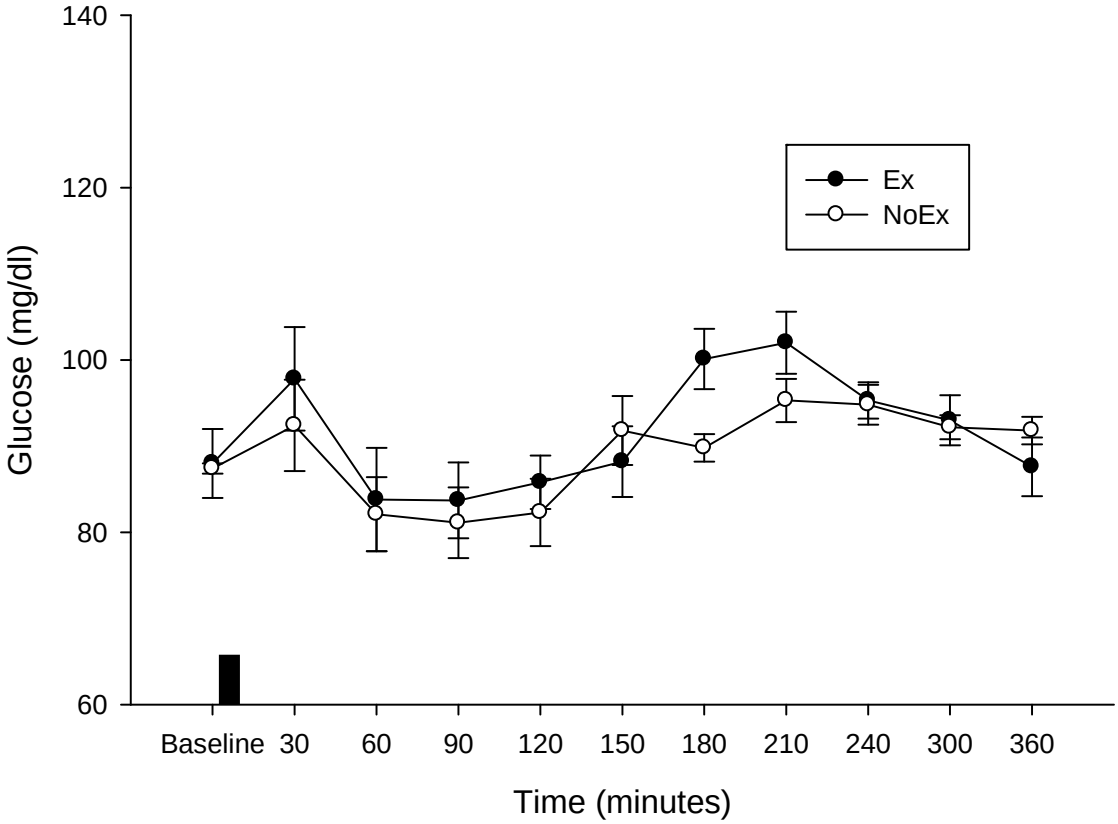

Figure 1. Plasma glucose concentrations for the EX and NonEX conditions immediately before and for 6 hours following a high fat, high sugar liquid meal challenge in 8 young adult women. Black rectangle indicates the time that the test meal was provided.

Fasting and Postprandial Plasma Insulin Concentrations

Fasting plasma insulin concentrations prior to the meal challenge were not different between EX and NonEX conditions. As expected there was a significant time effect owing to the sharp rise in insulin following the meal ingestion with a return to baseline concentrations by the end of the 6-h postprandial period. There was no main effect of condition, indicating the responses for EX and NonEx did not differ, and the lack of signficant time by treatment interaction indicates that the EX and NonEx insulin responses did not differ over time.

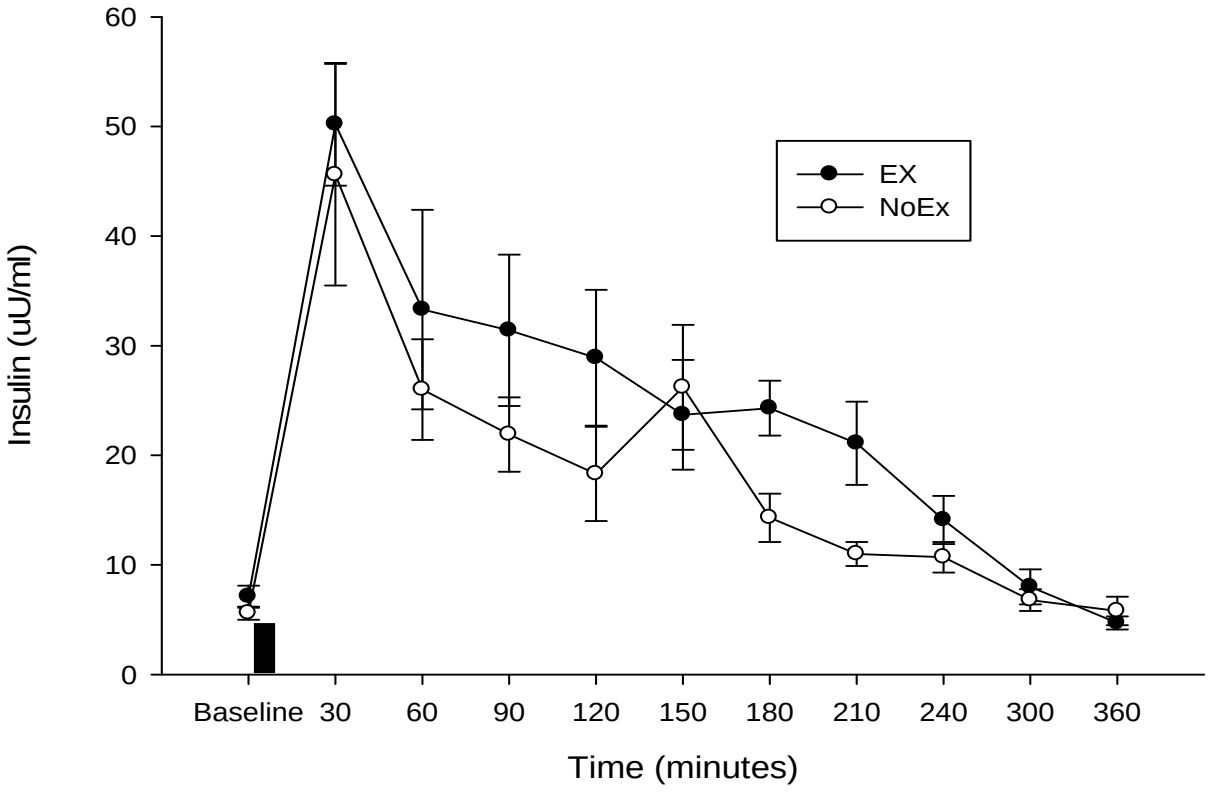

Figure 2 Plasma insulin concentrations for the EX and NonEX conditions immediately before and for 6 hours following a high fat, high sugar liquid meal challenge in 8 young adult women. Black rectangle indicates the time that the test meal was provided.

Fasting and Postprandial Plasma sVCAM-1 Concentrations

The sVCAM-1 data are provided in Figure 3. As can be readily seen in the graph, there was a significant condition effect such that the circulating soluble VCAM-1 concentrations were lower before and following the high fat, high sugar meal challenge for the EX compared to the NonEX condition. On average across all time points, the sVCAM-1 concentrations for the EX condition were 240 mg/dl lower than in the NonEX trial, $p < 0.001$. The meal challenge did not affect the VCAM-1 concentrations as evidenced by lack of significant time effect. There was no treatment by time interaction.

