2009

The Impact of Mental Challenge on Indicators of Endothelial Function in Obese Individuals

Chun-Jung Huang
Virginia Commonwealth University

School of Education
Virginia Commonwealth University

This is to certify that the dissertation prepared by Chun-Jung Huang entitled THE IMPACT OF MENTAL CHALLENGE ON INDICATORS OF ENDOTHELIAL FUNCTION IN OBESE INDIVIDUALS has been approved by his or her committee as satisfactory completion of the Dissertation requirement for the degree of Doctor of Philosophy

Dr. Edmund O. Acevedo; School of Education

Dr. Brent L. Arnold; School of Education

Dr. Ronald K. Evans; School of Education

Dr. Jennifer K. Stewart; College of Humanities and Sciences

Dr. John J. Ryan; College of Humanities and Sciences

Dr. Beverly J. Warren, Dean of the School of Education

Dr. F. Douglas Boudinot, Dean of the Graduate School

July 22nd 2009

THE IMPACT OF MENTAL CHALLENGE ON INDICATORS OF ENDOTHELIAL FUNCTION IN OBESE INDIVIDUALS

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

CHUN-JUNG HUANG
Master of Science, Indiana State University, 2005
Bachelor of Science, Fu-Jen Catholic University, Taiwan, 2000

Director: DR. EDMUND O. ACEVEDO
PROFESSOR, DEPARTMENT OF HEALTH AND HUMAN PERFORMANCE

Virginia Commonwealth University
Richmond, Virginia
July 2009

Table of Contents

List of Tables

Page

List of Figures

Page

Abstract

THE IMPACT OF MENTAL CHALLENGE ON INDICATORS OF ENDOTHELIAL FUNCTION IN OBESE INDIVIDUALS

By Chun-Jung Huang, M.S.

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

Virginia Commonwealth University, 2009

Major Director: Dr. Edmund O. Acevedo
Professor, Department of Health and Human Performance

A number of investigators have examined psychological stress-induced endothelial dysfunction, however, the underlying mechanisms for these responses have not been clearly elucidated. The purpose of this study was to compare the effects of mental challenge on forearm blood flow, total antioxidant capacity (a measure of oxidative stress), the release of norepinephrine (NE; stress induced neurotransmitter), and pro-inflammatory cytokine responses [both lipopolysaccharide (LPS)-stimulated TNF-α and IL-6 cytokine and mRNA] in lean and obese individuals. Twelve subjects who had a BMI above 30 kg/m^2 and were above 30% body fat were categorized as obese and twelve subjects with a BMI below 25 kg/m^2 and were below 25% body fat were categorized as lean subjects.

Blood samples were drawn and forearm blood flow was assessed prior to and following subjects' participation in a mental challenge protocol consisting of a computer-based Stroop Color-Word task and mental arithmetic task, for a total of 20 minutes. The mental challenge elicited an elevation in HR and NE in both the lean and obese groups. Furthermore, both lean and obese groups demonstrated an increase in FBF following the mental challenge, whereas no changes in total antioxidant capacity were observed. In addition, the lean group exhibited an increase in LPS-stimulated TNF-α cytokine production from baseline to following the mental challenge, whereas the obese group demonstrated a decrease in LPS-stimulated TNF-α cytokines. This corresponded with a decrease in LPS-stimulated TNF-α mRNA expression in the obese group, although the obese subjects maintained higher levels of both measurements (LPS-stimulated TNF-α cytokine and mRNA expression) compared with the lean group. Furthermore, in the LPS-stimulated IL-6 cytokine response, the obese group demonstrated a greater increase than the lean group following the mental challenge, even though both groups showed an increase in LPS-stimulated IL-6 mRNA expression. These findings suggest that the magnitude and direction of LPS-stimulated TNF-α cytokine response and mRNA expression and LPS-stimulated IL-6 cytokine response to acute stress may be dependent upon the effects of the additional percentage of body fat seen in obese individuals.

CHAPTER 1 REVIEW OF LITERATURE

Introduction

The epidemic of overweight and obesity has evolved and now includes 61.6% of American women and 70.5% of American men (National Center for Health Statistics, 2006). Obesity-related health expenses are derived from diabetes, hypertension, and cardiovascular diseases such as atherosclerosis (Quesenberry et al., 1998). One of the earliest sub-clinical stages in the atherosclerotic process is an impairment of endothelium-dependent vasodilation, also known as endothelial dysfunction (Singhai, 2005). The endothelium acts as a regulator of vascular homeostasis and maintains a balance between vasodilation and vasoconstriction, which can be disturbed by alterations in oxidative stress, inflammation, and obesity. One possible mechanism to explain obesity-induced endothelial dysfunction is the elevation of leptin. Obesity-related elevations in leptin can elicit elevations in oxidative stress (Bouloumıe et al., 1999; Considine et al., 1998) and have the ability to shift T-helper (Th) cell differentiation toward the Th1 subtype, a pro-inflammatory condition (Loffreda et al., 1998). Another factor that has been implicated as participating in the atherogenic process is psychological stress. It is well established that laboratory-induced psychological stress is capable of altering physiological homeostasis, and chronic stress has been demonstrated

to be a determinant of cardiovascular disease (Olinski et al., 2002). One potential mechanism that links psychological stress to endothelial dysfunction may be through the direct impact of stress hormones on oxidative stress. However, endothelial dysfunction may be exacerbated with acute and chronic psychological stress in obese individuals. Therefore, this review will discuss the main mechanisms of vasodilation within the endothelium and the impact of obesity on endothelial responses to psychological stress.

Mechanisms of Vasodilation within the Endothelium

The endothelium is a thin layer of cells that lines the blood vessel lumen which has been recognized as a major modulator of vascular tone. When these cells regulate vascular tone, either relaxing or constricting factors are released to induce vasodilation or vasoconstriction. In addition to regulating vascular tone, endothelial cells play an important role in preventing the development of cardiovascular diseases such as atherosclerosis. The development of atherosclerosis is involved in a complex interaction between the vascular endothelium, inflammatory cells, platelets, and vascular smooth muscle cells (Ross, 1999). Ross has demonstrated that the initial process in the pathogenesis of atherosclerosis is endothelial dysfunction, which may be caused by the reduction of endothelial-derived relaxing factors.

The primary endothelial-derived relaxing factors are nitric oxide (NO), prostacylin (PGI_2) while endothelial-derived constricting factors include endothelin-1

(ET-1), angiotensin-II, thromboxane A2, prostaglandins H2, and oxidant radicals (Clines et al, 1998). NO is the most potent endothelium-derived relaxing factor which maintains vascular tone and reactivity by opposing the endothelium-derived constricting factors such as ET-1 (Verma and Anderson, 2002). Thus, the balance between endothelium-derived relaxing and constricting factors can regulate vascular homeostasis (Figure 1). Luscher and Barton (1997) have stated that the endothelium is a regulator of vascular homeostasis and maintains a balance between vasodilation and vasoconstriction. Furthermore, NO can eliminate endothelin-induced arterial constriction and inhibit further release of endothelin from the endothelium (Luscher et al., 1990; Shepherd, 1995). A reduction in bioavability of NO could facilitate an exaggerated production of ET-1 (Verma and Anderson, 2002). Therefore, the endothelium-derived relaxing and constricting factors play a crucial role in preventing the occurrence of endothelial dysfunction.

