ABSTRACT

Interaction between Cell Adhesion Molecules and Stress Hormones following Different Intensities of Cycling Exercise in Obese Males

Jinkyung Park, Ph.D.

Mentor: Yunsuk Koh, Ph.D.

The purpose of the current study was to examine the interaction between exercise-induced stress hormones [epinephrine (E), norepinephrine (NE), and cortisol (COR)] and soluble cell adhesion molecules [soluble intercellular adhesion molecule 1(sICAM-1), soluble vascular cell adhesion molecule 1(sVCAM-1), and soluble endothelial selectin (sE-selectin)] over 24 hours following different intensities of exercise in obese men. In a randomised, cross-over design, 15 physically inactive (physical activity < 2 days per week) obese [body mass index (BMI) > 30 kg/m^2] men between the ages of 18-30 years performed a single bout of cycling exercise (average energy expenditure ~ 300 kcal) at two different intensities [lower-intensity (LI]: 50% of maximal heart rate and higher-intensity (HI) : 80% of maximal heart rate] in random order. Overnight fasting blood samples were collected at baseline, immediately post-exercise (IPE), 1-hr PE, and 24-hr PE. All data were analysed using an analysis of variance with repeated measures, along with Bonferroni multiple comparisons. A linear regression analysis was used to examine the interaction between exercise-induced stress hormones and soluble cell adhesion

molecules (sCAMs) ($p < .05$). sICAM-1, sVCAM-1, E or NE did not change, while sE-selectin at 1-hr PE (10.25±1.07 ng/mL) significantly decreased ($p = .045$) from baseline (12.22±1.39 ng/mL). COR at IPE (262.12±31.09 ng/ml) was significantly higher ($p = .001$) than 1-hr PE (189.35±31.11 ng/ml) during HI. In contrast, COR at IPE (187.52±31.09 ng/ml, $p = .009$) and 1-hr PE (156.24±31.11 ng/ml, $p = .001$) was significantly lower than baseline (259.75±23.07 ng/ml) during LI. COR and sICAM-1 had a negative relationship at 1-hr PE during LI ($r^2 = .34$, $p = .02$), whereas COR and sVCAM-1 had a positive relationship at IPE during HI ($r^2 = .36$, $p = .02$). Additionally, there was a positive relationship between E and sE-selectin at 1-hr PE during HI ($r^2 = .56$, $p = .01$) and a negative relationship between COR and sE-selectin at baseline during LI and HI ($r^2 = .32$, $p = .03$ and $r^2 = .70$, $p < .001$, respectively). An exercise-induced decrease in sE-selectin observed in the current study suggests sE-selectin may be an early marker of exercise-induced immunosuppression due to epinephrine. Although sICAM-1 and sVCAM-1 did not significantly change following exercise, a significant interaction between COR and sICAM-1 and sVCAM-1 suggests COR may play a critical role in the modulation of sICAM-1 and sVCAM-1.

Interaction between Cell Adhesion Molecules and Stress Hormones following Different Intensities of Cycling Exercise in Obese Males

by

Jinkyung Park, B.S., M.M.N.

A Dissertation

Approved by the Department of Health, Human Performance, and Recreation

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Paul M. Gordon, Ph.D., Chairperson

Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of

Doctor of Philosophy

Approved by the Dissertation Committee

_____

Yunsuk Koh, Ph.D., Chairperson

_____

Darryn S. Willoughby, Ph.D.

_____

Brian C. Leutholtz, Ph.D.

_____

Paul M. Gordon, Ph.D.

_____

Joon Jin Song, Ph.D.

Accepted by the Graduate School

August 2016

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J. Larry Lyon, Ph.D., Dean

Page bearing signatures is kept on file in the Graduate School.

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LIST OF ABBREVIATIONS

BMI– body mass index

CAM– cell adhesion molecule

cAMP– cyclic adenosine monophosphate

CATs– catecholamines

CCL2– chemokine ligand 2

COR– cortisol

DEXA– dual energy x-ray absorptiometry

E– epinephrine

HRmax– maximal heart rate

ICAM-1–intercellular adhesion molecule 1

LDL– low-density lipoprotein

LFA-1– lymphocyte function-associated antigen 1

MCP-1– monocyte chemotactic protein 1

NE– nor-epinephrine

RPE– ratings of perceived exertion

sE-selectin– soluble endothelial selectin

sICAM-1– soluble intercellular adhesion molecule 1

sVCAM-1– soluble vascular cell adhesion molecule 1

VCAM-1– vascular cell adhesion molecule 1

β2-ARs– β_2-adrenergic receptors

ACKNOWLEDGMENTS

I would never have been able to finish my dissertation without the guidance of my committee members, the help of my colleagues, and the support of my family and husband. I would like to express my deepest gratitude to my advisor, Dr. Yunsuk Koh, for his excellent guidance, care, and patience, and for providing me with an excellent atmosphere for doing research. I would like to thank Dr. Darryn Willoughby as my co-chair, who let me experience the research in his biochemistry lab, patiently corrected my writing, and supported my research. Both Dr. Yunsuk Koh and Dr. Darryn Willoughby have been wonderful mentors ever since I came to the Baylor University in 2011. I would also like to thank Dr. Leutholz, Dr. Song, and Dr. Gordon, from my dissertation committee, for their academic support and input. Special thanks goes to Dr. Gordon, who was willing to participate in my final defense committee. I would like to thank Sarah, Tom, Josh, Paul, and Elisa, from the laboratory of Dr. Willoughby, for helping me collect data. My research would not have been possible without their help, and it would have been a lonely lab without them. I would also like to thank my parents, who have always supported me and encouraged me with their best wishes. Finally, I would like to thank my husband, Hojae Lee, and my 10-month old son, Jayden Lee. Special thanks goes to my husband, who has always been there to cheer me up and has stood by me through the good times and bad.

CHAPTER ONE

Introduction

Atherosclerosis is the most common type of coronary artery disease and involves endothelial dysfunction and inflammation (Madamanchi et al., 2005). Increased leukocytes within the circulation in response to endothelial dysfunction or inflammation augment the expression of cell adhesion molecules (CAMs) on endothelia, including the integrin, immunoglobulin super-family, selectin, and cadherin families. (Nakashima et al., 1998; Wang et al., 2008). The selectins, including E-selectin and P-selectin, are receptors for carbohydrate ligands on the surface of leukocytes. Selectin function, by binding with leukocytes weakly, drawing them from circulation toward the endothelium and inducing them to decelerate and begin to “roll” across the surface of the endothelium (Bevilacqua, 1993). The immunoglobulin super-family, containing intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), interacts with integrins on the surface of the rolling leukocyte to promote its strong binding to the endothelium (Carlos & Harlan, 1994; Springer, 1990). Once bound, The leukocytes migrate into the vascular wall where they will consequently become foam cells, a hallmark of atherosclerosis (Galkina & Ley, 2007).

Increased expression of CAMs on endothelia stimulates CAMs being released into the circulation in soluble form (Pigott et al., 1992). In addition, circulating levels of soluble ICAM-1 (sICAM-1), soluble VCAM-1 (sVCAM-1), and soluble E-selectin (s E-selectin) are regarded as markers of endothelial injury or activation (Nakai et al., 1995). It

is presumed, therefore, that the soluble CAMs can be considered as important vascular inflammation biomarkers (Blann et al., 2002).

Several stress hormones including catecholamines [CATs; epinephrine (E) and norepinephrine (NE)] and cortisol (COR) are known to modulate CAMs (Fukao et al., 2010). When β-receptors on endothelia, known as catecholamine receptors, are activated in response to physical activity or chronic stress, cyclic adenosine monophosphate (cAMP) activity is elevated, which further increases leukocytes in the circulation (Croymans et al., 2014). At the same time, COR can also enhance the synthesis of CAMs (Alipour et al., 2008). The magnitude of changes in catecholamines and COR in response to exercise is dependent upon the intensity of exercise performed. The higher the exercise intensity, the greater the CATs and COR concentrations (Blake & Ridker, 2002).

In general, exercise can be of great benefit to the prevention of cardiovascular disease (Maldonado et al., 2006). However, there are some arguments regarding the effects of different intensities of exercise on soluble CAMs. Some studies have demonstrated that high-intensity exercise may increase circulating leukocytes and the expression of CAMs (Gleeson, 2007; Shephard, 2003), while low- to moderate-intensity exercise may not significantly change these molecules (Verde et al., 1992). In contrast, several studies have reported that high-intensity exercise may not significantly increase CAMs, but can provide beneficial effects on endothelial function and cardiovascular health (Miles et al., 1998; Petridou et al., 2007; Smith et al., 2000). The changes in CAMs may also depend on the type and duration of exercise (Shephard, 2003). Strenuous endurance exercise induces more marked changes in CAMs than resistance exercise (Smith et al., 2000), and the changes in CAMs appear to be influenced primarily by

intensity rather than duration. (Perez et al., 2001). However, it is still unclear how exercise-induced stress hormones following different intensities of exercise influence CAMs. In addition, examining the interaction between exercise-induced stress hormones and CAMs is important to understand whether exercise-induced stress hormones provide a negative effect on leukocyte migration, leading to an early stage of atherosclerosis.

Most studies have investigated the effect of different intensities of exercise on changes in CAMs in recreational athletes and trained individuals (Akimoto et al., 2002; Gabriel et al., 2012; Goebel & Mills, 2000; Jilma et al., 1997; Li et al., 1999; Miles et al., 1998; Nemet et al., 2002; Perez et al., 2001; Rehman et al., 1997; Simpson et al., 2006; Smith et al., 2000; Wang et al., 2005) rather than sedentary overweight or obese individuals (Petridou et al., 2007). There is only limited information available in regards to the relationship between exercise-induced stress hormones and CAMs in obese individuals. During obesity, excessive adipose tissue produces a number of inflammatory cytokines that contribute to acute and chronic inflammation. In addition, the major risk factors for cardiovascular disease include male sex, physical inactivity, and obesity (Mendis et al., 2011). Consequently, obese male individuals are at a greater risk of cardiovascular disease.

Therefore, it is important to understand the effect of exercise at different intensities on changes in stress hormones and CAMs in physically inactive obese males who are at high risk for cardiovascular disease. The results of the current study may also provide clinical importance for obese patients who have a high risk of immune impairment due to the acute inflammatory response.

Purpose of the Study

The main purpose of this study was to examine the response of soluble CAMs (sICAM-1, sVCAM-1, and sE-selectin) over a 24-hour period and exercise-induced stress hormones [CATs (E and NE) and COR] over a 1-hour period after cycling exercise at different intensities in obese men.

Hypotheses

The following null hypotheses were tested:

H_1: No significant differences would exist in soluble CAMs (sICAM-1, sVCAM-1, and sE-selectin) or stress hormones (E, NE, and COR) between each time point (soluble CAMs for baseline, immediately post-exercise, 1 hour post-exercise and 24 hours post-exercise and stress hormones for baseline, immediately post-exercise, 1 hour post-exercise).

H_2: No significant differences would exist in soluble CAMs (sICAM-1, sVCAM-1, and sE-selectin) or stress hormones (E, NE, and COR) between two different intensities (lower- and higher-intensity).

H_3: No significant interaction would exist between time and intensity in soluble CAMs (sICAM-1, sVCAM-1, and sE-selectin) or stress hormones (E, NE, and COR).

H_4: No significant relationships would exist between soluble CAMs (sICAM-1, sVCAM-1, and sE-selectin) or stress hormones (E, NE, and COR).

Delimitations

1. Fifteen physically inactive obese males [body mass index (BMI) $\geq$30 kg/m^2) between the ages of 18 to 30 years, who had not performed any forms of physical

activity (less than 2 days per week) for 6 months prior to the study, participated in the study.

2. Participants were recruited by flyers from Baylor University and within the surrounding Waco, TX area.
3. Participants were excluded from the study if they have consumed any medication for chronic medical conditions.
4. All participants were considered low risk for cardiovascular disease, with no contraindication to exercise as outlined by the American College of Sports Medicine (ACSM).
5. All participants were tested at the Baylor Laboratory for Exercise Science and Technology (BLEST) and Exercise Nutritional Biochemical Laboratory (EBNL) in accordance with Helsinki Code after signing university-approved informed consent documents.
6. Estimated energy expenditure of 300 kcal for each of the exercise trials were identical.

Limitations

1. The results of the study were only applicable to the larger population of physically inactive obese men between 18 and 30 years of age.
2. Inferences were limited to the time points at which samples were collected.
3. An equation for energy expenditure prediction was an indirect assessment of participants' net caloric goal (300 kcal).
4. Each participant's difference in inherent circadian rhythm due to sleep schedule and daily stresses may affect dependent variables (stress and inflammatory response).

Assumptions

1. All laboratory equipment was functioning properly to produce valid and reliable measurements. Proper calibration and the use of trained research staff were used to minimize any potential for errors.
2. Participants put forth-maximal effort during the HRmax (maximal heart rate) test, lower-intensity exercise trial, and higher-intensity exercise trial.
3. All participants followed the guidelines provided for completion of the study.
4. All participants refrained from any exercise during the study period other than that involved in the study.
5. Participants accurately answered all relevant questions regarding medical history, exercise experience, and other questionnaires used in the study.

Definitions

Atherosclerosis – a chronic inflammatory process that is characterized by the formation of plaques.

Beta-2 adrenergic receptor (β2 –ARs) – a receptor within a cell membrane which reacts with epinephrine.

Catecholamine – an amine-derived hormone causing physiological changes that prepare the body for physical activity such as fight or flight response under stress.

Cell adhesion molecules (CAMs) – proteins located on the cell surface involved in binding with other cells.

Cortisol (COR) – a steroid hormone secreted by the adrenal cortex in response to stress.

Cyclic adenosine monophosphate (cAMP) – a second messenger mediated by β2 –ARs.

Endothelium – a thin layer of cells that lines the interior surface of blood vessel.

Epinephrine (E) – one of a group of catecholamines that is secreted mainly by the adrenal medulla.

E-selectin – endothelia-leukocyte adhesion molecule that are receptors for carbohydrate ligands on the surface of leukocyte.

Higher-intensity exercise – 80% of maximum heart rate.

Hormone – a chemical messenger produced by glands that are released into the circulation to target distant organs to regulate physiology and behavior.

Integrins – transmembrane receptors that are the bridges for cell-cell adhesion molecules.

Intercellular adhesion molecule 1 (ICAM-1) – an endothelial- and leukocyte-associated transmembrane protein for facilitating leukocyte endothelial transmigration.

Leukocyte adhesion cascade – an inflammatory process, in which the leukocytes migrate in a series of steps: capture, rolling, firm adhesion and migration across the endothelium.

Leukocyte migration – a movement of leukocytes out of the circulation, towards the site of tissue damage or infection that is also known as leukocyte recruitment.

Lower-intensity exercise – 50% of maximum heart rate.

Maximum heart rate – a heart rate achieved at the point of maximal exhaustion (Keytel et al., 2005).

Norepinephrin (NE) – one of a group of catecholamines that is released from sympathetic nerve terminals and the adrenal medulla.

Obese male – an adult who is male and has a BMI of 30 (kg/m^2) or higher.

Selectins – transmembrane molecules of CAMs, expressed on the surface of leukocytes and activated endothelial cells mediate leukocyte. A key mediator for the leukocyte rolling along the blood vessel wall.

Soluble endothelial selectin (sE-selectin) – a soluble form of the E-selectin presenting in the circulation and an important biomarker for inflammatory process.

Soluble Intercellular adhesion molecule 1 (sICAM-1) – a soluble form of the intercellular adhesion molecule 1 presenting in the circulation and an important biomarker for inflammatory process.

Soluble Vascular cell adhesion protein 1(sVCAM-1) – a soluble form of the vascular cell adhesion protein 1 presenting in the circulation and an important biomarker for inflammatory process.

Vascular cell adhesion protein 1 (VCAM-1) – a protein mediating the adhesion of the circulating leukocytes to vascular endothelium, leading to development of an atherosclerosis.
Vascular intima – an innermost layer of blood vessel.

CHAPTER TWO

Literature Review

Cell Adhesion Molecules in Atherosclerosis

Atherosclerosis is an inflammatory disease, characterized as the accumulation of leukocytes, smooth vascular cells, and lipids in the arterial intima, which can lead to the formation of lesion that contributes to the narrowing of the arterial lumen (Hansson, 2005; Libby, 2002). In the early stage of atherosclerosis, the accumulation of lipids results in increased low-density lipoproteins (LDL) and subsequent oxidization by free radicals. The free radicals encounter an arterial wall and consequently damage the endothelium, leading to an increase in circulating leukocyte. The recruitment of the circulating leukocytes is a key factor for the accumulation of the leukocytes in atherosclerotic lesion, and CAMs in vascular tissues are known as potential markers for this atherosclerotic inflammatory process. CAMs, glycoproteins expressed on the surface of various cells such as vascular endothelia cells, leukocytes and platelets, mediate a recruitment of the leukocytes (Galkina & Ley, 2007). CAMs are expressed on the vascular endothelium and play a crucial role in the recruitment of the leukocytes from the blood stream into the vascular intima, which is considered a crucial step for the accumulation of the leukocytes in atherosclerotic lesion (Braunersreuther & Mach, 2006).

There are two major groups of CAMs including selectins and immunoglobulin superfamily that are responsible for four major steps of the leukocyte recruitment into the vascular intima (Galkina & Ley, 2007). The four major steps of adhesion cascade for

leukocyte recruitments consist of (1) capture, (2) rolling, (3) firm adhesion, and (4) transmigration (Huo & Ley, 2001) (Figure 1). In the beginning, inflammatory cytokines activate endothelial cells as a result of an infection or vascular injury, which leads to the upregulation of the CAMs in the endothelial cells. The first step of the adhesion cascade involves selectins, which induce leukocyte capturing and rolling. Selectins are composed of three subfamily members, including P-, E-, and L-selectins (Huo & Ley, 2001). P-selectin, which is stored in endothelial cells, quickly appears on endothelial cell surfaces in response to inflammatory cytokines. Inflammatory cytokines also induce synthesis of a second selectin, called E-selectin, which appears on the endothelial cell surface a few hours later. The interaction of P-selectin and E-selectin allows free flowing leukocytes to adhere to the vessel wall called “capture”, so that the adhesive leukocytes can “roll” along the endothelium. Another selectin, known as L-selectin, is expressed on circulating leukocytes and participates in the step of secondary capture, but the role of L-selectin in atherosclerosis is still incomplete. The second step of the adhesion cascade is the interaction between the leukocyte integrins and molecules that belong to the immunoglobulin superfamily including ICAM-1 and VCAM-1, which are also induced on the endothelial cellular surface by inflammatory cytokines. Following rolling, the leukocytes firmly adhere to the endothelium through binding of leukocytes integrins on ICAM-1 and VCAM-1. The final step is the leukocyte migration into the damaged vascular intima, leading to the formation of atherosclerotic lesion under the influence of pro-inflammatory chemokines and the interactions between integrins and the immunoglobulin superfamily of CAMs: ICAM-1 and VCAM-1. Therefore, CAMs (ICAM-1, VCAM-1, and E-selectin) are important in the regulation of the leukocyte

adhesion cascade and this CAMs-regulated process of leukocyte recruitment often results in endothelial cell dysfunction involved in early atherosclerosis.

