THESIS

THE EFFECTS OF A SINGLE BOUT OF EXERCISE, WITH ENERGY

REPLACEMENT, ON PLASMA CONCENTRATIONS OF TNF-α FOLLOWING

CONSUMPTION OF A HIGH-FAT, HIGH-CARBOHYDRATE MIXED MEAL.

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ABSTRACT

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CONSUMPTION OF A HIGH-FAT, HIGH-CARBOHYDRATE MIXED MEAL.

Background: Cardiovascular disease (CVD) is a major contributor to all-cause mortality in the United States and is a major cause of long-term disability. The risk for CHD typically includes a panel of fasting blood lipids including total, LDL-, and HDL-cholesterol. However, in recent years, the lipemic response to a meal and the accompanying pro-inflammatory molecules are recognized as independent risk factors for the development of CVD. Attention has focused on the vascular endothelium, where an increase in low-grade inflammatory processes leads to the development and progression of vascular insults ultimately contributing to an advanced, complicated atherosclerotic lesion. A large body of evidence suggests that improvements in postprandial lipemia, such as decreased triglycerides (TG) and improved insulin sensitivity can be achieved following an individual exercise session. However, limited data exists elucidating the effects of a prior acute bout of moderate intensity exercise on possible pro-inflammatory changes during the postprandial period.   Research consistently shows that an important determinant of the acute metabolic changes associated with a single session of exercise are energy-expenditure dependent.   It is of clinical importance to understand the

postprandial metabolic response after an acute bout of exercise in which the caloric cost of exercise has been balanced with additional energy intake, i.e. with energy replacement.

Purpose: Therefore, the purpose of this study was to determine the effects of a single bout of moderate intensity exercise, with energy replacement, on plasma tumor necrosis factor alpha (TNF-α) concentrations, measured before and after consumption of a high-fat, high-carbohydrate meal in young, non-obese, sedentary females.

Methods: Eight, non-obese, sedentary women participated in two seperate high-fat, high-carbohydrate meal tests after undergoing different treatments the following day. For the non-exercise condition (NoEx), the day prior to the postprandial challenge subjects refrained from exercise and consumed a prescribed diet. For the exercise condition (Ex) subjects consumed the same prescribed diet, and completed a cycle ergometer exercise bout at 65% peak heart rate, eliciting a net exercise energy expenditure of ~285 kcalories. Subjects then consumed an additional mixed-meal snack to account for the additional energy cost of the exercise. The following morning subjects reported to the laboratory in a fasted state at which time a venous blood sample was obtained. They were then fed a high-fat, high-carbohydrate meal (0.75g fat, 1.1g CHO, and 0.35 g protein/kg body weight), and postprandial venous blood samples were obtained 2, 4, and 6 hours after consumption meal for determination of plasma TNF-α concentrations.

Results: Plasma TNF-α concentrations were low in all subjects at baseline and dropped further by the second hour of the postprandial period returning toward baseline in both the Ex and NoEx conditions (time effect, $p < 0.05$). There was not a condition by time interaction on TNF-α concentration, nor a main effect of condition.

Conclusions: The results suggest that a single session of moderate intensity exercise, without an energy deficit, results in no significant effect on fasting or postprandial TNF-α plasma concentrations in young, non-obese, sedentary females. However, discretion is advised while interpreting the findings due to the very low concentrations of TNF-α measured in the plasma of these young women.

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CHAPTER I

INTRODUCTION

The dramatic rise in obesity over the last two decades has been labeled as a main contributor to the global increase in various chronic diseases including cardiovascular disease (CVD), diabetes, and the metabolic syndrome. From 1980 through 2008, obesity rates for adults have doubled and rates for children have tripled [1]. The obesity rates for all groups in society regardless of age, sex, race, ethnicity, socioeconomic status, education level, or geographic region have increased markedly as well during the past several decades [1]. Chronic diseases are the largest cause of death worldwide, led by CVD, cancer, chronic lung diseases, and type 2 diabetes [9] and are expected to increase substantially over the next 20 years [2]. Cardiovascular disease is broadly defined as those diseases that involve the heart or blood vessels. It encompasses a plethora of disorders including coronary heart disease (CHD), stroke, peripheral vascular disease, and atrial fibrillation. Between 1990 and 2020, mortality from heart disease in developing countries is expected to increase by 120% for women and 137% for men [10].

CVD Disorders are typically preceded by atherosclerosis, a condition which over time results in an advanced, complicated lesion within arterial walls. Evidence from research in recent years has emphasized a link between the atherosclerotic process and low-grade inflammation, revealing that atherosclerosis is not just the passive accumulation of lipids within the arterial wall. Oxidative components of modified

low-density lipoprotein (LDL) induce a chronic inflammatory process involving the arterial endothelium ultimately resulting in the complications of atherosclerosis [11]. Traditional risk factors associated with the development of coronary heart disease and progression of atherosclerosis include high plasma concentrations of: cholesterol, in particular those of low-density lipoprotein (LDL); triglycerides (TG); and low plasma concentration levels of high-density lipoprotein (HDL). Recently however, markers of inflammation, such as C-reactive protein (CRP), and leukocyte count, as well as pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) have been shown to be elevated in CHD [12]. Increased adiposity, specifically visceral adipose tissue, enhances production or TNF-α and IL-6 both of which can alter the normal homeostatic properties of the endothelium. Thus, inflammatory processes may therefore be potential targets of therapy in preventing or treating CHD.

Typically, clinical factors of CHD are measured after an 8- to 12- hour fast. However, considering a significant portion of life is spent in the postprandial state, recent research has demonstrated that the metabolic features of this period may be important in determining risk for atherosclerosis [13, 14]. Several studies have linked postprandial lipemia to the development of CVD, specifically focusing on the development and progression of atherosclerosis [13, 15]. These studies have linked CHD to the metabolic perturbations that follow ingestion of a meal including increased TG levels, elevated oxidative stress and pro-inflammatory markers, and reduced flow-mediated dilation [16, 17]. Pro-inflammatory markers are of interest specifically because a heightened pro-inflammatory response precedes and perpetuates the atherosclerotic process [18]. Ingestion of a high fat meal has been shown to increase levels of several

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pro-inflammatory markers, including TNF-α, IL- 6, and leukocytes, in healthy

individuals as well as those with increased risk for the metabolic syndrome and diabetes

[17, 19]. These markers have been added to an expanding list of emerging or

nontraditional risk factors for the atherosclerotic process as they mirror oxidative stress

and can promote vascular damage, endothelial injury, plaque rupture, and

atherothrombosis [20].

TNF-α, which is overproduced in obese patients and implicated in low-grade

systemic inflammation in both obese and normal weight individuals, plays a significant

role in these processes and can lead to the development and/or augmentation of various

chronic diseases. For example, TNF-α levels are markedly elevated in advanced heart

failure, and in experimental setting can induce left ventricular dysfunction and

cardiomyopathy [21, 22]. TNF-α has also been found to be elevated in obese and normal

weight individuals following a meal challenge [17]. Considerable data has shown that in

normolipemic individuals the postprandial response lasts upward of 6 hours [23], and

because an individual typically consumes three meals plus snacks in a day, one can

assume that ingestion of meals will overlap with the previous post-prandial period. This

continual insult from frequent meal challenges can potentially lead to a chronic pro-

inflammatory and dyslipidemic state, setting the stage for future disease development.

Physical activity is well known in offering protection against all-cause mortality,

primarily by protection against obesity, diabetes, and cardiovascular disease [9, 24].

Studies have shown that regular exercise contributes to an improved cardio-metabolic

risk profile following exercise by improving glucose tolerance and insulin sensitivity,

decreasing the postprandial rise in TG, rendering lipid profiles less atherogenic, and

decreasing the amount and/or activity pro-inflammatory biomarkers in both healthy and diseased individuals after a meal challenge [25, 26]. Research has also determined that there are beneficial effects of a single exercise bout on postprandial metabolism, typically related to the induced energy expenditure during the exercise session. However, as adult individuals often tend to increase energy intake when they exercise [7, 8], it is of clinical importance to understand the effects of exercise of postprandial metabolism when energy-intake is increased, thus negating the net cost of exercise.

Given that induction of low-grade systemic inflammation after a meal challenge may be involved in atherosclerosis and coronary heart disease pathogenesis, and given recent findings that a single session of moderate exercise with energy deficit may induce a decrease the systemic levels and/or activity of pro-inflammatory cytokines, it is important to understand if physical exercise will exert any protective effects on postprandial metabolism in the face of energy replacement. To date, no studies have investigated the effect on postprandial metabolism of a single bout of moderate exercise with energy replacement in females, and no studies to date have investigated the response in females to the pro-inflammatory cytokine TNF-α under the same conditions. Therefore, this study was undertaken to accomplish the following specific aims and investigate the subsequent hypotheses:

Specific Aims and Hypotheses:

Aim #1: To determine if fasting plasma TNF-α concentrations are different 12 hours following a single bout of moderate, aerobic exercise with energy replacement compared to a non-exercise control condition in healthy, sedentary, non-obese females.

Hypothesis #1: A single bout of moderate, aerobic exercise will not decrease the measured baseline plasma TNF-α values 12 hours after completion of exercise with energy replacement relative to the non-exercise condition in healthy, sedentary, non-obese females.

Aim #2: To determine if measured plasma TNF-α during a 6-hour postprandial period following consumption of a high-fat, high-carbohydrate meal is different 12 hours following a single bout of moderate, aerobic exercise with energy replacement in healthy, sedentary, non-obese females versus a non-exercise condition in the same individuals.

Hypothesis #2: A single bout of moderate, aerobic exercise will decrease the 6-hour postprandial plasma TNF-α concentrations relative to the non-exercise condition following ingestion of a high-fat, high-carbohydrate meal.

CHAPTER II

LITERATURE REVIEW

Obesity

Obesity is a multifarious, chronic condition that over the past few decades has become a global epidemic. It is associated with many co-morbidities related to health and is the second highest cause of preventable deaths in the United States - only tobacco use provides a higher preventable death rate. Obesity is not only a concern for adults, as the number of overweight and obese children has nearly doubled in the past two to three decades in the United States [28]. Body Mass Index (BMI) is an analytical measurement used to approximate body fatness based on the weight and height of an individual. It is defined as the weight of an individual in kilograms divided by the square of their height in meters. Table 1 shows the classification developed by a National Heart, Lung, and Blood Institute task force, along with the associated disease risk with increasing BMI [NIH]. BMI has been utilized by the WHO since the 1980's and is currently one of the most accepted method to screen for overweight and obesity because of the relative ease in obtaining the weight and height measurements necessary to calculate BMI.

The World Health Organization defines "overweight" as an individual with a BMI of 25 or more and "obese" as someone with a BMI of 30 or higher. It is projected that by 2018, in the United States alone, 43% or 103 million American adults will be considered obese [29]. Health care costs related to obesity – which is associated with conditions such as cardiovascular disease (CVD), hypertension, and diabetes - would total $344

billion in 2018, or more than one in five dollars spent on health care, if the trends continue [29]. Therefore, obesity remains a leading public health concern and the link between obesity and CVD is well recognized, with many studies concluding that obesity is strongly related to the development of CVD.

Obesity and Cardiovascular Disease

Obesity is an important determinant of cardiovascular disease. In fact, obesity is documented as being an independent risk factor for the development of CVD, and CVD risks have been documented in both obese adults and children [30]. Cardiovascular disease continues to be the principal cause of death in the United States, Europe, and much of Asia [11]. In 2006 in the United States alone, it was estimated that 831,000 lives were claimed by CVD and 81 million individuals had one or more forms of CVD [31]. The incidence of obesity in children and adolescents has more than tripled in the past 30 years. The prevalence of obesity in children aged 6-11 years old has increased from 6.5% in 1980 to 19.6% in 2008. The prevalence of obesity in children aged 12-19 years old increased from 5.0% to 18.0% [31]. In a population-based sample evaluating the prevalence of high body mass index (BMI) among children and adolescents in the United States, 70% of obese youth aged 5- to 17-year olds had at least one risk factor for cardiovascular disease [32, 33]. Regardless of age, overweight and obesity predispose individuals to an array of cardiac complications such as coronary heart disease, hypertension, stroke, and heart failure, due to the imposed stress on the cardiovascular system.