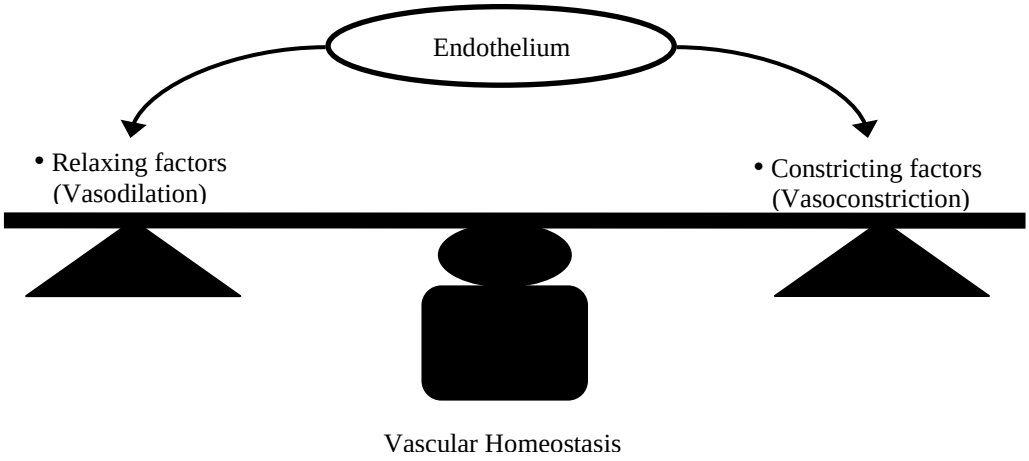


Figure 1. The role of endothelium in the vascular homeostasis.

When this balance is disrupted, the endothelium may result in expression of adhesion molecules, activations of platelet aggression, vascular inflammation, vasoconstriction, and stimulation of smooth muscle cell proliferation (Hamilton et al., 2004). Furthermore, Kazuhiro and Michel (1997) have demonstrated that the increase in enzymes from endothelial-derived relaxing factors can induce vascular smooth muscle relaxation, inhibition of vascular smooth muscle growth, modulation of endothelium-derived thrombogenic factors, and inhibition of platelet aggregation. Thus, endothelial dysfunction has been regarded as an imbalance between endothelium-derived relaxing and constricting factors.

A. Increased arterial blood pressure and sympathetic withdrawal

Studies have shown that the increase in blood flow and arterial blood pressure can stimulate endothelial cells to release acetylcholine. Then, acetylcholine can activate muscarinic receptors on endothelial cells and induce the release of NO to increase vasodilation (Halliwill et al., 1997) (Figure 2). Furthermore, although catecholamines are powerful vasoconstrictors due to the direct effect on vascular smooth muscle, they can also cause vasodilation. Harris et al. (2000) have stated that the release of endogenous catecholamines could increase the release of NO from endothelial cells and offset the direct vasoconstrictor effects of the catecholamines. This mechanism has been further demonstrated by Halliwill et al. showing that sympathetic withdrawal mediates the initial

vasodilation, which can be enhanced by catecholamines (e.g. epinephrine) via β_2-adrenergic receptor activation. The β_2-adrenergic vasodilation in the human forearm is dependent on nitric oxide synthases (Dawes et al., 1997). Glover et al. (1962) have shown that β_2-adrenergic blocker can reduce forearm vasodilation. In addition, an increase in plasma epinephrine levels may suggest that forearm vasodilation is due to the effects of β_2-adrenergic-receptor vasodilation on the skeletal muscle vasculature (Rowell and Seals 1990). Therefore, the contributions of catecholamines in vasodilation activated by β_2-adrenergic receptors may explain a withdrawal of sympathetic activity in vasoconstrictive nerve fibers and/or an activation of vasodilatory fibers.

Another possible mechanism to explain the sympathetic withdrawal is cardiopulmonary baroreceptor activity, which plays an important role in the control of blood pressure and vasodilation. Hamer et al. (2003) have found that cardiopulmonary baroreceptors are involved in the sympathetic withdrawal response during forearm vasodilation to stress, and the vasodilation response can be attenuated when these cardiopulmonary baroreceptors are inhibited. Moreover, Rea and Wallin (1989) stated that the increase of cardiac filling pressure can stimulate the cardiopulmonary receptors, which could induce the inhibition of sympathetic neural outflow and stimulation of parasympathetic neural outflow. Thus, the possibility of sympathetic withdrawal during stress-induced vasodilation may be due to excitation of cardiopulmonary baroreceptors, resulting in an increased inhibition of sympathetic neural outflow. Additionally, DiCarlo

et al. (1994) have demonstrated that the activation of cardiopulmonary baroreceptors is due to changes in cardiac contractility mediated through changes in circulating catecholamines. This may also suggest that the activated cardiopulmonary baroreceptors may increase sympathetic outflow during stress-induced vasodilation via an increased level of catecholamines.

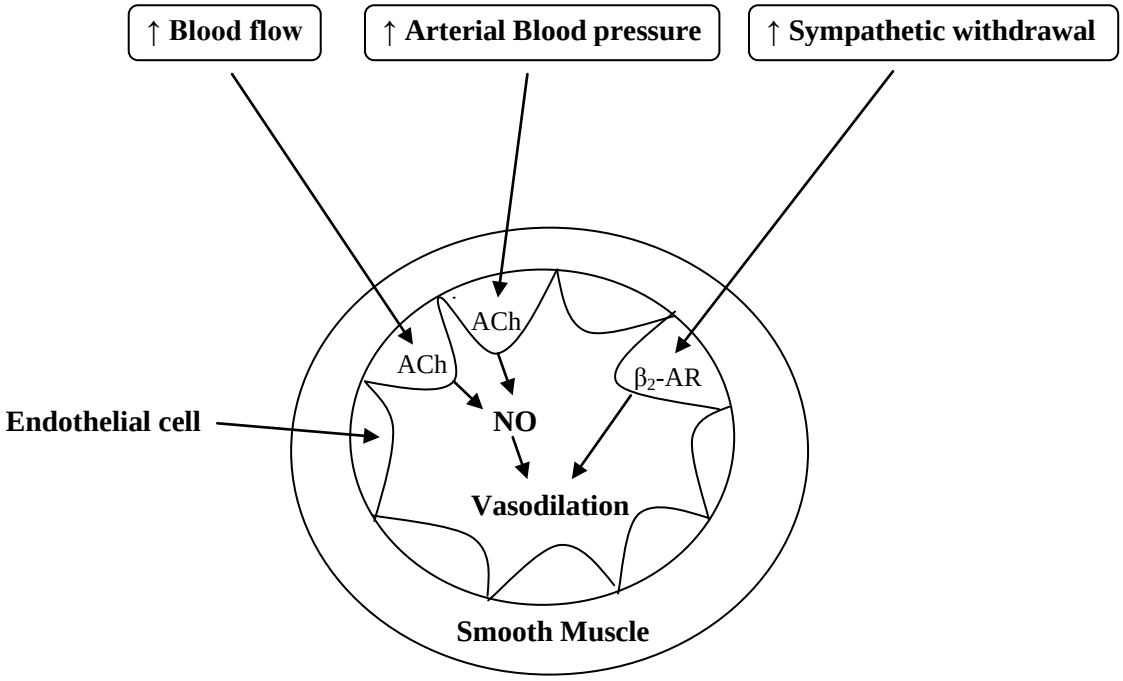


Figure 2. Adapted from Halliwill et al. (1997). The vascular response to stress. NO = nitric oxide; ACh = acetylcholine; β_2-AR = β_2-adrenergic receptor.