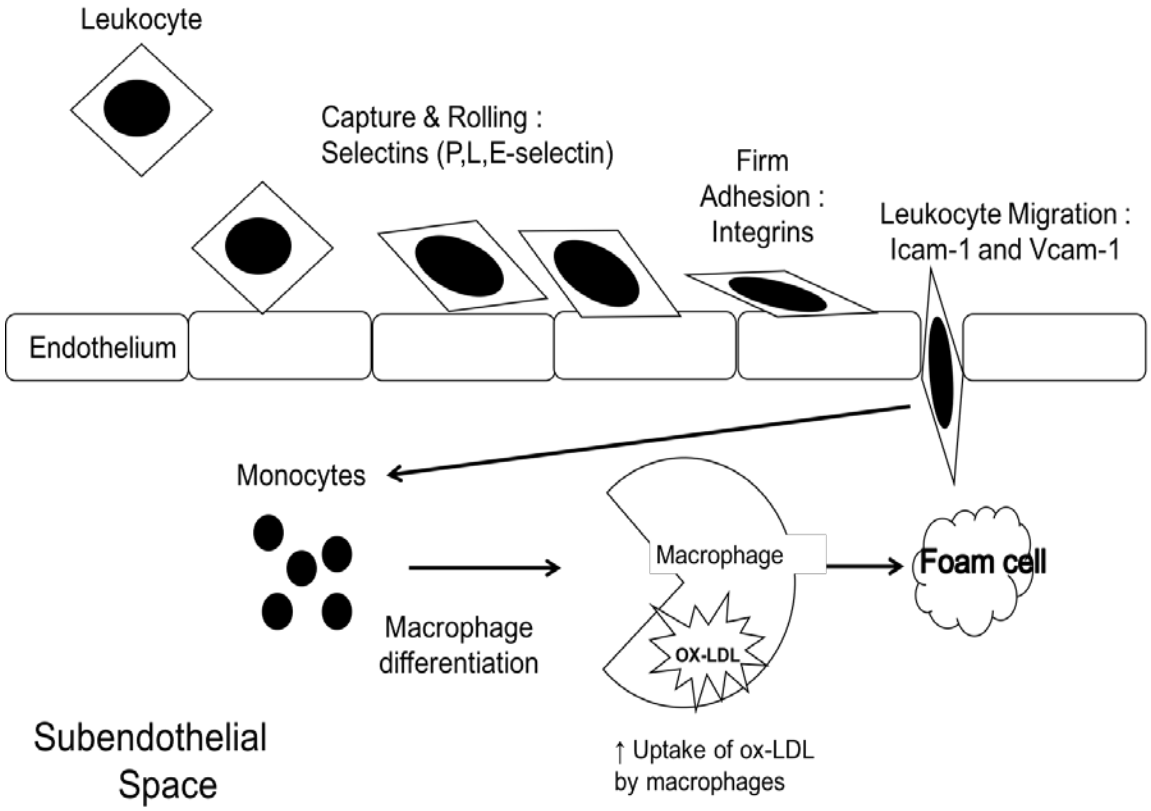

Figure 1. The role of CAMs in the leukocyte adhesion cascade during endothelial inflammation. Selectin family molecules predominantly function during leukocyte capture and rolling in the early inflammatory response, while ICAM-1 and VCAM-1 critically regulate leukocyte adhesion and migration. Activated leukocytes that migrate into inflammatory sites exhibit a high degree of asymmetry with flexibility to change from a spherical to a polarized shape such as a macrophage.

Obesity and Vascular Function

Obesity is an abnormal or excessive accumulation of adipose tissue and typically determined by the BMI, which is calculated as weight (in kilograms) divided by the square of the height (in meters) (WHO, 2014). According to the World Health Organization (WHO), adults with a BMI between 25.0 and 29.9 kg/m^2 are considered overweight, while those with a BMI of 30.0 kg/m^2 or greater are classified as obese

(WHO, 2014). Obesity has continued to grow in the United States and other countries in the world, and 2 in 3 adults are diagnosed as overweight or obese, which constitutes over 150 million adults in the United States (Ogden et al., 2014). Obesity is one of the leading risk factors for cardiovascular disease and endothelial dysfunction (Higashi et al., 2001; Lakka et al., 2001). Steinberg et al. illustrated a significant negative correlation between endothelial function and body fat content (Steinberg et al., 1996). They found endothelium-dependent vasodilatation was significantly reduced by 40-50% in obese subjects with greater than 28% body fat compared to normal weight subjects (Steinberg et al., 1996). Weil et al. (2011) examined the relationship between adiposity and endothelial function in overweight and obese men. The degree of endothelial vasodilator was almost identical in overweight and obese men with and without abdominal adiposity. It appears any excess adiposity, regardless of deposition pattern, is associated with endothelia dysfunction (Weil et al., 2011), suggesting a strong relationship between obesity and vascular function.

Obesity, Inflammation and Oxidative Reaction

Excess body fat increases oxidative reactions, which are crucial in all the events that lead to atherosclerosis (Avogaro & de Kreutzenberg, 2005). Recent data have indicated that endothelial dysfunction involves the interaction between inflammatory pathway and reactive oxygen species (ROS) signaling because of obesity (Hadi et al., 2005). ROS is a phrase used to describe a number of reactive molecules and free radicals derived from molecular oxygen. High levels of ROS is referred as “oxidative stress”, which can lead to accumulation of oxidative damage in cell constituents, including DNA, proteins, and lipids (Forman & Torres, 2002). When ROS are formed, they can act as

intracellular second messengers, modulating the responses as growth of vascular smooth muscle cells and fibroblasts (Droge, 2002). ROS can also regulate several classes of genes, including CAMs and chemotactic factors, antioxidant enzymes and vasoactive substances. Upregulation of CAMs and chemotactic molecules by oxidation reactions is of particular relevant to endothelial dysfunction since these molecules promote adhesion and migration of leukocytes into the vessel wall (Cooper et al., 2002).

Excess body fat triggers an inflammatory response including an increase in leukocyte accumulation (i.e. neutrophils, monocytes/macrophages, T-cells) (Chudek & Wiecek, 2006; Magalang et al., 2006; Zarkesh-Esfahani et al., 2001), secretion of adipose tissue-derived pro-inflammatory adipokines (i.e. leptin and resistin) (Lau et al., 2005), and inflammatory cytokines (i.e. TNF-α and IL-6) (Fain, 2006). In addition, production of anti-inflammatory adipokines and cytokines (i.e. adipokine and IL-10) is reduced in the presence of excess adipose tissue which exacerbates the obesity-induced inflammatory response (Lau et al., 2005). Pro-inflammatory adipokines and inflammatory cytokines are associated with the development and progression of atherosclerosis (Chudek & Wiecek, 2006; Meyers & Gokce, 2007). Increased circulating levels of pro-inflammatory and inflammatory cytokines act on leukocytes and endothelial cells producing ROS and inducing oxidative stress and, consequently, vascular disease (Chudek & Wiecek, 2006).

Exercise-induced Stress Hormones and Cell Adhesion Molecules

The leukocyte adhesion cascade can be mediated by several mechanisms. One of the important mediators involved in the CAMs adhesion cascade is stress hormones. Chronic exposure to stress hormones is associated with chronic inflammation associated

with the development of atherosclerosis, endothelial dysfunction, inflammatory reactivity and oxidative stress (Black & Garbutt, 2002). Additionally, physical activity evokes a strong stress response and immune response (Perna et al., 1997). Exercise-induced stress hormones such as CATs and COR are mainly involved in the immune response. CATs and COR released during exercise regulate the leukocyte migration.

The main CATs found in the body are E and NE (Ganong, 1989). E is secreted mainly by the adrenal medulla, and NE is released from sympathetic nerve terminals and the adrenal medulla (Ganong, 1989). CATs, primarily E, stimulate the leukocyte migration into vascular intima by activation of the leukocyte adhesion cascade. The lymphocyte, neutrophils, and endothelial cells all carry CATs receptors such as β_2-adrenergic receptors (β2–ARs), and the number of these receptors are increased by the exposure to E of CATs since E has higher affinity for β2–ARs than NE (Paur et al., 2012). The stimulation of β2–ARs activates adenylyl cyclase, which increases cAMP and promotes the leukocyte differentiation. As a result, this differentiation causes an increase in the number of leukocytes in the circulation leading to the expression of CAMs including ICAM-1 and VCAM-1, and E-selectin (Shephard, 2003). Therefore, the change in CATs is an important factor for regulating the CAMs.

COR is also central to the regulation of the immune response. Elevated COR levels in circulation can increase the leukocyte migration into vascular intima by upregulating chemokine receptors on the surface of the leukocytes and production of inflammatory cytokines such as interleukin (IL)-2, IL-4, and transforming growth factor (TGF)-beta (Okutsu et al., 2005). The upregulation of the chemokines on monocytes assists monocytes to migrate toward monocyte chemotactic protein-1/chemokine ligand2

(MCP-1/CCL2), which is expressed in damaged tissue (Rollins, 1996; Warren et al., 2004). Both CCL2 and MCP-1 are important for the leukocyte migration into a damaged tissue (Shireman et al., 2007; Warren et al., 2005; Warren et al., 2004) Cortisol also suppresses the immune response through a number of mechanisms (Coutinho & Chapman, 2011). For example, cortisol reduces the release of proinflammatory enzymes and reduces damage to vascular endothelium, thereby decreasing leukocyte migration (Coutinho & Chapman, 2011). Cortisol can promote increased transcription of anti-inflammatory genes and thus can down regulate the inflammatory response (Coutinho & Chapman, 2011). Most of the anti-inflammatory actions of cortisol are attributable either directly or indirectly to the transcriptional effects of glucocorticoid receptor (GR) agonism, which alters transcription of numerous genes in leukocytes (Coutinho & Chapman, 2011). It is clear that cortisol regulation of inflammation can vary from anti- to a pro-inflammatory in a concentration dependent GR mechanism. Therefore, exercise-induced stress hormones may have important effects on the regulation of inflammation.

It is well recognized that exercise elevates levels of stress hormones such as CATs and COR (Mastorakos et al., 2005). Additionally, the stress hormones are greatly influenced by exercise intensity (Mastorakos et al., 2005). For example, high-intensity exercise has been shown to induce the release of CATs and COR, with corresponding alterations in circulating leukocytes, leading to increase in sCAMs (Mastorakos et al., 2005). These findings indicate that exercise-induced acute stress enhances cellular immunity and that this alteration can be enhanced at higher intensities of physical exercise. However, the role of exercise-induced stress hormonal responses to exercise of intensity in the leukocyte adhesion cascade is controversial. Current evidence supports

that exercise-induced stress hormones can modulate expressions of CAMs such as ICAM-1, VCAM-1, and E-selectin (Black & Garbutt, 2002). Although moderate-intensity exercise may improve immune function (Jordan et al., 1997), recent evidence claims that high-intensity exercise may negatively influence the immune response by increasing CAMs (Gleeson, 2007). High-intensity exercise may also increase the density of β2–ARs in leukocytes and affect immune responses (Shephard, 2003). For example, high-intensity cycling exercise showed a strong correlation between the highest values of the leukocyte activation and the CATs concentration in trained men and untrained women (Ortega, 2003; Ortega Rincon et al., 2001). Exercise-induced COR increased the migration of the monocytes toward the chemokines, suggesting that COR may activate the leukocytes, which may influence inflammatory responses following exercise (Okutsu et al., 2008).

However, these findings did not extend the role of exercise-induced stress hormones in the altered expression of CAMs. The effects of exercise-induced CATs on the activation of the leukocytes and CAMs are still controversial. Following exercise to exhaustion at 110% of anaerobic threshold, trained individuals increased the density of CATs receptors and expression of inflammatory cytokines, while CAMs expressions were decreased (Gabriel & Kindermann, 1998). However, some studies suggested that CATs could stimulate the release of soluble CAMs from endothelial cells by CATs adrenoceptor mechanisms (Carlos & Harlan, 1994; Gearing & Newman, 1993). The CAMs express beta-aderenoceptors (β-ARs) and CATs that increase during exercise could influence β-ARs upregulation, resulting in the release of cells into the circulation (van Eeden et al., 1999).

The effect of exercise-induced COR on the leukocyte recruitment is also unclear. Some studies reported that the COR-induced leukocyte recruitments during exercise are comparable with increased release of leukocytes from bone-marrow (Carlson et al., 1989). However, others reported that there was no significant increase in the number of circulating leukocytes that were released from bone marrow during acute maximal exercise. Rather, circulating leukocytes numbers are proliferated by CATs response. The authors suggest that the increase in the density of β2 –ARs on the leukocytes and the CAMs on the surface of the endothelium may foster the leukocyte recruitments into the vascular intima (Nakagawa et al., 1998). In addition, vascular monocytes which differentiate macrophages within atherosclerotic plaque can express the highest levels of the COR receptors, and it is likely that elevated levels of COR by stress would enhance the leukocyte migration to vascular wall (Rickard & Young, 2009). At the same time, COR can induce the synthesis of CAMs directly, thereby promoting adhesion of vascular leukocytes migration into the vascular wall (Gu et al., 2012). On the other hand, a recent study concluded that COR regulation of inflammation is dualistic; it can augment and reduce inflammation due to a negative feedback on inflammatory cytokines which involve changes in sCAMs (Yeager et al., 2011; Zouhal et al., 2008).

Although, the role of exercise-induced stress hormones in the leukocyte adhesion cascade is still not fully elucidated, the effect of exercise-induced stress hormones may contribute to the leukocyte adhesion cascade by activated β2-receptors on the leukocytes and promote the leukocyte migration through COR-mediated inflammatory cytokines. Therefore, exercise-induced stress hormone may modulate vascular inflammation and it is associated with exercise-intensity.

Effect of Different Intensities of Exercise on Cell Adhesion Molecules

Exercise in general may improve vascular health by positively affecting CAMs (Pate et al., 1995). However, the effects of different intensities of exercise on CAMs are not clear. Based on the limited information, high-intensity exercise may influence the numbers of circulating leukocytes and CAMs, probably due to increased stress hormones secretion, while low-to moderate-intensity exercise does not affect the numbers of circulating leukocytes and CAMs (Jilma et al., 1997).

Low- to Moderate-Intensity Exercise

Some studies show that moderate-intensity exercise does not affect the number of leukocytes (Ortega et al., 1993; Shinkai et al., 1992; Suzui, 2002). For instance, cycle exercise at 50% of aerobic power did not affect either neutrophil adherence or the expression of adhesion molecules on neutrophils. After 15 minutes of exercise, neutrophil adherence was significantly stimulated, but it had returned to basal values (Ortega et al., 1993). In another study, 30 minutes of endurance exercise at 60% of maximal aerobic power did not change the expression of lymphocyte function-associated antigen 1 (LFA-1) integrin (Suzui, 2002), which is considered a crucial component for the firm adhesion of circulating leukocytes to endothelial cells expressing intercellular adhesion molecule 1 (ICAM-1) (Goebel & Mills, 2000). However, 60 minutes at the same intensity of exercise is associated with a decreased expression of L-selectin on both neutrophils and lymphocytes (Shinkai et al., 1992).

Other studies showed that low-to–moderate-intensity exercise improved vascular inflammation related to CAMs (Cuzzolin et al., 2000; Goebel & Mills, 2000; Jilma et al., 1997; Jordan et al., 1997). For example, acute cycling exercise significantly decreased the

percentage of CAMs at moderate-intensity in active males or did not affect CAMs at low-intensity in inactive males, suggesting that low-to moderate-intensity of exercise can prevent vascular inflammation (Cuzzolin et al., 2000). Acute moderate-intensity of cycling exercise also significantly decreased expression of L-selectin. However, the number of circulating leukocytes and the density of LFA-1 and integrin on the leukocytes were increased, indicating that although the exercise stressor led to an increase in the number of circulating leukocytes and the density of LFA-1, moderate-intensity exercise may have a beneficial effect on the regulation of the vascular CAMs such as L-selectin (Goebel & Mills, 2000). sICAM-1, sVCAM-1 and sE-selectin remain unchanged at 60 minutes of moderate-intensity cycling exercise in healthy men (Jilma et al., 1997). Moderate-intensity treadmill exercise also leads to a decrease of integrin receptors on leukocytes, which involves decreases in the leukocyte activation (Jordan et al., 1997).

High-Intensity Exercise

The results of the effects of high-intensity exercise on changes in CAMs are inconsistent. Some studies have reported that high-intensity exercise enhances the leukocyte adhesion and inflammatory CAMs proliferation. For instance, acute maximal cycling exercise increased expression of P-selectin and LFA-1 or ICAM-1, indicating that high-intensity exercise may cause intensity-dependent leukocyte activation (Goebel & Mills, 2000; Li et al., 1999). According to Granton et al. (1999), high-intensity exercise might be responsible for the changes of CAMs through the development of a marginated pool where the leukocytes are sequestered in the vessels from the circulating blood. Granton et al. (1999) also showed that acute maximal cycling exercise decreased L-selectin in the circulating leukocytes, suggesting the drop in L-selectin expression in the

circulating leukocytes might be associated with the increase of L-selectin expression in the marginated pool which plays an important role in the leukocyte-mediated tissue inflammation (van Eeden et al., 1999). Acute maximal treadmill exercise also significantly increased the plasma levels of sICAM-1 due to direct adrenoceptor stimulation on lymphocytes and endothelial cells (Rehman et al., 1997).

However, other studies suggest that high-intensity exercise may provide beneficial effects on vascular inflammation. Jilma et al. (1997) observed no change in sICAM-1, sVCAM-1, and sE-selectin with no significant difference between 60 minutes of endurance exercise and maximal cycling exercise. The higher leukocyte counts were found after a 60-minute endurance exercise than after maximal cycling exercise, indicating that the intensity of exercise has little influence on CAMs; instead, the duration of exercise might be a determinant of leukocyte counts. Previous studies suggest that changes in CAMs may be reflective of generalized physiologic changes of blood flow and shear stress during exercise (Arber et al., 1991; Miles et al., 1998; Smith et al., 2000). Exercise increases blood flow, inducing shear stress. An increase in shear stress reduces the adhesiveness of the leukocytes, and this effect of the shear stress inhibits atherogenesis (Niebauer & Cooke, 1996). Therefore, some studies that examined the beneficial effects of high-intensity exercise on changes in CAMs suggest that high-intensity exercise may produce beneficial effects on changes in CAMs due to shear stress (Miles et al., 1998; Smith et al., 2000). For instance, high-intensity resistance exercise reduced the soluble CAMs, P-selectin at 24 and 144 hours after exercise (Smith et al., 2000) or did not change L-selectin (Miles et al., 1998). High-intensity treadmill exercise has also shown improvements in sICAM-1 in overweight individuals (Roberts et al.,

2006). According to Roberts et al. (2006), ICAM-1 is greater in visceral fat within the obese individuals, compared to lean individuals; the reason for the reduction in sICAM-1 after high-intensity treadmill exercise could be caused by a reduction of visceral fat after exercise. Therefore, high-intensity exercise could provide beneficial effects on the changes of CAMs due to the increase in shear stress or the reduction in visceral fat; although, previous literature does not show clear evidence that the changes in CAMs are associated with shear stress or BMI levels.

Effect of Different Types and Durations of Exercise on Cell Adhesion Molecules

A majority of previous studies examined different intensities of exercise involving exercise-modulated changes in CAMs. Recent studies also suggest that different types and durations of exercise also affect the changes in CAMs.

Brief Endurance Exercise

Some studies showed that brief maximal exercise affects the changes in CAMs. Following a maximal exercise test, the plasma levels of sICAM-1 and sVCAM-1 in patients with intermittent claudication were elevated, suggesting exercise increased vascular inflammatory CAMs in the population at high risk for cardiovascular disease (Brevetti et al., 2001). Following maximal cycling exercise, there was an increase in the number of the circulating leukocytes expressing sICAM-1 and the density of LFA-1 on the lymphocytes in healthy men, indicating that exercise might be related to the activation of the immune system (Goebel & Mills, 2000). Following cycling exercise at 90% of maximal effort and 60 minutes of cycling exercise, there were no changes in CAMs, but higher numbers of neutrophil counts were observed after 60 minutes of cycling exercise

than after brief cycling exercise, indicating that the duration of exercise might be a determinant of neutrophil counts; although, both brief and 60 minutes of cycling exercise did not affect changes in CAMs (Jilma et al., 1997). Following brief treadmill exercise, an increase in sICAM-1 levels were observed and sICAM-1 levels were mitigated after treatment with the beta-adrenergic antagonists, suggesting that brief exercise leads to shedding of the CAMs via adrenergic mechanisms (Rehman et al., 1997).

Prolonged Endurance Exercise

Four of eight studies have found no changes in CAMs (De la Fuente et al., 1993; de la Fuente et al., 1990; Ortega et al., 1992; Rodriguez et al., 1991), whereas the rest of the studies have found an increase in sICAM1 or sVCAM-1. Following an ultra-marathon, significant increases of sVCAM-1, sE-selectin, and leukocytes were observed, and sVCAM-1 was significantly associated with running speed, supporting the positive relationship between vascular inflammation and exercise intensity due to an increase in shear stress and increased stress hormones (Jee & Jin, 2012). Jee and Jin (2012) suggested that changes in CAMs might be affected primarily by exercise intensity rather than exercise duration, and increased shear stress and stress hormones may increase the activation of CAMs to the risk level of vascular inflammation (Jee & Jin, 2012). All CAMs including sE-selectin, sP-selectin, sVCAM-1, and sICAM-1 were also increased following a marathon run, suggesting that prolonged and intensive endurance exercise altered CAMs due to a sustained increase in oxygen metabolism and upper respiratory tract infections (Nielsen & Lyberg, 2004). However, further research into the mechanisms underlying the altered CAMs responses to prolonged and intensive endurance exercise is necessary. Perez et al. (2001) compared the effect of 90 minutes of prolonged endurance

exercise versus 30 minutes of brief endurance exercise on change in CAMs. Contrary to their hypothesis, a shorter duration, single bout of exercise led to a marked increase in the numbers of the leukocytes and CAMs such as L-selectin and LFA-1 compared to prolonged endurance exercise. The authors suggested that the differences between the short duration of exercise and the long duration of exercise with respect to changes in the numbers of the leukocytes and CAMs can be explained by the intensity of the exercise, since despite the shorter duration, the intensity of the short duration of the exercise exceeded the intensity of the long duration of the exercise (Perez et al., 2001).