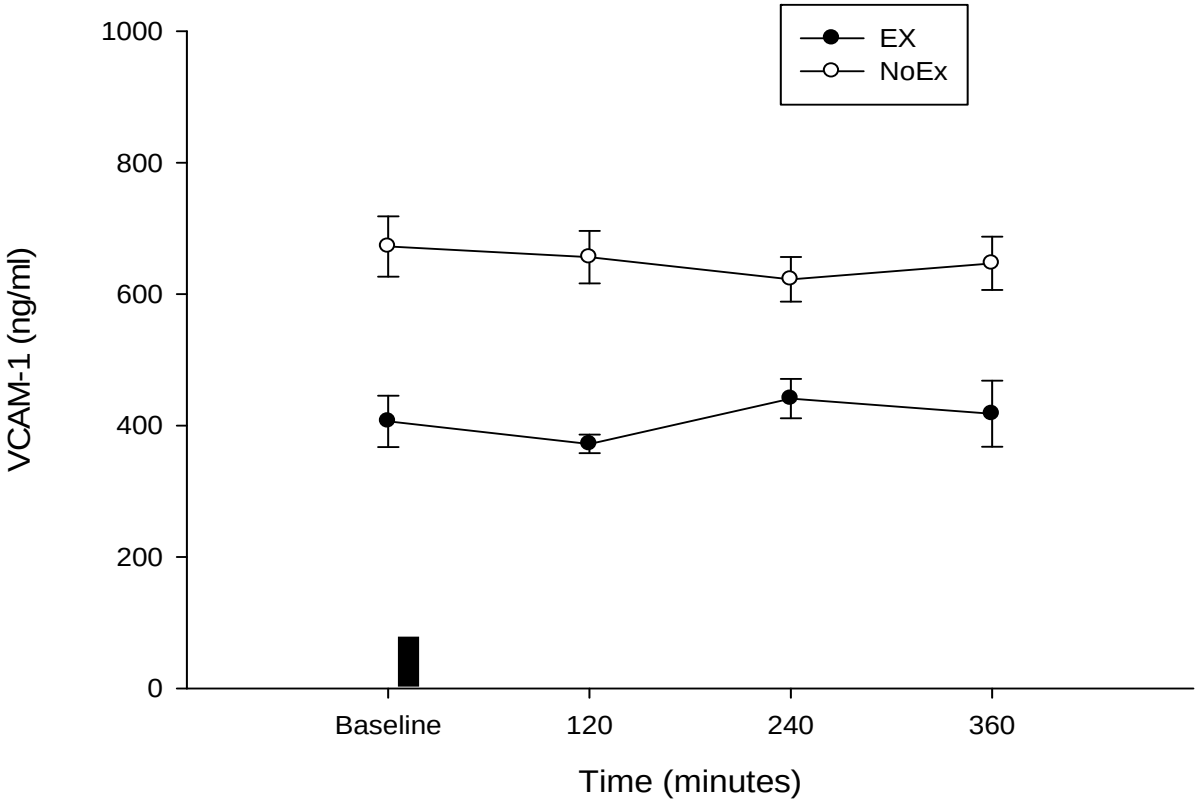

Figure 3 Plasma VCAM-1 concentrations for the EX and NonEX conditions immediately before and for 6 hours following a high fat, high sugar liquid meal challenge in 8 young adult women. Black rectangle indicates the time that the test meal was provided.

Discussion

Major Findings

Results from the current study demonstrate that a single session of moderate exercise, without an energy deficit, may reduce sVCAM-1 concentrations in young, sedentary females. However, for reasons discussed below, this finding must be viewed with caution and the assay repeated. The lower sVCAM-1 concentrations in the morning following the previous days' exercise bout compared to the condition without exercise was unrelated to glucose and insulin concentrations, as the postprandial glucose and insulin responses were not different for these two conditions.

Exercise and sVCAM-1

Exercise is known to confer protection against cardiovascular disease despite physiological stressors associated with it. These benefits, in part, may be due to a reduction in endothelial cell expression of VCAM-1, thus decreasing leukocyte recruitment and atherosclerosis development. Following a single bout of exercise, improvements in insulin sensitivity as well as reduction in postprandial lipemia have been shown [12, 14]. Additionally, a positive correlation between insulin resistance, type 2 diabetes and cardiovascular risk with increased levels of circulating VCAM-1 has been established [8]. Initial associations were primarily thought to be due to the hypertriglyceridemic state in these individuals, as opposed to direct association with insulin levels. However, more recent work with human umbilical vein endothelial cells have established greater understanding regarding the direct promotion of VCAM-1 expression by insulin as well as glucose [48, 49]. Specifically, when cells were exposed

to physiological levels of insulin, relevant for insulin resistance states, expression of cell surface VCAM-1 was about 1.8 fold greater compared to control, specifically through p38-mitogen-activated protein-kinase pathway (MAPK) [48]. Moreover, fluctuating glucose conditions, similar to daily changes of diabetic patients, also significantly induced VCAM-1 expression, likely due to the generation of oxidative stress [49]. The potential effects an acute bout of exercise could therefore have on decreasing VCAM-1 levels may be through the improvements in insulin sensitivity with subsequent improvement in glucose regulation particularly in the postprandial state. In addition, improvements in lipid oxidation following exercise could potentially decrease expression of VCAM-1 postprandially, likely related to greater TG clearance and utilization with subsequent decrease in cytokine release as well as increased vasodilation [16, 32] .

Decreased levels of fasting VCAM-1 may also ensue following a single bout of exercise. Potentially, increased insulin sensitivity and improvements in fat oxidation would decrease endothelial exposure and subsequent induction of VCAM-1 levels in a fasted state. However, prior work has demonstrated moderate exercise has little to no effect on fasting circulating sVCAM-1 levels [15, 16]. Earlier work by Jilma *et al.* investigating the influence of physical exercise on serum levels of circulating adhesion molecules found that 60-minutes of cycle ergometer exercise at 60% VO_{2max} had no significant impact on sICAM-1 or sVCAM-1immediately and up to 24-hours following the exercise bout [15]. Moreover, an **increase** in sVCAM-1 of approximately 11% was demonstrated immediately following graded ergometry exercises to exhaustion, although these results did not reach significance according to authors [15].

There is currently limited research regarding the effects of a single bout of moderate exercise on the postprandial response. The effect of prior exercise on postprandial lipemia and endothelial activation was also examined by MacEneaney *et al* who reported no differences in sVCAM-1 or sICAM-1 between exercise and no exercise treatments [12]. However, exercise did reduce postprandial TG response as evidenced by a 20% decrease in both the 6-hour postprandial total area under curve and incremental area under the curve [12].Results from these studies represent a disconnect between attenuating postprandial TG response and reduced activation of the endothelium.

The different results from these studies relative to our findings is not easily explained but may be related to the following: First, prior acute exercise has been shown to lower postprandial TG-rich lipoproteins, a phenomenon which could lower LDL production and oxidative stress, thus attenuating endothelial cell VCAM-1 expression. Second, investigations of acute exercise accompanied by complete energy replacement on adhesion molecules have not been adequately studied. Third, to our knowledge there are no studies that have examined the effect of acute exercise on postprandial VCAM-1 concentrations in females. Gender differences in responses to metabolic perturbations are becoming widely recognized. Specifically, women have been shown to exhibit a higher absolute rate as well as relative contribution of energy expenditure from lipids over a wide range of exercise intensities [37]. An increased rate of lipid metabolism during the exercise bout may have induced an increase in overall lipid metabolism within the subsequent 24-hour time period [37]. An increase in lipid oxidation with subsequent decrease in plasma lipoprotein has been shown to decrease inflammation, correlating with decreased levels of adhesion molecules. The acute triglyceride-lowering effects of exercise seems to be due to accelerated catabolism resulting from increased activity of lipoprotein lipase (LPL) [50]. Additionally, women have higher lipoprotein lipase activity, in general, when compared to

their male counterparts [50]. An increase in LPL activity within this female population, especially within skeletal muscle following a bout of exercise, could potentially increase lipid oxidation within myocytes even in the face of energy replacement. Thus, if females exhibit a higher rate of lipid oxidation during and following the same exercise intervention as male counterparts, they could exhibit greater TG clearance and utilization, resulting in lower VCAM-1 levels. However, this is entirely speculative and this argument is not supported by the TG (not reported here), insulin or glucose data in the present study. Also, the lower fasting and postprandial VCAM-1 concentrations in the exercise trial is in disagreement with much of the literature [12, 14-16]. Further caveats which may explain the effect of exercise on VCAM-1 levels will be discussed under the limitations section of this paper.