Cardiovascular disease is not a single disease or condition. Rather, it is a broad category that includes any diseases of the heart, arteries, and veins. One of the most common of all cardiovascular diseases is coronary heart disease (CHD) characterized by plaque accumulation in one or more coronary arteries. Traditional risk factors for CHD-positive family history, diabetes, high-blood pressure, obesity, lack of physical inactivity elevated cholesterol levels– explain a significant amount of morbidity and mortality, but do not fully account for the CHD burden [11, 34, 35]. The pathogenesis of obesity and its relationship with CHD is complex and not fully understood, but the two appear to be casually related based on the emergence of obesity as an independent risk factor for atherosclerosis. Atherosclerosis is a condition characterized by endothelial dysfunction, vascular inflammation, cholesterol deposition, and cellular debris within the intima of the vessel wall [35, 36]. Large and medium-sized arteries are particularly vulnerable [11] and atherogenesis in these vessels can lead to ischemia of the heart, brain, or extremities, resulting in infarction. The feature of this condition is an atherogenic dyslipidemic profile consisting of elevated triglycerides, high plasma concentrations of cholesterol, particularly in the form of low density lipoproteins (LDL), and low levels of high-density lipoprotein (HDL) [11]. There are also qualitative changes in the lipoprotein profile affecting the ability of HDL to promote cholesterol efflux and enhance the tendency of LDL to become easily oxidized [18]. Despite lifestyle changes and pharmacological approaches to lower plasma cholesterol in at-risk patients, CVD continues to affirm itself as a leading cause of mortality [37].

It is generally agreed that a heightened pro-inflammatory response precedes the atherosclerotic process [11, 38]. Recent studies have shown that circulating inflammatory

molecules are associated with disease activity and are linked with a transition from primal lesions to more advanced plaques [35]. Thus, the link between obesity and CVD may be pro-inflammatory cytokines released into circulation inducing unfavorable effects on the cardiovascular system. Studies have shown that obesity is associated with oxidative stress and elevations in the tissue expression and plasma concentrations of tumor necrosis alpha (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), vascular cell adhesion molecule-1 (VCAM-1), and plasminogen activator inhibitor-1 (PAI-1), all of which are pro-inflammatory mediators of inflammation [39]. CRP, for instance, has been shown predict and correlate independently with major cardiovascular end points, such as myocardial infarction [40]. In hypertensive patients, TNF-α and IL-6 were both independent predictors of cardiovascular events [41]. These inflammatory mediators mirror oxidative stress and generation of free radicals that can promote vascular damage, endothelial injury, and plaque rupture [20].

Atherosclerosis – An Inflammatory Disease

Atherosclerosis is a pathological condition that is responsible for underlying numerous major vascular events including coronary artery disease (CAD), stroke, peripheral artery disease, and myocardial infarction responsible for most of the mortality and morbidity present in the world today. Traditional factors for CVD – positive family history, smoking, hypertension, dyslipidemia – explain a bulk of the morbidity and mortality, especially when combined with abdominal obesity, sedentary lifestyle, and diet composition [18]. However, these traditional factors do not fully account for the entire CVD burden. Chronic low-grade inflammation is an ever-expanding area of research and

has been implicated at all stages of the development of atherosclerosis. Systemic chronic inflammation is involved in the process of repeated and incremental damage to the arterial wall that over time leads to vascular damage [18, 35]. The disease is characterized by the gradual assault of large- and medium-sized arteries mainly due to the accumulation of lipid-laden macrophages (primarily composed of oxidized LDL) that release a variety of pro-inflammatory mediators further enhancing the destruction of the vasculature. Although LDL remains the most important risk factor for atherosclerosis, numerous pathophysiologic observations in animals and humans have led to the conclusion that endothelial dysfunction, rather than the gradual wearing away of the vasculature, is the first major component to the progression of disease. In response to arterial injury, the endothelium is activated by a number of inflammatory substances, growth factors, and cytokines leading to the migration of these immunoresponsive cells. Inflammatory markers C-reactive protein (CRP), fibrinogen, the interleukins (IL's), TNF-α, and plasminogen activator inhibitor (PAI-I) have all been included in the listof non-traditional risk factors for the development of atherosclerosis. These markers can promote migration of immune cells, endothelial injury, vascular damage, plaque rupture, and finally an atherothrombotic event leading to myocardial infarction, stroke, or heart failure.

Although the cellular and molecular basis for the pathologic phenomena of atherosclerosis has not been fully elucidated, there is an increasing need to understand the process in order to identify key biomarkers that can facilitate the clinical diagnosis of CVD. The response-to-injury hypothesis of atherosclerosis, initially proposed that endothelial denundation was the first step in atherosclerosis [42]. The most recent

version of this hypothesis emphasizes endothelial dysfunction rather than denundation. The endothelial dysfunction that results from injury leads to compensatory responses that alter the normal homeostatic properties of the endothelium [11]. Atherosclerotic lesions begin to arise as a result of focal injury to the arterial endothelium, followed by adherence and aggregation of leukocytes. The attack of leukocytes (mainly mononuclear cells) is facilitated through an increase in chemotactic signals, leading to their adhesion, migration, and differentiation within the vessel wall. Endothelial dysfunction is also associated with high levels of oxidative stress and is driven by oxidized-LDL, which activates endothelial cells to express leukocyte adhesion molecules and facilitate migration into the subendothelial space. Over time, consistent resultant noxious stimuli only increase the damage sustained by the endothelium, eventually shifting the balance of the endothelial milieu toward one that promotes eventual failure of normal homeostatic repair mechanisms.
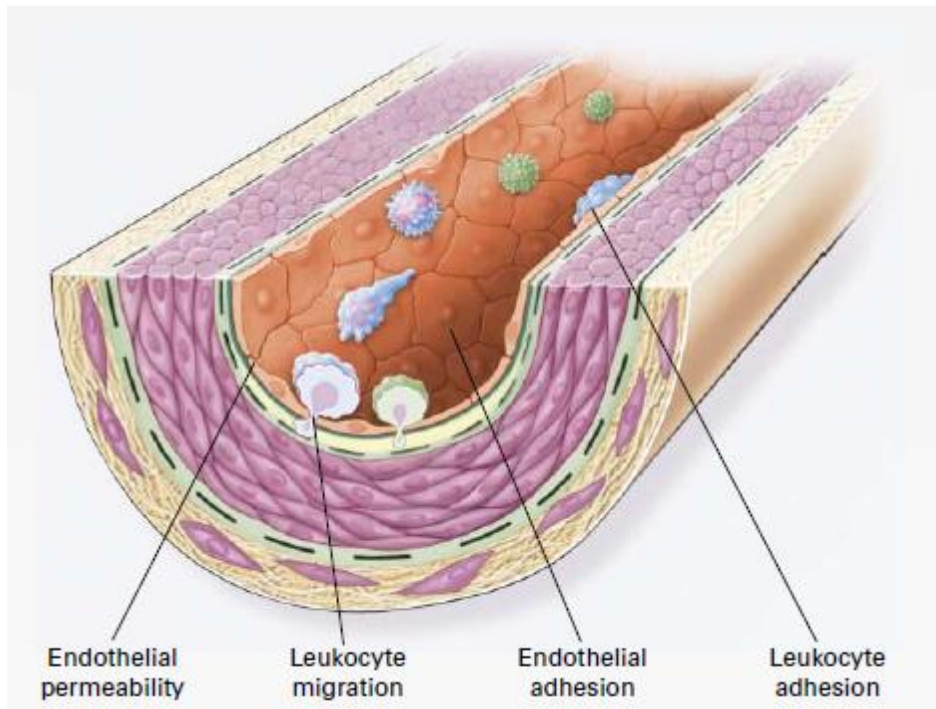
Figure 1. [Ross, R. 1999 *New England Journal of Medicine*]. An illustration of the earliest changes preceding the formation of the lesions of atherosclerosis resulting in endothelial dysfunction. Changes include increased permeability to lipoproteins and activation of the endothelium by a number of inflammatory substances, growth factors, and cytokines leading to the migration of immunoresponsive cells such as leukocytes.

The earliest type of lesion, the so-called "fatty streak", common to young infants and children, is a pure inflammatory lesion, consisting of monocytes and T lymphocytes [43]. The primary documented facilitator of monocyte migration and internalization is LDL remnant particles in plasma. Other mediators include free radicals caused by cigarette smoke, cytokines, hypertension, hyperglycemia, and diabetes. Monocytes enter through the arterial wall via transporter proteins where they differentiate into macrophages that stimulate the production of proinflammatory cytokines such as TNF-α and IL-6 [39]. These cytokines independently stimulate chemotactic proteins to recruit

more monocytes to the endothelial cell as well as increase the transcription of the LDL receptor gene [44]. Chemotactic proteins implicated in the atherosclerotic processes include MCP-1, intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1). Adhesion molecules are responsible for interactions between endothelial cells, leukocytes, and also have an important role in the biological activity of cytokines [45]. Macrophages are also capable of releasing growth factors and metalloproteinases, which lead to cell proliferation matrix degradation, respectively. If the initial inflammatory response does not effectively neutralize the offending stimuli, the process can happen indefinitely. Continued leukocyte adhesion and migration eventually increases endothelial permeability and facilitates the accumulation of even more atherogenic molecules into the vessel lumen.

When LDL becomes entrapped in the subendothelial space, particles undergo progressive oxidation leading to modified LDL (MM-LDL). MM-LDL can stimulate the production of numerous molecules implicated in the atherogenic process including monocyte chemotactic protein-1 (MCP-1), which promotes monocyte chemotaxis. Monocyte colony stimulating factor (M-CSF), a cytokine, is another molecule stimulated by MM-LDL and can promote the differentiation and proliferation of monocytes into macrophages [46]. The MM-LDL can then be internalized by these macrophages. Internalization of MM-LDL by macrophages leads to the formation of lipid peroxides and facilitates the accumulation of cholesterol esters, resulting in the formation of foam cells that are the hallmark of atherosclerosis. Foam cells, like macrophages, release proinflammatory cytokines and can encourage stimulation of vascular smooth muscle cell (SMC) migration and proliferation into the intima. Internalized smooth muscle cells can

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then ingest lipid themselves, become replaced with collagen and transform into foam cells as well. The combination of internalized LDL, macrophages, and foam cells all contribute to the continuous dysfunction of the artery. As foam cells and macrophages eventually die off, a protective fibrous cap then forms over this "fatty streak" leading to the appearance of an atherosclerotic lesion [35]. This fibrous cap, which overlies a cope or lipid and necrotic tissue, is known as an advanced and complicated lesion. Once the artery can no longer compensate by dilation the lesion may intrude into the lumen altering blood blow. The end result of this chronic process is usually an acute atherothrombotic vascular event.
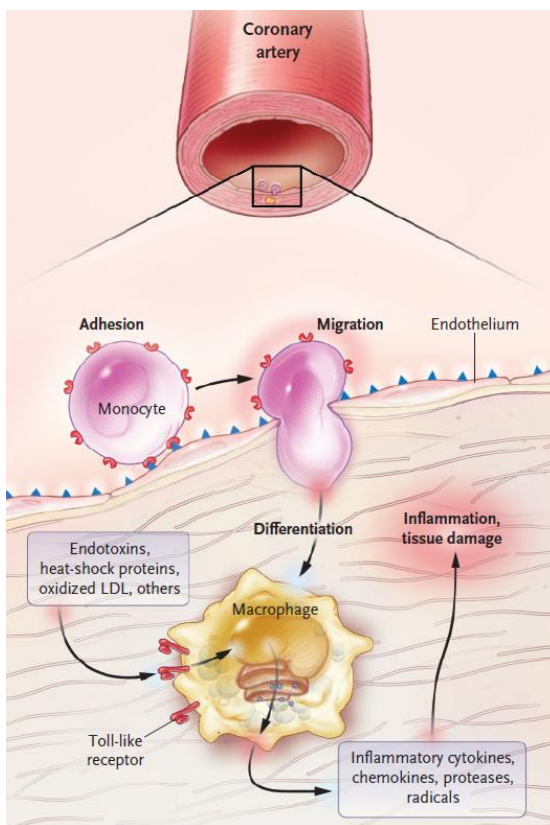

Figure 2. [Hansson, G. 2005 *New England Journal of Medicine*]

An attempt to independently link non-traditional biomarkers to the risk of atherosclerosis is an area of expanding research. If a biomarker is identified, and it

potentially provides significant prognostic value beyond established risk factors, this can possibly lead to health guidelines or pharmacological treatments in the prevention of the disease as well as augmentation of disease in progress [34]. In order to fully acknowledge an ideal biomarker, it should have the following characteristics: highly sensitive, specific, accessible, standardized, cost effective, and above all be easily interpretable by clinicians [35]. Further research will determine whether or not these biomarkers and risk factors are of clinical relevance and can be utilized in an effective manner.