B. Synthesis and role of nitric oxide (NO)

NO is a critical homeostatic regulator of the vessel wall and plays a role in the maintenance of vascular tone and reactivity. The impaired production of NO has been

considered in many cardiovascular disorders such as atherosclerosis and hypertension. Calver et al. (1992) have demonstrated that there is a significant reduction in the NO production in essential hypertension. The reduction in NO production leads to arterial vasoconstriction and hypertension (Nussler and Billiar, 1993), which reveal decreased vascular compliance with higher blood pressure and volume. These findings are consistent with an earlier study by Furchgott (1990) who demonstrated that NO has a half-life of 3 to 5 seconds and is a major factor resulting in vasodilation via activation of guanylate cyclase in vascular smooth muscle cells. In figure 3, NO syntheses catalyses the synthesis of NO from amino acid L-arginine and molecule oxygen (O_2) in generator cells with the L-citrulline by-product. NO binds and activates to the enzyme (guanylate cyclase) to produce cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP) in target cells to cause biological effects such as vasodilation. Haynes et al. (1996) have further stated that although the basal activity of guanylate cyclase is extremely low, when NO binds to guanylate cyclase, its activity is enhanced more than 400-fold. Therefore, an increase in the concentration of cGMP via guanylate cyclase in endothelium can cause the relaxation of vascular smooth muscle and cardiac myocytes, inhibition of platelet aggregation, and attenuation of white cell and platelet adhesiveness (Plumpton et al., 1995).

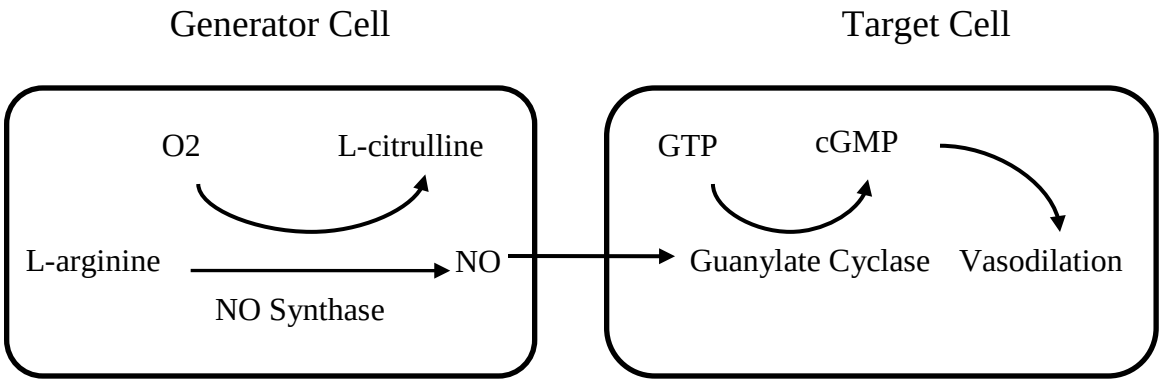


Figure 3. Adapted from Webb and Vallance (1997). Synthesis of nitric oxide (NO) and its effect in vasodilation. NO = nitric oxide; GTP = guanosine triphosphate; cGMP = cyclic guanosine monophosphate.

The NO synthases (NOS) which utilize L-arginine, oxygen, and cofactors [NADPH, tetrahydrobiopterin (BH_4), flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)] exist in three isoforms which are endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) (Kearney, 2007). The eNOS and nNOS are primarily expressed in endothelium and neurons, respectively. The eNOS and nNOS are calcium/calmodulin dependent and usually synthesize low amounts of NO (Webb and Vallance, 1997). NO produced by the eNOS in endothelial cells is used to regulate blood pressure and blood flow. In contrast, the iNOS is inducible in many cells such as macrophages and tissues by immunological stimuli such as cytokines (Nussler and Billiar, 1993) and binds to calmodulin tightly so that its activity is regulated by its rate of synthesis rather than by calcium concentration (Webb and Vallance, 1997). Thus, Dallaire and Marette (2004) have stated that the lack of calcium still allows iNOS to

produce high amounts of NO for longer periods of time than eNOS and nNOS. Additionally, Webb and Vallance (1997) have shown that when expressed iNOS is fully active, it can generate large amounts of NO. However, although the role of NO is to maintain normal blood pressure and blood flow, NO overproduction can cause damage to cells by reacting with one of the reactive oxygen species, superoxide (O_2^-).

C. Synthesis and role of reactive oxygen species (ROS)

Reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) are produced in vascular cells. Healthy vascular cells metabolize oxygen and ROS is generated; normally, the rate of ROS production is balanced by the rate of oxygen elimination (Vider et al., 2001). However, when ROS production is increased, the process of endothelial dysfunction occurs, resulting in damage to cell structures; this situation is also called oxidative stress. Ross (1999) has stated that ROS may act as a common intracellular messenger that leads to adhesion molecule expression.

The primary sources of ROS in the vasculature are NADPH oxidase, xanthine oxidase, and uncoupled eNOS (Hamilton et al., 2004). O_2^- has been recognized as the most potent reactive free radical (Webb and Vallance, 1997). Thus, Webb and Vallance have stated that NADH/NADPH oxidase and xanthine oxidase can induce O_2^-. Additionally, Tifenbacher (2001) has demonstrated that the uncoupling eNOS is a source

to produce O_2^-; for example, BH4 deficiency can induce an uncoupling eNOS, resulting in the powerful oxidant such as $O_2^-$and H_2O_2. However, NADPH oxidase has been found to be the most potent source of O_2^- in the human vasculature (Griendling and Ushio-Fukai, 1997) and could be activated by low-density lipoprotein and angiotensin II (Berry et al., 2000; Rajagopalan et al., 1996).

Oxygen (O_2) reacts with NADPH oxidase, resulting in O_2^- production which can further be converted to H_2O_2 by superoxide dismutases (SOD). Thus, there are two ways to increase O_2^- levels; one is an increase in O_2^- generating enzyme (NADPH oxidase) and the other is a decrease in the activity of SOD (Webb and Vallance, 1997). Furthermore, H_2O_2 can be converted to either H_2O and O_2 by catalase enzymes or OH^- by ferrous ion (Fe^{2+}) which can further induce lipid peroxidation and DNA damage (Figure 4). Therefore, the ROS-induced damage can be prevented by use of SOD and catalases (Belviranli and Gokbel, 2006). Although increased ROS production can damage cell structures, "an optimal ROS level is essential for the cell's survival; too much ROS may cause impaired physiological function due to either random cellular damage or programmed cell death (apotosis), whereas too few ROS may lead to decreased proliferative response and defective host capacity (Ji et al., 2006)."

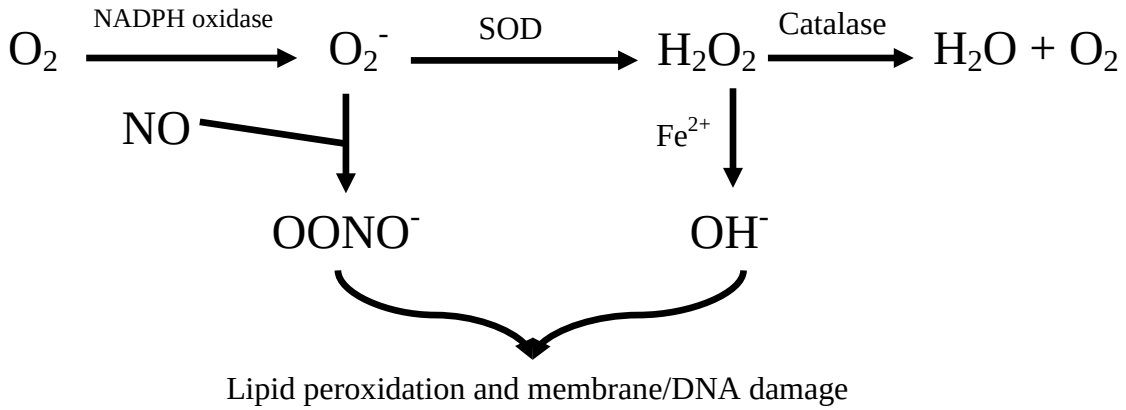


Figure 4. Adapted from Webb and Vallance (1997). ROS production and its interaction with NO in vascular cell. O_2^- = superoxide; NO = nitric oxide; H_2O_2 = hydrogen peroxide; $OONO^-$ = peroxynitrite; OH^- = hydroxyl radical. SOD = superoxide dismutase; Fe^{2+} = ferrous ion.