Interval Endurance Exercise

Intense interval endurance exercise produced a similar pattern of response to continuous endurance exercise. A post-exercise increase in CAMs expression such as LFA-1 has been previously demonstrated (Gray et al., 1993). Instead, ten 2-minute periods of heavy cycling exercise had a larger effect on changes in CAMs than a 90-minute soccer practice due to the differences of the intensity of exercise (Perez et al., 2001). However, Hovanloo et al. (2013) showed no significant differences in inflammatory markers between intense interval endurance exercise and moderate-intensity continuous endurance exercise; although, the effects of the two exercise protocols on changes in CAMs were not confirmed (Hovanloo et al., 2013).

Resistance Exercise

As with the endurance type of exercise, the high-intensity of resistance exercise increases the post-exercise expression of sICAM-1 (Nemet et al., 2004) and L-selectin on circulating leukocytes (Malm et al., 1999; Miles et al., 1998) and LFA-1 on monocytes

(Pizza et al., 1996). The high-intensity of resistance exercise also increases inflammatory cytokines, but these elevations are modest compared to strenuous endurance exercise (Smith et al., 2000). In addition, the magnitude of the changes in CAMs after resistance exercise are smaller than endurance exercise since the changes in vascular inflammatory CAMs are apparently related to the intensity and duration of exercise and seem to be most pronounced at least after strenuous endurance exercise (Natale et al., 2003; Wittels & Kanduth, 1995).

CHAPTER THREE

Methods

Participants

Fifteen apparently healthy, physically inactive obese males (BMI ≥30 kg/m^2) between the ages of 18 to 30 years, who had not engaged in any form of physical activity (< 2 days per week) for at least 6 months prior to the study, volunteered for this study. Enrollment was open to men of all ethnicities. Only participants, who were not on any medication for chronic medical conditions, were classified as low risk for cardiovascular disease, and had no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM), and who have not consumed any diet supplements for weight loss one month prior to the study were allowed to participate. All eligible participants signed university-approved informed consent documents, and the Institutional Review Board granted an approval for Human Subjects. Additionally, all experimental procedures involved in the study were conform to the ethical consideration of the Helsinki Code.

Study Site

All entry session assessments such as anthropometric and body composition Testing were conducted in the body composition lab at Baylor University. All study protocols including entry/familiarization sessions, exercise trials were conducted in the Baylor Laboratories for Exercise and Sport Technology (BLEST) at Baylor University. All sample analyses were completed in the Exercise, and Biochemical Nutrition Laboratory (EBNL) at Baylor University.

Study Design

In a randomized, cross-over design, participants visited the laboratory on five separate occasions in the following manner: visit 1 = entry/familiarization sessions, visit 2 = exercise trial 1, visit 3 = 24-hour follow-up trial 1, visit 4 = Exercise trial 2, visit 5 = 24-hour follow-up trial 2. After an initial entry session, participants performed two separate exercise trials consisting of a lower-intensity exercise (50% of maximum heart rate) protocol and a higher-intensity exercise (80% of maximum heart rate) protocol in random order. The exercise trials were randomly determined by a coin flip. Each exercise trial was performed at least 7 days apart to allow the participants to fully recover and return to basal conditions. During each exercise trial, the participants exercised on the cycle ergometer until they expended 300 kcal. Overnight fasting blood samples were collected from the antecubital vein at baseline, immediately post-exercise (IPE), 1 hour post-exercise (1-hr PE), and 24 hours post-exercise (24-hr PE) for each exercise trial to analyze the changes sICAM-1, sVCAM-1, and sE-selectin. Exercise-induced stress hormones including E, NE and COR were determined at baseline, IPE and 1-hr PE for each exercise trial.

Independent and Dependent Variables

The independent variables were 1) exercise intensity (lower- and higher-intensity) and 2) time (baseline, IPE, 1-hr PE, and 24-hr PE). Dependent variables were soluble CAMs (sICAM-1, sVCAM-1, and sE-selectin) and exercise-induced stress hormones (E, NE, and COR).

Entry and Familiarization Session

The participants expressing interest in participating in this study were initially interviewed on the phone and/or via e-mail to determine whether they were qualified to participate in this study. The participants that met all inclusion criteria were then invited to attend an entry session. Once reporting to the lab, the participants were provided with the detailed study protocol via a verbal and written explanation outlining the study design. Once the participants decided to volunteer for the study, they signed the informed consent form and completed a medical history questionnaire. During the entry session, body composition assessment and HRmax testing were conducted after the participants signed the informed consent form.

Anthropometric and Body Composition Testing (Entry Session)

Total body mass (kg) was determined by using a calibrated electronic scale with a precision of ± 0.02 kg (Detecto, Webb City, MO). To sufficiently define the anthropometric characteristics of the participants, percent body fat (% BF), fat mass (kg), and fat-free mass (kg) were determined using dual-energy x-ray absorptiometry [(DEXA) Hologic Discovery, Bedford, MA]. The participants were asked to lie in a supine position on the DEXA table in only shorts and a t-shirt and to remain motionless for approximately 6 minutes while the scan was being performed. The participants were exposed to a low dosage of radiation during the scan equal to approximately 1.5 mR of radiation. The maximal amount of x-ray radiation exposure per year for non-occupation exposure is 500 mR. The radiation exposure was not significantly more than the background radiation in the local Waco area. The DEXA scans were segmented into

regions (right & left arm, right & left leg, and trunk), and each of these segments were analyzed for total percent body fat (% BF), fat mass (kg), and fat-free mass (kg).

Maximal Heart Rate Test Protocol (Entry Session)

The HRmax test was conducted using a Monark cycle ergometer (Medgraphics, Monark, Ergomedic, Model No. 828E, St Paul, MN) based on the protocol developed by Keytel et al. (Keytel et al., 2005) to estimate the participants' HRmax (defined as heart rate achieved at the point of maximal exhaustion). The participants warmed up on the bike for 2 minutes with a workload of 2 Watts/kg of body weight. The participants kept pedaling at the speed of 70 revolutions per minute (rpm) throughout the test. After the warm-up, the participants began cycling at an exercise intensity of 3.33 Watts/kg of body weight for 150 seconds (stage 1). Upon the completion of the stage 1, the workload increased by additional 50 Watts for another 150 seconds (stage 2). Thereafter, the workload increased by 25 W every 150 seconds until the participants could no longer maintain 70 rpm or volitionally stopped the test. Prior to the HRmax testing, the participants' resting heart rate and blood pressure were measured after 15 minutes of rest in a supine position on the exam table. During the HRmax test, the participants wore a heart rate monitor (Polar Electro Inc., Lake Success, NY) around the chest, and heart rate was recorded every minute throughout the test. The workload, exercise time, heart rate, blood pressure, and ratings of perceived exertion (RPE) for each stage were recorded.

Heart Rate and Blood Pressure (Entry and Exercise trials)

At all visits, heart rate and blood pressure were measured and recorded. At the entry and familiarization sessions, these variables were obtained as part of the initial assessment. At visits 2 and 5, heart rate and blood pressure were obtained at each time

point where blood samples were obtained. Exercise heart rate was also obtained every stage during the exercise trials by using a heart rate monitor (Polar Electro Inc., Lake Success, NY). Resting heart rate and blood pressure were assessed in the supine position after resting for 5 minutes in a chair using a heart rate monitor and a mercurial sphygmomanometer, respectively, with standard procedures.

Exercise trials (Lower- and Higher-Intensity)

The predetermined HRmax from the HRmax test protocol was used to determine the appropriate target exercise heart rate for lower- (50% of HRmax) and higher-intensity (80% of HRmax) exercise trials and to estimate the duration of exercise for each exercise trial that allowed the participants to expend 300 kcal based on an equation for energy expenditure prediction by Keytel et al. (2005) (Keytel et al., 2005): gender X (-55.0969 + 0.6309 X heart rate + 0.1988 X weight + 0.2017 X age) + (1 – gender) X (-20.4022 + 0.4472 X heart rate – 0.1263 X weight + 0.074 X age). During both lower- and higher-intensity exercise trials, the participants wore a heart rate monitor (Polar Electro Inc., Lake Success, NY) around the chest and rested in a supine position for 15 minutes to measure resting heart rate and blood pressure at baseline. The participants then warmed-up for 2 minutes with 2.0 Watts/kg of body weight at 70 rpm. After the 2-minute warm-up, the appropriate work intensity, which was determined from the HRmax test, for each exercise trial (lower- or higher-intensity), was loaded to the cycle ergometer, and then the participants exercised until they expended 300 kcal for each exercise trial. During each exercise trial, heart rate was monitored and recorded every 3 minutes to ensure the appropriate target exercise intensity. In addition, blood pressure was measured every 10 minutes throughout the exercise trial. Immediately following the completion of each

exercise trial, blood samples (IPE) were collected, and then the participants rested for 1 hour in the laboratory where the participants were not allowed to perform any physical activity. At the end of a 1-hour resting period, another blood sample (1-hr PE) was collected, and the participants were allowed to leave. The participants were asked to refrain from any physical activity until the last blood sample was collected 24-hours post-exercise (24-hr PE).

Blood Analysis

Blood Sampling (Exercise trials)

The participants reported to the lab in the morning after an overnight fast, and venous blood samples (10 ml in one serum separator tube and 10 ml in one ethylenediaminetetraacetic acid (EDTA) - containing plasma tube) were obtained from the antecubital vein using standard venipuncture procedures. Blood samples in the plasma tube remained at room temperature for 10 minutes and then centrifuged at 1000g for 15 minutes to separate plasma. Blood samples in the serum separator tube remained at room temperature for 20 minutes to be clotted and then were centrifuged at 1000g for 20 minutes to separate serum. Aliquots of plasma and serum samples were pipetted into 1.5 mL polypropylene tubes and immediately be frozen at -80°C for later analyses. Blood samples were collected at four different time points [baseline, IPE, 1-hr PE, and 24-hr PE] for each exercise trial.

Analysis of Soluble Cell Adhesion Molecules and Stress Hormones

Cell Adhesion Molecules

sICAM-1 and sVCAM-1. Serum samples were analyzed in duplicate for sICAM-1 and sVCAM-1 (Cat# ZF000000AY, Bio-Rad Laboratories, Hercules, CA, USA) by a multiplex flow immunoassay (MFI). For a sample dilution of ICAM-1 and VCAM-1, 10 µl of serum sample was added to 30 µl of sample diluent. After the sample dilution, 50 µl of 1 x concentration of antibody-conjugated beads were added into a 96-well plate. The plate was washed two times with 100 µl of Bio-Plex wash buffer. 50 µl of standards, blanks, and diluted samples were added to the wells, and incubated at room temperature with shaking at 850 rpm (revolutions per minute). The plate was washed three times again with 100 µl of wash buffer, re-suspended in 125 µl assay buffer, and shaken at 850 rpm for 30 seconds. The concentration of sICAM-1 and sVCAM-1 were determined by median fluorescent intensity (MFI) using a five parameter logistic (5-PL) curve-fit.

sE-selectin. Serum samples in duplicate were analyzed for sE-selectin (Kit# EK0501, BOSTER, Fermont, CA, USA) by an enzyme-linked immunosorbent assay (ELISA). The serum samples were diluted 1:10 with provided diluent buffer. A sE-selectin standard was provided to generate a standard curve for the assay. One hundred µL of standards or diluted samples were pipetted into a pre-coated 96-well plate. After incubation at 37°C for 90 minutes, 100 µL of biotinylated antibody was added. After 1-hour incubation at 37°C, the plate was washed three times with 0.01 TBS. One hundred µL of an avidin-biotin-peroxidase complex (ABC) working solution was added to the wells, and they were incubated at 37°C for 30 minutes. After washing five times with

0.01M TBS, 100 µL of tetramethyl benzene (TMB) color developing agent was added. After incubation at 37°C for 15 minutes, the optical density was read at 450 nm by the spectrophotometer (SmartSpec Plus, Bio-Rad, Hercules, CA, USA) and calculated by a semi-log Fit.

Stress Hormones

E and NE. Serum samples in duplicate were analyzed for E and NE (Kit# EA613/192, DLD Diagnostika GmbH, Adlerhost, Hamburg, Germany) by a BI-CAT ELISA. The test was conducted in two separate steps. First, samples were prepared. Briefly, 300 µL of sample and 50 µL of extraction buffer were added to an extraction plate. After 1-hour incubation and washing steps, 150 µL of acylation buffer and 50 µL of acylation reagent were added to the wells. After 20 minutes of incubation at room temperature and a washing step, 200 µL of 0.025 M HCL was added to the wells. After another 20 min incubation at room temperature, the supernatant was removed. For the measurement of NE, 20 µL of enzyme mix and 15 µL of standards or samples were added to the wells. After 30 minutes of incubation at room temperature, 100 µL of adrenaline-antiserum was added to the wells, and they were incubated overnight at 4°C. After four washes, 100 µL of peroxdiase (POD)-conjugate was added to the wells, and they were incubated for 30 minutes at room temperature. Subsequently, 100 µL of tetramethylbenzidine (TMB) substrate was added to the wells, and following color development (20 minutes), 100 µL of 0.3 molar (M) sulphuric acid stop solution was added. The optical density was then immediately read at 450 nm by the

spectrophotometer (SmartSpec Plus, Bio-Rad, Hercules, CA, USA) and calculated by a 4-parameter logistic fit.

For the measurement of E, 20 µL of enzyme mix and 100 µL of standards or samples were added to the wells. After 30 minutes incubation at room temperature, 100 µL of adrenaline–antiserum was added to the wells and they were incubated overnight at 4°C. After four washes, a 100 µL of POD-conjugate was added to the wells and they were incubated for 30 minutes at room temperature. Subsequently, 100 µL of TMB substrate was added to the wells, and following color development (20 minutes), 100 µL of 0.3M sulphuric acid stop solution was added. The optical density was then immediately read at 450 nm by the spectrophotometer (SmartSpec Plus, Bio-Rad, Hercules, CA, USA). The amount of E and NE were calculated based on standard curves using a 4 parameter logistic fit.

COR. Plasma samples were analyzed in duplicate for COR (Kit# DKO001, DiaMetra, Segrate, MI, Italy) by an ELISA. COR standard was provided to generate a standard curve for the assay. 20 µl of standards or diluted samples were pipetted into a pre-coated microtiter plate. Two hundred µl of cortisol-peroxidase conjugate was added to the standards and samples in the wells. After a 1-hour incubation period, the plate was washed and 100µl of TMB substrate was added. After 15 minutes of incubation, 100 µL of 0.15M sulphuric acid stop solution was added. the color reaction was read at 450 nm by the spectrophotometer (SmartSpec Plus, Bio-Rad, Hercules, CA, USA) and calculated based on standard curves using a 4 parameter logistic fit.

Statistical Analyses

A crossover design was used where each participant served as his own control. The "Shapiro-Wilk test" procedure was employed to determine if data were normally distributed. The dependent variables were soluble CAMs (sICAM-1, sVCAM-1, and sE-selectin) and stress hormones (E, NE and COR). The independent variables were intensity (LI and HI) and time (baseline, IPE, 1-hr PE, and 24-hr PE). Paired sample t-tests were used to compare the dependent variables at baseline between intensities. A 2 x 4 (intensity x time) factorial analysis of variance (ANOVA) with repeated measures was used to test the effects of the lower- and higher-intensity of exercise on sCAMs at baseline, IPE, 1-hr PE and 24-hr PE. In addition, a separate 2 x 4 (intensity x time) ANOVA with repeated measures was used to examine any differences in macronutrients and total calorie intake between lower- and higher-intensity exercise. A 2 x 3 ANOVA with repeated measures was used to test the effects of the lower- and higher-intensity of intensity of exercise on stress hormones (E, NE, and COR) at baseline, IPE and 1-hr PE. The reason for this is that acute stress responses develop during exercise and the levels of the stress hormones peak at IPE before returning to baseline levels 1-hr PE.

The Bonferroni pairwise comparisons were conducted as post hoc tests to locate the significant mean differences. If a significant interaction between intensity and time was found, the follow-up simple effects test was conducted. Linear regression was used to examine the relationship between sCAMs and stress hormones. All statistical procedures were performed using IBM SPSS Statistics software (Version 21.0) and a level of significant was set at 0.05.

Effect sizes were determined based on previous studies (Nielsen & Lyberg, 2004; Olson et al., 2007; Wang et al., 2005) which showed in Table 1. Sample sizes calculations for a two-tailed study design using the Gpower computer program indicated that the sample sizes for sICAM-1 and sVCAM-1 were 6 and 5, respectively, detecting large effects (d= 2.44 and 1.61, respectively) and the sample size of 10 for sE-selectin was needed to detect medium effect (d = .41) with 80% power using a within-between interaction for repeated measures multivariate ANOVAs with alpha at .05. Therefore, a sample size in the current study (n = 15) may have played a role in the significance of statistical comparisons conducted.

Table 1. Effect sizes from previous studies.

References	Variables	Intervention	Baseline	Post-Exercise	F-Value	Effect Size (*d*)	Sample Size (n)
Wang et al., 2005	sICAM-1	LI	147 ± 10	148 ± 11	49.55	2.44	6
		MI	136 ± 7	142 ± 8			
		HI	151 ± 11	159 ± 7			
	sVCAM-1	LI	185 ± 3	182 ± 2	2.93	.59	6
		MI	173 ± 3	175 ± 4			
		HI	173 ± 4	176 ± 3			
	sE-selectin	LI	38.6 ± 6.1	38.9 ± 5.1	1.29	.39	10
		MI	38.1 ± 5.2	38.5 ± 4.6			
		HI	37.2 ± 4.5	40 ± 5			
Olson et al., 2007	sICAM-1	MI	231.5 ± 44	231.2 ± 51.3	6.69	1.03	5
		Con	218.8 ± 49.7	229.8 ± 60			
	sVCAM-1	MI	572.9 ± 143.6	615.5 ± 184.9	7.97	1.12	5
		Con	571.8 ± 150.3	628.8 ± 149.9			
	sE-selectin	MI	24.9 ± 7.5	25.8 ± 8.5	.5	.28	34
		Con	31.7 ± 12.6	31.7 ± 14.2			
Nielsen & Lyberg, 2004	sICAM-1	FM	188 ± 8	198 ± 9	6.12	.96	5
		HM	258 ± 11	279 ± 13			
	sVCAM-1	FM	472 ± 30	574 ± 29	17.24	1.61	5
		HM	504 ± 22	575 ± 25			
	sE-selectin	FM	32 ± 3	37 ± 3	1.11	.41	9
		HM	43 ± 4	46 ± 4			

Note: All data are presented as mean ± standard deviation. d = Cohen's d; n = number; sICAM-1 = soluble intra cellular adhesion molecule-1; sVCAM-1 = soluble vascular cellular adhesion molecule-1; sE-selectin = soluble endothelial selectin; LI = low-intensity exercise; MI = moderate-intensity exercise; HI = high-intensity exercise; Con = control; FM = full marathon; HM = half marathon.

CHAPTER FOUR

Results

Anthropometric and HRmax Data

Physical, anthropometric, and HRmax data of the participants are expressed in Table 2.

Table 2. Anthropometric and HRmax Data.

Variable	Mean ± SE
Age (Years)	21.73 ± .47
Height (cm)	177.09 ± 2.27
Bodyweight (kg)	107.88 ± 4.83
BMI (kg/m^2)	34.25 ± 1.17
Body fat (%)	31.56 ± 1.17
Lean mass (kg)	64.62 ± 2.37
Fat mass (kg)	31.77 ± 2.5
HRmax (bpm)	190.93 ± 3.35

Note: SE = standard error; cm = centimeters; BMI = body mass index; kg = kilograms; m^2 = square meter; % = percent; HRmax = maximal heart rate; bpm = beat per minute.