Tumor Necrosis Factor Alpha (TNF-α)

Among many inflammatory markers, TNF-α has emerged as a key cytokine that influences intermediary metabolism and contributes to proatherogenic changes in lipid metabolism [47]. First recognized as an endotoxin induced serum factor that caused necrosis of tumors [48] and cachexia [49], it is now recognized that TNF-α may participate in the etiologic and pathologic processes related to future cardiovascular complications. Only recently when the role of inflammation in the development of atherosclerosis was documented and its importance accepted, were proinflammatory cytokines such as TNF-α regarded as potential players in the development of disease. The first reported relationship between inflammatory cytokines and metabolic disturbances were in obesity, recognized by increased concentrations of TNF-α in adipose tissue and in plasma [50]. Additional early studies showed that high-fat feedings in primary cultured human adipocytes induced the release of TNF-α [51].

TNF-α is a cytokine with a wide range of proinflammatory activities and is a member of a growing family of peptide mediators compromising at least 19 other cytokines [52]. It is a multifunctional protein that has been implicated in a number of metabolic disorders including obesity, dyslipidemia, insulin resistance, type II diabetes, and atherosclerosis. Synthesis and secretion of TNF-α is mainly governed by macrophages, but a wide variety of other cells (monocytes, T-cells, smooth muscle cells, adipocytes, and fibroblasts) can also participate [15, 28, 53]. Like other cytokines, TNF-α has tremendously diverse functions within the body including induction of apoptotic cell death, lysis of tumor cells, growth promotion and inhibition, suppression of lipoprotein lipase (LPL), and stimulation of other proteins, such as M-CSF, IL-1, VCAM-1, ICAM-1, and MCP [54, 55]. Because tumor necrosis factor plays such a diverse role in numerous metabolic processes, both in normal and pathophysiological conditions, it would be extremely difficult to describe all the actions appropriately. Therefore, the focus of this review will concentrate of TNF-α as a proinflammatory cytokine involved in intermediary metabolism and the pathogenesis of atherosclerosis.

Biological responses to TNF-α are mediated by ligand binding via two structurally distinct receptor isoforms: TNF receptor type 1 (TNF-R1; p55) and TNF receptor type 2 (TNF-R2; p75), which are both present on the membrane of all cell types except erythrocytes [56]. The two receptors differ significantly in their binding affinities as well as the intracellular pathways they activate or inhibit [36]. For example, Mackay and colleagues discovered that the TNF-R1 receptor was in control of TNF-induced cell adhesion to human endothelial cells [57]. This receptor also regulates activation of the NK-kB pathway, expression of ICAM-1, E-selectin, and VCAM-1 [57], and the lipolytic

effect of TNF-α on TGs [58]. TNF-R2 on the other hand has been found to play an important role in obesity associated CHD by neutralizing TNF-α actions, therefore attenuating the inflammatory cascade associated with atherosclerosis [59]. It has also been established that patients with peripheral vascular disease or MI survivors were found to have increased plasma TNF-RII concentrations compared to TNF-RI concentrations [60].

Although there are described differences in receptor response, there also seems to be important crosstalk between the two, responsible for the net response of a cell to TNF-α stimulation. Further research will elucidate the exact mechanisms each receptor stimulates and the implications of said activation. In addition to the activities the membrane receptors play in TNF-α signaling, both receptors are can be proteolytically cleaved to yield two soluble receptors (sTNFR1 & sTNFR2) both capable of binding systemic TNF-α [61]. Porteau and colleagues were the first to induce the systemic appearance of sTNFR from polymorphonuclear cells by stimulation with physiological peptides and pharmacological agents [62]. Data from hypoxia trials in the human macrophage cell line THP-1 and LPS administration into healthy volunteers also both revealed an increase in sTNFR levels systemically [63].

Even with the increase in appearance of sTNFR in numerous experiments, so far the exact function of the protein is unknown. Circulating sTNFR was first suggested to represent a buffer system against the excess release of TNF-α into circulation during inflammatory situations [61]. In line with this hypothesis, Bertini et al. showed that administration of sTNFR can protect mice from lethal effects of TNF-α and septic shock [64], suggesting the protective effect of sTNFR. However, Chu et al. found that

administration of a TNF-α neutralizing antibody containing sTNFR domains actually increased the biological effects of TNF-α [65]. Compared to circulating TNF-α levels, systemic sTNFR levels remain high for longer periods of time suggesting that measurements of sTNFR may be more useful for monitoring an inflammatory response. However, the discrepancy in the data calls for more research dedicated to an explanation of the exact mechanisms between TNF-α and sTNFR1 and 2 before using sTNFR as a measure of TNF-α activity.

TNF-α and Mechanisms of Atherosclerosis

The primary mechanisms by which TNF-α directly exacerbates atherosclerotic lesions and the risk of future adverse cardiovascular events is by upregulation of chemotactic proteins, vasoactive molecules, and growth factors all of which are involved in eventual endothelial dysfunction. TNF-α is also known to support the development of the atherosclerosis indirectly by interfering with the metabolic pathways of TGs and cholesterol leading to increased lipid and cholesterol deposition within arterial walls.

The plasma concentration of TNF-α and its two soluble receptors are a result of cytokine production from a number of tissues, including monocytes and macrophages [66]. A very early step by which TNF-α increases atherothrombotic disease is by enhancing expression of cellular adhesion molecules, causing adherence of circulating leukocytes to dysfunctional endothelial cells with ensuing transendothelial migration [67]. A number of adhesion molecules within the endothelium induced by TNF-α are implicated in this process including; P-selectin, E-selectin, ICAM-1, and VCAM-1. The expression of E- and P-Selectin and their ligands are responsible for the recruitment of

leukocytes along the endothelium. A number of small animal studies have demonstrated that E- and P-selectin mRNA transcription and protein expression is inducible by TNF-α in vivo and in vitro [40, 68]. The responsibility of ICAM-1 & VCAM-1 is to then freeze the leukocytes on the endothelium preparing them for transendothelial migration. TNF-α is one of the most potent inducers of VCAM-1 and ICAM-1 in a wide range of cell types. Ohta et al. demonstrated the importance of TNF-α induced adhesion molecules in vivo when TNF-α deficient ApoE$^{-/-}$ mice showed a reduction in lesion formation, with a concomitant decrease in VCAM-1 and ICAM-1 expression [69]. Transmigration of leukocytes through the intracellular junctions of endothelial cells into injured tissue appears to be mediated by platelet/endothelial cell adhesion molecule-1 (PECAM-1) and MCP-1 [38].

Numerous human and animal models of obesity researched to date have demonstrated an increase in macrophage infiltration of muscle and adipose tissue with a concomitant abnormal production of TNF-α mRNA and protein compared to their lean counterparts [50]. In human obesity the expression of TNF-α was in strong positive correlation with the degree of obesity (BMI) and the level of hyperinsulinemia. Obesity is also associated with an increase in FFA production and circulation, resulting in an acute increase of plasma TGs. Research has shown that administration of TNF-α and endotoxin (LPS) to mice and humans resulted in an acute increase of plasma TG concentration of ~85% [70]. Although lipid changes can be beneficial for the host during times of acute inflammatory conditions, prolonged TNF-α induced exposure to an increase in plasma TGs will increase atherosclerotic progression and subsequent morbidity and mortality.

There are several mechanisms behind the TNF-α induced increase in plasma TGs: The first is by increasing plasma FFA (the substrate for TG synthesis) production from both adipose tissue and liver [58]. Gual et al. demonstrated that TNF-α may also inhibit early insulin receptor signaling within cells, thereby thwarting the antilipolytic role of the hormone [71]. TNF-α has also been found to downregulate the expression of perillipins, a protective coating utilized by lipid droplets in adipocytes [72]. This increases lipid substrate availability and enhances the cleavage activity of hormone sensitive lipase (HSL), one of the main lipolytic enzymes in adipose tissue. A second mechanism by which TNF-α increases plasma TGs is by increasing VLDL production while decreasing VLDL clearance. TNF-α can increase hepatic TG synthesis by increasing hepatic levels of citrate, and allosteric activator of acetyl-coA carboxylase, the rate-limiting enzyme in FFA synthesis [73]. Numerous studies [47, 73] have also shown a decrease in VLDL clearance due to downregulation of LPL activity. In vivo and in vitro studies in both humans and rodents have shown that inhibition of LPL is seen at both the transcriptional and post-transcriptional level, thereby promoting decreased triglyceride clearance. Finally, besides increasing the concentration of VLDL particles, TNF-α may also alter their composition. Memon et al. demonstrated that the content of sphingolipids has been shown to increase with TNF-α administration [70]. Sphingolipid modification not only can lead to a decreased clearance in circulation, but can also facilitate uptake by the LDL receptor on macrophages, leading to an increase in the formation of foam cells.

Not only has TNF-α been demonstrated to interfere with metabolic modifications affecting TG metabolism, but it also has been shown to directly interfere with cholesterol regulatory pathways. In the context of cholesterol production, studies have only shown

an increase in serum concentrations of total cholesterol and hepatic cholesterol synthesis in rodents [47, 74]. Humans on the other hand, show either no change or a decrease in serum cholesterol and LDL-cholesterol levels [75]. The mechanism underlying this species difference is not known, but recognizes that TNF-α does not augment systemic levels of cholesterol; a metabolite when overproduced can increase the risk of cardiovascular disease. However, TNF-α may decrease hepatic cholesterol catabolism and excretion [36]. Cholesterol is oxidized in the liver and the main pathway that regulates its elimination is through conversion into bile acids. The pathway is regulated by the enzyme cholesterol-7α-hydroxylase (CYP7A1), a cytochrome P450 heme enzyme. A study by De Fabiani et al. indicated that TNF-α inhibited the expression and activity of CYP7A1, thereby decreasing the conversion of cholesterol into bile acids and reducing elimination [76]. If the elimination of cholesterol from the body is prohibited through its most important pathway, excessive build up can occur systemically promoting potentially harmful effects.
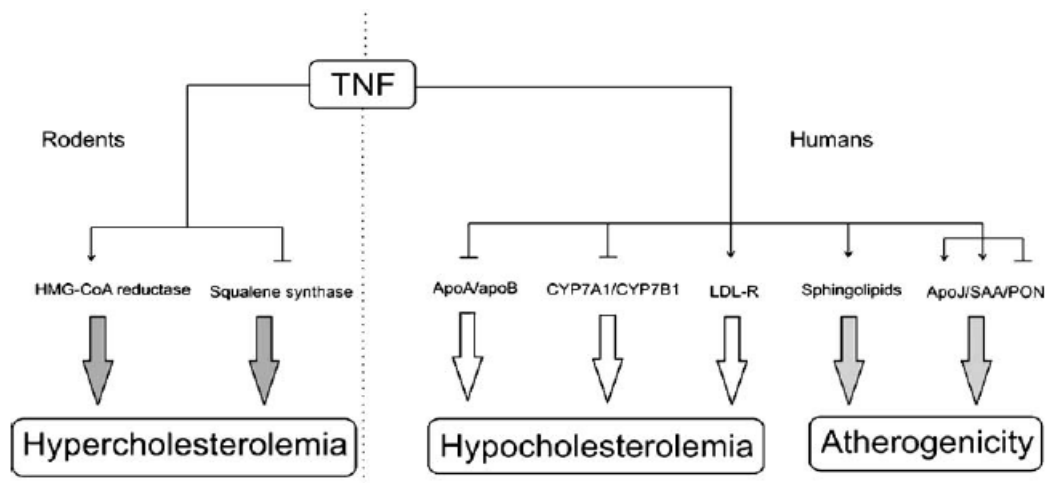

Figure 3. [Popa et al, 2007 *Journal of Lipid Research*] Effects of TNF-α on cholesterol metabolism in rodents and humans.