Postprandial VCAM-1

We hypothesized that VCAM-1 would increase significantly during the postprandial period following consumption of the high fat, high sugar liquid meal. A significant rise in postprandial response of sVCAM-1, between 9-20% from baseline, subsequent to high-fat meal intake has been demonstrated in previous investigations [8, 33]. Specifically, in healthy, normal weight individuals peak postprandial elevation has been demonstrated to occur within 2-4 hours following high-fat meal ingestion [8, 33]. Additionally, an increase in sVCAM-1 of approximately 9% has been observed when an oral glucose load in addition to a high-fat meal was administered [33]. Results from these studies demonstrate a significant correlation between an acute high-fat meal challenge and endothelial activation as determined by increases in circulating sVCAM-1. Endothelial activation, or functional changes to the endothelium, may be induced through various stimuli such as inflammatory cytokines. Increased circulation of inflammatory markers such as TNF-α and IL-6 are likely mediators to activation of endothelial

cells with subsequent induction of adhesion molecule expression. Furthermore, data from previous work also suggests independent and cumulative effects of postprandial hyperglycemia and hypertriglyeridemia on the concentrations of circulating adhesion molecules, which is likely due to oxidative stress [33].

Why we saw no increases in VCAM-1 in response to the high fat, high sugar liquid meal is unclear. Possibly in these young women, VCAM-1 rose quickly during the early postprandial period, and had returned to baseline concentrations by 120 minutes following meal consumption. If this were the case, our first postprandial measurement of VCAM-1 at 120 minutes would have missed its zenith. It is also possible that other dietary constituents, including antioxidant vitamins in the liquid meal could have blunted the post-prandial rise in VCAM-1. [8]. Nappo *et al* demonstrated that the significant rise of both VCAM-1 and ICAM-1 following a high fat meal were attenuated when the meal was supplemented with vitamins E and C [8]. Data from these studies suggest the possibility that supplementation with vitamins A, C and E may dampen the oxidative stress of a high fat meal and attenuate its effects on adhesion molecule levels. The authors suggest that antioxidant properties of such vitamins normalize endothelial activation following a high-fat meal in normal and diabetic subjects [8, 51]. Mechanistically, these antioxidants reduce susceptibility of isolated LDL to oxidation and inhibit secretion of proinflammatory cytokines, known inducers of VCAM-1 expression [8, 20]. In these prior studies, , an attenuated postprandial response was observed, such that postprandial VCAM-1 levels were not significantly different then baseline [12, 51]. Given these published observations, it is possible that antioxidants within our test meal may have attenuated the postprandial VCAM-1 response. However, further analysis of our test meal revealed substantially lower vitamin content compared to Nappo and colleagues who supplemented with 800 IU of Vitamin E and 1,000 mg ascorbic acid [8]. Nutritional analysis of our test meal provided 2.1 IU Vitamin E and

1.0 mg Vitamin C. Although retinal concentrations were not provided within either study, an average of 544 mcg or 78% of the RDA was contained within our test meal and may have effected the postprandial response, A similar study by MacEneany *et al* also reported no change in postprandial VCAM-1 levels despite marked increases in serum TG and plasma IL-6 within young, healthy male subjects [12]. The authors speculated that the amount of retinal within the test meal may have attenuated the response. Our use of a dairy and whey protein based supplement containing retinal and tocopherols may help explain the absence of a postprandial response among our study subjects. However, we have no direct evidence to support this argument as the role of antioxidant on VCAM-1 was not directly investigated. Further research is warranted to better understand this effect within young, normal weight females.

It is also possible that a simple venous blood sampling protocol was insufficiently sensitive in these young women to detect changes in sVCAM-1. Using a more sensitive test such as forearm blood flow technique may be necessary to examine endothelial function within this population [12]. However, the concentrations of sVCAM-1 measured within this study are within range to previous published work establishing physiological concentrations of healthy individuals. Specifically, blood levels of circulating VCAM-1 concentrations within healthy individuals ranges has been reported at 504±278 ng/ml [24]. Additionally, higher fasting, baseline concentrations ranging from 650-900 ng/ml have also been established within healthy populations of both men and women [8, 12, 15, 33, 44]. The range of concentrations presented in previously published work support the current data such that the mean concentrations of 406.3±110.5ng/ml and 672.3±129.9ng/ml, for Ex and Non-Ex conditions respectively, are both within physiological ranges. Although day-to-day intersubject variability of 8% is considered "normal", the 40% change in baseline concentrations between trials suggests that a true change

was captured. However, due to caveats discussed below the assays must be repeated prior to drawing conclusions on these data.

Limitations

There are several limitations to the present investigation. The plasma VCAM-1 samples were determined in duplicate following manufacture instructions (R&D Systems, Minneapolis); however, due to investigator error, the VCAM-1 concentrations for EX and NoEX conditions were run on different plates, respectively. Thus without using the same standard curve for each of the two conditions for each subject, the VCAM-1 values for EX and NoEX are not as directly comparable as they should be. This unfortunate approach to this assay is a likely source of systematic bias and unwanted variability, and could be the reason that the VCAM-1 data are different between the EX and NonEx conditions. However, the assays were run with 24-hours of each other and the same controls were used on each plate. Additionally, R^2 values were similar between EX and NonEx plate, 0.996 and 0.949 respectively. These assays will need to run again with samples for each of the two conditions for each subject run on the same plate. Another limitation of the study is related to the failure to accurately specify the exercise intensity and duration necessary to achieve a net caloric cost of 400 kcal for each subject. Although subjects underwent VO_2max testing with recorded heart rate values, such calibration curves were not developed and utilized. The use of resting metabolic rate, peak VO2, minutes of exercise and 4.9 calories per minute expended allowed for a gross estimate of the net caloric expenditure within each subject. On average subjects expended approximately 285 calories during the exercise session, with immediate consumption of 380 calories from mixed meal snack, thus placing individuals into a positive, net energy balance of approximately 100 calories. The consequence of inducing an overfed state may help explain our finding that exercise failed to attenuate the

postprandial changes in insulin that have been clearly seen in other studies when subjects experienced an acute energy deficit or zero energy balance [14].

Conclusions

In conclusion, the results of the present study indicate that a moderate exercise bout in comparison to no exercise prior to a high fat, high-carbohydrate meal may reduce fasting and postprandial circulating VCAM-1 levels, in normal weight, premenopausal females. However, the method for determination of the plasma VCAM-1 concentrations suggests this finding is tenuous, and the assay must be repeated using more appropriate methods.

References

1. **S. Blackenberg, S.Barbaus,L.Tiret, *Adhesion molecules and atherosclerosis.* Atherosclerosis, 2003. 170: p. 191-203.**

2. **G.K. Hansson, MD, PhD, *Mechanisms of Disease: Inflammation, atherosclerosis, and coronary artery disease.* the New England Journal of Medicine, 2005. 352: p. 1685-1695.**

3. **Organization, W.H., *Cardiovascular Disease Fact Sheet.* 2009.**

4. **Association, A.H., *Women and Cardiovascular Diseases- Statistics.* 2010.**

5. **National Heart, L.A.B.I. 2010; Available from: http://www.nhlbi.nih.gov/.**

6. **C.F. Ebenbichler, R.Kirchmair., C. Egger, and J.R. Patsch, *Postprandial state and atherosclerosis.* Current Opinion in Lipidology, 1995. 6(5): p. 286-290.**

7. **F Muntwyler, G.Sutsch, J.H. Kim, H. Schmid, F. Follath, W. Kiowski, F.W. Amann, *Post-prandial lipemia and endothelial function among healthy men.* Swiss Medicine Weekly, 2001. 131: p. 214-218.**

8. **F. Nappo, MD, K. Esposito, MD, M. Cioffi, MD, G. Giugliano, A.M. Molinari, MD, G. Paolisso, MD, R. Margella, MD, D. Giugliano, MD, *Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals.* Journal of the American College of Cardiology, 2002. 39(7): p. 1145-1150.**

9. **G.J. Blake,P.M.Ridker, *Inflammatory bio-markers and cardiovascular risk prediction.* Journal of Internal Medicine, 2002. 252: p. 283-294.**

10. **P.M. Ridker, C.H.Hennekens, B.Roitman-Johnson, M.J. Stampfer, J. Allen, *Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men.* The Lancet, 1998. 351: p. 88-92.**

11. **W. Chen, D.B.Jump, M. B. Grant, W.J. Esselman, J.V. Busik, *Dyslipidemia, but not hyperglycemia, induces inflammatory adhesion molecules in human retinal vascular endothelial cells.* Investigative ophthalmology and visual science, 2003. 44(11): p. 5016-5022.**

12. **O.J. MacEneaney, M.Harrison, D.J. O'Gorman, E.V. Pankratieva, P.L. O'Connor, N. M. Moyna, *Effect of prior exercise on postprandial lipemia and markers of inflammatin and endothelial activation in normal weight and overweight adolescent boys.* European Jouran of Applied Physiology, 2009. 106: p. 721-729.**