Dietary Intake

All participants recorded their food intake over 3 consecutive days (from 2 days prior to exercise to the day of exercise) for each exercise trial. There were no significant differences in total calories, fat, carbohydrate, or protein content between exercise trials ($p > .05$). Data for total calories, fat, carbohydrate, and protein content for each exercise trial are presented in Table 3.

Table 3. Dietary Intake of Participants.

Intensity	Variables	-48 hr PRE	-24 hr PRE	Exercise
LI	Total calories (kcals/day)	1811.89 ± 178.72	2134.68 ± 133.58	1690.99 ± 196.62
	Fat (kcals/day)	637.19 ± 126.70	864.04 ± 440.23	749.71 ± 122.21
	Carbohydrate (g/day)	257.70 ± 32.89	233.25 ± 44.01	166.35 ± 37.66
	Protein (g/day)	86.43 ± 13.69	165.48 ± 59.43	73.38 ± 12.48
HI	Total calories (kcals/day)	1820.48 ± 184.99	1833.27 ± 138.27	1739.02 ± 203.52
	Fat (kcals/day)	1073.56 ± 126.70	1402.44 ± 440.23	700.32 ± 122.21
	Carbohydrate (g/day)	217.03 ± 32.89	266.85 ± 44.01	228.56 ± 37.66
	Protein (g/day)	109.71 ± 13.69	95.69 ± 59.43	82.81 ± 12.48

Note: All data are presented as mean ± standard error. LI = lower-intensity; HI = higher-intensity; g = grams; kcals = kilocalories; PRE = prior to exercise; hr = hour.

Workload, Heart Rate, and Time

Each exercise trial was performed at the similar exercise volume (total energy expenditure of 300 kcal). Significant differences were found between LI and HI in workload (p = .001), heart rate (p = .001), and time (p = .001). Data for workload, heart rate, and, time for each exercise trial are presented in Table 4.

Table 4. Workload, Heart Rate and Time for Each Exercise Trial.

Variable	LI	HI	*P* -value
Workload (kp)	1.51 ± .05	2.42 ± .09	.001
Heart rate (bpm)	98.8 ± 1.82	154.2 ± 2.76	.001
Time (min)	42 ± 1.77	19 ± .53	.001

Note: All data are presented as mean ± standard error. LI = lower-intensity; HI = higher-intensity; kp = kilopond; min = minutes; bpm = beats per minute.

Soluble Cell Adhesion Molecules Concentrations

Responses of sCAMs to each exercise trial over time are presented in Table 5. The results of a repeated measures ANOVAs for sCAMs are presented in Table 6.

sICAM-1 and sVCAM-1

Mauchly's tests of sphericity for sICAM-1 and sVCAM-1 indicated that the assumptions of sphericity had been violated for time [sICAM-1: χ^2 (5) = 26.57, p = .001 and sVCAM-1: χ^2 (5) = 24.87, p = .001]; therefore, Greenhouse-Geisser corrections was applied (sICAM-1: ε = .63 and sVCAM-1: ε = .68). There were no statistically significant interactions between time and intensity for sICAM-1 and sVCAM-1 concentrations [sICAM-1: F (1.89, 52.91) = 1.86, p = .17 and sVCAM-1: F (2.05, 57.46) = 2.09, p = .13]. Therefore, analyses of the main effects for time and intensity were performed. The main effects of time revealed no statistically significant differences in sICAM-1 and sVCAM-1 concentrations between time points [sICAM-1: F (1.89, 52.91) = .52, p = .59 and sVCAM-1: F (2.05, 57.46) = .9, p = .41]. The main effects of intensity demonstrated no statistically significant differences in sICAM-1 and sVCAM-1 concentrations between trials [sICAM-1: F (1, 28) = .56, p = .46 and sVCAM-1: F (1, 28) = 1.47, p = .24].

sE-selectin

There was no statistically significant interaction between time and intensity for sE-selectin concentration [F (3, 84) = 1.90, p = .14]. Therefore, analyses of the main effects for time and intensity were performed. The main effect of time revealed statistically significant differences in sE-selectin concentration between time points [F (3, 84) = 2.74, p = .048]. The main effect of intensity demonstrated no statistically

significant difference in sE-selectin concentration between trials [F (1, 28) = .09, p = .76]. The Bonferroni multiple comparisons test showed that sE-selectin was decreased at 1-hr PE as compared to baseline and IPE (10.25 ± 1.07 ng/ml vs. 12.22 ± 1.39 ng/ml and 12.20 ± 1.55 ng/ml, respectively), which was statistically significant (p=.01, and p=.029, respectively) (Figure 2.)

Table 5. sCAMs Concentrations for Each Time Point by Exercise Intensity.

Intensity	Variables	Baseline	IPE	1-hr PE	24-hr PE
LI	sICAM-1 (ng/ml)	126.80 ± 7.27	120.43 ± 8.86	118.64 ± 7.99	126.17 ± 8.25
	sVCAM-1 (ng/ml)	119.01±7.00	112.25 ± 8.25	107.08 ± 6.21	116.19 ± 5.83
	sE-selectin (ng/ml)	12.99±8.85	11.36 ± 6.55	9.6 ± 4.81	10.48 ± 6.67
HI	sICAM-1 (ng/ml)	127.52 ± 7.27	137.97 ± 8.86	129.50 ± 7.99	127.84 ± 8.25
	sVCAM-1 (ng/ml)	119.87 ± 7.00	130.87 ± 8.25	122.74 ± 6.21	121.34 ± 5.83
	sE-selectin (ng/ml)	11.45 ± 6.13	13.04 ± 10.1	10.9 ± 6.76	12.14 ± 5.83

Note: All data are presented as mean ± standard error. LI = lower-intensity; HI = higher-intensity; sICAM-1 = soluble intra cellular adhesion molecule-1; sVCAM-1 = soluble vascular cellular adhesion molecule-1; sE-selectin = soluble endothelial selectin; ng = nanogram; ml = milliliter; IPE = immediately post-exercise; PE = post-exercise; hr = hours.

Table 6. Repeated Measures ANOVAs for sCAMs.

Variables	Source	SS	df	MS	F	*p*
sICAM-1	Intensity	1777061278.61	1	1777061278.61	.56	.46
	Error	88939063544.08	28	3176395126.57		
	Time	401296278.49	1.89	212373512.59	.52	.59
	Time x Intensity	1438175776.82	1.89	761109578.69	1.86	.17
	Error	21596211818.32	52.91	408182860.72		
sVCAM-1	Intensity	3043357635.12	1	3043357635.12	1.47	.24
	Error	58164987352.45	28	2077320976.87		
	Time	692294625.11	2.05	337333383.20	.90	.41
	Time x Intensity	1600881833.60	2.05	780059335.22	2.09	.13
	Error	21463711632.02	57.46	373521113.61		
sE-selectin	Intensity	179534.16	1	179534.16	.09	.76
	Error	54241235.79	28	1937186.99		
	Time	785007.79	3	261669.26	2.74	.04
	Time x Intensity	542859.36	3	180953.12	1.9	.14
	Error	8017573.90	84	95447.31		

Note: sICAM-1 = soluble intra cellular adhesion molecule-1; sVCAM-1 = soluble vascular cellular adhesion molecule-1; sE-selectin = soluble endothelial selectin; SS = sum of square; df = degree of freedom; MS = mean of square; F = F-value; *p* = p-value.

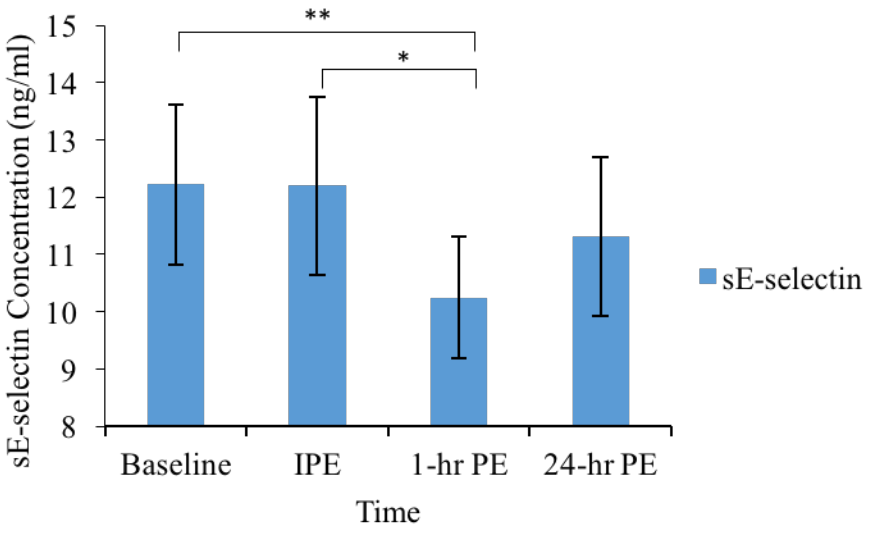

Figure 2. sE-selectin Concentration for Each Time Point.
sE-selectin = soluble endothelial selectin; ng = nanogram; ml = milliliter; IPE = immediately post exercise; hr = hours; PE = post-exercise; * = a significant difference between IPE and 1-hr PE (p = .03). ** = a significant difference between baseline and 1-hr PE (p = .01).

Stress Hormones Concentrations

Responses of stress hormones to each exercise trial over time are presented in Table 7. The results of a repeated measures ANOVAs for stress hormones are presented in Table 8.

Plasma E

There was no statistically significant interaction between time and intensity for plasma E concentration [F (2, 44) = .43, p = .65]. Therefore, analyses of the main effects for time and intensity were performed. The main effect of time revealed no statistically significant differences in plasma E concentration between time points [F (2, 44) = .39, p = .68]. The main effect of intensity demonstrated no statistically significant difference in plasma E concentration between trials [F (1, 22) = .01, p = .91].

Plasma NE

There was no statistically significant interaction between time and intensity for plasma NE concentration [F (2, 42) = 2.6, p = .09]. Therefore, analyses of the main effects for time and intensity were performed. The main effect of time revealed no statistically significant differences in plasma E concentration between time points [F (2, 42) = 1.88, p = .17]. The main effect of intensity demonstrated no statistically significant difference in plasma NE concentration between trials [F (1, 21) = .09, p = .77].

Serum COR

There was a statistically significant interaction between time and intensity for serum COR concentration [F (1.43, 56) = 4.94, p = .02]. Simple effects tests showed that COR was higher at IPE as compared to 1-hr PE for HI (262.12 ± 31.09 ng/ml vs. 189.35 ± 31.11 ng/ml, respectively p = .001). For LI, COR was higher during baseline as compared to IPE and 1-hr PE (259.75 ± 23.07 ng/ml vs. 187.52 ± 31.09 ng/ml and 156.24 ± 31.11 ng/ml, respectively), which was statistically significant (p = .009 and .001, respectively). The responses of COR over 1-hour period between LI and HI are presented in Figure 3.

Mauchly's test of sphericity indicated that the assumption of sphericity had been violated for time [χ^2 (2) = 13.62, p = .001]; therefore, a Greenhouse-Geisser correction was applied (ε = .72). The main effect of time revealed statistically significant differences in serum COR concentration between time points [F (1.43, 56) = 15.48, p = .001]. The main effect of intensity demonstrated no statistically significant difference in serum COR concentration between trials [F (1, 28) = .68, p = .42].

Table 7. Stress Hormones Concentrations for Each Time Point by Exercise Intensity.

Intensity	Variables	Baseline	IPE	1-hr PE
LI	E (pg/ml)	44.07 ± 6.78	41.19 ± 9.06	41.80 ± 7.85
	NE (pg/ml)	257.97 ± 34.99	246.25 ± 49.93	299.87 ± 26.46
	COR (ng/ml)	259.74 ± 23.07*****	187.52 ± 31.09	156.24 ± 31.11
HI	E (pg/ml)	44.29 ± 8.02	48.35 ± 10.72	38.05 ± 9.29
	NE (pg/ml)	214.71 ± 41.40	335.67 ± 59.08	257.39 ± 31.31
	COR (ng/ml)	243.75 ± 23.07	262.12 ± 31.09$^{\#}$	189.35 ± 31.11

Note: All data are presented as mean ± standard error. LI = lower-intensity; HI = higher-intensity; E = epinephrine; NE = norepinephrine; COR = cortisol; pg = picogram; ml = milliliter; ng = nanogram; IPE = immediately post-exercise; PE = post-exercise; hr = hours; ***** = significantly different from IPE and 1-hr PE for LI (p = .009 and .001, respectively); $^{\#}$ = significantly different from 1-hr PE for HI (p = .001).

Table 8. Repeated Measures ANOVAs for Stress Hormones.

Variables	Source	SS	*df*	MS	F	*p*
E	Intensity	25.71	1	25.71	.014	.91
	Error	40167.14	22	1825.78		
	Time	325.74	2	162.87	.39	.68
	Time x Intensity	355.21	2	177.6	.43	.65
	Error	18224.52	44	414.19		
NE	Intensity	2991.2	1	2991.2	.09	.77
	Error	687455.42	21	32735.97		
	Time	47340.13	2	23670.07	1.88	.17
	Time x Intensity	65437.06	2	32718.53	2.6	.09
	Error	528347.56	42	12579.7		
COR	Intensity	21026.87	1	21026.87	.68	.42
	Error	861249.78	28	30758.92		
	Time	96656.22	1.43	67474.06	15.48	.001
	Time x Intensity	30846.2	1.43	21533.21	4.94	.02
	Error	174795.89	56	3121.36		

Note: E = epinephrine; NE = norepinephrine; COR = cortisol; sE-selectin = soluble endothelial selectin; SS = sum of square; df = degree of freedom; MS = mean of square; F = F-value; *p* = p-value.

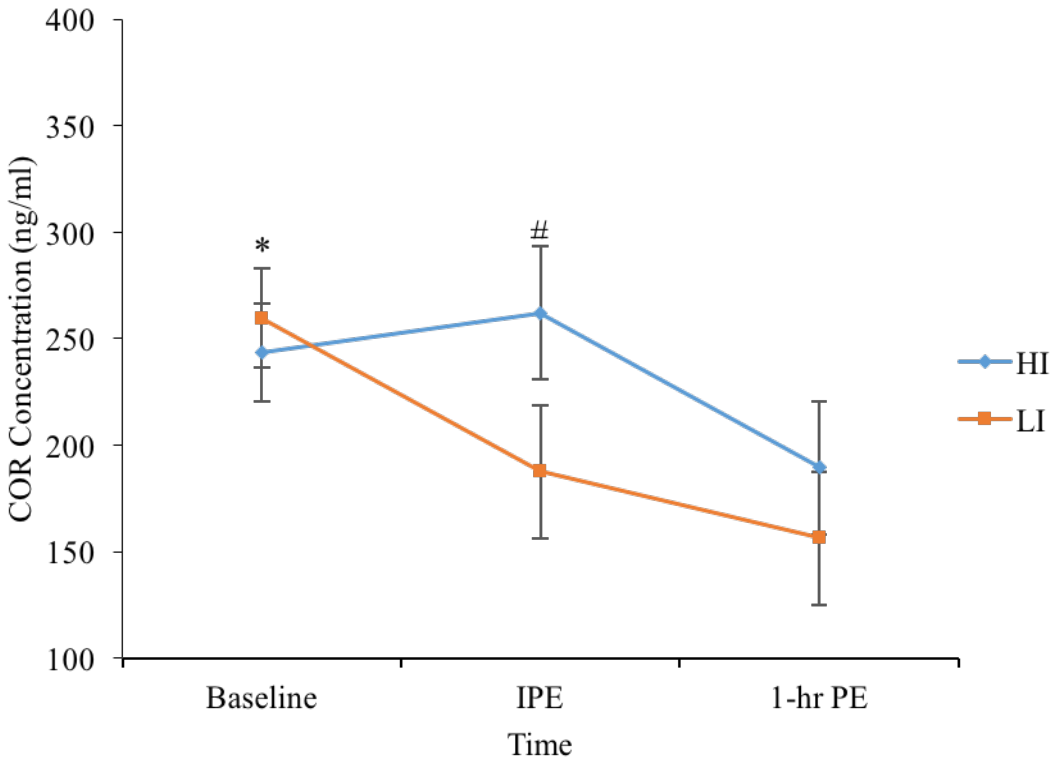

Figure 3. Serum COR Concentration for Each Time Point following Different Intensities. COR = cortisol; ng = nanogram; ml = milliliter; IPE = immediately post exercise; hr = hours; PE = post-exercise; HI = higher-intensity; LI = lower-intensity; * = significantly different from IPE and 1-hr PE for LI (p = .01 and .001, respectively); # = significantly different from 1-hr PE for HI (p = .001).

Relationships between sCAMs and Stress Hormones following Exercise of Different Intensities

LI

Relationships between sCAMs and stress hormones following LI are presented in table 9. There was no statistically relationship between sVCAM-1 and stress hormones at any time following LI. However, a linear regression established that COR could significantly fit sICAM-1 concentration at 1-hr PE for LI [F (1, 13) = 6.73, p = .02] and sE-selectin concentration at baseline for LI [F (1, 13) = 5.96, p = .03]. The COR accounted for 34.1% of the explained variability in sICAM-1 concentration at 1-hr PE for

LI and 31.5% of the explained variability in sE-selectin concentration at baseline for LI. The regression equations were fitted sICAM-1 concentration = 143197 -157.16 x (COR at 1-hr PE for LI) and fitted sE-selectin concentration = 2956.07 – 6.37 x (COR at baseline for LI). The results of the linear regression are presented in Figure 4 and 5.

HI

Relationships between sCAMs and stress hormones following HI are presented in table 10. There was no statistically relationship between sICAM-1 and stress hormones at any time following HI. However, a linear regression established that COR could statistically significantly fit sVCAM-1 concentration at IPE for HI, F (1, 13) = 7.31, p = .02, and sE-selectin concentration at baseline for HI, F (1, 13) = 29.83, p = .001. The COR accounted for 36% of the explained variability in sVCAM-1 concentration at IPE for LI and 69.7% of the explained variability in sE-selectin concentration at baseline for HI. The regression equations were fitted sVCAM-1 concentration = 97231.58 +128.34 x (COR at IPE for HI) and fitted sE-selectin concentration = 2398.98 -5.14 x (COR at baseline for LI). Therefore, there was a positive relationship between COR and sVCAM-1 at IPE for HI and a negative relationship between COR and sE-selectin at baseline for HI. The results of a linear regression for COR are presented in Figure 6 and 7. Additionally, there was a positive relationship between E and sE-selectin at 1-hr PE for HI [F (1, 9) = 11.25, p = .008]. The E accounted for 55.6 % of the explained variability in sE-selectin concentration at 1-hr PE for HI. The regression equation was fitted sE-selectin concentration = 143.08 + 25.13 x (E at 1-hr PE for HI). The result of the linear regression for E is presented in Figure 8.

Table 9. Relationships between sCAMs and Stress Hormones for Lower-Intensity.

Intensity	Independent Variables	Time	Dependent Variables	R^2 (%)	P-value
LI	sICAM-1	Baseline	E	0	1
			NE	11.8	.21
			COR	1.6	.65
		IPE	E	1.3	.68
			NE	0	1
			COR	2.9	.54
		1-hr PE	E	6.4	.39
			NE	1.9	.64
			COR	34	.02*
	sVCAM-1	Baseline	E	.3	.86
			NE	5.2	.41
			COR	7.6	.32
		IPE	E	8.4	.3
			NE	4.7	.44
			COR	10.2	.25
		1-hr PE	E	7.3	.35
			NE	.5	.81
			COR	5.3	.41
	sE-selectin	Baseline	E	.1	.89
			NE	2.3	.59
			COR	31.5	.03*
		IPE	E	1.4	.67
			NE	5.7	.39
			COR	10.1	.24
		1-hr PE	E	11.8	.22
			NE	19	.12
			COR	7.4	.32

Note: LI = lower-intensity; sICAM-1 = soluble intracellular adhesion molecule-1; sVCAM-1 = soluble vascular adhesion molecule-1; sE-selectin = soluble endothelial selectin; E = epinephrine; NE = norepinephrine; COR = cortisol; IPE = immediately post-exercise; PE = post-exercise; hr = hours; * $p < .05$.