Effect of Postprandial Response on Cardiovascular Function

Based on typical Western eating habits, an individual eats anywhere from 4-5 times a day, with each meal containing anywhere from 30-80 grams of fat [31]. Data has shown that in normolipemic individuals, an exaggerated postprandial lipemic response is present for 6 hours past baseline after test meals containing 30 – 80g fat and above have been consumed [77]. Therefore, it can be expected that all circulating TGs from the previous meal have not been fully cleared from circulation before ingestion of the next meal. [5, 14]. This leads to a continuous challenge for the endothelium by atherogenic lipoproteins and their remnants. It is thus possible that a chronically increased and extended macronutrient intake leads to a pro-oxidative and pro-inflammatory state in normal weight, overweight, and obese individuals.

The importance of postprandial metabolism to the development of atherosclerosis was formulated almost 30 years ago by Zilversmit [78]. A growing body of scientific research suggests that exaggerated postprandial lipemia, especially after a high-fat meal is linked to the progression of the atherosclerotic process. Certainly, this is a possibility with numerous epidemiological studies linking poor dietary habits to the increased risk of atherosclerosis. When hypertriglyceridemia is present, highly atherogenic Chylomicron remnant's and small-dense LDL are generated leading to direct deposition of remnants onto artery surfaces, eventually relocating to the sub-endothelial space [79]. These particles are highly damaging because they are capable of being oxidized, glycated, and associated with proteoglycans all leading to major injury of the endothelium and underlying smooth muscle [80]. Inflammatory markers, coupled with hypertriglyceridemia and dyslipidemia, play a role in the atherosclerotic process by

upregulating the oxidation and glycation of remnant lipoproteins, ultimately leading to endothelial dysfunction. A prevalence of small dense LDL is very common in patients with diabetes, insulin resistance, and the metabolic syndrome [81]. These observations raise fundamental issues about the relation between food intake, oxidative damage, inflammation, atherosclerosis, and about the ideal way to eat and modify lifestyle [15].

Following food ingestion, triglycerides are transported from the small intestines via chylomicrons through the bloodstream. Catabolism of the chylomicrons is catalyzed by the endothelial bound enzyme lipoprotein lipase (LPL), which transforms these particles into atherogenic triglyceride-rich remnant lipoproteins. The chylomicrons and chylomicron remnant concentrations both typically increase to their peaks by approximately 4 hours and decline shortly thereafter [82]. Another form of triglyceride-rich lipoproteins, termed very-low density lipoproteins (VLDL), are secreted continuously from the liver in response partly to hepatic uptake of lipid (chylomicron remnants) and free fatty acids (FFA's). In insulin resistant states, insulin is unable to attenuate the release of FFA's from adipose tissue, leading to an increase in VLDL output. These endogenous lipids and exogenous lipids share the same metabolic pathway, e.g. LPL that hydrolyzes both into glycerol and FFA's. Increased levels of NEFA's (non-esterified fatty acids) as a result of uncontrolled release, obesity, and increased caloric intake is one of the prime components of dyslipidemia leading to diseases such as the metabolic syndrome, CVD, and diabetes [83]. In a hypercaloric state, the postprandial phase is thus marked by an increase and accumulation in both exogenous and endogenous lipid due to competition of LPL availability, leading to an accumulation of atherogenic lipoproteins [3]. It has been suggested that of all patients

with premature CHD, 40% have normal fasting plasma lipids [84], whereas most of these patients have impaired clearance of postprandial lipoproteins [85]. Atherosclerosis has therefore been considered to be a postprandial phenomenon.

The importance of high serum cholesterol, especially a high-level of LDL cholesterol, as a risk for CVD has been well established in numerous prospective and case-control studies [86, 87]. Evidence for the atherogenicity of LDL derives from studies in experimental animals, clinical trial in humans, and genetic forms of hypercholesterolemia. There is also a vast amount of research documenting the decrease in incidence of coronary artery disease with the use of drug therapy to lower the levels of serum LDL [24, 88]. It should be noted that endogenous very low density lipoprotein (VLDL) and exogenously derived chylomicrons behave in the same way as LDL, acutely changing the blood lipid profile and ultimately ending up proinflammatory and proatherogenic after modification. For example, Tyldum et al. found a marked reduction in basal arterial diameter (~10%) following ingestion of a high-fat meal compared to baseline [89]. There is however some controversy as to whether or not chylomicrons themselves are proatherogenic directly via the deposition of postprandial remnants onto artery surfaces, or whether they simply compete for clearance with endogenous lipids such as VLDL, IDL, and LDL. A study by Karpe et al. in CHD patients found that endogenous TRL constituted 96-97% of all TRL in the fasting state; this figure was only reduced to 91-96% in the postprandial state following an oral fat loading test [13]. If this is true, and exogenous TRL does not directly affect the proatherogenic state during the postprandial phase, they still may indirectly affect vascular damage by increasing VLDL production and competing with VLDL for lipolytic pathways. The end result is increased

proatherogenic endogenous remnant lipoproteins due to delayed clearance. However, Proctor et al. used fluorescent labeled chylomicron remnants and found that these remnants of post-prandial lipoproteins rapidly penetrate arterial tissue, and that efflux is not always complete, which leads to entrapment of particles within the subendothelial space [90]. Further research is warranted in respect to exogenous lipoproteins and how important they are to the development of atherosclerosis.

Previous prospective and case-control studies have concluded that endothelial dysfunction is due to elevations of fasting TGs, NEFA levels, as well as increased tissue/plasma concentrations of proinflammatory cytokines [86, 87]. These studies have given no regard to systemic fluctuations that occur during the postprandial period. Measurements of these markers are traditionally determined in the fasting state and treatment strategies for the prevention of cardiovascular disease are constructed despite the fact that most of our lives are spent between the consumption of meals. Current national guidelines recommend that blood for lipid profiles be drawn after an 8- to 12-hour fast [91]. The increasing amount of experimental evidence implicating macronutrient intake in increased oxidative stress and pro-inflammatory markers has now pushed for recommendations considering the measurement of metabolic perturbations generated following a meal. This transient impairment in endothelial function induced by hypertriglyceridemia and inflammatory markers may very well play a role in the atherosclerotic disease process.

Numerous studies have led to measuring potential markers within the plasma that ultimately can lead to an abnormal cardiovascular response. Alijada et al. reported that intake of a 900-kcal mixed meal in normal weight subjects induced a significant increase

in TGs and intranuclear NF-kB binding activity in mononuclear cells, which is responsible for transcription of pro-inflammatory genes such as TNF-α, CRP, and IL-6 [15]. It is also of interest to note that proinflammatory stimuli such as endotoxin and cytokines (TNF-α) can induce an increase in intranuclear NK-kB [92]. The proinflammatory changes in this study occurred within 1 hour of meal consumption and lasted for less than or equal to 3 hours signifying the persisting effects of the postprandial response. Esposito et al. demonstrated that in overweight subjects with the metabolic syndrome, ingestion of a 760-kcal high-fat meal resulted in higher levels of circulating TGs and TNF-α, and a reduced endothelial function compared to normal weight controls [16]. This study demonstrated that intake of a high-fat meal in patients with the metabolic syndrome, already exhibiting abnormal levels of TNF-α, TGs, and endothelial function at baseline, promoted further increases of all adverse factors measured. Finally, a study by Nappo et al. induced lipemia by a high-fat feeding and found that TNF-α concentrations rose significantly in both healthy and diabetic individuals [17]. These studies offer insight in that the postprandial response does in fact cause metabolic perturbations, demonstrating that fasting level values of certain risk factors may provide an inadequate representation of vascular risk [93].

However, not all studies have implicated the postprandial response to an increase in inflammatory markers related to cardiovascular impairment. Poppitt et al. conducted a study on the acute effects of a high-fat test meal and the potential inflammatory response associated with it in healthy young men [19]. They found that a single high-fat meal did not elicit any changes in circulating levels of TNF-α or CRP, but did induce an increase in circulating levels of IL-6. An intriguing finding when looking at this study is that the

percent energy as fat was markedly higher (71%) compared to the studies above; Alijada et al. (50%), Esposito et al. (59%), and Nappo et al. (59%). Without a high-carbohydrate arm for comparison, it is difficult to postulate why the energy percentage of fat contained in the meal (71%) failed to elicit an acute response in inflammatory markers. A study by Derosa et al. provided evidence that ingestion of pure glucose during an oral glucose tolerance test (OGTT) increased biomarkers of endothelial dysfunction such as CRP, TNF-α, ICAM-1, and VCAM-1 [94]. Therefore, macronutrient composition may be important to consider when examining acute dietary load interventions affecting certain inflammatory biomarkers.

Effect of Prior Exercise on Postprandial Cardiovascular Function

It is well established that regular physical activity affords protection against all-cause mortality, primarily through the prevention or delay of diseases such as atherosclerosis, insulin resistance, obesity, and cardiovascular disease. Less is known about the acute effects of exercise and the benefits bestowed upon the individual after consumption of a meal. Considering that atherogenesis has been proposed to be a "postprandial" phenomenon, it is important to develop potential clinical interventions that can reduce the magnitude of the postprandial response. In recent years, a large body of evidence has suggested that a single exercise session is enough to offer a protective effect postprandially, particularly with respect to lipid metabolism, insulin/glucose dynamics, energy substrate utilization, and inflammatory biomarkers, with energy expended being an important determinant of the magnitude [4]. Therefore, it can be postulated that

events that can shorten the duration of the postprandial response will have a preventative effect on the atherogenic process.

Numerous studies have looked at the benefits of an acute bout of exercise on the postprandial response in a variety of conditions related to the timing of exercise. These studies suggest that the maximal-exercise induced benefits related to the postprandial response occur after a delay of some hours, rather than during or immediately post-exercise [95]. For instance, Zhang et al. reported that exercising 1 hour and 12 hours before ingestion of a high-fat meal decreased the triglyceride response by 38% and 51% respectively [96]. This improvement was not found when exercise was conducted 1hr after ingestion of the high-fat meal. Beneficial adaptations of a single session of exercise 4-24 hours prior to a high-fat meal include, but are not limited to, lower levels of postprandial lipemia [26], increased TG clearance [97], and a decrease in the insulinemic response [98]. These beneficial effects of exercise appear to be related to the energy expended during individual session rather than the intensity achieved [96, 98]. A study by Tsetsonis and Hardmon demonstrated that exercise at 90 minutes at 60% VO_2max resulted in twice the reduction in subsequent postprandial lipemia than exercise at 30% VO_2max for the same amount of time [99]. However, a later study conducted by the same group found that three hours of exercise at 30% VO_2max induced the same reduction in postprandial lipemia as 90min at 60% VO_2max [100], suggesting that the mitigation of the lipaemic response is related to the energy expenditure, rather than the intensity of the preceding exercise. Other studies have reported the same effect, with some even providing evidence that exercise, whether performed in single or multiple sessions, will attenuate the postprandial response to the same degree [4].