13. **J.Q. Zang, T.R.Thomas, S.D. Ball, *The effect of exercise timing on postprandial lipemia and HDL cholesterol subfractions. .* Journal of Applied Physiology, 1998. 85: p. 1516-1522.**

14. **F.L. Burton, D.Malkova, M.J. Caslake,J.M.R. Gill, *Energy replacement attenuates the effects of prior moderate exercise on postprandial metabolism in overweight/obese men.* International Journal of Obesity, 2008. 32: p. 481-489.**

15. **B. Jilma, H.GEichler, P. Stohlawetz, E. Dirnberger, S. Kapiotis, O.F. Wagner, W. Schutz, K. Krejcy, *Effects of exercise on circulatin vascular adhesion molecules in healthy men.* Immunobiology, 1997. 197: p. 505-512.**

16. **S.A. Marsh, J.S.Coombes, *Exercise and the endothelial cell.* International Journal of Cardiology, 2005. 99: p. 165-169.**

17. **G. Ranković, B.M., T. Savić‡, B. Đinđić||, Z. Mančev| |, G. Pešić§, *Effects of physical exercise on inflammatory parameters and risk for repeated acute coronary syndrome in patients with ischemic heart disease.* VOJNOSANITETSKI PREGLED, 2009. 66(1): p. 44-48.**

18. **R.A. Vogel, MD, M.C. Corretti, MD, G.D. Plotnick, MD, *Effect of a single high-fat meal on endothelial function in healthy subjects.* American Journal of Cardiology, 1997. 79: p. 350-354.**

19. **J.Steffel, T.F. Luscher, *Predicting the Development of Atherosclerosis.* Circulation: Journal of the American Heart Association, 2009. 119: p. 919-921.**

20. **R. De Caterina, J.K.Liao,P. Libby, *Fatty acid modulatin of endothelial activation.* American Journal of Clinical Nutrition, 2000. 71: p. 213-223.**

21. **T.A. Springer, *Adhesion receptors of the immune system.* Nature, 1990. 346: p. 425-434.**

22. **P.M Ridker, C.H.Hennekens, B. Roitman-Johnson, M.J. Stampfer, J.Allen, *Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men.* The Lancet, 1998. 351: p. 88-92.**

23. **D.T. Price, MD,J.Loscalzo, MD, PhD, *Cellular adhesion molecules and atherogenesis.* American Journal of Medicine, 1999. 107: p. 85-97.**

24. **A.J.H.Gearing, W.Newman, *Circulating adhesion molecules in disease.* Immunology Today, 1993. 14(10): p. 506-512.**

25. **E. Stefanadi, D.Tousoulis, N. Papageorgiou, A. Briasoulis, C. Stefanadis, *Inflammatory biomarkers predicting events in atherosclerosis.* Current Medicinal Chemistry, 2010. 17: p. 1690-1707.**

26. **A.C Newby, *Studying mechanisms underlying shedding of endothelial membrane proteins could help patients at risk for myocardial infarction.* Cardiovascular Research, 2005. 67: p. 4-5.**

27. **S. Blankenberg, H.H.Ridker, C.B., *Circulating cell adhesion molecules and death in patients with coronary artery disease.* Circulation: Journal of the American Heart Association, 2001. 104: p. 1336-1342.**

28. **L.E. Rohde, R.T.L., L. Rivero, M. Jamacochian, L.H. Arroyo, W. Briggs, N. Rifai, P. Libby, M.A. Creager , P.M Ridker, *Circulating cell adhesion molecules are correlated with ulrasoundpbased assessment of cartic atherosclerosis.* Arteriosclerosis, Thromobosis, Vascular Biology, 1998. 18: p. 1765-1770.**

29. **M.I. Cybulsky, K.Iiyama, H. Li, S. Zhu, M. Chen, M. Iiyama, V. Davis, J.C. Gutierrez-Ramos, P.W. Connelly, D.S. Milstone, *A major role of VCAM-1, but not ICAM-1, in early atherosclerosis.* The Journal of Clinical Investigation, 2001. 107(10): p. 1255-1262.**

30. **S.J. Hwang, C.M.B., A.R. Sharrett, L.C. Smith and C.E. Davis, *Circulating adhesion molecules VCAM-1, ICAM-1, and E-selecin in coratid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study.* Circulation: Journal of the American Heart Association, 1997. 96: p. 4219-4225.**

31. **S.S. Gropper, J.L.Smith, J.L. Groff, *Advanced Nutrition and Human Metabolism*. 2005, Peter Marshall: Belmont, CA. p. 137-164.**

32. **Schiffrin, D.H.E..E.L., *Endothelial Dysfunction.* Journal of American Society of Nephrology, 2004. 15: p. 1983-1992.**

33. **A. Ceriello, L.Quagliaro, L. Piconi, R. Assaloni, R. Da Ros, A. Maier, K. Esposito, D. Giugliano, *Effect of postprandial hypertriglyeridemia and hyperglycemia on circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment.* Diabetes, 2004. 53: p. 701-710.**

34. **H.E. Aldred, I.C.Perry, A.E.Hardman, *The effect of a single bout of brisk walking on postprandial lipemia in normolipidemic young adults.* Metabolism-Clinical and Experimental, 1994. 43(7): p. 836-841.**

35. **K. Hansen, T.S., D. Schoeller *The effets of exercise on the sotragef and oxidation of dietary fat.* Sports Medicine, 2005. 35: p. 363-373.**

36. **J.M.R. Gill, S.L. Herd, N.V. Tsetsonis, A. E. Hardman, *Are the reductions in triacyglycerol and insulin levels after exercise related?* Clinical Science, 2002. 102: p. 223-231.**

37. **M.C. Venables, J.Achten.A.E.Jeukendrup, *Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study.* Journal of Applied Physiology, 2003. 98: p. 160-167.**

38. **J.M.R Gill A.E. Hardman, *Postprandial lipemia: effects of exercise and restirction of energy intake compared.* American Journal of Clinical Nutrition, 2000. 71: p. 465-471.**

39. **E. Ekblom-Bak, *Independent associations of physical activity and cardiovascular fitness with cardiovascular risk in adults.* European Journal of Cardiovascular Prevention & Rehabilitation. 17(2): p. 175-180.**

40. **E.J. Shiroma I.M.Lee, *Physical activity and coronary heart disease in men: the Harvard alumni Health Study.* Circulation: Journal of the American Heart Association, 2000. 102: p. 975-980.**

41. **J. Myers, M.P., V. Froelicher, D. Do, S. Partignton, J.E. Atwood., *Exercise capacity and mortality among men referred for exercise testing.* New England Journal of Medicine, 2002. 346: p. 793-801.**

42. **A.M. Malek, M., PhD; S.L. Alper, MD, PhD; S. Izumo, MD, *Hemodynamic shear stress and its role in atherosclerosis.* Journal of the American Medical Association, 1999. 282(21): p. 2035-2042.**

43. **S. Adamopoulos, J.P., C. Kroupis, M. Georgiadis, D. Karatzas, G. Karavolias, K. Koniavitou, A.J.S. Coats and D. Th. Kremastinos, *Physical training reduces peripheral markers of inflammation in patients with chornic heart failure.* European Heart Journal, 2001. 22: p. 791-797.**

44. **M. Harrison, R.P.Murphy, P.L. O'Connor, D.J. O'Gorman, N.McCaffrey, P.M. Cummins, N.M. Moyna, *The endothelial microparticle response to a high fat meal is not attenuated by prior exercise.* European Jouran of Applied Physiology, 2009. 106: p. 555-562.**

45. **D.M. Garner, M.P.Olmsted, Y Bohr, P.E. Garfinkel, *The eating attitude test-phychometric features and clinical correlates.* psychological medicine, 1982. 12(4): p. 871-878.**

46. **J.B. Weir, *New methods for calculating metabolic rate with special reference to protein metabolism.* Journal of Physiology, 1949. 109: p. 1-9.**

47. **P.Trumbo, P.S.S., PhD; A.A. Yates, Phd, RD M. Poos, PhD, *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids.* Journal of the American Dietetic Association, 2002. 102(11): p. 1621-1630.**

48. **R. Madonna, A.Pandolfi, M. Massaro, A. Consoli, R.De Caterina, *Insulin enhances vascular cell adhesion molecule-1 expression in human cultured endothelial cells through the pro-atherogenic pathway mediated by p38 mitogen-activated protein-kinase.* Diabetologia, 2004. 47: p. 532-536.**