D. Destruction of NO by ROS

NO production and bioavailability are regulated by eNOS but can be dysregulated by reacting with ROS. Cai and Harrison (2000) have stated that the production of ROS such as O_2^- within the vascular wall plays an essential role in the progress of endothelial dysfunction; for example, the NO-induced toxic effect is through its interaction with O_2^-. A large quantity of NO has been shown to have the capacity to react with O_2^- (Cai and Harrison, 2000). This reaction results in the generation of peroxynitrite ($OONO^-$) which is a powerful reactive oxidant and can further induce tissue damage and endothelial dysfunction due to lipid peroxidation and DNA damage when O_2^- levels maintain high and NO is produced (Beckman et al., 1990; Cai and Harrison, 2000) (Figure 4). Thus, NO is both antioxidant and oxidant dependant on the relative concentration of NO and

ROS such as O_2^- .

Further evidence by Garcia et al. (1995) has stated that the reaction between NO and O_2^- is faster than the reaction between O_2^- and SOD. H_2O_2 is a stronger oxidant than O_2^-, but $OONO^-$ is much more powerful than H_2O_2. Therefore, SOD plays a critical role in preventing the production of $OONO^-$ from damage in vascular cells. An increased destruction of NO by diminished scavenging O_2^- via SOD has been demonstrated to induce cardiovascular diseases such as hypertension (Tschudi et al., 1996).

E. Regulation of nuclear factor-kappa B (NF-kB) by NO and ROS

Although many signaling pathways regulate endothelial function, nuclear factor-kappa B (NF-kB) has been considered to be the most important to cope with ROS. The NF-kB family is divided into two groups based on the structure and function. The first group consists of p65 (RelA), RelB, and c-Rel, and the second group consists of p50 (NF-kB1) and p52 (NF-kB2). Under normal conditions, IkB, an inhibitory protein, binds and maintains NF-kB inactive. Following the stress-induced ROS stimulation, IkB is phosphorylated by IkB kinase (IKK), resulting in dissociation of IkB from NF-kB. Then, the activated NF-kB is translocated into the nucleus and binds DNA to activate gene expression. Many expressions of proteins are dependent on NF-kB to induce a change in cell function such as expression of adhesion molecules (Figure 5). This is supported by Marui et al. (1993) who demonstrated that ROS such as $OONO^-$ and H_2O_2 provides a

pathway for activation of NF-Kb. Furthermore, Khan et al. (1996) stated that the inhibitor of NO, NG-nitro-L-arginine methyl ester (L-NAME), could activate NF-kB resulting in the vascular cell adhesion molecule-1 (VCAM-1) expression. However, although an increase in NO production has been shown to inhibit the activation of NF-kB, NO does not suppress IKK activity (Spiecker et al., 1998). Therefore, NO may use a different pathway on the suppression of NF-kB rather than IkB phosphorylation by IKK.

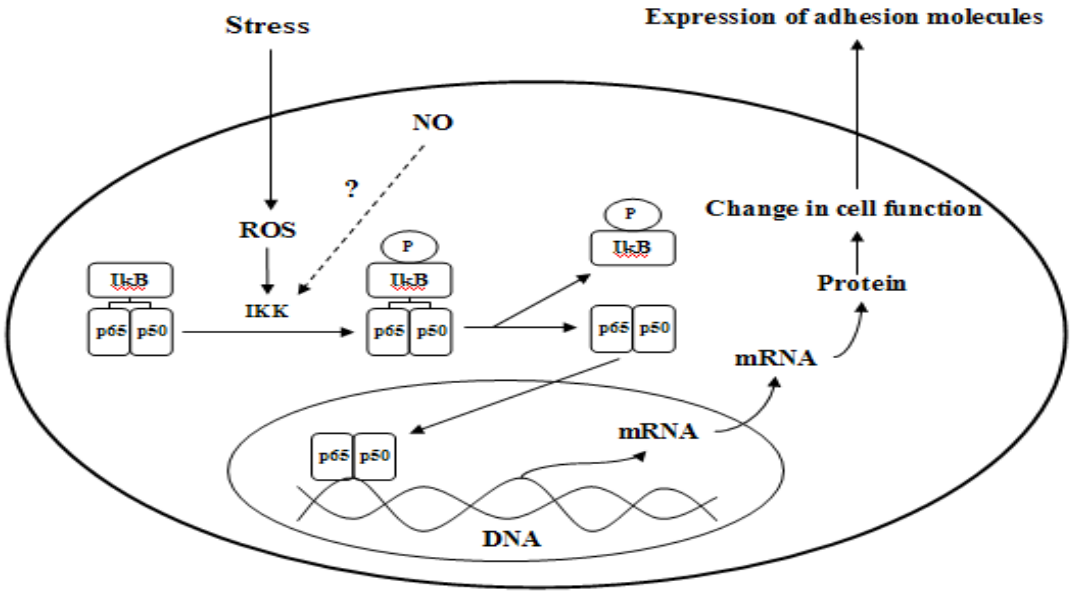


Figure 5. Activation of NF-kB by ROS. ROS = reactive oxygen species; NO = nitric oxide; IKK = IkB kinase; P = phosphorylation.

Inflammation and Endothelial Response

A. Process of inflammatory response

Immune cells circulate in the blood stream to protect against pathogenic organisms and to ensure that the bodies' immune response is efficient. A stress-immune interaction is capable of causing an inflammatory response, which is important to host anti-viral defenses and allows the elimination of invading microorganisms (Paulose et al., 1998).

The initial step in the development of vascular inflammation is the activation of endothelial cells (Ross, 1993), which can be generated by production of cytokines induced by immune cells such as macrophages and T cells. This activated endothelium can further express cell adhesion molecules (Cybulsky and Gimbrone, 1991). This process of inflammatory response consists of four steps: rolling adhesion, tight binding, diapedesis, and migration. For example, during inflammation, T cells are released in the blood and bind with cell adhesion molecules such as E-selectin in the vessel wall. Furthermore, T cells roll along the vessel wall and may bind tightly with other cell adhesion molecules such as VCAM-1. In diapedesis, T cells migrate between adjacent endothelial cells. Then, chemokines such as interleukin-8 (IL-8) promote T cells to sites of infection, and these activated T cells ingest and kill bacteria and produce other cytokines which can further recruit additional immune cells. Therefore, cytokines

promote immune-endothelial cell interaction and play important roles in the initiation and propagation of vascular inflammation.

B. Pro-inflammatory cytokines and endothelial function

The reduction of NO, the formation of ROS, and increased expression of cell adhesion molecules in the vessel wall are early markers for atherosclerosis (Khan et al., 1996). These relationships have been investigated with pro-inflammatory cytokines. Many studies (De Caterina et al., 1995; Freeman, 1993; Khan et al., 1996; Moncada and Higgs, 1991; Peng et al., 1995) have shown that NO functions as an immunomodulator of the vessel wall because it attenuates the immune cells to adhere to endothelial wall and decreases pro-inflammatory cytokine-induced expression of cell adhesion molecule such as VCAM-1. Pober et al. (1993) further demonstrated that this suppression of cytokine-induced expression of VCAM-1 is via elevation of cGMP. The primary pro-inflammatory cytokines are interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), which are mainly secreted by macrophages. IL-1 and TNF-α are involved in the development of inflammation through an increased expression of endothelial cell adhesion molecules such as E-selectin and VCAM-1(Chudek and Wiecek, 2006; Cotran, 1999) by NF-kB activation (Li, 2008; Marui et al., 1993; Paulose M et al., 1998).