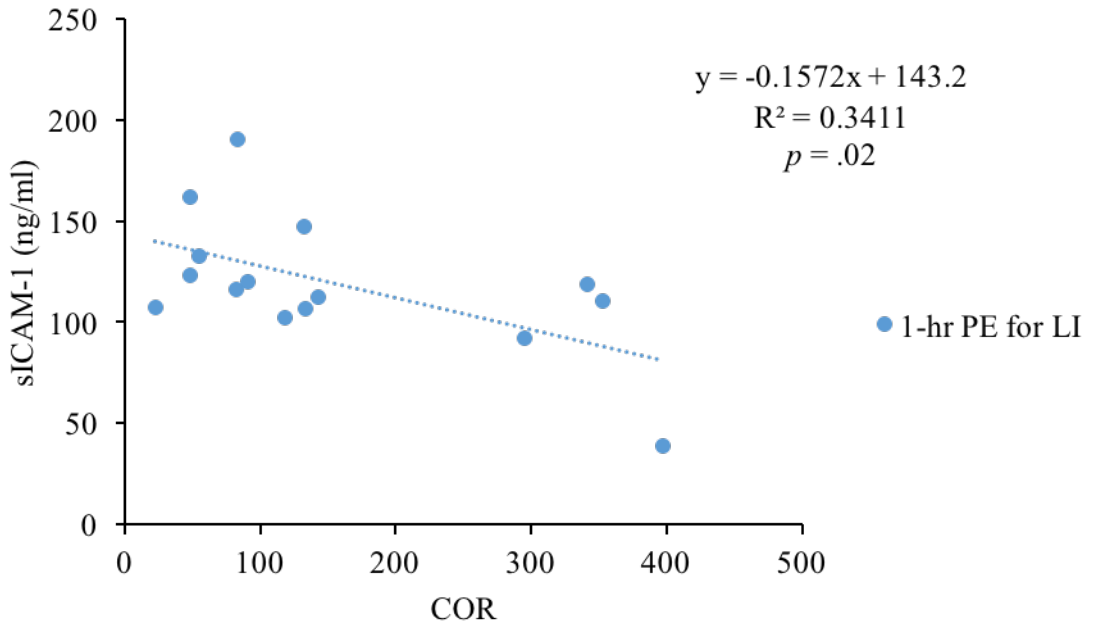

Figure 4. A Relationship between COR and sICAM-1 Concentration at 1-hour Post Exercise for Lower-Intensity. COR = cortisol; sICAM-1 = soluble intracellular adhesion molecule-1; hr = hours; PE = post exercise; LI = lower-intensity; ng = nanogram; ml = milliliter.

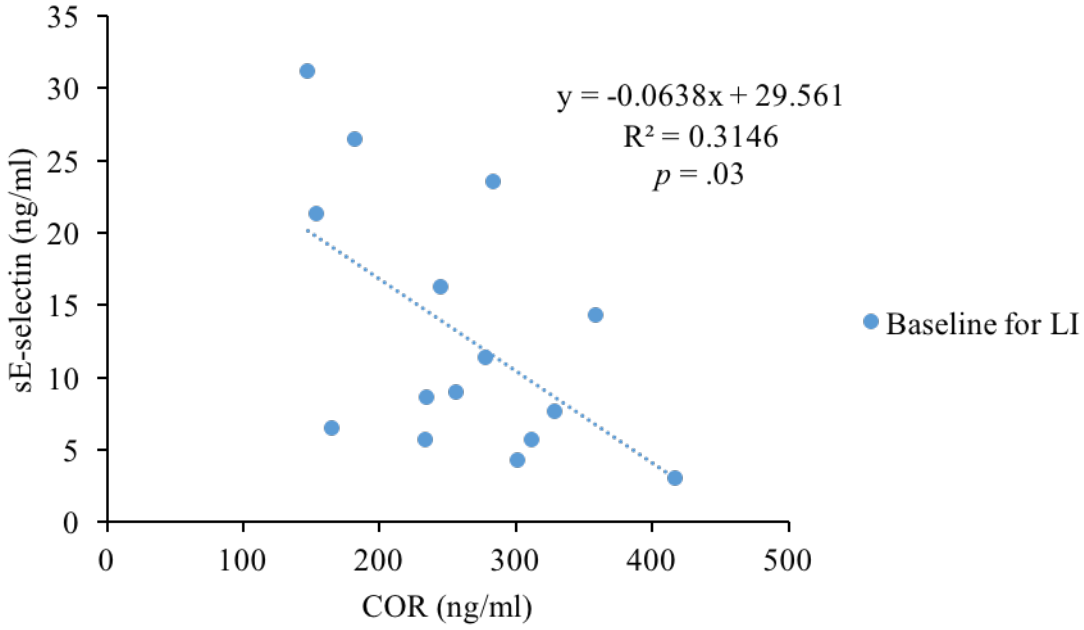

Figure 5*:* A Relationship between COR and sE-selectin Concentration at Baseline for Lower-Intensity. sE-selectin = soluble endothelial selectin; hr = hours; LI = lower-intensity; ng = nanogram; ml = milliliter.

Table 10. Relationships between sCAMs and stress hormones for Higher-Intensity.

Intensity	Variables	Time	Predictors	R^2 (%)	P-value
HI	sICAM-1	Baseline	E	2.8	.57
			NE	.6	.79
			COR	5.7	.39
		IPE	E	16.8	.15
			NE	2.5	.59
			COR	0	.97
		1-hr PE	E	1.4	.73
			NE	.2	.90
			COR	.4	.81
	sVCAM-1	Baseline	E	3.2	.54
			NE	2.7	.57
			COR	11.4	.22
		IPE	E	19.3	.10
			NE	6.5	.36
			COR	36	.02*
		1-hr PE	E	13.3	.27
			NE	19.5	.17
			COR	23.2	.07
	sE-selectin	Baseline	E	.3	.85
			NE	1.3	.69
			COR	69.7	.001*
		IPE	E	7.2	.33
			NE	18.7	.10
			COR	13.3	.18
		1-hr PE	E	55.6	.008*
			NE	19.1	.17
			COR	4.6	.44

Note: HI = high-intensity; sICAM-1 = soluble intracellular adhesion molecule-1; sVCAM-1 = soluble vascular adhesion molecule-1; sE-selectin = soluble endothelial selectin; E = epinephrine; NE = norepinephrine; COR = cortisol; IPE = immediately post-exercise; PE = post-exercise; hr = hours; * $p < .05$.

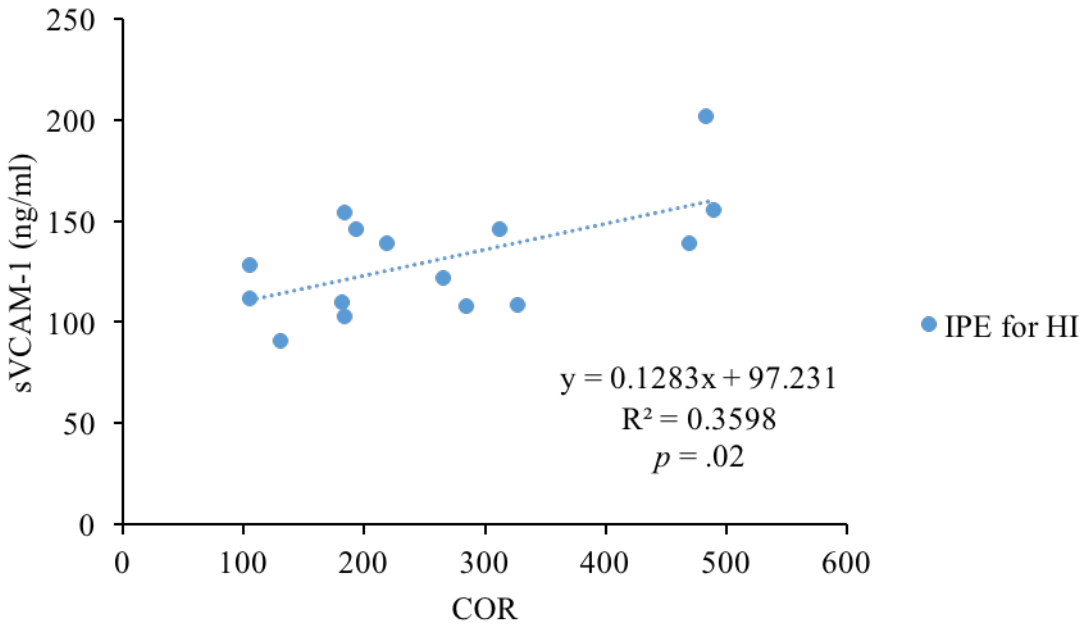

Figure 6. A Relationship between COR and sVCAM-1 Concentration at Immediately Post Exercise for Higher-Intensity. COR = cortisol; sVCAM-1 = soluble vascular adhesion molecule-1; IPE = immediately post exercise; HI = higher-intensity; ng = nanogram; ml = milliliter.

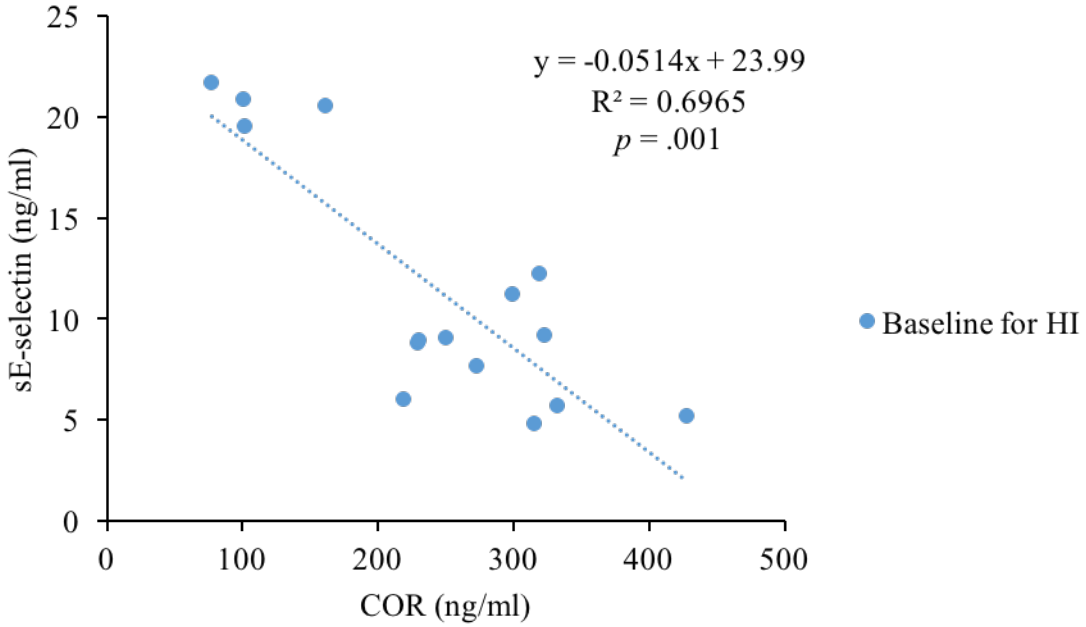

Figure 7. A Relationship between COR and sE-selectin Concentration at Baseline for Higher-Intensity. COR = cortisol; sE-selectin = soluble endothelial selectin; hr = hours; PE = post exercise; HI = higher-intensity; ng = nanogram; ml = milliliter.

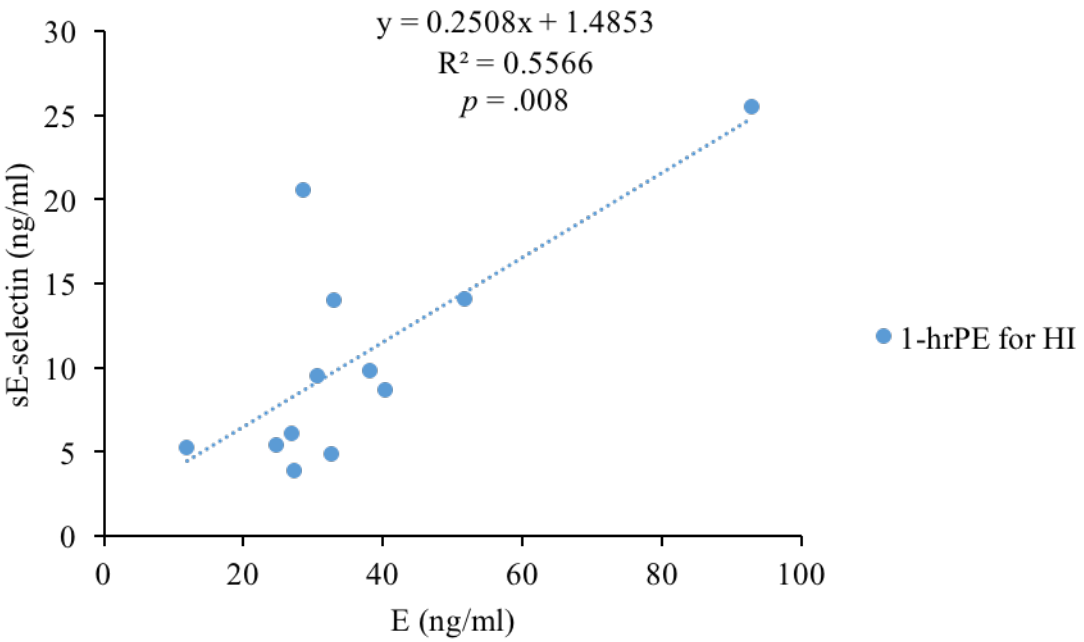

Figure 8. A Relationship between E and sE-selectin Concentration at 1-hr PE for Higher-Intensity. E = epinephrine; sE-selectin = soluble endothelial selectin; hr = hours; PE = post exercise; HI = higher-intensity; ng = nanogram; ml = milliliter.

CHAPTER FIVE

Discussion

Introduction

The obese are more likely to develop vascular inflammations than people of normal weight due to increases in vascular inflammation markers such as sICAM-1, sVCAM-1, and sE-selectin (Hatunic et al., 2007). Furthermore, vascular inflammation markers produced specifically in endothelium through stress hormones generate a pro-inflammatory environment in endothelium related to an initial stage of atherosclerosis (Black & Garbutt, 2002). Although it is well known that stress hormones modulate vascular inflammation (Black & Garbutt, 2002), it is unclear how stress hormones in response to exercise, affect vascular inflammation and yet, there is no experimental data regarding the relation between exercise-induced stress hormones and vascular inflammation markers clearly found in obese individuals who are at high risk for cardiovascular disease. Therefore, the current study examined the relation between exercise-induced stress hormones and vascular inflammation markers following different intensities of cycling exercise in obese men.

Participants

According the result of participants' characteristics, the mean BMI and body fat percentage for the participants categorized them as obese. Other than having a lack of physical activity and obesity, they were apparently healthy. None of them reported signs or symptoms suggestive of cardiovascular disease. Each of the participants was

sedentary, reporting average of less than 2 hours per week of any physical activity. As intended, the participants represented college-aged, sedentary, obese men.

Primary Findings

The major findings of the present study demonstrate that the different intensities of cycling exercise do not affect significant changes in sICAM-1 and sVCAM-1; however, sE-selectin was significantly decreased regardless of the exercise intensity. For COR, a significant interaction effect was apparent. COR decreased in a time-dependent manner following LI, while it was increased at IPE following HI. Additionally, there were some significant relations between sCAMs and exercise-induced stress hormones following different intensities of exercise trials. COR and sICAM-1 displayed a negative relationship at 1-hr PE during LI, while COR and sVCAM-1 had a significant positive relationship at IPE during HI. COR and sE-selectin have significant negative relationships at baseline during both LI and HI. E and sE-selectin had a significant positive relationship at 1-hr PE during HI.

sICAM-1 and sVCAM-1

Although there were not statistically significant, the responses of both sICAM-1 and sVCAM-1 showed the similar pattern for each different exercise intensity. For instance, sICAM-1 and sVCAM-1 at baseline increased at IPE, which then returned to baseline value following 1-hr PE during HI, whereas baseline sICAM-1 and sVCAM-1 decreased at IPE, which then returned to baseline value following 1-hr PE during LI. These findings are similar to others reported in the literature. In healthy male volunteers, the level of sICAM-1 increased by 24% at IPE and returned to baseline at 1-hr PE

following a VO_2 max test (Rehman et al., 1997). In patients with peripheral arterial disease, the levels of sICAM-1 and sVCAM-1 increased by 10% at IPE following a brief maximal walking test, and returned to baseline at 15 minutes post exercise (Brevetti et al., 2001). In untrained healthy males, the levels of sICAM-1 and sVCAM-1 were increased by 10% at IPE following a maximal cycling exercise and returned to baseline after 2-hr PE, but there were minor changes (sICAM-1: 5% and sVCAM-1: 1%) following 60% of maximal ergometer exercise (Jilma et al., 1997). In overall, most studies had the absence of significant changes in sCAMs with exercise in healthy subjects (Brevetti et al., 2001; Ciuffetti et al., 1999; Jilma et al., 1997; Wang et al., 2005), which are in agreement with findings in the current study. A common characteristic of these studies is the short volume and duration. Thus, acute exercise might have a short-lived effect on sCAMs, but not affect the changes in sCAMs.

In contrast to our findings, there are reports of significant changes in sCAMs after exercise. In healthy males, sICAM-1 increased after a 42-km run (12%) and a 30-min downhill running (14%), but not after cycling exercise at 80% of HRmax (Akimoto et al., 2002). Akimito et al. suggest that exercise intensity does not seem to influence the sICAM-1, but exercise type may be a critical factor in determining the level of sICAM-1. Furthermore, they found muscle damage was associated with a high-level of sICAM-1, suggesting exercise type could induce inflammation and muscle damage (Akimoto et al., 2002). Nielsen and Lyberg also found increased levels of sICAM-1 and sVCAM-1 after long-distance running (Nielsen & Lyberg, 2004). The common characteristic of these results have a high exercise volume, suggesting that the high exercise volume such as

prolonged strenuous activity may have a negative influence on vascular inflammation by increasing sCAMs.

Therefore, it is possible that if both the current exercise trials had been of lesser volumes, the differences in sCAMs would have been lower between time points, even though the levels of sCAMs at IPE decreased during LI or increased during HI. Indeed, there is evidence that for hours subsequent to heavy exertion, several components of both the innate and adaptive immune system exhibited suppressed function (Nieman, 1994; Nieman & Pedersen, 1999), which means exercise volume may be an important factor for determining sCAMs. Furthermore, Pedersen and Ullum (1994) proposed the "open-window" hypothesis, which indicates that moderate exercise strengthens the immune system, whereas severe exercise is followed by a period of immune suppression (Pedersen & Ullum, 1994), which may explain the temporary decrease in sCAMs at IPE during LI and the temporary increase in sCAMs at IPE during HI in the current study.

sE-selectin

Unlike sICAM-1 and sVCAM-1 involving a final step of a leukocyte migration, sE-selectin is only detected on the activated endothelium, mediating an initial step of the leukocyte migration and rolling (Bevilacqua et al., 1994; Tedder et al., 1995). Additionally, inflammatory stimuli such as pro-inflammatory cytokines induce maximum expression of E-selectin on the surface of endothelial cells, and they have suggested that E-selectin is required to inflammatory stimuli (Lasky, 1992). Therefore, it is reasonable to expect change differences in soluble concentrations of CAMs. The question remains as to why only sE-selectin decreased. sE-selectin is unique among the CAMs in that it is derived solely from endothelial cells, while the others derived from multiple sources,

including the surface of leukocytes and nonvascular cell types (Demerath et al., 2001). Therefore, sE-selectin is a sensitive indicator of initial inflammatory response on the endothelial cells. Since the primary effect of exercise can reduce pro-inflammatory cytokines related to increases in sE-selectin, this may explain why only sE-selectin decreased. Further work on the effect of exercise on the pro-inflammatory cytokines is needed. Therefore, the decrease in sE-selectin following different intensities of exercise can explain why exercise may inhibit activation of endothelium.