During a single session of moderate, aerobic exercise (generally defined as an intensity between 30-60% VO_2max) individuals derive energy from a plethora of substrate including NEFA's, glucose, glycogen, and intra-muscular TGs [101]. The utilization of substrates induces energy deficits within different major tissues of the body including skeletal muscle, adipose tissue, and liver. During periods of recovery and re-feeding, energy deficits are then restored rapidly by the uptake of substrate into the same tissues. It is generally thought that the mechanisms mediating the TG lowering effects of exercise are attributable to increased stimulation of LPL enzyme activity, thereby reducing TG clearance and directing hepatic NEFA flux toward oxidation rather than re-esterification, leading to decreased VLDL production [95].

LPL, the main enzyme responsible for TG hydrolysis, liberates fatty acids from both chylomicrons and VLDL. These fatty acids are then released into circulation where they are taken up into adipose tissue, skeletal muscle, and the liver for oxidation or re-esterification into TG. Although skeletal muscle accounts for less than 50% of body mass, it is the largest component of the metabolically active cell mass and is the main determinant of TG clearance rate at rest [100]. Thus after an acute bout of exercise, one would assume that an increase in LPL activity would further increase TG clearance rates after a meal challenge. Prolonged acute exercise studies in endurance trained individuals have shown a 46-74% increase in skeletal muscle LPL activity 18 hours post-exercise, whereas changes after more modest exercise (yet enough to decrease postprandial lipemia) have been much smaller [102]. Others have shown that exercise induces human LPL gene expression predominantly in skeletal muscle and that the intensity of exercise potentially determines the extent of production [103]. However, more recent reports have

demonstrated in untrained individuals, that when exercise is of moderate duration and intensity, there is no increase in skeletal muscle LPL activity [104]. Although the data are conflicting regarding increased LPL activity, it seems increased LPL-mediated clearance of TG in skeletal muscle is an important contributor to the attenuated TG response.

A second potential mechanism to the TG lowering effects of prior exercise is decreased hepatic synthesis of VLDL. Although no direct human evidence exists supporting a reduction in VLDL, animal studies and indirect evidence in humans conform to this view [6, 105]. The idea is that after an acute bout of exercise utilizing stored substrate from the liver, this energy deficit can shift the hepatic fatty acid flux toward β-oxidation and ketone body production and away from re-esterification and VLDL production. Indirect evidence in humans rests upon measured circulating levels of hydroxybutyric acid (3-OHB). 3-OHB is a ketone body produced in the liver and increased circulating concentrations provide a marker of hepatic fatty acid oxidation. Human studies examining the postprandial response to an acute bout of exercise have reported a correlation between the exercise-induced attenuation of the TG response and increases in the 3-OHB response [101]. While these data do not provide powerful conclusions on the mechanism related to decreased VLDL production, the findings are consistent with the idea that a reduced secretion of VLDL potentially is a player in attenuation of the postprandial response.

The fact that exercise energy expenditure seems to be the primary determinant in respect to the attenuation of postprandial TG concentrations, suggests that the exercise-induced depletion of energy reserves mentioned above, and their subsequent

replenishment, play a key role in the lipemic-lowering process. Less is known about whether or not the favorable effects of exercise on postprandial metabolism persist when there is an increase in energy intake following exercise that negates the energy deficit induced by the exercise bout. A review by Blundell and King found that only 19% of intervention studies report an increase in energy intake after exercise, while 65% showed no change [106]. However, most of these studies included men only and research has shown that food intake patterns differ between genders [107]. Stubbs et al. demonstrated in a group of free-living men that even with high-intensity exercise bouts over 7 consecutive days, there was no energy compensation observed throughout the week [108]. However, when the same group studied a group of free-living women, there was a significant, but partial compensation in energy intake of ~30% of the energy expended over 7 days [8]. The study included both a high-intensity (70% peak O_2 uptake) and moderate-intensity group (40% peak O_2 uptake) and concluded that the high-intensity group consumed significantly more daily energy, as well as more energy immediately after exercise.   These studies, as well as future studies, are of clinical and mechanistic importance as some (not all) individuals tend to increase energy intake when they exercise thus eliminating any improvements in their cardio-metabolic profile.

The transient impairment of the endothelium postprandially as mentioned above is not due simply to circulating levels of TG depositing cholesterol and lipid into different tissue. It is the subsequent cellular and molecular responses to that infiltration that lead to the pathophysiologic observations and feed-forward mechanisms of vascular dysfunction. Not only is exercise associated with the attenuation of lipemia following a meal, but also regular exercise has been shown to potentiate anti-inflammatory actions

and may offer protection against the pro-inflammatory cascade following a meal. A decrease in chronic low-grade systemic inflammation can potentially attenuate the continuous pro-inflammatory cytokine insult on different tissue through local, central, or peripheral actions.

Although an acute bout of physical activity in itself can initiate a potent immune response resulting in low grade systemic inflammation and changes in levels of pro-inflammatory cytokines such as IL-1, TNF-α, and IL-6 [109], these effects are usually acute and rapidly decline during the post-exercise period [110]. There is also evidence that following pro-inflammatory cytokine release, there is an increase in circulating levels of well-known anti-inflammatory cytokines, cytokine inhibitors, such as IL-1ra and sTNFR [111]. Studies have shown that these two anti-inflammatory cytokines can be induced by IL-6, demonstrating that IL-6 exhibits anti-inflammatory effects as well [9]. In vitro [112] and animal studies [113] have also found that IL-6 is a potent inhibitor of TNF-α production. It has even been suggested that due to these actions, IL-6 should be classified as an "anti-inflammatory" cytokine. This further develops the potential role TNF-α has as a main catalyst in the link between dyslipidemia and atherosclerosis. In order to determine if an acute bout of exercise could downregulate TNF-α suggesting an anti-inflammatory effect, Starkie et al. developed a model of low grade inflammation by injecting endotoxin (a potent inducer of TNF-α) to healthy subjects [114]. Subjects were randomized to either rest, rhIL-6 infusion in the amount expected to be released during 3 hours of moderate intensity cycling, or 3 hours of cycling before endotoxin injection. The results demonstrated that in resting subjects, endotoxin induced a two-threefold increase in circulating TNF-α compared to the other two groups. The exercise group as

well as the rhIL-6 group both displayed a total attenuation of the TNF-α response. This study provides direct evidence that exercise (a potent inducer of IL6) and infusion of rhIL-6 by itself mediates anti-inflammatory activity by suppressing TNF-α.

Not only can a single bout of exercise potentially offer anti-inflammatory benefits, but regular exercise training induces anti-inflammatory benefits and has been linked to lower levels of systemic and locally produced pro-inflammatory cytokines within tissue. Studies have linked chronic physical inactivity and low grade systemic inflammation in healthy individuals, as well as individuals with chronic disease [9, 115]. However, these studies only provide an association between inactivity and inflammation, and do not provide direct causality. In response to regular exercise, several longitudinal and intervention studies have noted a decrease in systemic levels of cytokines such as CRP [116], TNF-α [25], and IL-6 [117] in response to regular training, suggesting that consistent physical activity may suppress low grade systemic inflammation. A study by Gokhale et al. looked at the cytokine response to strenuous exercise in athletes and non-athletes and the role regular training has on it [117]. The majority of athletes and non-athletes both demonstrated an increase in IL6 and a decrease in TNF-α levels. An important finding within the study was that athletes displayed a smaller magnitude of change in cytokine levels following a longer duration of exercise than non-athletes, proposing that chronic exercisers have a diminished cytokine response. Moreover, this study found that in athletes the resting level of IL-6 in exercise was found to significantly impact resting & post-exercise TNF-α levels. Those with higher IL6 levels at baseline were also found to have lower TNF-α levels, indicating a mechanism by which regular exercise can afford protection against chronic disease. Several other studies are also in

agreement suggesting that regular exercise can lower baseline TNF-α values potentially offering protection against TNF-α induced atherosclerosis [9, 118].

In summary, lifestyle factors such as physical activity and diet can have a profound protective effect on vascular function, this risk for atherosclerosis. To date, only one study has investigated the effects of an acute bout of exercise on the postprandial pro-inflammatory TNF-α cytokine response [119]. A cross-over design was employed, and ten adolescent boys (overweight vs. non-overweight) either rested the night before an oral fat tolerance test or exercise at an intensity of 65% VO_2max in order to elicit a 600kcal deficit. The results indicated no change in postprandial or baseline values of TNF-α even with substantial reductions in TG after an acute bout of exercise. However, it was noted that plasma TNF-α was higher in the overweight group compared to the non-overweight group suggestive of chronic low grade inflammation in the overweight subjects. The exclusion of female subjects from this investigation is a major limitation and limits the generalizability of the study. So far there are no data available on the effects of prior moderate exercise on mitigating the postprandial response of the pro-inflammatory cytokine TNF-α in young, non-obese, sedentary females. Due to this absence of previous research, it is not entirely known if sedentary, but otherwise healthy, young females experience attenuation of the postprandial inflammatory response after an acute bout of exercise. Therefore, the following investigation was conducted to assess the effects of a single bout of moderate exercise, followed by energy replacement on the pro-inflammatory cytokine TNF-α in response to a high-fat, high-carbohydrate meal in sedentary, non-obese females.

CHAPTER III

METHODS AND PROCEDURES

Subjects eligible for the participation of this study were determined following
preliminary screenings tests and health questionnaires. All subjects were informed of the
test procedures, dietary and exercise terms of the study, and time commitment required
for participation. Written and verbal consent was obtained from each research volunteer
and each subject received a copy of the informed consent document (Appendix A) prior
to participation. Throughout the study individuals were informed that they could
withdraw from the study at any time. The study protocol was approved by the Colorado
State University Human Research Committee.

Subjects

A total of eight subjects were recruited locally from the Fort Collins area for
participation in the study via student FYI emails, flyers, and word of mouth. All
volunteers for the study were apparently healthy, sedentary, eumenorrheic females
between the ages of 18-40 years of age. Subject inclusion criteria included the following:
fasting blood glucose <126 mg/dl, resting blood pressure <140/90mmHg, no past or
present history of endocrine disorders, weight stable during the previous 6 months, body
mass index (BMI) between 21-29.9 kg/m^2, and were not exercise trained (exercise <2
times/wk). Subjects were excluded from participation if they were pregnant, vegetarians,

had diabetes mellitus, used tobacco, exhibited extreme dietary patterns, or were lactose-intolerant. No subjects were taking medications thought to interfere with insulin action or lipid metabolism. It should also be noted that despite findings in literature that the effect of menstrual cycle phase on postprandial lipemia is too small to be detected [120], care was taken to ensure that female subjects performed each condition between day 6 and 14 (mid-follicular phase) of their menstrual cycle in order to remove any confounding issues of hormonal changes affecting the endpoints of this study.

Experimental Design

Following initial screening to determine eligibility for the study, participants completed two trials, Exercise (EX) and No-Exercise (No-EX), in random order separated by an interval of at least 7 days. Each trial consisted of two days in which the subjects completed specific procedures set forth by the experimenter. On the evening of day 1, subjects either participated in normal activities of daily living (No-EX) or reported to the laboratory to complete an exercise intervention on a cycle ergometer (EX). The exercise bout was arranged to elicit a net energy expenditure of 400 kcalories. In order to achieve this, subjects exercised at 65% of peak heart rate determined by a VO2max test prior to the trial. Following this caloric deficit accomplished by exercise, a mixed macronutrient snack (58% CHO, 28% Fat, 21% Pro) containing approximately 380 calories was consumed immediately after cessation of activity. Unfortunately, an error was made in the exercise protocol by basing the intensity on 65% of peak heart rate rather than 65% of peak VO_2. This resulted in an estimated energy expenditure of approximately 285 kcalories rather than the target of 400kcal.