Furthermore, the formation of ROS is associated with levels of TNF-α, which is a potent activator for NADPH oxidase (Umeki, 1994). ROS is also involved in TNF-α-

induced VCAM-1 expression (Yu et al., 2006) which can further activate NADPH oxidase (Tudor et al., 2001). Other studies have shown that ROS acts as a second messenger to stimulate NF-kB-dependent expression of pro-inflammatory cytokines such as TNF-α (Schreck et al., 1992; Toledano and Leonhard, 1991). In addition, a strong correlation exists between TNF-α and endothelial dysfunction in patients with coronary heart diseases (Sinisalo, 2000). Thus, pro-inflammatory cytokines such as IL-1 and TNF-α play a major role in inducing the expression of endothelial cell adhesion molecules by reacting with NO and ROS. An understanding of NO-ROS interaction and their effects on the expression of pro-inflammatory cytokines and endothelial cell adhesion molecules may explain the link between inflammation and cardiovascular diseases.

C. Th1/Th2 cytokines and endothelial function

(a) Th1/Th2 activation

Stress (physical or psychological) influences many aspects of the central nervous system (CNS), immune system and endocrine system. There is coordinated communication between the CNS and the immune system through the hypothalamic–pituitary–adrenal (HPA) axis, the autonomic nervous system, and the mediators of immune function (cytokines). The initial response to a physical or psychological stress causes the release of corticotrophin-releasing hormone from the hypothalamus. This initiates the release of adrenocorticotropin hormone via the blood to the adrenal cortex

which releases cortisol intothe peripheral circulation. Cortisol prompts production of cytokines induced by immune cells such as T-helper (Th) cells to prepare for a possible antigen invasion. In response to stress, catecholamines (norepinephrine and epinephrine) are also secreted from the adrenal medulla and sympathetic nerve endings. These powerful modulators of sympathetic activity also interact with the immune system in response to stressful stimuli and are thought to alter pro-inflammatory and anti-inflammatory cytokine release.

Under normal conditions, a balance exists between pro-inflammatory and anti-inflammatory cytokine production and release. This balance is mediated through activation of Th cells and their subsequent differentiation to either Th1 subtypes, associated with inducing cellular immunity, or Th2 subtype, associated with promoting humoral immunity. Th cells are produced from precursors designated naïve T cells that have not encountered antigen-presenting cells (APCs) such as macrophages. When the APCs are activated, Th1 subtypes release pro-inflammatory cytokines (e.g., IL-2 and IFN-γ) that stimulate the cytotoxic T cells, natural killer cells, and macrophages while Th2 subtypes release anti-inflammatory cytokines (e.g., IL-4 and IL-10) that stimulate eosinophils, B cells and mast cells (Elenkov, 2002 and 2004). Th1 and Th2 subtypes exhibit inhibitory cross-regulation via their cytokine release (Elenkov and Chrousos 2002) (Figure 6).

In addition, elevated cortisol (CORT), norepinephrine (NE), and epinephrine (EPI) are thought to inhibit differentiation of Th cells toward the Th1 subtype, resulting in the release of anti-inflammatory cytokines (Kidd, 2003). In general, CORT, NE, and EPI inhibit pro-inflammatory cytokine synthesis and have immunosuppressive potential by a shift of Th1 to Th2 (Elenkov, 2004).

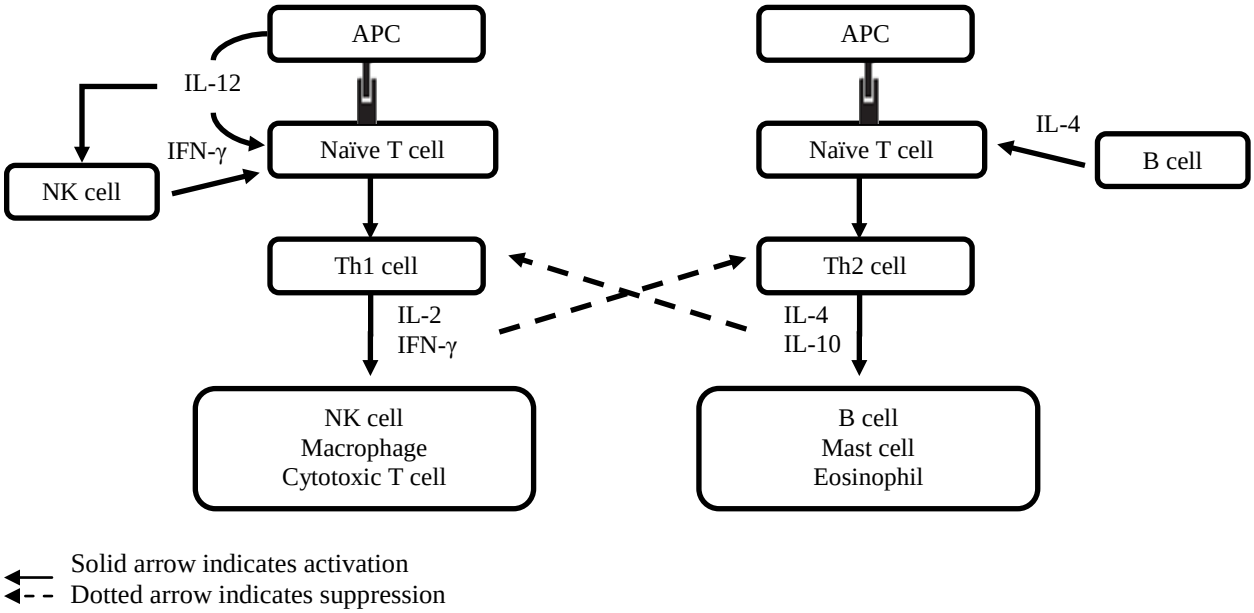


Figure 6. Adapted from Elenkov and Chrousos (2002). Th1/Th2 diagram. APC = antigen-presenting cell.

(b) Th1/Th2 cytokines, NO, and ROS

Macrophages are important sources for NO production due to the activation by Th1 cytokines (Murr C et al., 2005). Niedbala et al. (2006) have stated that the increased cGMP by NO is associated with the enhanced Th1 cell activation. Furthermore, Moran et al. (2006) have shown that Th1 cytokines induce the iNOS, but Th2 cytokines suppress

the iNOS. For example, Th1 cytokines such as IFN-γ and IL-2 up-regulate iNOS, resulting in the production of large amounts of NO (Nathan, 1997; Oswald and James, 1996; Verma and Anderson, 2002; Xiao et al., 2008; Xie et al., 1992;). Xiao et al. (2008) has further stated that a deficiency of IFN-γ may reduce NO concentration in iNOS-deficient mice. In addition, Th2 cytokines such as IL-4 and IL-10 have been shown to decrease the induction of iNOS (Verma and Anderson, 2002). Thus, the level of iNOS expression is likely dependant on the Th1/Th2 cytokine balance.

Furthermore, NO has been considered a major macrophage-immunosuppressive factor for T-cell immunity (Taylor-Robinson, 1990; Tomioka and Saito, 1991). Large amounts of NO have been demonstrated to inhibit the secretion of Th1 cytokines such as IL-2 and IFN-γ, but not Th2 cytokines such as IL-4 (Taylor-Robinson, 1990). This suggests that high levels of NO are produced by Th1-stimulated macrophages, resulting in the suppression of T cell proliferation (Van der Veen et al., 2000; Xiao et al., 2008). Niedbala et al (1999) have further supported that although high levels of NO are detrimental, low levels of NO increase the Th1 differentiation, but not Th2. Other studies have also shown that NO deficiency in mice enhances Th1 response, producing more IFN-γ and less IL-4 (MacLean et al., 1998; McInnes et al., 1998; Wei et al., 1995). It is likely that there is a negative feedback regulation between NO and Th1 cells. Therefore, NO has the potential ability to regulate Th1 and Th2 cytokine balance.