Relation with Stress hormones and sCAMs

According to the literature on CATs, it is generally agreed that stress, such as physical exercise, stimulates CATs, which could influence the sCAMs (Shephard, 2003). However, in the present study we found there were no significant changes in CATs following either LI or HI. One of possible explanations for this discrepancy may be due to obesity, which may cause the lower responses of CATs at rest and following exercise. For instance, CATs responses are significantly lower in obese-people than in non-obese, both at rest and in response to physical exercise (Zouhal et al., 2013). Indeed, several studies examined the relationship between obesity and CATs, suggesting obesity may modify adrenergic receptor sensitivity, leading to low CATs responses in obese subjects (Bougneres et al., 1997; Ravussin, 1993; Ravussin et al., 1988). Therefore, the fact that we demonstrated no significant differences in CATs in response to LI and HI could be possibly due to obesity-mediated decrease in adrenergic receptor sensitivity.

Intense exercise is a physiological stress capable of inducing the interaction of leukocytes with endothelial cells and their migration into vascular tissue. Exercise increased the recruitment of leukocytes through beta-adrenergic stimulation that interacts

with E in endothelial tissue and induced an increase in the expression of CAM like E-selectin (Shephard, 2003). Previous studies clearly showed that beta-adrenergic stimulation is required for upregulation of E-selectin, which indicates changes in E-selectin are mainly related to E (Flach et al., 2013). Therefore, afore-mentioned mechanism supports our result showing a positive relationship between E and sE-selectin at 1-hr PE during HI.

A COR response is dependent on exercise-intensity, while the concentration of COR decreased after one hour of recovery (Shephard, 2013). We observed a decrease in COR at 1-hr PE for both LI and HI as seen in a previous review (Shephard, 2013); COR decreased at 1-hr PE compared to baseline and IPE for LI. Additionally, COR decreased at 1-hr PE compared to IPE for HI. According to Shephard (2013), the increase in COR following brief exercise is influenced by the intensity of the exercise and the level of COR because intensities above 60% VO_2 max show either no changes in COR levels or increases in COR, while exercising below 50% VO_2 max seems to reduce COR due to an enhanced elimination and a suppressed secretion (Shephard, 2013). The same pattern was seen in sCAMs levels. Interestingly, we found a statistically significant relationship between sCAMs and COR known as the possible mediator for sCAMs. However, only sVCAM-1 at IPE had a significant positive relationship with COR at IPE during HI. According to a previous review, there were no additional anti-inflammatory effects at high concentrations of COR by a negative feedback regulation on inflammatory cytokines, which may increase the pro-inflammatory cytokines while decreasing the anti-inflammatory cytokines (Tian et al., 2014; Yeager et al., 2011). Additionally, anti-inflammatory cytokines such as Interleukin (IL)-4 and IL-13 modulate only the

expression of VCAM-1 (Hosokawa et al., 2006). Therefore, our results suggest that the high concentrations of COR following HI may affect sVCAM-1, primarily due to the decrease in anti-inflammatory cytokines such as IL-4 and IL-13.

On the other hand, COR, in a glucocorticoid class of hormones, is known to have potent anti-inflammatory effects due to a glucocorticoid used to suppress inflammation (Guilpain & Le Jeunne, 2012). Glucocorticoids have an inhibitory effect on the expression of ICAM-1 by switching off activated ICAM-1 gene through glucocorticoid receptors (GRs). More importantly, particularly, at lower concentrations the glucocorticoids are likely to be relevant in a decrease in the inflammatory gene transcription (Barnes & Adcock, 2003). Thus, a low concentration of COR for a certain condition such as a lower-intensity exercise may suppress ICAM-1 due to a decrease in an inflammatory cytokine such as IL-1β modulating the expression of ICAM-1 (Hosokawa et al., 2006), and this can explain our result showing the negative relationship between COR and sICAM-1 at 1-hr PE during LI. Additionally, the pattern of the COR during LI was the same with sCAMs patterns during LI. Therefore, COR level during LI is associated with a decrease in sCAMs, supporting the evidence that low level of COR had anti-inflammatory effect as seen in previous studies (Gleeson et al., 2011; Guilpain & Le Jeunne, 2012).

Although no previous studies have examined the relation between COR and sCAMs following exercise, previous reviews proposed COR action that describes a concentration-dependent, bi-phasic (both stimulatory and suppressive) on the inflammation (Nieman, 1999; Yeager et al., 2011). According to Yeager et al., there was a complex relationship between COR and immune-mediated inflammation. Effects of COR

on a stimulus-induced inflammatory response can be either suppressive or stimulatory in a concentration- and time-dependent manner (Yeager et al., 2011). Thus, current findings showing the positive relation with Svcam-1 at IPE following HI and the negative relation with sICAMs at 1-hr PE following LI suggest that the exercise-induced COR may have a bi-phasic effect on vascular inflammation and a central role on the regulation of sCAMs.

Additionally, sE-selectin levels at baseline were higher than all other time points for both LI and HI since sE-selectin is more related to obesity-associated adipose tissue inflammation than other sCAMs such as sICAM-1 and sVCAM-1 (Flach et al., 2013). Furthermore, it has been reported that high BMI is associated with elevated sE-selectin concentrations in males (Ferri et al., 1999). However, COR is only released after physical stress such as exercise (Mastorakos et al., 2005), which may explain low level of COR at baseline observed in the current study. Therefore, increased sE-selectin and low level of COR at baseline can explain the negative relation between sE-selectin and COR following both LI and HI.

Conclusion

Regardless of intensities, cycling exercise can decrease sE-selectin, which may be related to the positive relation with E. Despite the fact that exercise-induced COR may affect sICAM-1 and sVCAM-1, the current study did not show any significant changes in sICAM-1 and sVCAM-1, which may be due to a low exercise volume. Therefore, our findings suggest that sE-selectin is the most sensitive marker of vascular inflammation in response to exercise in obese men, and it is assumed that E plays a critical role in changes in sE-selectin. COR may have an anti-inflammatory or a pre-inflammatory effect depending on exercise-intensity and engage in modulation of sICAM-1 and sVCAM-1.

Future research should determine if different intensities of chronic exercise alter the influences of the exercise-induces stress hormones on CAMs metabolism in obese men and if the positive effect of exercise-induced stress hormones on CAMs metabolism applies to cardiovascular patients to improve the vascular health.

APPENDICES

APPENDIX A

Informed Consent Form

Baylor University
Health, Human Performance, & Recreation

Consent Form for Research

PROTOCOL TITLE: **Interaction between cell adhesion molecules and stress hormones following different intensities of cycling exercise in obese males.**

PRINCIPAL INVESTIGATOR: Yunsuk Koh Ph.D.
Department of HHPR, Baylor University

CO-INVESTIGATORS: Darryn S. Willoughby, Ph.D.
Jin Park. M.S.
Department of HHPR, Baylor University

SUPPORTED BY: **Baylor University**

Introduction

Please read this form carefully. The purpose of this form is to provide you with important information about taking part in a research study. If any of the statements or words in this form are unclear, please let us know. We would be happy to answer any questions. You have the right to discuss this study with another person who is not part of the research team before making your decision whether or not to be in the study.

Taking part in this research study is up to you. If you decide to take part in this research study we will ask you to sign this form. We will give you a copy of the signed form.

The persons in charge of this study are **Yunsuk Koh, Darryn S Willoughby, and Jin Park.** We will refer to these persons as the "researchers" throughout this form.

Why is this study being done?

The purpose of this study is to **examine the responses of blood vessel inflammatory markers and stress hormones over 24 hours following exercise at different intensities in obese males that do not regularly exercise.**

Heart disease is the number cause of death in America. One of the main contributors of heart disease is atherosclerosis, which is mainly caused by an excess of fatty materials (fats,

cholesterol, and other substances), called plaques, in the walls of blood vessels in the heart. There are several specific cells in the blood indicating the atherosclerotic events. These cells are called blood vessel inflammatory markers. Inflammation is a localized protective reaction of tissues to irritation, injury, or infection. Elevated levels of these blood vessel inflammatory markers are associated with high risk of atherosclerosis in the future. Additionally, people that do not regularly exercise or are overweight have increased risk for heart disease including atherosclerosis as compared with other people who exercise regularly and maintain normal body weight.

We are asking you to take part in this study because you are **one of 15 apparently healthy, physically inactive (defined as any forms of physical activity performed < 2 days per week) males between the ages of 18 and 30 years with body mass index (BMI) ≥ 30 kg/m^2.**

About **15** of subjects will take part in this research study at Baylor University.

How long will I take part in this research study?

We expect that you will be in this research study for about **three weeks**. During this time, we will ask you to make **5** study visits to **Exercise and Biochemical Nutrition Laboratory (EBNL) at Baylor University).**

What will happen if I take part in this research study?

If you agree to take part in this study, we will ask you to sign the consent form before we do any study procedures.

Description of the exercise protocol and blood draw

You will perform three exercise trials (maximal heart rate test, low-intensity exercise trial and high-intensity exercise trial) over the 5 study visits. We will take blood from your arm 4 times during the low- and high-intensity exercise trials. The procedure is described in more detail below.

1. Maximal heart rate testing
You will wear a heart rate monitor around the chest, and your resting heart rate will be measured after 15 minutes of rest in a supine position on the exam table. After the measurement of resting heart rate, you will be familiarized with testing procedures. You will warm up for 2 minutes. After the warm-up, you will begin the cycling maximal heart rate testing until you cannot maintain pedaling or volitionally stop the test.

2. Low- and high- intensity exercise trials
You will wear a heart rate monitor to measure resting heart rate at baseline. You will then warm up for 2 minutes on the bike. After the 2-minute warm-up, you will exercise on the bike at pre-determined exercise intensity (low-intensity exercise trial: 60% of your maximal heart rate and high-intensity exercise trial: 80% of your maximal heart rate) until you burn 300 kcal. During each exercise trial, your heart rate will be monitored and recorded every 3 minutes. Your blood pressure will also be measured every 10 minutes throughout the exercise trial. At least 7 to 10 days will separate each exercise trial to give you full recovery.

Immediately following the completion of each exercise trial (low- and high-intensity exercise trial), blood samples will be collected, and then, you will rest for 1 hour in the laboratory. You will not perform any physical activity while resting. At the end of a 1-hour rest, another blood sample will be collected, and you will leave. You will be asked to refrain from any physical activity until the last blood sample at 24 hours after you finish each exercise trial.

3. Blood draw
We will draw about 20 milliliters (about 4 tea spoonful) of blood from your forearm, using a sterile needle and blood tubes by an experienced phlebotomist four times (prior to exercise, immediately post-exercise, 1 hour post-exercise, and 24 hours post-exercise) per each exercise trial.

Study Visit 1

Visit 1 will take about **60 minutes** to complete. At this visit, we will ask you to do the following procedures:

- Give you some questionnaires to fill out about **your physical health**
- Ask about your medications
- Measure your vital signs (blood pressure and heart rates)
- Interview you about your experiences with **exercise history**
- Give you a DXA (or DEXA) scan. A DXA is a type of x-ray used to measure bone strength. During this test, X-ray pictures of your body will measure how much fat and muscle are present. You will lie flat on a table and a machine will take pictures of different areas of the body. This test will last about **6 minutes**.
- Test your maximal heart rate

You will perform two different exercise trials on the bike, and these trials will be randomly assigned using a coin toss. One exercise trial will be **a low-intensity exercise trial** and the other exercise trial will be **a high-intensity exercise trial.** You and the researcher cannot choose your exercise trials in order. You will have an **equal chance** of being assigned to either exercise trial. **During visits two through five, you will report to the lab in the morning with an overnight fast (no food or liquid except water after 10:00 PM the night before your study visit).**

Study Visit 2

Visit 2 will take about **60 minutes** to complete. At this visit, we will ask you to do the following procedures:

- Measure your vital signs (blood pressure and heart rates)
- Perform exercise trial 1
- Collect blood sample (prior to exercise, immediately post exercise and 1 hour post exercise)

Study Visit 3

Visit 3 will take about **30 minutes** to complete. At this visit, we will ask you to do the following procedures:

- Measure your vital signs (blood pressure and heart rates)
- Collect blood sample (24 hours post exercise trial 1)

Study Visit 4

Visit 4 will take about **60 minutes** to complete. At this visit, we will ask you to do the following procedures:

- Measure your vital signs (blood pressure and heart rates)
- Perform exercise trial 2
- Collect blood sample (prior to exercise, immediately post exercise and 1 hour post exercise)

Study Visit 5

Visit4 will take about **30 minutes** to complete. At this visit, we will ask you to do the following procedures:

- Measure your vital signs (blood pressure and heart rates)
- Collect blood sample (24 hours post exercise trial 2)

What are the risks of taking part in this research study?

No foreseeable risks: To the best of our knowledge, taking part in this study will not hurt you.

Blood Draw Risks
Risks of having blood drawn are soreness and/or a black and blue mark at the site from where the blood is drawn. Sometimes, people feel uncomfortable at the time of the blood draw. Occasionally people feel lightheaded or faint. There is also a small risk of infection whenever blood is drawn.

Risks from Radiation
DXA scans will be used during this research study. The cumulative radiation exposure from these tests is considered small and is not likely to adversely affect you **or your disease**. However, the effects of radiation add up over a lifetime. It is possible that having several of these tests may add to your risk of injury or disease. When deciding to enter this study, think about your past and future contact with radiation. Examples of contact with radiation include x-rays taken for any reason or radiation therapy for cancer treatment.

Risks of Completing Exercise Trials
There is minor muscular soreness associated with the cycling exercise protocol, which is common for individuals who do not perform cycling exercises on a regular basis. The muscle soreness will naturally go away after two or three days of resting.

Risks of an Overnight Fast
An overnight fast may cause a light headache, constipation, or stress. Drinking plenty of water will reduce these symptoms. Eating food with fruit and vegetables helps reduce constipation.

Loss of Confidentiality
A risk of taking part in this study is the possibility of a loss of confidentiality. Loss of confidentiality includes having your personal information shared with someone who is not on the study team and was not supposed to see or know about your information. The researcher plans to protect your confidentiality. Their plans for keeping your information private are described later in this consent form.

Incidental Findings
Although the procedures you will have in this study are being undertaken for research purposes only, it is possible that researchers may notice something that could be important to your health. If so, we will contact you to explain what was noticed. If you so desire, we will also talk with your private physician. If you do not have a private physician, we will refer you to an appropriate clinic for follow-up. It will be your choice whether to proceed with additional tests and/or treatments to evaluate what we observed, and you or your insurer will be responsible for these costs.

Are there any benefits from being in this research study?

You will be given free blood assessments, body composition, and maximal heart rate testing during the course of the study, and may receive information regarding the results of these tests if you desire. Please check the appropriate space indicating whether or not you would like a copy of your results for being a participant in the study.

_____ I would like to receive a copy of my study results.

_____ I would NOT like to receive a copy of my study results.

Storing Study Information for Future Use

We would like to store your study information for future research related to **exercise and cardiovascular disease.** We will label all your study information with a code instead of your name. The key to the code connects your name to your study information. The researcher will keep the code in a **password-protected computer/locked file.**

Future use of study information is **optional** for this study**.** If you do not want your information to be used for future research, you should not be in this study.

Storing Samples and Health Information for Future Use

We would like to store some of your samples and health information for future research related to **exercise and cardiovascular disease**. We will label your samples and health information with a code instead of your name. The key to the code connects your name to your samples and health information. The study doctor will keep the key to the code in a **password protected computer/locked file**.

Your samples will be stored at **Exercise and Biochemical Nutrition Laboratory** (**EBNL)**. Your sample will be kept for **a maximum of 5 years.** After that time, the sample will be destroyed by methods in accordance with laboratory or institution procedures.

Future use of samples and health information is **optional** for this study**.** If you do not want your samples and health information to be used for future research, you should not be in this study.

How Will You Keep My Study Records Confidential?

We will keep the records of this study confidential by **storing in a locked cabinet in Dr. Koh office. Only Yunsuk Koh, Ph.D. will have access to the key.** We will make every effort to keep your records confidential. However, there are times when federal or state law requires the disclosure of your records.

Reporting risk of harm to self or others: If, during your participation in this study, we have reason to believe that you are at risk for harming yourself or others, we are required to take the necessary actions. This may include notifying your doctor, your therapist, or other individuals. If this were to occur, we would not be able to assure confidentiality.

The following people or groups may review your study records for purposes such as quality control or safety:

- The Researcher and any member of **his/her** research team
- Authorized members of Baylor University who may need to see your information, such as administrative staff members from the Office of the Vice Provost for Research and

members of the Institutional Review Board (a committee which is responsible for the ethical oversight of the study)
- Federal and state agencies that oversee or review research (such as the HHS Office of Human Research Protection or the Food and Drug Administration)

The study data will be stored **at Exercise and Biochemical Nutrition Laboratory** (**EBNL).**

The results of this study may also be used for teaching, publications, or presentations at professional meetings. If your individual results are discussed, your identity will be protected by using a code number or pseudonym rather than your name or other identifying information.

Study Participation and Early Withdrawal

Taking part in this study is your choice. You are free not to take part or to withdraw at any time for any reason. No matter what you decide, there will be no penalty or loss of benefit to which you are entitled. If you decide to withdraw from this study, the information that you have already provided will be kept confidential. You cannot withdraw information collected prior to your withdrawal.

If students are enrolled: You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or your grades at Baylor University. You will not be offered or receive any special consideration if you take part in this research study.

If Baylor faculty or employees are enrolled: You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your job status at Baylor University. You will not be offered or receive any special consideration if you take part in this research study.

If the researcher can withdraw the subject: The researcher may take you out of this study without your permission. This may happen because:
- The researcher thinks it is in your best interest
- You can't make the required study visits
- Other administrative reasons

Will I get paid for taking part in this research study?

You will be paid $100 for completing the entry/familiarization and all experimental testing sessions (5 visits). However, if you withdraw from the study after the completion of the first exercise trial, you will be paid $50.

What will it cost me to take part in this research study?

There are no costs to you for taking part in this research study.

What happens if I am injured as a result of participating in this research study?

If you become ill or injured as a result of your participation in the study, you should seek medical treatment from your doctor or treatment center of choice. You should promptly tell the researcher about any illness or injury.

There are no plans for Baylor University to pay you or give you other compensation for your injury or illness. You do not give up any of your legal rights to seek compensation by signing this form.

What if I have any questions or concerns about this research study?

You can call us with any concerns or questions about the research. Our telephone numbers are listed below:

- **Yunsuk Koh, Ph.D. (Principal investigator, Department of Health, Human Performance & Recreation, 121 Marrs McLean Gymnasium, Baylor University, phone: 254-710-4002)**

- **Jin Park, MS (Co-investigator, Department of Health, Human Performance & Recreation, 120 Marrs McLean Gymnasium, Baylor University, phone: 254-710-4012**

If you want to speak with someone **not** directly involved in this research study, you may contact the Baylor University IRB through the Office of the Vice Provost for Research at 254-710-1438. You can talk to them about:

- Your rights as a research subject
- Your concerns about the research
- A complaint about the research

Indicate your decision for the below optional research discussed earlier in this form:

Consent for future research with study information:

Do you agree to let us store your study information for future research related to **atherosclerosis**?

_____YES _____NO _____INITIALS

Consent for future research with samples and health information:

Do you agree to let us store your samples and health information for future research related to **exercise and cardiovascular disease**?

_____YES _____NO _____INITIALS

Future Contact

We may like to contact you in the future either to follow-up to this study or to see if you are interested in other studies taking place at Baylor University.

Do you agree to let us contact you in the future?

_____YES _____NO _____INITIALS

Statement of Consent

SIGNATURE OF SUBJECT:

I have read the information in this consent form including risks and possible benefits. I have been given the chance to ask questions. My questions have been answered to my satisfaction, and I agree to participate in the study.

_____ _____
Signature of Subject Date

Signature of Person Obtaining Consent:

I have explained the research to the subject and answered all his/her questions. I will give a copy of the signed consent form to the subject.

_____ _____
Signature of Person Obtaining Consent Date

APPENDIX B

IRB Proposal

Proposal

Title of the research project/teaching exercise: Interaction between cell adhesion molecules and stress hormones following different intensities of cycling exercise in obese males.

Are you using subjects in research? Yes (yes or no)
Are you using subjects in teaching exercises? No (yes or no)

Part 1: Expedited Review Request (if applicable)

The Baylor University Committee for Protection of Human Subjects in Research (Institutional Review Board or (IRB) has agreed to perform expedited reviews of certain research proposals that involve only survey research that poses minimal risk to research subjects. Proposals handled through the expedited review process are held to the same standard as those that go through the normal review process.