On Day 2 of each trial, subjects arrived at the Nutrition and Fitness Laboratory at Colorado State University following a 12 hour overnight fast. Blood pressure and resting pulse rate were recorded and subjects were asked to relax in a supine position for several minutes. An intravenous catheter was placed in the antecubital vein for the collection of blood samples. Two baseline blood samples were collected 5-min apart from one another at -10 minutes and -5 minutes, respectively. Baseline samples were then followed by the consumption of a high-fat, high-sugar liquid test meal under the supervision of the investigator. The test meal consisted of whole milk, heavy whipping cream, whey protein powder, chocolate syrup, and sugar. The macronutrient composition of the test meal consisted of 872 ± 109 kcal of energy, 51 ± 6 g of fat, 74 ± 9 g of carbohydrate (CHO), 24 ± 3 g of protein. Study participants each received a relative dose of the test shake based on their body weight (12.5kcal/kg body weight), with a macronutrient intake of 0.75g fat/kg body weight, 1.1g CHO/kg body weight, and 0.35 g protein/kg body weight. The macronutrient composition as a percentage of kcalories in the liquid test shake was 53% fat, 34% carbohydrate, and 11% protein. Subjects were asked to finish the test meal within a 15 minute time period. Postprandial blood specimens were then collected at time 30, 60, 90, 120, 150, 180, 210, 240, 300, and 360 minutes.

Procedures:

Anthropometric Measurements

Body weight measurements were made using a balance beam scale to the nearest 0.1kg. Body height was measured with a wall-mounted stadiometer to the nearest 0.1cm. From this, BMI was calculated as: weight (kg) x height $(m)^2$. Waist and hip

circumferences were measured to the nearest 0.1cm using a non-stretchable tape, and waist to hip ratio was calculated. Fasting blood glucose was measured by fingerstick using a glucometer. The percentage of body fat was measured using dual-energy x-ray absorptiometry (DEXA) (Hologic, Discovery, QRD series). Short scans (6 minutes) were used for all subjects and body composition was then analyzed using computer software provided by the manufacturer Hologic.

Resting Metabolic Rate (RMR) and Dietary Control

Resting metabolic rate was determined in each subject prior to the beginning of the experiment in order to determine each subject's daily energy requirements. RMR was necessary in order to provide subjects with the appropriate caloric intake to remain in energy balance the day before each limb of the experiment involving ingestion of the high fat, high-carbohydrate meal. Subjects arrived at the Nutrition and Fitness Laboratory following a 12 hour overnight fast. Indirect calorimetry (Parvo medics, Model No. MMS 2400, Sandy, UT) was used to determine resting metabolic rate. Subjects were instructed to lie comfortably in a supine position on a bed, and then were fitted with a hooded enclosure over the head in order to obtain resting VO_2 and VCO_2 values. The Weir equation was used to convert gas exchange values into kilocalories [121]. Care was taken to ensure calibration of the cart during the measurement. After 30 minutes of gas analysis, an average of the last 10 minutes was used to determine each subject's RMR.

On the day before each test meal, diets were strictly controlled to ensure consistent macronutrient intake and to approximate energy balance. This was

accomplished by preparing all meals in the Department of Food Science and Human

Nutrition metabolic kitchen and these were provided to the subjects. Subjects were

instructed to consume all foods provided and return any uneaten portions for collection

and analysis. Ad libitum water intake was allowed and subjects were instructed to stop

eating 12 hours before testing to ensure a fasted condition. Diet composition was

individualized to each subject's RMR multiplied by a physical activity level of 1.4 [122],

which corresponds to the activity level of a non-active adult. Average macronutrient

composition consisted of 57% carbohydrate, 24% fat, and 20% protein provided to the

subjects in the form of fresh fruits, vegetables, whole grains, lean meats, and dairy.

Peak VO_2 Determination

In order to determine the sub-maximal intensity required for the EX-trial,

maximal oxygen consumption was determined via an incremental cycling ergometer

protocol in each subject (Medgraphics, Monark Ergomedic, Model No. 8082E, St Paul,

MN). Oxygen consumption, carbon dioxide production, pulmonary ventilation, and the

respiratory exchange ratio (RER) were determined via a breath-by-breath computer

assisted metabolic system (Parvo medics, Model No. MMS 2400, Sandy, UT). Extreme

care was taken by the investigators in order to familiarize each subject with the protocol.

Following a brief warm up period, the workload was set to 1kp and subjects were

instructed to pedal at a cadence between 70-100rpm. The workload was then increased

approximately 0.5kp every two minutes until volitional exhaustion or one of the three

following criteria were met: RER $\geq$ 1.10, subject's heart rate was within 15 beats of age

predicted max (220-Age), plateau in oxygen consumption values despite an increase in

workload. Heart Rate (Polar Target, Hong Kong) was recorded every 30 seconds during testing. Subjects were included in the study if they had a VO2max <40 ml/kg/min.

During the exercise intervention (EX), subjects arrived at the Nutrition and Fitness Laboratory the day prior to the postprandial experiment, approximately 14-16 hours before the consumption of the test meal. Subjects were instructed to cycle at 65% max heart rate for a time duration required to result in a net exercise caloric expenditure of 400kcal. As previously mentioned however, exercise intensity was mistakenly based on 65% of peak heart rate rather than 65% VO2max. This resulted in a net energy cost from exercise of only approximately 285kcal. The caloric expenditure resulting from the exercise intervention was determined based on each individual's oxygen consumption at 65% maxHR as determined by the incremental cycling ergometer protocol. Oxygen consumption (measured at 65% of maxHR) was then multiplied by 4.95kcal/L to determine how many calories would be expended per minute of exercise. The resulting value was then subtracted from each subject's resting metabolic (kcal/min) in order to determine the *net cost* of calories expended exclusively from exercise. This value was then multiplied by each individual's exercise duration, generating the kcal deficit we elicited in each subject. Heart rate was monitored during exercise to ensure subjects stayed within the recommended intensity. After completion of exercise, the acute energy deficit was replaced with a 380 kilocalorie meal consisting of 57% carbohydrate, 24% fat, and 20% protein.

Blood Assays

Blood samples were centrifuged at 2500rpm for 10 minutes in order to separate plasma. Plasma was then isolated and stored immediately at -80ºC for analysis. Plasma samples were used to determine the 6 hour postprandial glucose and insulin area under the curve (AUC) as well as identify concentrations of Tumor Necrosis Factor alpha (TNF-α). Plasma glucose & insulin assays were performed at the University of Colorado Clinical and Translational Sciences Laboratory. Glucose concentrations were measured by the glucose oxidase method (YSI 2300, YSI Inc. Yellow Springs, OH). Plasma insulin concentrations were measured by an immunoenzymatic ("sandwich") assay for use with the Beckman-Coulter Access Immunoassay System (Beckman Coulter, Inc. Fullerton, CA). Plasma TNF-α concentrations were determined using a high-sensitivity Enzyme-Linked Immunosorbent Assay (ELISA) procedure (R&D Systems, Minneapolis). Note that the plasma glucose, insulin, and triglyceride concentration data have already been published in two separate CSU M.S. theses (Smith W and Krause M, Department of Food Science and Human Nutrition, Colorado State University, 2010). These data are included here simply to provide the reader with a more comprehensive view of the postprandial responses, and also to determine if the TNF-α response was correlated with the glucose, insulin, and TG responses.

Statistics

SPSS version 18.0 was used to analyze data. Using a 2x2 repeated measures of analysis variance (ANOVA), differences in circulating TNF-α, TG, insulin, and glucose

were examined to determine the main effects of condition, time, and their possible

interactions. Statistical significance was set at $p < 0.05$.

CHAPTER IV

RESULTS

Subject Characteristics

      A total of eight female participants completed the protocol. Table 1 provides the physical characteristics of all subjects at baseline. In general, subjects were young, non-obese adults who displayed relatively low cardiorespiratory fitness levels. Fasting blood glucose levels were normal for all individuals. There were no significant changes in weight or BMI during the course of the study.

Table 1: Subject Physical Characteristics at Baseline.

Characteristics	Mean	Standard Deviation
Age (years)	19.6	1.3
Weight (kg)	68.2	8.4
Height (cm)	166.4	7.1
BMI (m^2/kg)	24.6	1.8
Body Fat %	29.8	3.2
Waist (cm)	77.2	7.0
WHR	0.84	0.06
Fasting Blood Glucose (mg/dl)	80.0	15.4
Resting Energy Expenditure (REE)	1502	169
VO_2Max (ml/kg/min)	31.3	2.9

Exercise Perturbation

During the acute exercise protocol, subjects cycled an average of 70±15 (SD) minutes. The average heart rate for subjects was 127±15 (SD) beats per minute.