The levels of ROS can also be produced by macrophages and are dependent on T

cell activation during antigen presentation (Gelderman et al., 2007; Komatsu et al., 2006; Snelgrove et al., 2006A and 2006B). Murr et al. (2005) have stated that ROS such as O_2^-, H_2O_2, and OH^- which are released by macrophages can enhance Th1 cell activation (Figure 7). Within the Th1 immune response, the most potent trigger for macrophage-induced ROS production is IFN-γ (Nathan et al., 1983). The expression of other Th1 cytokines such as IL-2 can also be stimulated by macrophage-induced ROS (Miesel, 1995B). These Th1-derived cytokines are capable of inhibiting the Th2 response.

Furthermore, many studies have shown that ROS produced by macrophages promotes the development of Th1-induced immune diseases including rheumatoid arthritis. The generation of ROS by macrophage is enhanced in rheumatic patients (Miesel R et al., 1994). The patients with rheumatic disease have10-fold greater levels of ROS compared to normal healthy individuals (Miesel, 1995A). In addition, decreased severity in arthritis has been shown less Th1 response in the presence of macrophage-mediated ROS (Snelgrove et al., 2006). It indicates that ROS by macrophages plays a crucial role in the pathogenesis of arthritis and has an immunosuppressive potential in altering Th1 and Th2 immunity.

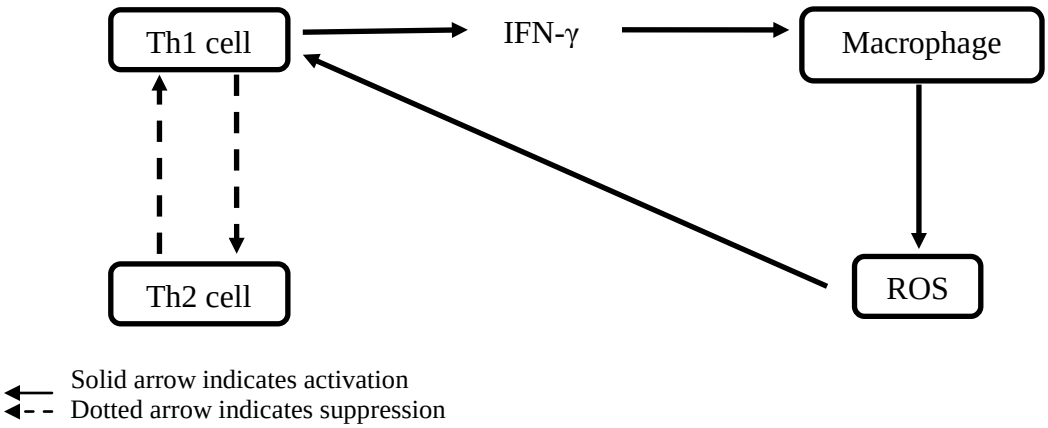


Figure 7. Th1 cell activation by ROS. ROS = reactive oxygen species.

The Impact of Obesity on Endothelial Responses to Psychological Stress

The epidemic of overweight and obesity has evolved and now includes 61.6% of American women and 70.5% of American men (National Center for Health Statistics , 2006). Obesity-related health expenses are derived from diabetes, hypertension, and cardiovascular diseases such as atherosclerosis (Quesenberry et al., 1998). One of the earliest sub-clinical stages in the atherosclerotic process is an impairment of endothelium-dependent vasodilation, also known as endothelial dysfunction (Singhai, 2005). Obesity is also associated with endothelial dysfunction, and one mediator of the process of obesity-induced endothelial dysfunction is the level of oxidative stress, assessed as elevations in free radicals (Schafer and Bauersachs, 2008; Timimi et al., 1998). Furthermore, the proinflammatory state of the vessel can negatively impact oxidative stress and may play a crucial role in the pathogenesis of obesity-related

diseases. Another possible mechanism to explain obesity-induced endothelial dysfunction is the elevation of leptin. Obesity-related elevations in leptin can elicit elevations in oxidative stress (i.e., elevations in free radicals including H2O2 and OH-) (Bouloumıe et al., 1998; Considine et al., 1996) and have the ability to shift T-helper (Th) cell differentiation toward the Th1 subtype, a pro-inflammatory condition (Loffreda et al., 1998). Another factor that has been implicated as participating in the atherogenic process is psychological stress. It is well established that laboratory-induced psychological stress is capable of altering physiological homeostasis and chronic stress has been demonstrated to be a determinant of cardiovascular disease (Olinski, 2002). One potential mechanism that links psychological stress to endothelial dysfunction may be through the direct impact of stress hormones on oxidative stress. However, there is limited information investigating the impact of psychological stress on the oxidative stress and inflammation responses of obese individuals. The possible interaction (additive or synergistic) of obesity and psychological stress on the development of endothelial dysfunction has not been investigated.

A. Oxidative Stress and Inflammation

Oxidative stress is an imbalance between antioxidants (e.g. nitric oxide [NO]) and reactive oxygen species (ROS) (e.g. superoxide [$O2^-$], hydrogen peroxide [H_2O_2]) (Sies, 1997). Under normal conditions, NO is a critical homeostatic regulator of the vessel wall

and plays a role in the maintenance of vascular tone and reactivity (Verma and Anderson TJ, 2001). Healthy vascular cells metabolize oxygen and ROS is generated; normally, the rate of ROS production is balanced by the rate of oxygen elimination (Vider et al., 2001). However, when ROS production is elevated, the process of cell damage leading to endothelial dysfunction (e.g. vasoconstriction/lack of vasodilation) occurs (Ji et al., 2006). In obese individuals, blood flow response to shear stress at rest (a dragging frictional force generated by blood flow in the vasculature) has been shown to be attenuated (Arcaro et al., 1999). This subsequent attenuation of shear stress may reduce the activation of endothelial NO synthase (eNOS), resulting in the reduction of NO (Halliwill et al., 1997).

Furthermore, obese individuals have demonstrated elevated levels of markers for ROS, including urinary 8-Isoprostane level (Keaney et al., 2003). Further evidence has demonstrated that several mechanisms may explain elevated oxidative stress seen in obese individuals (Lopes et al., 2003). For example, a decreased antioxidant defense, represented by lower antioxidant enzymes (e.g. superoxide dismutases and catalase), has been found in obese population (Olusi, 2002). However, in obese insulin-resistant individuals, the effect of insulin on eNOS is impaired and inducible NO synthase (iNOS) is stimulated, resulting in NO overproduction (Dallaire and Marette, 2004). Evidence has demonstrated that when expressed iNOS is fully active, it can generate large amount of NO which has been shown to have the capacity to react with $O2^{-}$ (Webb and Vallance,

1997). This results in the generation of peroxynitrite which is a powerful reactive oxidant and can further induce tissue damage, resulting in endothelial dysfunction, due to lipid peroxidation and DNA damage (Beckman et al., 1990).