☐ I have reviewed the research or teaching exercise listed above. In my opinion, this proposal meets all three of the following criteria required for expedited review by the Baylor University Committee for Protection of Human Subjects in Research:

1. The only involvement of research subjects in the proposed research/teaching activity is response to written, oral, or electronic surveys;
2. The information requested in these surveys does not include any highly personal or sensitive information (reports of criminal activity or sexual behavior); and
3. The activity poses minimal physical and psychological risk to the research participant.

Part 2: Introduction and Rationale

Describe the research background and rationale for the project:
(Limit 500 words)

Atherosclerosis is the most common type of coronary artery disease (CAD) and is involved in endothelial dysfunction and inflammation (1). Increased leukocytes in the circulation in response to endothelial dysfunction or inflammation augment the expression of cell adhesion molecules on endothelia, such as intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). An increase in expression of ICAM-1 and VCAM-1 is considered the initial step of atherosclerotic events (2,3) since these cell adhesion molecules facilitate the migration of leukocytes into the intima, which later are differentiated to macrophages (4) and consequently become foam cells, a hallmark of atherosclerosis (5). Several stress hormones including, catecholamines (epinephrine and norepinephrine) and cortisol, are known to modulate cell adhesion molecules (6). When β-receptors on endothelia, known as catecholamine receptors are activated in response to physical activity or chronic stress, cyclic adenosine monophosphate (cAMP) activity is elevated, which further increases leukocytes in the circulation (7). At the same time, cortisol can also enhance the synthesis of cell adhesion molecules (8). The magnitude of changes in catecholamines and cortisol in response to exercise is dependent upon the intensity of exercise performed. The higher the exercise intensity is, the greater catecholamines and cortisol concentrations are (9). In general, exercise can be of great benefit to the prevention of atherosclerosis. However, there are some arguments regarding the effects of different intensities of exercise on ICAM-1 and VCAM-1. Some studies have demonstrated that high-intensity exercise may increase circulating leukocytes and the expression of ICAM-1 and

VCAM-1 (10, 11), while low- to moderate-intensity exercise may not significantly change these molecules (12). In contrast, some studies have reported that high intensity exercise may not significantly increase ICAM-1 or VCAM-1, but can provide beneficial effects on endothelial function and cardiovascular health (13, 14, 15). There is only limited information available in regard to the relationship between exercise-induced stress hormones and cell adhesion molecules (ICAM-1 and VCAM-1) in obese individuals that are at high risk of cardiovascular disease. Therefore, it is important to clearly understand the relationship between exercise-induced stress hormones and cell adhesion molecules that are involved in many chronic disease processes.

Clearly outline the questions being addressed:
(Limit 250 words)

The specific aim of the current study is to examine the responses of cell adhesion molecules (ICAM-1 and VCAM-1) and the exercise-induced stress hormones, catecholamines and cortisol, over 24 hours following cycling exercise at different intensities in obese men. The independent variables are 1) different exercise intensities (Low vs. High) and 2) time [baseline, immediately post-exericse (IPE), 3-hrs PE, and 24-hrs PE]. The dependent variables are 1) the cell adhesion molecules, ICAM-1 and VCAM-1 and 2) the exercise-induced stress hormones, epinephrine, norepinephrine, and cortisol.

Describe any expertise you have in this area or research or teaching:

I have previously conducted several research projects involving a very similar experimental design as in the proposed study.

Cite relevant research (including your own) in a bibliography:

1. Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arterioscler Thromb Vasc Biol 25(1):29-38, 2005.
2. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. Arteriosclerosis, thrombosis, and vascular biology 18(5):842-51, 1998.
3. Wang C-A, Liu Y-C, Du S-Y, Lin C-W, Fu H-W. < i> Helicobacter pylori</i> neutrophil-activating protein promotes myeloperoxidase release from human neutrophils. Biochemical and biophysical research communications 377(1):52-6, 2008.
4. Saha P, Modarai B, Humphries J, Mattock K, Waltham M, Burnand KG, Smith A. The monocyte/macrophage as a therapeutic target in atherosclerosis. Current opinion in pharmacology 9(2):109-18, 2009.
5. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. Arterioscler Thromb Vasc Biol 27(11):2292-301, 2007.
6. Fukao K, Shimada K, Naito H, Sumiyoshi K, Inoue N, Iesaki T, Kume A, Kiyanagi T, Hiki M, Hirose K, Matsumori R, Ohsaka H, Takahashi Y, Toyoda S, Itoh S, Miyazaki T, Tada N, Daida H. Voluntary exercise ameliorates the progression of atherosclerotic lesion formation via anti-inflammatory effects in apolipoprotein E-deficient mice. J Atheroscler Thromb 17(12):1226-36, 2010.
7. Croymans DM, Krell SL, Oh CS, Katiraie M, Lam CY, Harris RA, Roberts CK. Effects of resistance training on central blood pressure in obese young men. J Hum Hypertens 28(3):157-64, 2014.
8. Alipour A, van Oostrom AJ, Izraeljan A, Verseyden C, Collins JM, Frayn KN, Plokker TW, Elte JWF, Cabezas MC. Leukocyte activation by triglyceride-rich lipoproteins. Arteriosclerosis, thrombosis, and vascular biology 28(4):792-7, 2008.
9. Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. J Intern Med 252(4):283-94, 2002.
10. Gleeson M. Immune function in sport and exercise. Journal of applied physiology 103(2):693-9, 2007.
11. Shephard RJ. Adhesion molecules, catecholamines and leucocyte redistribution during and following exercise. Sports Med 33(4):261-84, 2003.
12. Verde TJ, Thomas SG, Moore RW, Shek P, Shephard RJ. Immune responses and increased training of the elite athlete. Journal of applied physiology (Bethesda, Md. : 1985) 73(4):1494-9, 1992.

13. Miles MP, Leach SK, Kraemer WJ, Dohi K, Bush JA, Mastro AM. Leukocyte adhesion molecule expression during intense resistance exercise. Journal of applied physiology 84(5):1604-9, 1998.
14. Petridou A, Chatzinikolaou A, Fatouros I, Mastorakos G, Mitrakou A, Chandrinou H, Papassotiriou I, Mougios V. Resistance exercise does not affect the serum concentrations of cell adhesion molecules. British journal of sports medicine 41(2):76-9, 2007.
15. Smith L, Anwar A, Fragen M, Rananto C, Johnson R, Holbert D. Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise. European journal of applied physiology 82(1-2):61-7, 2000.
16. Keytel, L., Goedecke, J., Noakes, T., Hiiloskorpi, H., Laukkanen, R., Van Der Merwe, L., & Lambert, E. (2005). Prediction of energy expenditure from heart rate monitoring during submaximal exercise. Journal of Sports Sciences, 23(3), 289-297.

Part 3: Methodology

Thoroughly describe the methodology to carry out the project/teaching exercise:

Experimental Approach
Fifteen participants will perform 2 separate exercise trials in random order [one at low-intensity: 50 % of maximum heart rate and one at high-intensity: 80% of maximum heart rate)] on the cycle ergometer. The exercise trials will be randomly determined by a coin flip. Each exercise trial will be performed at least 7 days apart to allow the participants to fully recover and return to basal conditions. During each exercise trial, the participants will exercise on the cycle ergometer until they expand 300 kcal. Blood samples will be collected from the antecubital vein at baseline, immediate post-exercise (IPE), 3-hrs PE, and 24-hrs PE for each exercise trial to analyze the changes in catecholamine, cortisol, ICAM-1 and VCAM-1.

Participants
Fifteen obese [body mass index (BMI) > 30 kg/m2] males that are apparently healthy, physically inactive (defined as any forms of physical activity performed < 2 days per week), between the ages of 18-30 years will volunteer to participate in this study. Enrollment will be open to men from all ethnic groups. Only participants considered as low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) will be included. All experimental procedures involved in the study will conform to the ethical consideration of the Helsinki Code and be reviewed and approved by the Institutional Review Board for Human Subjects of Baylor University. All eligible participants will be asked to provide university-approved informed consent prior to performing any study protocols.

Study Site
All experimental procedures will be conducted in the Baylor Laboratories for Exercise and Sport Technology (BLEST) and Exercise and Biochemical Nutrition Laboratory (EBNL) at Baylor University. All sample analyses will be completed in the EBNL at Baylor University.

Independent and Dependent Variables
Independent variables will be 1) exercise intensity (Low and High) and 2) time (baseline, immediately post exercise (IPE), 3-hr PE, and 24-hr PE). Dependent variables will be cell adhesion molecules (ICAM-1 and VCAM-1) and exercise-induced stress hormones (epinephrine, norepinephrine, and cortisol).

Entry Session
The participants expressing interest in participating in this study will be initially interviewed on the phone and/or via e-mail to determine whether they are qualified to participate in this study. The participants that met all inclusion criteria will then be invited to attend an entry session. Once reporting to the lab, the participants will be provided with the detailed study protocol via a verbal and written explanation outlining the study design. Once the participants decide to volunteer for the study, they will sign the informed consent form and complete a medical history questionnaire. During the entry session, body composition assessment and maximal heart rate (HRmax) testing will be conducted after the participants signed the informed consent form.

Body Composition Assessment

Total body mass (kg) and height (cm) will be determined on a standard dual-beam balance scale with a height measuring rod (Detecto 439). Body fat percent (%), fat mass (kg), and fat-free mass (kg) will be determined using a dual-energy x-ray absorptiometry (DEXA; Hologic Discovery Series W). The DEXA procedures will be performed by a primary or co-investigator who has undergone radiation safety training by the Environmental, Health, and Safety office at Baylor University. During the body composition assessment, the participants will lie down on their back in a standardized position in a pair of shorts/t-shirt or a gown and remain until the test is completed. The DEXA segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass.

Maximal Heart Rate (HRmax) Test Protocol

The HRmax test will be conducted using a Monark cycle ergometer (Medgraphics, Monark, Ergomedic, Model No. 828E, St Paul, MN) based on the protocol developed by Keytel et al. (16) to determine the participants' HRmax (defined as heart rate achieved at the point of maximal exhaustion). The participants will warm up on the bike for 2 minutes with work load of 2 Watts/kg of body weight. The participants will keep pedaling at the speed of 70 revolutions per minute (rpm) throughout the test. After the warm-up, the participants will begin cycling at an exercise intensity of 3.33 Watts/kg of body weight for 150 seconds (stage 1). Upon the completion of the stage 1, the work load will increase by additional 50 Watts for another 150 seconds (stage 2). Thereafter, the work load will increase by 25 W every 150 seconds until the participants no longer maintain 70 rpm or volitionally stop the test. Prior to the HRmax testing, the participants' resting heart rate and blood pressure will be measured after 15 minutes of rest in a supine position on the exam table. During the HRmax test, the participants will wear a hear rate monitor (Polar Electro Inc., Lake Success, NY) around the chest, and heart rate will be recorded every minute throughout the test. The work load, exercise time, heart rate, blood pressure, and ratings of perceived exertion (RPE) for each stage will be recorded.

Blood Sampling

The participants will report to the lab in the morning after an overnight fast, and venous blood samples, 10 ml in one serum separator tube and 10 ml in one ethylenediaminetetraacetic acid (EDTA) - containing plasma tube, will be obtained from the antecubital vein using the standard venipuncture procedures routinely performed in our laboratory. Blood samples in the plasma tube will remain at the room temperature for 10 minutes, and then will be centrifuged at 1000g for 15 minutes to separate plasma. Blood samples in the serum separator tube will remain at room temperature for 20 minutes to be clotted, and then will be centrifuged at 1000g for 20 minutes to separate serum. Aliquots of plasma and serum samples will be pipetted into 1.5 mL polypropylene tubes and immediately be frozen at -80°C for later analyses. Blood samples will be collected at four different time points for each exercise trial [baseline, immediately post exercise (IPE), 3-hr PE, and 24-hrs PE.

Exercise trials (Low- and High-intensity)

The predetermined HRmax from the maximal heart rate (HRmax) test protocol will be used to determine the appropriate target exercise heart rate for low- (50% of HRmax) and high-intensity (80% of HRmax) exercise trials and to estimate the duration of exercise for each exercise trial that allows the participants to expend 300 kcal (energy expenditure prediction equation by Keytel et al. (16): gender X (-55.0969 + 0.6309 X heart rate + 0.1988 X weight + 0.2017 X age) + (1 – gender) X (-20.4022 + 0.4472 X heart rate – 0.1263 X weight + 0.074 X age). During both low- and high-intensity exercise trials, the participants will wear a heart rate monitor (Polar Electro Inc., Lake Success, NY) around the chest, and rest in a supine position for 15 minutes to measure resting heart rate and blood pressure at baseline. The participants will then warm up for 2 minutes with 2.00 Watts/kg of body weight at 70 rpm. After the 2-minute warm-up, the appropriate work load, which was determined from the HRmax test, for each exercise trial (low- or high-intensity) will be loaded to the cycle ergometer, and then the participants will exercise until they expand 300 kcal for each exercise trial. During each exercise trial, heart rate will be monitored and recorded every 3 minutes to ensure the appropriate target exercise intensity. In addition, blood pressure will be measured every 10 minutes throughout the exercise trial. Immediately following the completion of each exercise trial, blood samples (IPE) will be collected, and then the participants will rest for 3 hours in the laboratory, but will not be allowed to perform any physical activity while resting. At the end of 3-hour resting, another blood sample (3-hr PE) will be collected, and the participants will be allowed to leave. The participants will be also asked to refrain from any physical activity until the last blood sample 24-hours post exercise trial (24-hr PE) is collected.

Laboratory Analysis of Blood Samples

All blood samples obtained from each exercise trial will be analyzed for catecholamines (Kit#ABIN770662, Antibodies, Atlanta, GA, USA), cortisol (Kit#ABIN649057, Antibodies, Atlanta, GA, USA), ICAM-1 (Kit# EK0370, BOSTER, Fremont, CA, USA), and VCAM-1 (Kit# EK0537, BOSTER, Fremont, CA, USA) using a commercially available enzyme-linked immunoabsorbent assay (ELISA) kit with a microplate reader (X-Mark, Bio-Rad, Hercules, CA).

Statistical Analyses
Statistical analyses will be performed by utilizing 2 x 4 (trials x time) factorial analysis of variance (ANOVA) with repeated measures. The Bonferroni pairwise comparisons will be conducted as post hoc tests to locate the significant mean differences. If a significant interaction between trials and time is found, the follow-up simple effects test will be conducted. All statistical procedures will be performed using IBM SPSS Statistics software (Version 21.0) and a probability level of < 0.05 will be set for the statistical significance.

How many subjects will be used? 15

How will the subjects be recruited?

Recruitment
Fifteen apparently healthy, physically inactive males (defined as any forms of physical activity performed < 2 days per week), between the ages of 18-30 years and with a body mass index (BMI) equal to or greater than 30 kg/m2 will volunteer to participate in the study. Enrollment will be open to men of all ethnicities. A recruitment flyer will be posted on campus and is attached to this application.

Inclusion Criteria
The pariticpants will be allowed to be participate in this study if they
1. are males, age between 18-30 years
2. are apparently healthy and physically inactive (defined as any forms of physical activity performed < 2 days per week)
3. have a body mass index (BMI) > 30 kg/m2
4. are non-smokers
5. are free from the following chronic medical conditions : heart disease, arrhythmias, diabetes, thyroid disease, bleeding disorder, history of pulmonary disease, hypertension, hepatorenal disease, musculoskeletal disorder, neuromuscular/neurological disease, autoimmune disease, cancer, peptic ulcers, anemia or chronic infection (HIV).
6. are free from the orthopedic conditions that would hinder physical activity
7. have not taken any heart, pulmonary, thyroid, anti-hyperlipidemic, hypoglycemic, anti-hypertensive, endocrinologic (e.g, thyroid, insulin, etc), emotional/psychotropic (e.g., Prednisone, Ritalin, Adderall), neuromuscular/neurological, or androgenic medications (anabolic steroids)
8. have low risk for cardiovascular disease and with no contraindications to exercise as outlined by the American College of Sports Medicine (ACSM) will be included.

Compensation or Incentives
The participants who successfully complete all requirements of the study will be paid $100. However, participants who withdraw after the first testing session will receive $50. The participants may receive information regarding results of these tests if they desire. If the participants are Baylor students, they will not receive any academic credit for participating in this study.

Possible risks to the subjects (both physical and psychological):

Potential Risks
The participants who meet eligibility criteria will be subjected to an aerobic cycling exercise involving dynamic muscle contractions. The participants may experience short-term muscle soreness, moderate fatigue, and muscle strains/pulls. As a result of the exercise protocol, the participants will most likely experience short-term muscle fatigue. In addition, they will likely experience muscle soreness in their thigh area for up to 24 to 48 hours after exercise. This soreness is normal and should be commensurate with the type of muscle soreness the participants may have felt after doing unaccustomed physical activity. Muscle strains/pulls resulting from the dynamic exercise

protocol are possible. During the familiarization session, the participants will be informed of the cycling exercise protocol. In addition, only Yunsuk Koh, Ph.D., Darryn Willoughby, Ph.D., and Jin Park, M.S. will conduct the testing and exercise procedures. The participants will be made aware of the intensity and duration of the expected soreness due to the exercise trials. However, there are minor risks of muscular pain and soreness associated with the exercise protocol required in this study which are not uncommon to any exercise program especially for individuals who do not exercise on a regular basis.

The participants will donate about approximately 20 milliliters (4 tea spoons) of venous blood a total of 8 times (baseline, immediately post exercise (IPE), 3-hr PE, and 24-hr PE for low- and high-intensity exercise trials) during the study by way of standard phlebotomy using sterile techniques by an experienced phlebotomist using standard procedures. These procedures may cause a small amount of pain when the needle is inserted into the vein as well as some bleeding and bruising. However, proper pressure will be applied upon removal to reduce bruising. The participants may also experience some dizziness, nausea, and/or faint if they are unaccustomed to having blood drawn. Body composition: body fat percent (% BF), fat mass (kg), and lean body mass (kg) will then be determined using a calibrated Hologic 4500W dual energy x-ray absorptiometry (DEXA) by study personnel who have all received training on radiation safety from Baylor's Department of Risk Management.

The DEXA body composition test will involve having the participant lie down on their back in a standardized position in a pair of shorts/t-shirt or a gown. A low dose of radiation will then scan their entire body for approximately six (6) minutes. The DEXA segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass. Radiation exposure from DEXA for the whole body scan is approximately 1.5 mR per scan. This is similar to the amount of natural background radiation a person would receive in one month while living in Waco, TX. The maximal permissible x-ray dose for non-occupational exposure is 500 mR per year. Total radiation dose will be less than 5 mR for the entire study. Even though the DEXA scan only provides minimal radiation, there is an accumulative effect over a lifetime. Thus, if a participant has had numerous x-rays over the course of the last year, or years, etc. they will need to take this into account when deciding whether or not to participate in the study.

Trained, non-physician exercise physiologists certified in CPR will supervise the participants undergoing testing and assessments. A telephone is in the laboratory in case of any emergencies, and there will be no less than two researchers working with each participant during testing sessions. In the event of any unlikely emergency one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor's campus police at extension 2222. Instructions for emergencies are posted above the phone in the event that any other research investigators are available for assistance. The participants will be informed to report any unexpected problems or adverse events they may encounter during the course of the study toYunsuk Koh, Ph.D. and Darryn S. Willoughby, Ph.D. If clinically significant side effects are reported, the participants will be referred to their physician for medical follow-up. New findings and/or medical referrals of unexpected problems and/or adverse events will be documented, placed in the participants research file, and reported to the Baylor IRB committee.

Method(s) to limit risks:

Assessment of Risk
While there are risks associated with the blood sampling and also the cycling exercise protocol, all of these procedures are done so often in the BLEST and EBNL, that they are considered routine. Drs. Koh and Willoughby are skilled and competent in performing the bicycle testing protocol. Relative to the blood sampling, to date there have been hundreds performed in the EBNL with no untoward events. The greatest risk associated with participating in this study will likely be from the muscle soreness the participants will experience from participating in the cycling exercise protocol. However, the intensity of the exercise protocol will be no more than when individuals engaged heavily in a new or different form of physical activity. Therefore, the potential benefits of participating in this study outweigh the potential risks.