49. **L. Piconi, L.Quagliaro, R. Da Ros, R. Assaloni, D. Giugliano, K. Esposito, C. Szabos, A. Ceriello, *Intermittent high gluocse enhances ICAM-1, VCAM-1, E-selectin and interleukin-6 expression in human umbilical endothelial cells in culture: the role of poly(ADP-ribose) polymerase.* Journal of Thrombosis and Haemostasis, 2004. 2: p. 1453-1459.**

50. **W.L. Haskell,*The influence of exercise on the concentrations of triglyceride and cholesterol in human plasma.* Exercise Sports Science Review, 1984. 12: p. 205-244.**

51. **D. Rubin, S.Claas, M. Pfeuffer, M. Nothnagal, U.R. Foelsch and J. Schrezenmeir, *s-ICAM-1 and s-VCAM-1 in healthy men are strongly associated with traits of metabolic***

syndrome, becoming evident in the postprandial response to a lipid-rich meal. **Lipids in Health and Disease, 2008. 7(1): p. 32-38.**

Appendix I

Consent to Participate in a Research Study

Colorado State University

TITLE OF STUDY: Interaction of Diet and Exercise on Chronic Disease Risk

PRINCIPAL INVESTIGATOR: Matt Hickey, PhD

CO-PRINCIPAL INVESTIGATOR: Chris Melby, DrPH, Stacy Schmidt, MS, Whitney Smith, Dan Warro

SPONSOR OF THE PROJECT: Colorado Agricultural Experiment Station

WHO IS DOING THE STUDY? The Departments of Food Science & Human Nutrition and Health & Exercise Science

WHAT IS THE PURPOSE OF THIS STUDY? The purpose of the present study is to determine how a single session of exercise affects your blood glucose and blood fats after you eat a meal.

WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST? The study will take place on the CSU campus in the Health & Exercise Science Department and the Food Science & Human Nutrition department.

PROCEDURES/METHODS TO BE USED: After completing initial screening tests, eligible subjects will participate in 2 separate trials. The only difference between the 2 trials will be that for one trial you will exercise for 60-90 minutes on a stationary bicycle the evening before the trial (EX) and the other trial you will not exercise at all. On the day prior to each trial, you will be asked to eat the food we provide. On each trial day, you will consume a test meal and remain in the lab for an additional 6 hours. During this time, blood samples will be drawn. If you are eligible to be in the study, your participation will require a time commitment of about 17 hours during a 6-7 day period. There are a number of "exclusion criteria" (things that will make you ineligible for the study such as having diagnosed diabetes, certain types of medication, etc). Should you meet any of these exclusion criteria during the screening period, we will fully inform you as to the reason you cannot be in the study.

DAYS 1-2: PRELIMINARY SCREENING: During one of the initial days of the study, you will complete the following screening tests to help us determine if you are eligible to go to the next phases. These tests will require about 2 hours of your time and will be completed several days before beginning one of the two trials.

Health and Medical History Questionnaire- You will need to answer questions about your medical history and personal health habits. Time: 15 minutes

Ethnicity Questionnaire- You will need to identify your ethnic background. Time: 5 minutes

Food Preferences Questionnaire- You will need to answer questions about foods you like and don't like. You will also be asked to list any food allergies you think you might have.

Time: 10 minutes

Eating Disorders Questionnaire- You will be asked to complete a form that screens for eating disorders. Time: 5 minutes

Exercise Questionnaire- You will need to answer questions about your exercise habits.

Time: 10 minutes

Pregnancy test- All women in the study will be asked to take a pregnancy test. If the pregnancy test is positive, you cannot be in this study. It is important that you do not become pregnant during the study. This will stop you from continuing the study. Time 10 minutes

Food Intake Record: You will be asked to record your food intake on 3 consecutive days prior to beginning the experimental trials. Time: 15 minutes.

Body measurements- Your height will be measured without you wearing shoes. Body weight will be measured on a normal balance scale. This will include the weight of light indoor clothing minus shoes. Your waist and hip circumference will be measured using a measuring tape. Time: 10 minutes.

Blood pressure and blood glucose tests- Following a 12-h fast (no food or beverages except water for 12 hours) you will have your blood pressure taken using normal procedures while you sit quietly in a comfortable chair. You will then have a small amount of blood taken from your fingertip (one drop). From this we will measure your blood sugar levels. If your blood pressure is greater than 140/90 or your blood sugar level higher than 126 mg/dl, you will not be able to participate in the study and you will be told to see your doctor to check for high blood pressure or diabetes. Time: 10 minutes.

Body composition (fat and lean tissue) - This will be performed using a machine called a dual energy X-ray absorptiometer (DEXA). This unit uses 2 low energy X-rays to determine the amount of body fat you have. You will be exposed to some radiation. But, the amount of radiation exposure in this procedure is very low, about 1/1,000 of the normal radiation exposure you receive yearly from what is called "background" radiation from the environment. Put another way, the exposure from a DEXA scan is less than the normal exposure in a flight from Denver to Chicago, and about $1/40^{th}$ the exposure from a normal stomach X-ray you might receive at a hospital. This test will be performed in room 124 in the Human Performance Clinical Research Laboratory (HPCRL) located near Moby Gym. You will be asked to lie quietly on a bed in shorts and a T-shirt for about 6 minutes while the scan is performed. Time: 30 minutes.

Resting Metabolic Rate - This test involves reporting to the HPCRL or to room 216 Gifford after a 12-hour overnight fast. You will be asked to lay on a bed for 20 minutes with a plastic canopy over your head or fitted with a mouthpiece to breathe into. Tubes connected to the canopy or mouthpiece measure how much air you breathe in and out. This measures how many calories you are burning while at rest. Time: 30 minutes

Physical Fitness: For this test you will ride a stationary bicycle. It will be conducted in either Gifford 216 or the HPCRL. The exercise will be easy at first and then get harder by adjusting the pedal tension on the bike. We will ask you to exercise until it gets too hard for you to continue. The total time you will be exercising on the bike will be less than 15 minutes. During this test, you will be asked to breathe through a mouthpiece so we can measure the amount of oxygen your body uses. We will also measure your heart rate (using a heart rate monitor, which is like a small elastic belt you wear around your chest) and your blood pressure (using a small cuff that fits around your upper arm). Time: 30 minutes.

DAYS 3-6: EXPERIMENTAL PROTOCOL:

You will participate in 2 separate experimental trials. The only difference between the 2 trials will be whether you remain sedentary throughout the trial (SED) or perform 60-90 minutes of moderate intensity cycling in the evening prior to the trial (EX). The order of the trials will be randomized, which means the order will be determined by flipping a coin. The two trials will be separated by at least 3 days. For females, the trials will be performed during days 6-14 of the menstrual cycle or while taking birth control. The day before each trial, you will be provided a standardized diet. The next morning, you will come to the lab having fasted for 12 hours. This means you will not have eaten any food or have drunk any beverages except water during the previous 12 hours. You will lie on a bed. A catheter (a hollow needle/plastic tube) will be put in your forearm (or back of your hand if your veins are better there) for blood sampling. First about 2 teaspoons of blood will be taken from the tube in your arm. We will later determine how much glucose, fats (like cholesterol), hormones and specific proteins are in your blood. After this blood sample has been taken, you will consume a meal over 15 minutes. This meal will be high in fat and sugar. After you have consumed the meal, blood samples will be collected from the catheter every 30 minutes over a 6 hour period. This test will estimate your body's ability to move dietary fats and sugars from your blood into your cells. After blood is taken from the catheter in your arm each time, a small amount of sterile salt-water will be used to flush the catheter to keep your blood from clotting inside the tubing.