Glycemic Response

Plasma glucose concentrations (baseline and 6 hours postprandial) for each of the two conditions are provided in Figure 1. Fasting baseline plasma glucose concentrations were not significantly different for either Ex or NonEx trials. Over the course of the 6 hour meal challenge, there was a significant time effect ($p < 0.001$). However, there was not a significant main effect observed for condition ($p = 0.59$) or a condition by time interaction ($p = 0.17$).
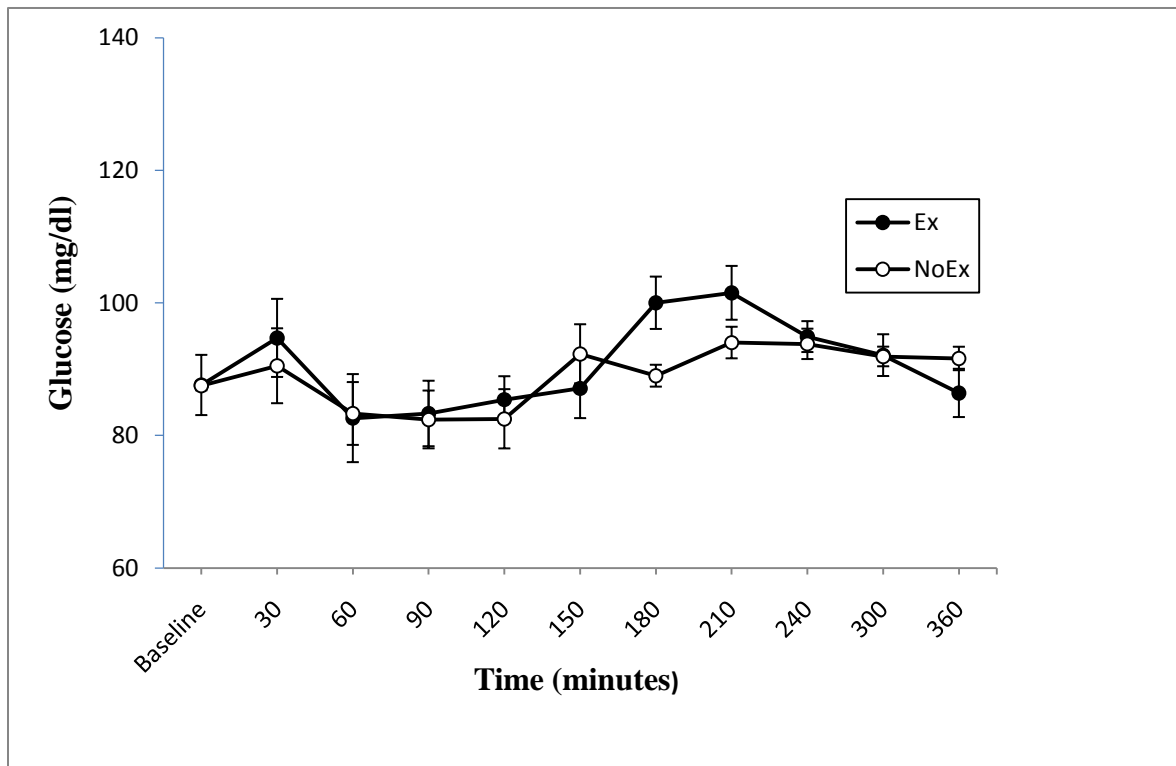
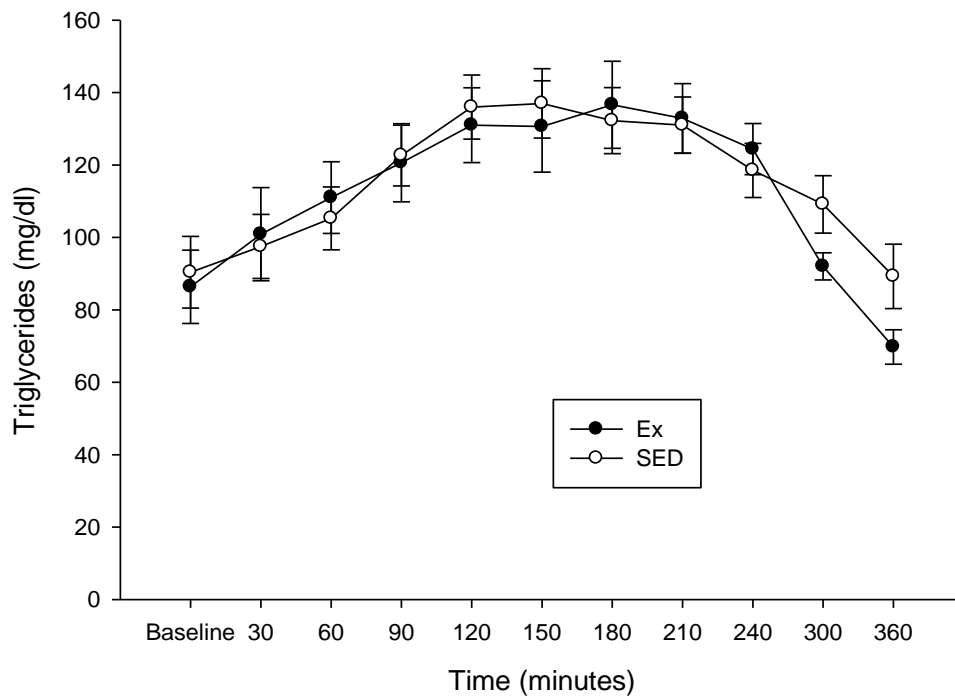**Figure 1:** Fasting and Postprandial Glucose Concentrations During Control and Post-Exercise Conditions in Non-Obese, Sedentary Females (Mean ± Standard Error).

Insulin Response

Fasting and postprandial plasma insulin concentrations for each of the two conditions are provided in Figure 2. As expected, insulin concentrations increased rapidly for both conditions, peaking at 30 minutes at which time it decreased quite rapidly. Due to this sharp increase, a significant time effect ($p<0.001$) was observed during the 6 hour period after the meal challenge. There was no main effect of condition ($p=0.119$), indicating the responses between both conditions did not differ. There was also no significant condition by time interaction ($p=0.545$), indicating no differences between insulin responses for Ex and NonEx over time.
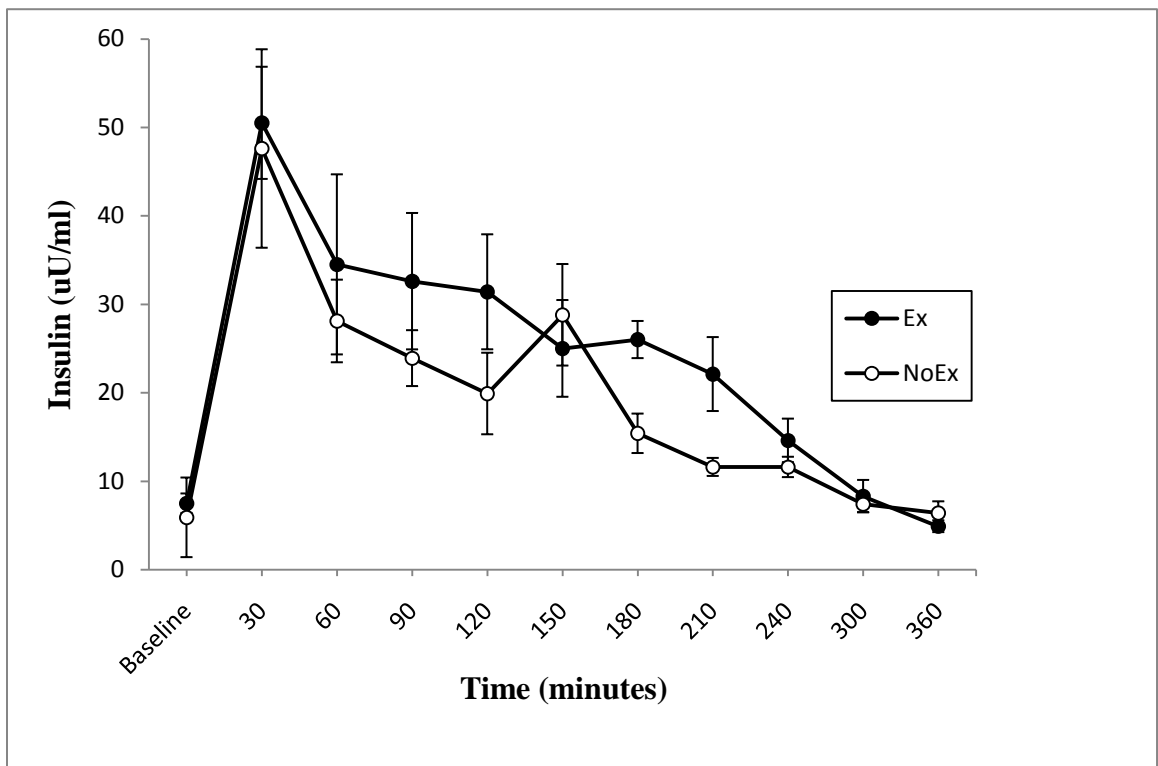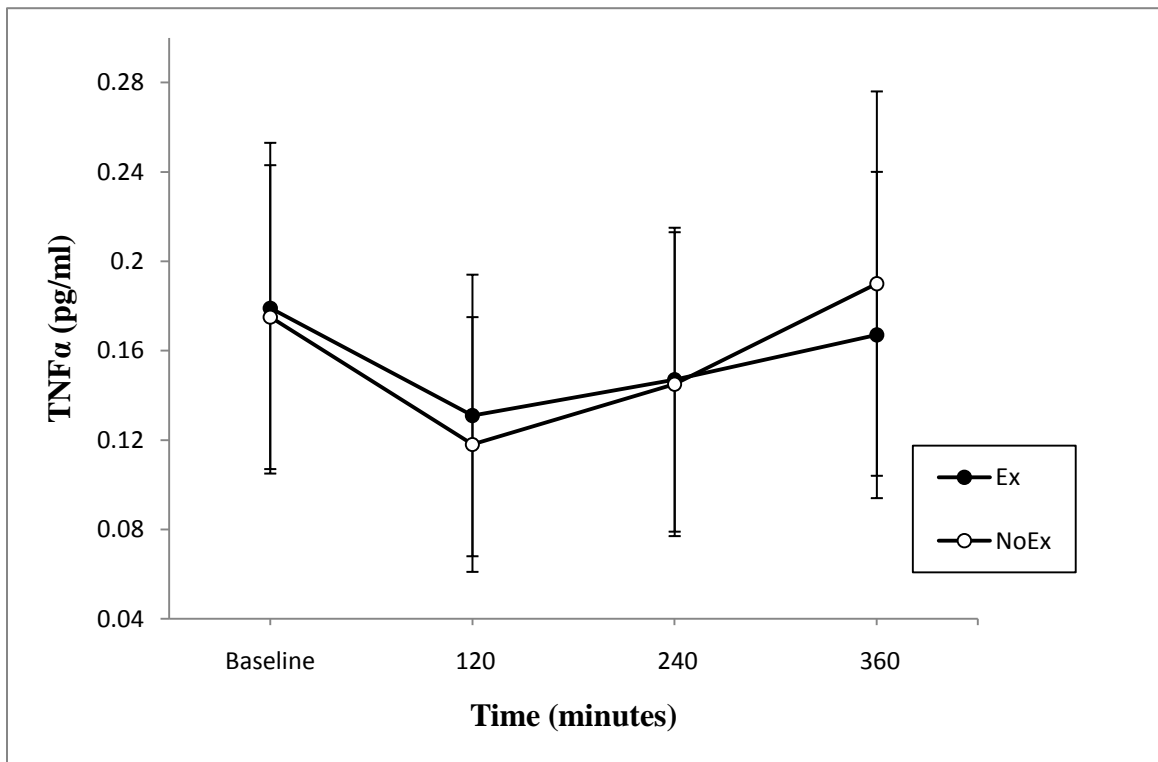

Figure 2: Fasting and Postprandial Insulin Concentrations During Control and Post-Exercise Conditions in Non-Obese, Sedentary Females. (Mean ± Standard Error).

Triglyceride Response

Plasma triglyceride concentrations for each of the two conditions are provided in Figure 3. The baseline fasting triglycerides prior to the meal challenge were not significantly different between Ex and NonEx trials. There was a significant time effect, owing to a rise in plasma TG concentrations, peaking at 3 hours and returning to baseline within the 6 hour measurement period for both groups. During the first 4 hours of the postprandial period, there were no group differences in plasma TG responses. However, during the final 2 hours of the postprandial period, the TG concentrations were significantly lower for Ex compared to NonEx.

Figure 3: Fasting and Postprandial Triglyceride Concentrations During Control and Post-Exercise Conditions in Non-Obese, Sedentary Females. (Mean ± Standard Error).

TNF-α Response

Figure 4 shows baseline and postprandial plasma TNF-α concentrations for both Ex and NonEx conditions. Comparison of the conditions revealed a significant time effect ($p < 0.05$) owing to the decrease in TNF-α plasma concentrations at 2 hours, and the steady increase back toward baseline by 6 hours. There was not a significant main effect for condition, indicating no difference in the postprandial TNF-α response between Ex and NonEx conditions ($p = 0.891$), nor was there a condition by time interaction ($p = 0.763$). The TNF-α response was not correlated with the glucose, insulin, and TG responses for either condition (data not shown).

Figure 4: Fasting and Postprandial TNF-α Concentrations During Control and Post-Exercise Conditions in Non-Obese, Sedentary Females. (Mean ± Standard Error).

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CHAPTER IV

DISCUSSION

This study was designed to ascertain potential differences in the postprandial TNF-α concentration following ingestion of a high-fat, high-carbohydrate meal, with or without a single session of moderate, aerobic exercise in which young, non-obese, sedentary females were kept in energy balance. The data revealed no statistically significant differences in circulating baseline TNF-α concentrations 12 hours following Ex and NonEx conditions in which subjects were kept in energy balance. Data also revealed no significant differences in 6-hour postprandial circulating TNF-α concentrations between interventions 12 hours following the same above mentioned conditions. While these findings contradict several studies showing an increase in TNF-α concentrations following a meal challenge with or without prior exercise [16, 17], it also is in agreement with several studies demonstrating no increase in concentrations of TNF-α postprandially [19, 119]. However, it should be noted though that none of the above studies, or studies to this researchers knowledge, have looked at the TNF-α plasma response following a single session of moderate intensity aerobic exercise with energy replacement or following a high-fat, high-carbohydrate meal in women. Therefore, this is the first investigation to examine: 1) Fasting TNF-α concentrations 12 hours following an acute bout of moderate intensity, aerobic exercise with energy replacement; and 2) 6-hour postprandial TNF-α response following a high-fat, high-carbohydrate meal 12 hours after an acute bout of moderate intensity, aerobic exercise with energy replacement in

women. However, due to researcher error, the current findings must be interpreted with caution and the assay repeated before definitive conclusions can be determined from the present study.