Inflammation and its subsequent impact on oxidative stress may play a crucial role in the pathogenesis of obesity-related diseases. The development of vascular inflammation is the activation of endothelial cells which can be generated by production of cytokines induced by immune cells such as macrophages and T cells (Ross, 1993). The formation of ROS is associated with the levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) which is a potent activator for NADPH oxidase (Umeki, 1994) and maintains a strong correlation with endothelial dysfunction in patients with coronary heart diseases (Sinisalo et al., 2000). Furthermore, interleukin-17 (IL-17), a pro-inflammatory cytokine, is mainly secreted by memory activated CD4+ and CD4+CD451RO+ memory T cells (Yao et al., 1995), and increased expression of IL-17 has been related to a number of inflammatory diseases (Ziolkowska et al., 2000). This increased IL-17 has been shown to up-regulate the expression of TNF-α (Jovanovic et al., 1998). Additionally, Miljkovic et al. (Miljkovic et al., 2003) demonstrated that IL-17 induces the activation of iNOS in the rodent endothelial cells. iNOS-induced NO production can be further elevated in the presence of both IL-17 and TNF-α (Miljkovic et al., 2003).

B. Obesity-induced Leptin and Endothelial Dysfunction

Another possible mechanism to explain obesity-induced endothelial dysfunction is the interactions of leptin with oxidative stress and inflammation. Leptin, an adipocyte-derived hormone, plays an important role in metabolism, adiposity, and vascular inflammation, and recently has been implicated in the development of coronary heart disease (Wannamethee et al., 2007). At rest, obese individuals have demonstrated elevated plasma leptin concentrations (up to 10-fold greater than lean individuals) (Considine et al., 1996). Recent evidence (Bouloumıe et al., 1999) has demonstrated that leptin is also associated with elevated oxidative stress. For example, in vitro stimulation of cultured human endothelial cells with leptin has induced an increased accumulation of ROS, including H_2O_2 (Bouloumıe et al., 1999). This leptin-induced oxidative stress may contribute to the development of vascular diseases.

TNF-α has been shown to increase resting serum leptin levels in humans (Zumbach et al., 1997). Evidence has shown up to three-fold increases in both TNF-α mRNA and plasma levels in obese individuals compared with non-obese individuals (Schachinger et al., 2000) and this high level of TNF-alpha is correlated with variables associated with metabolic syndrome (Moon et al., 2004). Additionally, under normal physiological conditions, a balance exists between two major types of T-helper (Th) cells: Th1 and Th2 which can exhibit inhibitory cross-regulation via their cytokine release (Elenkov and Chrousos, 2002). Th1 cytokines (e.g. IFN-y and IL-2) have been

demonstrated to induce the iNOS, but Th2 cytokines (e.g. IL-4) suppress iNOS (Xie et al., 1992). Leptin has been shown to have an ability to shift Th cell differentiation toward the Th1 subtype as a result of increased IL-2 and IFN-γ production and release (Loffreda et al., 1998). The study of leptin and its effects on oxidative stress and inflammation in obesity-related endothelial dysfunction warrants further investigation.

C. Psychological Stress and Obesity

Psychological stress has been proposed as a major factor that can impact the pathogenesis and the progression of cardiovascular diseases (Olinski et al., 2002). Although a number of investigators have expressed interest in examining the mechanisms underlying psychological stress-induced endothelial dysfunction, mechanisms that explain this relationship have not been clearly elucidated. However, previous investigations have shown that psychological stress may contribute to the development of atherosclerosis by eliciting an elevation in ROS, which can further induce oxidative DNA damage, a notion supported by an elevation in the DNA damage biomarker, 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Olinski et al., 2002). Initial evidence that psychological stress can induce damage to nuclear DNA was in the liver cells of rats exposed to a conditioned emotional stimulus (Adachi et al., 1993). Subsequently, in a study on medical students, Sivonova et al. (2004) demonstrated greater nuclear DNA damage in lymphocytes on the day of an examination (stress condition) compared with during the

time between two examination periods (non-stress condition). One explanation for this increase in oxidative stress during psychological stress could be the negative effect of high circulating levels of stress hormones (cortiosl, epinephrine and norepinephrine). Furthermore, Flint and colleagues (2007) has demonstrated that cortisol, norepinephrine, and epinephrine released during psychological stress can induce DNA damage within 10 minutes.

Recent studies have shown that elevated cortisol responses to acute psychological stress are associated with increased waist-hip ratio (Epel et al., 2000). Furthermore, people who undergo acute psychological stress demonstrate increases in leptin, and these increases are positively correlated with waist circumference (Otsuka et al., 2006). This suggests that endothelial dysfunction may be exacerbated with psychological stress in obese individuals. Another potential mechanism that links psychological stress to endothelial dysfunction may be the increase of oxidative stress through the activation of inflammatory cytokines. However, there is limited information investigating the link between psychological stress, oxidative stress, inflammation, and endothelial dysfunction in obese individuals. An understanding of the mechanisms that explain possible differences in lean and obese populations may enhance our understanding of the link between obesity, psychological stress and cardiovascular disease.

Conclusion

Obesity is associated with endothelial dysfunction, and one regulator of the process of obesity-induced endothelial dysfunction is the level of oxidative stress. The pro-inflammatory state of the vessel can negatively impact oxidative stress, assessed as elevations in free radicals and may play a crucial role in the pathogenesis of obesity-related diseases. Furthermore, another possible mechanism to explain obesity-induced endothelial dysfunction is the interactions of elevated leptin with oxidative stress and inflammation. Obesity-related elevations in leptin can elicit elevations in oxidative stress (i.e., H_2O_2 and OH^-) and have the ability to shift T-helper (Th) cell differentiation toward the Th1 subtype, a pro-inflammatory condition. Therefore, an understanding of the interaction between oxidative stress and inflammatory cytokines may explain the link between endothelial and immune function, especially in obese population. Furthermore, relationships between elevations in leptin found in obese individuals and its effects on oxidative stress and inflammation in obesity-related endothelial dysfunction may provide insight into the mechanisms that explain the link between obesity and heart disease.

The possible interaction (additive or synergistic) of obesity and psychological stress on the development of endothelial dysfunction has not fully understood. Therefore, the future study should attempt to expand the understanding of the mechanisms contributing endothelial dysfunction that links between obesity, psychological stress and cardiovascular disease.

CHAPTER 2 PILOT STUDY

Title: Stress Hormones and Immunological Responses to a Dual Challenge in Professional Firefighters

Abstract

The purpose of this study was to examine the changes in heart rate (HR), catecholamines (norepinephrine [NE] and epinephrine [EPI]), pro-inflammatory cytokines (interleukin-2 [IL-2] and interleukin-6 [IL-6]), and lymphocytes (CD8+ and CD56+) in firefighters exposed to a decision-making challenge (firefighting strategies and tactics drill) while participating in moderate intensity exercise. Nine professional male firefighters participated in two counterbalanced exercise conditions on a cycle ergometer: (1) 37 min of cycle ergometry at 60% VO_{2max} (exercise alone condition; EAC) and (2) 37 min of cycle ergometry at 60% VO_{2max} along with 20 min of a computerized firefighting strategies and tactics decision-making challenge (firefighting strategies condition; FSC). FSC elicited significantly greater HR, NE, EPI, and IL-2 when compared to EAC. These elevations may suggest that the addition of a mental challenge to physical stress can alter the hormonal and immunological responses during

firefighting. In addition, this evidence provides insight into the possible mechanisms that explain the link between physical activity, psychology stress, and stress-related diseases.