We will later analyze your blood's cholesterol, fat, insulin, and sugar levels. The amount of blood taken at each sample is about ½ teaspoon. Altogether, we will take about 7 teaspoons of blood. You will probably not feel any pain when the blood is collected from the catheter in your arm. Time: 6.5 hours **This procedure will be performed 2 times: once during the SED trial and once during the EX trial at least 3 days apart.**

STUDY TIMELINE:

	Prelim. Testing	SED Day 1	SED Day 2	EX Day 1	EX Day 2
Screening	X				
BP/ Glucose	X				
Weight	X				
Diet Analysis	X				
Fitness test	X				
DEXA	X				
RMR	X				
Exercise				X	
Standard Diet		X		X	
Meal Test			X		X
6-h Blood Testing			X		X
Time (hours)	2		6.5	1.5	6.5

TOTAL TIME COMMITMENT:

approximately 17 hours

RETENTION OF BLOOD SAMPLES

You should understand that we plan to keep any extra blood samples that are not used in the analysis for this study. In other words, if we have any "extra" blood we will keep them in a freezer in our lab. It is very possible that we will use all of the blood obtained in this study and will have none left, but in the event that we do, we would like your permission to keep the samples in the event that they can be used for further research. We will use these samples in the future solely for additional research on obesity and metabolism; specifically, all future research will simply be an extension of what we hope to accomplish with the current study. We may simply analyze your blood for the presence of other hormones or metabolites. Your stored samples will be coded in such a way that your confidentiality will be maintained. Only the Principal Investigators (Professors Melby and Hickey) will have access to the coding system for your samples. There is a possibility that your samples may be shipped to other departments on the CSU campus, or to colleagues at other Universities for assistance with analysis. Under such circumstances, the same coding system will be used, so researchers in other labs will not be able to identify you. We do not anticipate ANY commercial product development from your tissue, the samples will be used solely for research purposes. You should be

advised that we do NOT have plans to re-contact you in the future regarding any additional analyses, but will seek full approval of the CSU Regulatory Compliance Office prior to initiating any further research on your samples.
By checking "Yes" below and signing on the accompanying line, you are agreeing to allow the investigators retain any blood samples obtained during this study. If you do not wish the investigators to retain any samples, please check the box marked "No" and also sign on the accompanying line.

The investigators may keep any blood samples obtained during the course of this study for future research on obesity and metabolism YES ☐ NO ☐

_____

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

1). DEXA: The risks associated with the DEXA are very low. The radiation you will receive in this study is less than 1/3000th of the FDA limit for annual exposure. Put another way, you could receive 3000 DEXA scans in a single year and still not meet the FDA limit for radiation exposure. In this study, you will receive only a single scan. The more radiation you receive over the course of your life, the more is the risk of having cancerous tumors or causing changes in genes. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risks is not known. Women who are pregnant or could be pregnant should receive no unnecessary radiation and should not participate in this study.

2). Blood Samples: The risks associated with blood drawing include bruising, vein inflammation, slight risk of infection, local soreness, and fainting. These are all very minor risks and if present, are generally resolved within a few days.

3). Resting Metabolic Rate measurement: There is no known risk associated with this procedure. You may experience some minor discomfort associated with this measurement if you have claustrophobia, but this is very unlikely. The canopy used is a large, see-through plastic bubble. There is adequate space and breathing is unrestricted, whether you are in the canopy or you use a mouthpiece.

4). Cardiorespiratory Fitness and Exercise: The exercise test is a standard test for determining the presence of heart and lung problems. 1 in 10,000 individuals with cardiovascular disease may die and 4 in 10,000 may have abnormal heart beats or chest pain. The exercise session on the EX trail will be less intense than your fitness test, so the risks are less. However, as with any exercise, there is the possibility of muscle soreness and muscle, bone, or joint injury.

5). Research Diets: There is a small risk that you could get a food-borne illness from the research diets. The food will be prepared under the supervision of a nutritionist, trained in food safety. Meal preparation will occur in nutrition laboratories in the Department of Nutrition at CSU or in kitchens which supply meals to campus residence halls. All food preparation will be done in accordance with standard procedures designed to minimize the risk of illness. Thus, the likelihood of a food-borne illness is remote.

It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

BENEFITS:

You will receive detailed diet and body composition data, and information on the role of diet and exercise in maintenance of health. You will receive 2 days of food at no cost to you.

COMPENSATION

The expectation is for you to complete the entire study. You will be paid $50 upon completion of both trials. If you finish one trial and elect to not do the second trial, you will be paid only $25.

DO I HAVE TO TAKE PART IN THE STUDY? Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

WHO WILL SEE THE INFORMATION THAT I GIVE?
We will keep private all research records that identify you, to the extent allowed by law. Your information will be combined with information from other people taking part in the study. When we write about the study to share it with other researchers, we will write about the combined information we have gathered. You will not be identified in these written materials. We may publish the results of this study; however, we will keep your name and other identifying information private.
We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places under lock and key.

CAN MY TAKING PART IN THE STUDY END EARLY? As mentioned, we are aware that this study requires a significant time commitment from you as a volunteer. It is very important to the study that you not miss scheduled visits with study personnel. In the event that something comes up that will make you miss a visit, please call and let us know. Please also note that we may call you if a visit is missed, simply to check and make sure everything is OK. If you have conflicts that require you to miss more than 10% of your scheduled visits, we will have to remove you from the study. If this happens, we will contact you and let you know the reason why you will not be allowed to continue, and make arrangements to pay you for the portion(s) of the study you have completed. Should our testing reveal information that suggests you need to be referred for medical care and you are a CSU student, we will put you in contact with Dr.Risma, at the Hartshorn Health Service at Colorado State University, who is the physician contact for this study. If you are not a CSU student, you will be referred to your primary care physician.

WHAT HAPPENS IF I AM INJURED BECAUSE OF THE RESEARCH? The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury. In light of these laws, you are encouraged to evaluate your own health and disability insurance to determine whether you are covered for any injuries you might sustain by participating in this research, since it may be necessary for you to rely on individual coverage for any such injuries.

WHAT IF I HAVE QUESTIONS?
Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions about the study, you can contact the investigators, Chris Melby at 970-491-6736 or Matthew Hickey at 970-491-5727. If you have any questions about your

rights as a volunteer in this research, contact Janell Barker, Human Research Administrator at 970-491-1655. We will give you a copy of this consent form to take with you.

PARTICIPATION:
Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled. Your signature acknowledges that you have read the information stated and willingly sign this consent form.

_____ _____
Signature of person agreeing to take part in the study Date

_____
Printed name of person agreeing to take part in the study

_____ _____
Name of person providing information to participant Date

Appendix II

EATS-26

Please place and (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.

PARTICIPANT ETHNICITY IDENTIFICATION FORM

Nutrition and metabolic Fitness Laboratory, Colorado State University

ID #_____ Date: _____

1. Please identify your ethnicity: _____ A. Mexican American G. Other Spanish
B. Mexican/Mexicano H. Caucasian
C. Puerto Rican I. Black
D. Cuban J. Asian/Pacific
E. Other Latin American Islander
F. Native American

2. What are your parents' surnames? Father: _____
Mother: _____

3. What are your parents' countries of origin? Father: _____
Mother: _____

4. Please identify the ethnicity of your 4 grandparents: Father's father: _____
(Use the letters from Question #1) Father's mother: _____
Mother's father: _____
Mother's mother: _____

5. What is your primary (first) language spoken?

Food Preferences and Acceptability Questionnaire

Please circle the number that corresponds to your response.