Our study found no difference in the fasting plasma concentrations of TNF-α between the Ex and NonEx conditions following a single bout of moderate exercise. Work by MacEneany et al. [119], is the only study that has attempted measurements of TNF-α both in a control and exercise group, in the fasting and postprandial period. There was no change in TNF-α concentrations during the postprandial period relative to fasting values. Fasting levels of TNF-α the following morning were also unaltered in response to an acute bout of exercise. However, two separate studies report a decrease in plasma TNF-α levels following long-term regular exercise in women demonstrating that exercise can indeed attenuate systemic TNF-α concentrations [25, 26]. Both studies demonstrated that regular physical activity of moderate intensity can decrease TNF-α system activity and that decrease may play an important role in improving glucose metabolism parameters. TNF-α is known to exhibit an inhibitory effect on LPL in adipocytes [47], therefore directing lipoproteins to tissue not designed for lipid storage. For instance, increased intracellular accumulation of lipid intermediates within skeletal muscle, such as TGs, diacylglycerols, ceramides, has been linked to defects in the insulin signaling cascade [50]. Increased lipid accumulation, particularly in the form of LDL, can also have negative effects within endothelial cells creating a pro-atherogenic environment as previously discussed in the literature review. Notably, both studies concluded that physical activity had an independent effect in the regulation of the TNF system, which was not attributed to changes in body fat. Considering adipose tissue has been found to

overexpress the TNF-α gene [25] and levels of TNF-α are positively correlated with visceral adiposity [123], it is important to understand that the decrease in TNF-α concentrations is not simply an issue of weight loss.

While we hypothesized that TNF-α would increase significantly during the postprandial period following consumption of the high-fat, high-carbohydrate meal, we found nothing to support this hypothesis. Previous data have shown associations between a hypertriglyceridemic response and increases in TNF-α following a single high-fat meal [15-17]. These studies found that a single-high fat meal acutely affected the level of TGs, TNF-α, and other pro-inflammatory biomarkers in both healthy and diseases individuals. Why our group found no increases in the postprandial plasma concentrations of TNF-α is unknown. The subjects used in the three studies mentioned above in which, TNF-α increased after a high-fat meal, were on average between 40 and 50 years of age. Although the measurement time points for postprandial TNF-α concentrations were fairly similar to our measured time points, the difference in age may have accounted for different peaks of TNF-α in plasma. Our first measured time point took place at 120 minutes, and if the plasma concentrations of TNF-α increased sharply and decreased quickly, we would have missed this change. Although there is no direct evidence supporting this argument, further research is warranted in the area to understand appropriate measurement points. Nappo et al. [17] found that supplementation with high doses of vitamin E and ascorbic acid markedly attenuated the TNF-α response possibly through decreased susceptibility to LDL oxidation thus inhibiting mechanisms related to the secretion of pro-inflammatory cytokines. Other studies have also demonstrated that antioxidants have the ability to reduce the postprandial pro-inflammatory response within

an individual [124, 125]. Bogani et al. demonstrated that various polyphenols found in olive oil were very protective against postprandial inflammation after a fat-rich meal administered to 12 normolipemic, healthy subjects. [125]. The test meal administered by our group consisted of a number of ingredients including heavy whipping cream, 2% milk, whey protein, chocolate syrup, and sugar. Human and small animal studies have both demonstrated that whey protein, either directly or indirectly, may possess anti-inflammatory or anti-cancer properties [126, 127]. Two percent milk also contains vitamin E and carotenoids such as beta-carotene and lutein, all found to possess anti-inflammatory effects *in vivo* and *in vitro* [17, 128]. Therefore, one can speculate that any number of antioxidants within our test meal could have affected the postprandial TNF-α response possibly by attenuating the oxidation of LDL, as modified LDL leads to increases in TNF-α concentration.

TNF-α concentrations were also much lower in our female subjects than expected, given that previous findings in women have shown circulating levels of TNF-α anywhere between 2.0pg/ml and 9.0pg/ml for lean and overweight/obese subjects [25, 26]. In these studies, the average age of the women (over 40+ years) plus the associated chronic inflammation associated with the obese/overweight group may have led to the measureable values of TNF-α concentration. In contrast, the subject population of our study included young (average age 19.6±1.3 yrs) and healthy women. Therefore our intervention may not have been appropriate to elicit increased concentrations of TNF-α within plasma potentially because young, healthy individuals can prevent an acute increase in systemic low-grade inflammation. Subjects were found to exhibit surprisingly low values of TNF-α (Ex: 0.16±0.07pg/ml and NonEx: 0.16±0.07pg/ml) even after meal

ingestion and reasons for this phenomenon are unclear at present. In fact, our study

revealed a decrease in TNF-α concentrations at 2 hours inconsistent with data on the

postprandial inflammatory response [16, 17]. To this researchers knowledge no other

studies have measured TNF-α in young, non-obese, females and therefore further

research is necessary to determine if any of these arguments hold any value or merit.

Limitations

There are a number of limitations to the present investigation. The exclusion of a

third intervention trial looking at the effects of exercise with an energy deficit (which is

well established in literature with respect to TG attenuation postprandially) limited our

ability to compare the effects of exercise on postprandial metabolism when there is an

increase in energy intake to compensate for energy expenditure caused by exercise. Not

only were the subjects all female, limiting the generalizability of our results, but they

were young, non-obese, healthy adults, and therefore their risk for increased pro-

inflammatory markers typically found in already metabolically compromised individuals,

may have been much lower.

Methodological limitations that could account for the lack of any difference in

TNF-α concentrations include the fact that TNF-α circulates in very low concentrations

and systemic changes can be extremely difficult to detect [50]. Our group measured

TNF-α concentrations before meal ingestion (baseline), and after meal ingestion (2, 4, 6

hours). These gaps in time points may have potentially allowed the failure to measure

significant TNF-α peak concentrations, if in fact one existed. Perhaps the peak in TNF-α

concentrations occurred shortly before the significant decrease from baseline we saw 2

hours postprandially. TNF-α is known to be inactivated rapidly by the already mentioned sTNFR and cleared from circulation quickly through the kidneys [129]. It can be hypothesized that as we age, we see decreases in the ability to clear and inactivate circulating TNF-α systemically. This is pure speculation however, and future studies are needed to validate or invalidate such a proposition.

Another limitation to the study was failure to accurately determine the correct exercise intensity and duration needed to achieve a net caloric exercise cost of 400 kcal in each subject. Although subjects underwent baseline measurements of VO_2max with peak heart rate values recorded, there was a failure to utilize the VO2max values in order to establish the appropriate exercise intensity necessary to produce our net caloric goal. After gross calculations were made for each subject's net caloric cost, taking into account resting metabolic rate, peak VO_2, minutes of exercise, and 4.9 calories per minute expended, it was determined that each subject expended approximately 285 calories. This net caloric cost was then offset by immediate consumption of approximately 380 calories from a mixed meal snack, thus putting each subject into a positive energy balance of approximately 100 calories. Whether or not a positive energy balance of 100 calories is of significance is unknown, and owing to well-known difficulties in measuring energy balance, it is not clear if this estimated 100 additional kcal has any relevance. Nevertheless, a state of overfeeding could possibly explain how exercise failed to attenuate the postprandial changes in both insulin and TG that have been clearly demonstrated in other studies with subjects displaying energy balance or in energy deficit.

Conclusions

A single bout of moderate intensity exercise compared to no exercise preceding a high-fat, high-carbohydrate meal resulted in no significant beneficial effects on fasting or postprandial TNF-α plasma concentrations in young, non-obese, sedentary females.

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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 1/40th 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). <u>Time: 30 minutes.</u>

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. <u>Time: 6.5 hours</u> **This procedure will be performed 2 times: once during the SED trial and once during the EX trial at least 3 days apart.**

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. <u>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.</u> 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 <u>blood drawing</u> 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.

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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?

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
	Food Preferences and Acceptability Questionnaire										
	Please circle the number that corresponds to your response.										
☐	**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_____

<u>GENERAL MEDICAL HISTORY</u>

	YES	NO	
Do you have any current medical conditions? explain:	☐	☐	If Yes, please

	YES	NO	
Have you had any major illnesses in the past? explain:	☐	☐	If Yes, please

	YES	NO	
Have you ever been hospitalized or had surgery? explain: (include date and type of surgery, if possible)	☐	☐	If Yes, please

	YES	NO	
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:

        ☐   ☐

<u>Medication</u>	<u>Reason</u>	<u>Times taken per Day</u>	<u>Taken for how long?</u>

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? explain:	YES	NO	If yes, please

Age at diagnosis_____

	☐	☐	

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

<u>FAMILY HISTORY</u>

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	☐	☐

GASTROINTESTINAL HISTORY

	YES	NO
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)   CURRENT TOBACCO USE (if applicable)

					# per day
None	☐		Cigarette		_____
Quit	☐	(when)_____	Cigar		_____
Cigarette	☐		Pipe		_____
Cigarette	☐		Chew Tobacco		_____
Pipe	☐		Snuff		_____
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 ($\leq$ 126 mg/dl)	
Blood Pressure (<140/90 mmHg)	
Pulse	
Pregnancy Test	
Date of Birth	

BMI (21-30)   =   <u>Weight (kg)</u>   =   <u>   kg   </u>   =   <u>   </u>

                  Height (m²)   m²

Sample Diet ~2,000calories

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

<u>Lunch</u>: 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

<u>Aims</u>:
> Appropriate Volume
> 700-1,000 kcal
> 40-60 g Fat
> 20-40% CHO (Closer to 40% and preferably sucrose)

<u>Proposed calculation</u>:
> 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	45 g fat	52.5 g fat	60 g fat	67.5 g fat
337.5 kcals	405 kcals	472.5 kcals	540 kcals	607.5 kcals
54% Fat	**54% Fat**	**54% Fat**	**54% Fat**	**54% Fat**
55 g CHO	66 g CHO	77 g CHO	88 g CHO	99 g CHO
220 kcals	264 kcals	308 kcals	352 kcals	396 kcals
35% CHO	**35% CHO**	**35% CHO**	**35% CHO**	**35% CHO**
17.5 g Pro	21 g Pro	24.5 g Pro	28 g Pro	31.5 g Pro
70 kcals	84 kcals	98 kcals	112 kcals	126 kcals
11% Pro	**11% Pro**	**11 % Pro**	**11% Pro**	**11% Pro**
627.5 kcals	**753 kcals**	**878.5 kcals**	**1,004 kcals**	**1129.5 kcals**

<u>Shake Ingredients</u>:

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

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

<1g Fat, 2g CHO, 16g 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
- o Repeat calibration if the difference between low and high values on Flowmeter Calibration Conformation screen is greater than 0.5 or 5%.
- o Save calibration data.

- **Test**
  - o Adjust bike seat to subject.
  - o Fit headgear and mouth piece to subject.
  - o Attach breathing tube to subject's mouth piece.
  - o Select VO_2/Metabolic Testing from left hand side of the screen.
  - o Enter subject ID, age, sex, height and weight.
  - o Enter your initials under Tech and select Maximal for Test Degree.
  - o At "Ready to Start Testing" window, wait 1 minute or until $FECO_2 > 3\%$
  - o Set cadence at 50 rpm
  - o Females
    - Initial workload is 1 kp for 2 mins
    - Increase the workload by 0.5 kp each 2 minutes
  - o Males
    - Initial workload is 2 kp men for 2 minutes
    - Increase the workload by 1 kp each 2 minutes