Proposed safeguards to protect the subjects' right to privacy:

Confidentiality
Information obtained from this research (including questionnaires, medical history, laboratory findings, or physical examination) will be kept confidential to the extent permitted by law. However, according to FDA regulations, records will be open to FDA representatives to review if necessary. This may include questionnaires, medical history, laboratory findings/reports, statistical data, and/or notes taken throughout this study. Records of the research may also be subpoenaed by court order or may be inspected by federal regulatory authorities. Data derived from this study may be used in reports, presentations and publications. The participants in this study will not be individually identified unless they give their written consent. All participants will have a number to identify their results. Only the study personnel will know the subject numbers. Only study personnel will have access to the data. All data will be stored in a locked cabinet in Dr. Koh's office and only he will have access to the key. All evidence of primary data will be stored for exactly three years after the completion of the study. At this time data will be destroyed in a manner that instills complete privacy to all participants of the study. Analyzed blood samples will be discarded in an appropriately-labeled biohazard waste disposal container. However, unused blood samples will be kept in a locked freezer for no longer than one year. If any subsequent analysis occurs with the samples, they will be re-coded to instill confidentiality further.

Outline the method(s) to be used to obtain the data, to analyze the data, and to disseminate the results of the research project:

The investigators will obtain all of the data through one entry session and 2 exercise trials all conducted at the Baylor Laboratories for Exercise and Sport Technology and Exercise Biochemical Nutrition Laboratory (Baylor University; Waco, TX) per the protocols described above.

Statistical analyses will be performed by utilizing 2 x 4 (trials x time) factorial analysis of variance (ANOVA) with repeated measures. The Bonferorri pairwise comparisons will be conducted as post hoc tests to locate the significant mean differences. If a significant interaction between trials and time is found, the follow-up simple effects test will be conducted. All statistical procedures will be performed using IBM SPSS Statistics software (Version 21.0) and a probability level of < 0.05 will be set for the statistical significance.

Data will be presented at an appropriate scientific conference (e.g., American College of Sports Medicine, Experimental Biology, etc.) and published in a peer reviewed scientific journal (e.g., Medicine & Science in Sport and Exercise, Journal of Applied Physiology, etc.).

Part 4: Informed Consent Form Checklist

When using humans as subjects in research you must obtain their informed consent. Please upload a copy of your Informed Consent Form before submitting your proposal

I verify that the following items appear on my Informed Consent Form:

☒ A statement explaining the purpose of the research.

☒ A statement of the expected duration of the subject's participation.

☒ A description of the procedures to be followed.

☒ A description of any reasonable foreseeable risks or discomforts to the subject, including invasion of privacy.

☒ A description of any benefits resulting from the research, either to the subject or to others.

☒ A statement that informs subject of his/her right not to be a subject in a research project that is also a teaching exercise.

☒ A statement informing subject about how his/her anonymity will be guarded; i.e., that their confidentiality will be protected by assigned code numbers, by limiting access to data, by locked storage of files, etc.

☒ A statement that the subject's participation is voluntary, and that his/her refusal to participate will involve no penalty or loss benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

☐ A disclaimer, if applicable, regarding the use of the Internet to collect data.

☒ For research involving more than minimal risk, an explanation regarding the availability of any compensation or any medical treatments if injury occurs (if applicable, see OHRP Reports).

☒ If written informed consent is required, a place for the subject to sign and date the form and a statement that a copy of the signed consent form will be given to the subject for his/her records.

☐ If the subject is a minor, a statement of parental responsibility in consenting to the child's participation in the study with a place for the parent to sign and date the form in addition to the participant's signature.

☒ The name, address, and telephone number of the principal investigator of the research project, and his/her affiliation with Baylor University. If the principal investigator is a graduate student, the name and telephone number of the faculty advisor is also required.

☒ A statement informing subject that inquiries regarding his/her rights as a subject, or any other aspect of the research as it relates to his/her participation as a subject, can be directed to Baylor's University Committee for Protection of Human Subjects in Research.

Part 5: Research Instrument(s)

Please upload any non-standard, newly developed interview or questionnaire instrument (one that has not been previously published) that will be used

also

Upload as appendices any other information pertinent to the proposal, such as consent letters from participating agencies, etc.

IMPORTANT:

You must share your proposal with your Faculty Advisor and Department Chair using the "Share this Project" feature in IRBnet. If your Faculty Advisor or Department Chair is not listed as an IRBnet user, contact them and have them register with IRBnet so you can share your project with them. Your Faculty Advisor and Department Chair must sign your project within IRBnet before submitting the proposal to the IRB.

APPENDIX C

IRB Approval

INSTITUTIONAL REVIEW BOARD – PROTECTION OF HUMAN SUBJECTS IN RESEARCH

NOTICE OF FULL BOARD APPROVAL – INITIAL REVIEW

Principal Investigator: Yunsuk Koh
Study Title: Interaction between cell adhesion molecules and stress hormones following different intensities of cycling exercise in obese males
IRB Reference #: 669721

Date of Conditional Approval: 10/23/2014
Date Response Accepted: 10/31/2014
Date of Expiration: 10/23/2015

Date of IRB Meeting: 10/23/2014

The above referenced human subjects research project has been approved by the Baylor University Institutional Review Board (IRB). Specifically, the IRB reviewed and approved the following documents:

- Protocol, submitted 10/09/2014
- Consent Form, dated 10/16/2014
- Phone and Email scripts
- Flyer
- Medical History Inventory
- Exercise History Questionnaire
- Data Collection Forms

This approval is limited to the activities described in the approved protocol and application, and extends to the performance of these activities at each respective site identified in the IRB Application. In accordance with this approval, the specific determinations for the conduct of this research are listed below. General conditions for the conduct of research are attached.

The IRB has made the following determinations for the conduct of this research study:

- Signed, informed consent of adult participants is required.

Per your submission, your approved enrollment is: **15**.

Any change to the approved research (including changes to targeted enrollment), must receive prior IRB approval.

For questions concerning this approval, contact Deb Penney at 254-710-3708 or Debbie_Penney@Baylor.edu

Sincerely,

David W. Schlueter, Ph.D.
Chair, Baylor IRB

General Conditions of Approval and Investigator Responsibilities

- The principal investigator (PI) is responsible for personally conducting or supervising the conduct of the research and for protecting the rights, safety, and welfare of the enrolled subjects. All human-subjects research must be conducted in an ethical manner and in accordance with all federal, state, and local laws and regulations, institutional policies, and requirements or determinations of the IRB.
- The PI should have a plan for supervision and oversight of the research. The PI may delegate study-related tasks to research personnel qualified by training and experience to perform the delegated tasks, but must adequately supervise personnel to whom tasks are delegated.
- The PI or another specific qualified individual must be available to subjects to answer questions or provide care during the research.
- The research should not be initiated without adequate resources and should be stopped if the necessary resources become unavailable. These resources might include research personnel, space, equipment, time, and availability of medical or psychological care for problems that arise during participation in the research.
- The PI must ensure that:
    - IRB approval is obtained prior to initiation of the research;
    - The research is conducted in accordance with the IRB-approved protocol, including, when applicable, the approved recruitment and consent procedures;
    - When informed consent is required, informed consent is obtained prior to the initiation of any study-related procedures and documented using the current IRB-approved research consent form;
    - When FDA-regulated products are being investigated or used, they are managed and controlled as required by institutional policy and FDA regulations;
    - Changes to the IRB-approved protocol and/or the research consent form are not initiated without IRB approval unless necessary to eliminate apparent immediate hazards to the subject;
    - Unanticipated problems involving risks to subjects or others (including adverse events) are reported promptly to the IRB;
    - When applicable, Data and Safety Monitoring Board/Data Monitoring Committee or other monitoring group reports are submitted promptly to the IRB for review;
    - Continuing review is conducted prior to expiration of IRB approval;
    - Should IRB approval lapse, research procedures, such as recruitment and enrollment of subjects, study procedures on currently enrolled subjects, review of health/medical records, collection of tissue or other samples, or analysis of data, are not conducted until the IRB re-approves the research or until special permission is obtained from the IRB to continue previously enrolled subjects because it is in their best interests to do so;
    - When the research has been completed or is being closed out prior to completion, a Research Closure Form is submitted to the IRB;
    - Adequate and accurate research records are kept and retained as required by the IRB and, when applicable, by the sponsor or FDA; and
    - Research records are made available to the IRB, the Office of the Vice Provost for Research, the sponsor, and when applicable, the Office for Human Research Protections (OHRP), and the Food and Drug Administration (FDA) upon request for monitoring and oversight of the research.

APPENDIX D

Recruitment Flyer

Test your Cardiovascular endurance!

Sedentary men
Needed for a Cycling Exercise Study

Researchers in the Exercise & Biochemical Nutrition Lab in the Department of Health, Human Performance, and Recreation at Baylor University are recruiting ***15 healthy, inactive men (BMI of 30 or over) between the ages of 18-30 years*** *to participate in a study designed to evaluate the effect of different intensities of cycling exercise on cell adhesion molecules and stress hormones. Participants will be required to engage in* ***2 separate cycling exercise trials****. Participants will be required to undergo heart rate, blood pressure, body composition testing, and also provide blood samples. Eligible subjects will receive* ***$100*** *for completing the study. Participants will also receive important information regarding their health including* ***body composition, bone mineral density, and aerobic fitness exercise testing.***

For more information email or call:

Jin Park, M.S.
Exercise & Biochemical Nutrition Lab
Jin_Park@baylor.edu
254-710-4012

Jin_Park@baylor.edu
Phone:254-710-4012

APPENDIX E

Medical History Inventory

Directions. The purpose of this questionnaire is to enable the staff of the Exercise and Biochemical Nutrition Laboratory to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is **CONFIDENTIAL** as described in the **Informed Consent Statement.**

Name:_____ Age _____Date of Birth_____

Name and Address of Your Physician:_____

MEDICAL HISTORY

Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in blank).

____	Heart murmur, clicks, or other cardiac findings?	____	Asthma/breathing difficulty?
____	Frequent extra, skipped, or rapid heartbeats?	____	Bronchitis/Chest Cold?
____	Chest Pain of Angina (with or without exertion)?	____	Cancer, Melanoma, Skin Lesions?
____	High cholesterol?	____	Stroke or Blood Clots?
____	Diagnosed high blood pressure?	____	Emphysema/lung disease?
____	Heart attack or any cardiac surgery?	____	Epilepsy/seizures?
____	Leg cramps (during exercise)?		
____	Rheumatic fever?		
____	Chronic swollen ankles?	____	Scarlet fever?
____	Varicose veins?	____	Ulcers?
____	Frequent dizziness/fainting?	____	Pneumonia?
____	Muscle or joint problems?	____	Anemias?
____	High blood sugar/diabetes?	____	Liver or kidney disease?
____	Thyroid Disease?	____	Autoimmune disease?
____	Low testosterone/hypogonadism?	____	Nerve disease?
____	Glaucoma?	____	Psychological Disorders?

Do you have or have you been diagnosed with any other medical condition not listed?

_____
_____

Please provide any additional comments/explanations of your current or past medical history.

_____
_____

Please list any recent surgery (i.e., type, dates etc.).

_____
_____

List all prescribed/non-prescription medications and nutritional supplements you have taken in the last 3 months.

_____

What was the date of your last complete medical exam?

_____

Do you know of any medical problem that might make it dangerous or unwise for you to participate in this study (including strength and maximal exercise tests) _____ If yes, please explain:

Have you been involved in any type of weight loss program within the past 6 months? ____ If yes, please explain:

Recommendation for Participation

____ No exclusion criteria presented. Subject is ***cleared*** to participate in the study.

____ Exclusion criteria is/are present. Subject is ***not cleared*** to participate in the study.

Signed: _____ Date: _____

APPENDIX F

Exercise History Questionnaire

Baylor University

Exercise and Biochemical Nutrition Laboratory

Personal Information

Name:

Address:

City: _____ State: _____ Zip Code _____

Home Phone: (___) _____ Work Phone: (___)_____

Cellular : (___) _____ E-mail address: _____

Birth date: _____ / _____ / _____ Age: _____ Height: _____ Weight: _____

Exercise & Activity Questionnaire

1. Describe your typical occupational activities.

2. Describe your typical recreational activities (non-structured exercise activities, i.e. basketball, other sports). Approximately how many hours do you spend doing these activities per week?

3. Describe any exercise training (i.e. running, cycling, elliptical, weight lifting etc.)? If so, approximately how many days per week do you train? On average, approximately how many hours of exercise do you perform per week?

How long (years/months) have you been consistently training (aerobic and/or resistance exercise)

APPENDIX G

Diet Logs

Baylor University
Exercise & Biochemical Nutrition Laboratory

NAME _____ **Date** _____

INSTRUCTIONS

1. Record everything you eat for 3 days. If you eat pretzels, record how many. If you eat a bag of chips, record the number of ounces. For drinks, record the number of cups or ounces. Record everything you drink except water.
2. Record the Food, Amount, Brand Name, and Preparation Methods. For example: baked vs. fried chicken; 1 cup of rice; 2 teaspoons of margarine; 1 cup of 2% milk; McDonald's, Healthy Choice, or Frosted Flakes.
3. Record immediately after eating. Waiting until that night may make it difficult to remember all foods and quantities.

Food (include brand)	Method of Preparation	Quantity (cups, oz., no.)
BREAKFAST:		
LUNCH:		
DINNER:		
SNACKS:		

APPENDIX H

Data Collection Form

Subject Code:	

Data collection form
Effect of cycling exercise on cell adhesion molecules and stress hormones

Entry session (Date:)

1) Paper work

IC signed:	
Med history:	
Personal info:	

2) Resting Hemodynamics

Heart Rate:	
SBP:	
DBP:	

3) Anthropometrics

Height:	inch	cm
Weight:	lb	kg
DEXA:	bf%	BMI

4) *W*peak Assessment

PRED 50% WL:	kp
PRED 50% HR:	bpm
PRED 80% WL:	kp
PRED 80% HR:	bpm
Max WL:	kp
Max HR:	bpm

5) Workout order (LI/HI)

1st trial	
2nd trial	

6) Age-predicted HRmax

DOB:	/ /
Age:	
APMHR:	bpm

7) Post-Exercise Resting Hemodynamics

Heart Rate:	
SBP:	
DBP:	

Time (sec)	Workload (Watts, kp)		HR (bpm)	BP (mm Hg)	RPE	Signs/Symptoms
Stage0 **0-2:00**	2.00 Watts/kg					
	watts =#kp*6*70	kp				
30						
60						
90						
120						
Stage 1 **2:00-4:30**	3.33 Watts/kg					
	watts	kp				
30						
60						
90						
120						
150						
Stage 2 **4:30-7:00**	stage 1 + 1kp					
	watts	kp				
30						
60						
90						
120						
150						
Stage 3 **7:00-9:30**	stage 2 + 0.5kp					
	watts	kp				
30						
60						
90						
120						
150						
Stage 4 **9:30-12:00**	stage 3 + 0.5kp					
	watts	kp				

30						
60						
90						
120						
150						
Stage 5 **12:00-15:30**	Stage 4 + 0.5kp watts	kp				
30						
60						
90						
120						
150						

APPENDIX I

Poster Presentation

Mima Fondong, **Jin K. Park**, Stanley Ly, Yunsuk Koh. March 2nd, 2016. Relationships of Cellular Adhesion Molecules and Stress Hormones in Obese Males Following Exercise. Texas Chapter meeting of the American College of Sports Medicine, College station, TX.

Relationship of Cellular Adhesion Molecules and Stress Hormones in Obese Males Following Exercise

MIMA FONDONG, JIN K. PARK, STANLEY LY, and YUNSUK KOH

Department of Health, Human Performance, and Recreation, Baylor University, Waco, TX

Category: Undergraduate

Koh, Yunsuk (Yunksuk_Koh@baylor.edu)

ABSTRACT

The development of atherosclerosis is associated with a steady accumulation of inflammatory molecules. Exercise-induced hormones such as cortisol and catecholamines (epinephrine and norepinephrine) may play a role in endothelial inflammation. METHODS: Fifteen obese (BMI > 30 kg/m2) sedentary (less than 2 days per week of physical activity) male volunteers, the ages between 18 and30, participated in the study. The participants performed a single bout of cycling exercise (average energy expenditure ~ 300 kcal) at two different intensities in random order [low-intensity: 50% of maximal heart rate and high-intensity: 80% of maximal heart rate]. Overnight fasting blood samples were collected at baseline, immediate post-exercise (IPE), 1-hr PE, and 24-hr PE for each intensity of exercise to determine the responses of soluble cell adhesion molecules [intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1), and E-selectin (sE-selectin)] and exercise-induced stress hormones. Data were analyzed by an analysis of variance with repeated measures along with the Bonferroni multiple comparisons. The linear regression analysis was used to examine the interaction between exercise-induced hormones and vascular inflammation markers ($p < .05$). RESULTS: There exhibited no significant change in sICAM-1, sVCAM-1, E or NE, while sE-selectin at 1-hr PE (10.25±1.07 ng/mL) significantly decreased ($p = .045$) from baseline (12.22±1.39 ng/mL). COR at IPE (262.12±31.09 ng/ml) was significantly higher ($p = .001$) than 1-hr PE (189.35±31.11 ng/ml) during high-intensity exercise. In contrast, COR at IPE (187.52±31.09 ng/ml, $p = .009$) and 1-hr PE (156.24±31.11 ng/ml, $p = .001$) were significantly lower than baseline (259.75±23.07 ng/ml) during low-intensity exercise. COR and sICAM-1 had a negative relationship at 1-hr PE during low-intensity exercise ($r2 = .34$, $p = .02$), whereas COR and sVCAM-1 had a positive relationship at IPE during high-intensity exercise ($r2 = .36$, $p = .02$). CONCLUSION: sE-selectin was favorably reduced following exercise, and changes in cortisol were exercise-intensity dependent. Although sICAM-1 and sVCAM-1 did not significantly change following exercise, a significant interaction between cortisol and these cell adhesion molecules suggests that cortisol is one of the responsible exercise-induced hormones that may be associated with cell adhesion molecule metabolism.

International Journal of Exercise Science www.tacsm.org

Jin K. Park, Mima L. Fondong, Yunsuk Koh. June 2, 2016. Interaction Between Vascular Inflammation Markers and Exercise-induced Stress Hormones in Obese Males. American College of Sports Medicine International Meeting, Boston, MA.

Interaction Between Vascular Inflammation Markers and Exercise-induced Stress Hormones in Obese Males

Author Block Jin K. Park, Mima L. Fondong, Yunsuk Koh. *Baylor University, Waco, TX.*

Abstract:

PURPOSE: To examine the interaction between exercise-induced stress hormones [epinephrine (E), norepinephrine (NE) and cortisol (COR)] and vascular inflammation markers [soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble E-selectin (sE-selectin)] following different intensities of exercise in obese men.

METHODS: As a cross-over design, 15 physically inactive (physical activity < 2 days per week) obese (BMI > 30 kg/m^2) men between the ages of 18-30 years participated in the study. Participants performed a single bout of cycling exercise (average energy expenditure ~ 300 kcal) at two different intensities in random order (low: 50% and high: 80% of maximal heart rate). Overnight fasting blood samples were collected at baseline, immediate post-exercise (IPE), 1-hr PE, and 24-hr PE. All data were analyzed by an analysis of variance with repeated measures along with the Bonferroni multiple comparisons. The linear regression analysis was used to examine the interaction between exercise-induced hormones and vascular inflammation markers ($p < .05$).

RESULTS: sICAM-1, sVCAM-1, E or NE did not change, while sE-selectin at 1-hr PE (10.25±1.07 ng/mL) significantly decreased ($p = .045$) from baseline (12.22±1.39 ng/mL). COR at IPE (262.12±31.09 ng/ml) was significantly higher ($p = .001$) than 1-hr PE (189.35±31.11 ng/ml) during high-intensity exercise. In contrast, COR at IPE (187.52±31.09 ng/ml, $p = .009$) and 1-hr PE (156.24±31.11 ng/ml, $p = .001$) were significantly lower than baseline (259.75±23.07 ng/ml) during low-intensity exercise. COR and sICAM-1 had a negative relationship at 1-hr PE during low-intensity exercise ($r^2 = .34$, $p = .02$), whereas COR and sVCAM-1 had a positive relationship at IPE during high-intensity exercise ($r^2 = .36$, $p = .02$).

CONCLUSION: sE-selectin was favorably reduced following exercise, and changes in cortisol were exercise-intensity dependent. Although sICAM-1 and sVCAM-1 did not significantly change following exercise, a significant interaction between cortisol and these cell adhesion molecules suggests that cortisol is one of the responsible exercise-induced hormones that may be associated with cell adhesion molecule metabolism.

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