Have not tried item (check box)	Food Item	Like Extremely	Like Very Much	Like Moderately	Like Slightly	Neither Like Nor Dislike	Dislike Slightly	Dislike Moderately	Dislike Very Much	Dislike Extremely
☐	**Apple**	1	2	3	4	5	6	7	8	9
☐	**Banana**	1	2	3	4	5	6	7	8	9
☐	**Bell Peppers**	1	2	3	4	5	6	7	8	9
☐	**Broccoli**	1	2	3	4	5	6	7	8	9
☐	**Carrots**	1	2	3	4	5	6	7	8	9
☐	**Cheerios**	1	2	3	4	5	6	7	8	9
☐	**Chicken**	1	2	3	4	5	6	7	8	9
☐	**Chocolate Chip Cookies**	1	2	3	4	5	6	7	8	9
☐	**Cucumber**	1	2	3	4	5	6	7	8	9
☐	**French Bread**	1	2	3	4	5	6	7	8	9
☐	**Granola Bar**	1	2	3	4	5	6	7	8	9
☐	**Honey Nut Cheerios**	1	2	3	4	5	6	7	8	9
☐	**Italian Dressing**	1	2	3	4	5	6	7	8	9
☐	**Lettuce**	1	2	3	4	5	6	7	8	9
☐	**Mayonaise**	1	2	3	4	5	6	7	8	9
☐	**Mozzarella Cheese**	1	2	3	4	5	6	7	8	9
☐	**Mustard**	1	2	3	4	5	6	7	8	9
☐	**Oatmeal**	1	2	3	4	5	6	7	8	9
☐	**Oreo's**	1	2	3	4	5	6	7	8	9
☐	**Parmesan Cheese**	1	2	3	4	5	6	7	8	9
☐	**Parsley**	1	2	3	4	5	6	7	8	9
☐	**Peaches**	1	2	3	4	5	6	7	8	9
☐	**Peanut Butter**	1	2	3	4	5	6	7	8	9
☐	**Pears**	1	2	3	4	5	6	7	8	9
☐	**Pepperoni**	1	2	3	4	5	6	7	8	9
☐	**Pizza**	1	2	3	4	5	6	7	8	9
☐	**Provolone Cheese**	1	2	3	4	5	6	7	8	9
☐	**Pudding**	1	2	3	4	5	6	7	8	9
☐	**Red Onion**	1	2	3	4	5	6	7	8	9
☐	**Romano Cheese**	1	2	3	4	5	6	7	8	9
☐	**Sausage**	1	2	3	4	5	6	7	8	9
☐	**Skim Milk**	1	2	3	4	5	6	7	8	9
☐	**String Cheese**	1	2	3	4	5	6	7	8	9
☐	**Tomato**	1	2	3	4	5	6	7	8	9
☐	**Tomato Sauce**	1	2	3	4	5	6	7	8	9
☐	**Triscuit's**	1	2	3	4	5	6	7	8	9
☐	**Turkey**	1	2	3	4	5	6	7	8	9
☐	**Wheat Thins**	1	2	3	4	5	6	7	8	9
☐	**Whole Wheat Bread**	1	2	3	4	5	6	7	8	9
☐	**Whole Wheat Pasta**	1	2	3	4	5	6	7	8	9
☐	**Whole Wheat Tortilla**	1	2	3	4	5	6	7	8	9
☐	**Yogurt**	1	2	3	4	5	6	7	8	9

Please list any food allergies you have:

Appendix III

Colorado State University
CONFIDENTIAL HEALTH HISTORY QUESTIONNAIRE

Study_____ Date_____ Subject ID_____

Reviewed by PI: _____

PLEASE PRINT

Current Age_____ Height_____
Weight_____

GENERAL MEDICAL HISTORY

	YES	NO	
Do you have any current medical conditions? explain:	☐	☐	If Yes, please
Have you had any major illnesses in the past? explain:	☐	☐	If Yes, please
Have you ever been hospitalized or had surgery? explain: (include date and type of surgery, if possible)	☐	☐	If Yes, please
Have you ever had an electrocardiogram (EKG)? explain: (a test that measures your heart's activity using an electrical tracing)	☐	☐	If Yes, please

Are you currently taking any medications, including aspirin, hormone replacement therapy, or other over-the-counter medications? YES ☐ NO ☐ If yes, please explain:

Medication	Reason	Times taken per Day	Taken for how long?

PI Initials_____

Are you currently taking any nutritional supplements, such as Ginko, St. John's Wort, or others?

YES ☐ NO ☐ If Yes, please explain:

Supplement	Reason	Times taken per Day	Taken for how long?

Have you been diagnosed with diabetes? YES ☐ NO ☐ If yes, please explain:

Age at diagnosis_____

Have you been diagnosed with a thyroid disorder? YES ☐ NO ☐ If yes, please explain:

FAMILY HISTORY

Please indicate the current status of your immediate family members.

	Age (if alive)	Age of Death	Cause of Death
Father	_____	_____	_____
Mother	_____	_____	_____
Brothers/Sisters	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____

_____ _____ _____

Do you have a family history of any of the following: (Blood relatives only, please give age at diagnosis if possible)

	YES	NO	Relation	Age at Diagnosis
a. High Blood Pressure	☐	☐	_____	
b. Heart Attack	☐	☐	_____	
c. Coronary bypass surgery	☐	☐	_____	
d. Angioplasty	☐	☐	_____	
e. Stroke	☐	☐	_____	
f. Diabetes	☐	☐	_____	
g. Obesity	☐	☐	_____	
h. Other (Please List)	☐	☐	_____	

PI Initials_____

MUSCULOSKELETAL HISTORY

	YES	NO
Any current muscle injury or illness?	☐	☐
Any muscle injuries in the past?	☐	☐
Muscle pain at rest?	☐	☐
Muscle pain on exertion?	☐	☐
Any current bone of joint (including spinal) injuries?	☐	☐
Any previous bone or joint (including spinal) injuries?	☐	☐
Painful joints?	☐	☐
Swollen joints?	☐	☐
Edema (fluid build up)?	☐	☐
Pain in your legs when you walk?	☐	☐

If you checked YES to any of the above, you will be asked to clarify your response by an investigator so we can be sure to safely determine your ability to participate

NEUROLOGICAL HISTORY	YES	NO
History of seizures	☐	☐
Diagnosis of epilepsy	☐	☐
History of fainting	☐	☐

YES NO

GASTROINTESTINAL HISTORY

History of ulcers	☐	☐
History of colitis	☐	☐
History of chronic diarrhea	☐	☐
History of chronic constipation	☐	☐

REPRODUCTIVE HISTORY	YES	NO
Currently pregnant	☐	☐
Think you might be pregnant	☐	☐
Planning on becoming pregnant in the near future	☐	☐
Currently using Oral Contraceptives	☐	☐
History of Menstrual cycle irregularities	☐	☐
Hysterectomy	☐	☐

PI Initials_____

TOBACCO HISTORY (check any that apply)

None	☐	
Quit	☐	(when)_____
Cigarette	☐	
Cigarette	☐	
Pipe	☐	
Chew Tobacco	☐	
Snuff	☐	

CURRENT TOBACCO USE (if applicable)

	# per day
Cigarette	_____
Cigar	_____
Pipe	_____
Chew Tobacco	_____
Snuff	_____

Total Years of tobacco use_____

CARDIORESPIRATORY HISTORY

	YES	NO
Presently diagnosed with heart disease	☐	☐
History of heart disease	☐	☐
Heart murmur	☐	☐
Occasional chest pain or pressure	☐	☐
Chest pain or pressure on exertion	☐	☐
Heart valve problem	☐	☐
Abnormal heart rhythm	☐	☐

Edema (fluid build up)	☐	☐
High Cholesterol	☐	☐
History of rheumatic fever	☐	☐
Episodes of fainting	☐	☐
Daily coughing	☐	☐
High blood pressure	☐	☐
Shortness of breath	☐	☐
At rest	☐	☐
Lying down	☐	☐
After 2 flights of stairs	☐	☐
Asthma	☐	☐
Emphysema	☐	☐
Bronchitis	☐	☐
History of bleeding disorders	☐	☐
History of problems with blood clotting	☐	☐

If you checked YES to any of the above, you will be asked to clarify your response to an investigator so we can be sure to safely determine your ability to participate.

PI Initials_____

DIET HISTORY

	YES	NO
Have you ever dieted?	☐	☐

If YES, have you dieted within the past 12 months or are you currently on a diet?

	YES	NO
	☐	☐

If you have dieted within the past 12 months, please describe the diet:

a). Name (if applicable): _____

b). Prescribed by a Physician/nutritionist	YES ☐	NO ☐
c). Have you lost weight?	YES ☐	NO ☐

d). Duration of the diet?_____

What was your weight 12 months ago?_____

What was your weight 6 months ago?_____

	YES	NO
Have you dieted other than in the past 12 months?	☐	☐

If YES, please answer the following:

a). How many times have you dieted?

b). How old were you?

C). Weight loss (amount)?

	YES	NO
History of eating disorders?	☐	☐

EXERCISE HISTORY

How many times a week do you participate in moderate to high intensity exercise? (examples include jogging, biking, aerobics, basketball, swimming, etc.)_____

How long do these exercise sessions last? _____

You may be asked to complete a more detailed diet survey if you are volunteering for a research study.

Appendix IV

Initial Screening Data Sheet

Measurements: Day 1

Height (cm)	
Weight (kg)	
Hip circumference (cm)	
Waist circumference (cm)	
Blood Glucose (≤ 126 mg/dl)	
Blood Pressure (<140/90 mmHg)	
Pulse	
Pregnancy Test	
Date of Birth	

BMI (21-30) = Weight (kg) = _____ kg = _____

Height (m²) m²

Sample Diet ~2,000calories

Breakfast: 1 Medium Banana, 1 c Honey Nut Cheerios, 1 c Skim Milk
(Substitutions: Orange, Oatmeal, Yogurt)

Lunch: 3 oz Turkey, 2 slices Whole Wheat Bread, 2 Medium Lettuce Leafs, 2 Thin Slices Tomato, 1 Mustard Packet, 1 T Kraft Light Mayo, 10 Triscuit's, 1 c Canned Peaches in Light Syrup
(Substitutions: Chicken, Whole Wheat Tortilla, Cucumber, Italian Dressing, Wheat Thins, Canned Pears)

Dinner: 1.5 c Frozen Broccoli with Cheese Sauce (add option below)
(Substitutions: Frozen Carrots and Cheese Sauce)

Snacks: 1 c Skim Milk, 3 Nabisco Snackwell's Chocolate Chip Cookies, 1 Nature Valley Chewy Granola Bar
(Oreo's, Pudding)

Above Totals ~ 1671 calories, 51 g Fat, 238 g CHO, 81 g Pro (MyPyramid Analysis)

Healthy Choice Frozen Dinner Options:

1. Gourmet Supreme Pizza: 360 calories, 4 g Fat, 56 g CHO, 22 g Pro

2. Four Cheese Pizza: 370 calories, 3 g Fat, 58 g CHO, 25 g Pro

Daily Total with Dinner 1 = 2031 calories, 55 g Fat, 294 g CHO, 103 g Pro
58% CHO, 24% Fat, 20% Pro

Daily Total with Dinner 2 = 2041 calories, 54 g Fat, 296 g CHO, 106 g Pro
58% CHO, 24% Fat, 21% Pro

400 Calorie Addition:
Yoplait Original Yogurt, Kraft String Cheese (Red. Fat Mozz), Kashi Trail Mix Bar
= 380 calories, 10.5 g Fat, 54 g CHO, 19 g Pro
= 57% CHO, 25% Fat, 20% Pro

Test Shake

Aims:

Appropriate Volume
700-1,000 kcal
40-60 g Fat
20-40% CHO (Closer to 40% and preferably sucrose)

Proposed calculation:

0.75g Fat/kg
1.1g CHO/kg
0.35g Pro/kg

50 kg (110#)	**60 kg (132#)**	**70 kg (154#)**	**80 kg (176#)**	**90 kg (198#)**
37.5 g fat 337.5 kcals **54% Fat**	45 g fat 405 kcals **54% Fat**	52.5 g fat 472.5 kcals **54% Fat**	60 g fat 540 kcals **54% Fat**	67.5 g fat 607.5 kcals **54% Fat**
55 g CHO 220 kcals **35% CHO**	66 g CHO 264 kcals **35% CHO**	77 g CHO 308 kcals **35% CHO**	88 g CHO 352 kcals **35% CHO**	99 g CHO 396 kcals **35% CHO**
17.5 g Pro 70 kcals **11% Pro**	21 g Pro 84 kcals **11% Pro**	24.5 g Pro 98 kcals **11 % Pro**	28 g Pro 112 kcals **11% Pro**	31.5 g Pro 126 kcals **11% Pro**
627.5 kcals	**753 kcals**	**878.5 kcals**	**1,004 kcals**	**1129.5 kcals**

Shake Ingredients:

Whole Milk
1 cup (236g):
160kcals, 70kcals from fat
7.8g Fat, 4.5g Sat Fat, 13g CHO, 8g Pro
cholesterol

Heavy Whipping Cream
1T (14.333g):
50kcals, 50kcals from fat
5g Fat, 3.5g Sat Fat, 20mg

Whey Protein Powder
1 scoop (21g):
80 kcals, 5kcals from fat

<1g Fat, 2g CHO, 16g Pro

Hershey's Chocolate Syrup
1 T (39g)
100kcal, 24g CHO, <1g Pro

Sugar

Carbohydrate Breakdown:

11 g lactose per 1 cup milk
g sugar = g sucrose
Whey Protein Powder: residual lactose and maltodextrin (no fructose or glucose)
Hershey's: 50.2% Sugar per 1 T
(21.3% Dextrose, 13.7% Fructose, 8.3% Maltose, 7.4% Sucrose)

_____kg subject. Test Shake will consist of:

Shake	70 kg Subject
#kg x 3.37 = g Whole Milk	70 x 3.37 = 235.9g
#kg x 1.64 = g Heavy WC	70 x 1.64 = 114.8g
#kg x 0.30 = g Whey Protein	70 x 0..30 = 21g
#kg x 1.11 = g Choc Syrup	70 x 1.11 = 77.7
#kg x 0.20 = g Sugar	70 x 0.20 = 14

Example: 60kg subject

Shake #3

Ingredient	Amount	Kcals	Fat	CHO	Pro
Whole Milk	236g (1 c)	160	7.8g 70.2 kcals	13g	8g
Heavy WC	114.7g (8 T)	400	44.4g 400 kcals	--	--
Whey Protein	21g (1 scoop)	80	<1g 5 kcals	2g	16g
Chocolate Syrup	78g (2 T)	200	--	48g	--
Sugar	14 g	56	--	14	--
Total g			**52.2g** x9 = 469	**77g**	**24g**
Total kcals		**896**	**475.2** **53% Fat**	**308** **34% CHO**	**96** **11% Pro**

Comparison for 70kg subject:

Proposed Calculation	Test Shake
878.5 kcals	896 kcals
52.5 g Fat (54% Fat)	52.5 g (53% Fat)
77 g CHO (35% CHO)	77 g (34% CHO)
24.5 g Pro (11% Pro)	24 g (11% Pro)

Appendix V

VO_2 Max – Met Cart Instructions

- **Warm Up:** If first test to be run allow 30 minutes for cart to warm up.
- **Remove white flow detection box from the back of the mixing chamber.**
- **Gas Calibration –** Double click on TrueOne32 Exercise
    - Select Gas Calibration from left hand side of screen.
    - Connect tubing from gas tank attached to met cart (Exercise – 4.01% CO_2, 15.98% Oxygen) to the cal gas slot on the back of the analyzer module.
        - Two black switches below cal gas slot:
            - O_2 range should stay on 25%
            - Input Select should stay on Polar
    - Enter temperature (Celsius), barometric pressure (mm), and humidity (%)
        - To change units on black weather box first select measurement and push the units button twice.
    - Click Ok and follow prompts to "Turn on Cal Gas to 3psi"
        - Turn black handle on top of gas tank counter-clockwise 90 degrees.
    - Room Air is sampled for 15 seconds to achieve steady flow.
    - Room Air is sampled for 5 seconds.
    - Cal Gas is sampled for 15 seconds to achieve steady flow.
    - Cal Gas is sampled for 5 seconds.
    - Follow prompt to "Turn off Cal Gas"
        - Turn black handle on top of gas tank clockwise to off position.
    - If CO_2 is greater than 1%, repeat gas calibration.
    - Save updated gas calibration parameters.
- **Flowmeter Calibration**
    - Select Flowmeter calibration from left hand side of screen.
    - Connect breathing tube to clear attachment of Hans Rudolph 2-way valve (half inch covering) and attach the small white portion of the 2-way valve to the calibration syringe.
    - Enter temperature (Celsius), barometric pressure (mm), and humidity (%).
    - Sample Baseline.
    - Turn off green power switch on back of dilution pump.
    - To complete calibration:
        - Complete one detection stroke
        - Complete four flushes using a quick, steady stroke

        - Complete 5 strokes:
            - Between 50-80
            - Between 100 and 199
            - Between 200 and 299
            - Between 300 and 399
            - Between 400 and 499
    - Repeat calibration if the difference between low and high values on Flowmeter Calibration Conformation screen is greater than 0.5 or 5%.
    - Save calibration data.
- **Test**
    - Adjust bike seat to subject.
    - Fit headgear and mouth piece to subject.
    - Attach breathing tube to subject's mouth piece.
    - Select VO_2/Metabolic Testing from left hand side of the screen.
    - Enter subject ID, age, sex, height and weight.
    - Enter your initials under Tech and select Maximal for Test Degree.
    - At "Ready to Start Testing" window, wait 1 minute or until $FECO_2 > 3\%$
    - Set cadence at 50 rpm
    - Females
        - Initial workload is 1 kp for 2 mins
        - Increase the workload by 0.5 kp each 2 minutes
    - Males
        - Initial workload is 2 kp men for 2 minutes
        - Increase the workload by 1 kp each 2 minutes