1. Introduction

Numerous occupations (e.g., firefighting, military operations, law enforcement) are subject to inherent physical and psychological stress. Intense and prolonged physical activity alone has been shown to increase the incidence of upper respiratory tract infection (Nieman, 1994) and asthma (Cooper et al., 2007). Furthermore, psychological stress has been proposed as a major contributor to the progression of cardiovascular diseases (Dimsdale, 2008; Olinski et al., 2002). Interestingly, it has been proposed that psychological stress may impact that pathogenesis of cardiovascular disease through pathways that include elevated oxidative stress (Adachi, 1993; Sivonova et al., 2004). One explanation for this increase in oxidative stress may be the elevated levels of stress hormones (e.g. norepinephrine [NE]) and epinephrine [EPI]) and pro-inflammatory cytokines (e.g. interleukin-2 [IL-2] and interleukin-6 [IL-6]) in response to psychological stress (Sivonova et al., 2004; Cosentino et al., 2004; Wassmann et al., 2004). For example, Flint and colleagues (2007) have demonstrated that NE and EPI released during psychological stress can induce DNA damage within 10 minutes. Furthermore, IL-2 has been found to be one of most potent triggers for macrophage-induced reactive oxygen species (ROS production (Nathan et al., 1983), and IL-6 is thought to induce oxidative

stress and play a major role in the pathogenesis of atherosclerosis (Wassmann et al., 2004). Nevertheless, elevated NE and EPI could inhibit IL-2 but induce IL-6, thus enhancing the potential for immunosuppression (Elenkov, 2004).

Physical and psychological stressors, when induced separately, have been shown to elicit increases in IL-2 and IL-6 cytokines dependent on the acute or chronic nature of the stimuli. The magnitude and direction of the IL-2 and IL-6 cytokine responses to acute exercise are variable and dependent upon intensity and duration. For example, Akimoto et al. (2000) examined the effects of brief anaerobic maximal exercise and found that plasma IL-12 increased significantly after exercise. IL-12 has been demonstrated to generate the IL-2 response, further enhancing the activity of CD8+ and CD56+ lymphocytes; Kobayashi et al., 1989; Morel & Oriss, 1998; Stern et al., 1990). In addition, Steensberg et al. (2001) found that IL-6 increased following 2.5-hours of treadmill exercise at 75% of maximal oxygen uptake. Thus, intense exercise may result in initial increases in IL-2 followed by increases in IL-6.

Utilizing a more prolonged stress model, Uchakin et al. (2001) have examined several inflammatory cytokines following 24 hours of an examination in medical students, and found that IL-2 levels were lower than the control group, but no changes were observed in interferon-gamma (IFN-y) and IL-10. Furthermore, Kang and Fox (2001) also examined chronic academic stress during examinations and found that decreased IL-2 (in both peripheral blood mononuclear cell [PBMC] and whole blood

measures) and IFN-y (only PBMC) levels were observed whereas an elevation was seen in IL-6. IL-4 and IL-5 did not change following the stress associated with exams. Further study of the time course responses of IL-2 and IL-6 to varying stressors may provide greater understanding of the impact of acute and chronic stress in cell-mediated and humoral immunity. Furthermore, there are no studies that have examined IL-2 and IL-6 responses following combined physical and psychological stress.

An examination of stress hormones and pro-inflammatory cytokines following a combination of physical and psychological challenge may provide additional insight into the mechanisms that explain the link between physical activity, psychological stress, and stress-related diseases. Occupations such as military service, law enforcement and firefighting provide a paradigm to investigate the mechanisms that explain stress-induced immunological disorders and diseases. Therefore, the purpose of this study was to examine the changes in heart rate (HR), catecholamine (NE, EPI), pro-inflammatory cytokines (IL-2, IL-6), and lymphocytes (CD8+, CD56+) values in firefighters exposed to a computerized firefighting strategies and tactics decision-making challenge while participating in moderate intensity exercise (firefighting strategies condition; FSC). Furthermore, this study examined the possible relationships among catecholamines (NE and EPI) and cytokines (IL-2 and IL-6) and among IL-2, lymphocytes (CD8+ and CD56+) following the FSC. It was hypothesized that FSC would elicit greater HR, NE, EPI, IL-2, and IL-6. Finally, we expected that CD8+ and CD56+ lymphocytes would

increase in both exercise alone condition (EAC) and FSC, and their peak levels would be positively correlated to changes in IL-2.

2. Methods

2.1. Participants

Nine professional male firefighters were recruited following approval to recruit participants from the Fire Chief (Table 1 for Participant Descriptive Characteristics).

Table 1. Participant descriptive characteristics (N = 9)

Variable	Mean	SD	Minimum	Maximu
Age (years)	33.4	5.1	28.3	42.9
Height (cm)	178.	8.0	165.0	196.0
Weight (kg)	88.1	9.5	72.7	107.3
Years as firefighter	11.3	7.5	4.5	23.0
VO_{2max} (ml/kg/min)	36.9	5.6	27.2	47.4

Participants provided informed consent and completed a medical history questionnaire prior to data collection. All experimental procedures were approved by the University of Mississippi's Institutional Review Board.

Participants in this study were (1) free of cardiorespiratory and metabolic disorders, (2) free of any known blood disorders (e.g. anemia, hemophilia), (3) without

hearing or vision problems (including color-blindness), (4) free of a history of psychological disorders and/or chronic illnesses, (5) native English speakers (6) not having used any prescription or nonprescription medication or tobacco products within the previous eight hours, (7) non-smokers and consuming an average of less than ten alcoholic beverages per week, (8) having not experienced any major life events within 30 days of participation (e.g. death in family, divorce, wedding), and (9) not engaged in actual fire suppression tasks within the previous 72 hours. Additionally, prior to each testing session, participants were asked to fast overnight for at least eight hours and to abstain from alcohol consumption for at least 48 hours. Participants were also instructed to maintain their normal physical activity levels throughout the duration of their involvement in the data collection procedures.

In addition, all firefighters involved in this study had participated in advanced fire training, beyond the basic firefighter I/II instruction required of professional firefighters in the State of Mississippi. Thus, all participants were qualified to assume the duties of Incident Commander at a fire scene.

2.2. Instrumentation

During all testing sessions, a ParvoMedics TrueOne 2400 integrated metabolic measurement system was used to assess metabolic variables, and a Quinton Q-4500 EKG was used to assess heart rate. The EKG was integrated into the ParvoMedics system with

a Measurement and Computing Model A-to-D board. Exercise was performed on a CompuTrainer Pro Cycle ergometer system with workload controlled by the CompuTrainer Coaching Software (Version 1.1). Utilizing this software, a program for controlling workload was specifically written for each participant.

2.3. Lab induced stressor

Firefighters participated in a computer-based Firefighting Strategies and Tactics Drill (FSTD) that served as a mental challenge. The FSTD was created using Fire Studio Version 3.0 (Digital Combustion, Inc., 2004) and Authorware 5.0 (Macromedia, 1999) software. Twelve minutes after the initiation of exercise in the treatment session, the subject began to respond to the FSTD and continued for a total of 20 minutes. For the FSTD, firefighters were asked to respond as the Incident Commander to multiple choice questions that based upon a fire scene presented on a 1.32 meter (52”) monitor. These questions were relevant to the fire scene that was being depicted on an adjacent monitor equal in size. Thus, firefighters were presented with two adjacent monitors; one depicting the fire scene and one presenting relevant multiple choice questions. A numeric keypad placed at handlebar level on the right side of the subject within finger reach was utilized so that participants could respond to each question by pressing the number of the corresponding correct answer on the keypad.

Throughout the FSTD, participants were provided information typical to actual fire suppression pertaining to the context of the fire that was presented on the adjacent monitor. Participants were required to acknowledge having read these pieces of information by pressing a specified key on the numeric keypad before continuing on with the relevant multiple choice questions. If the participant took greater than 30 seconds to respond, a confederate was present to prompt the participant to make a choice from the options presented. To verify the validity of the emergent fire scenes and questions, two independent experts (Fire Director, Memphis, TN and senior training officer, MS State Fire Academy) evaluated the scenes and questions for accuracy of fire behavior and appropriateness of the questions and sequence. Each participant was erroneously informed that his fire chief would be provided with the results of his performance on the FSTD. At the termination of the study, all participants were informed that the Fire Chief would not in fact be provided with the performance results on the FSTD, and that this false information was intended to enhance the challenge of the FSTD. A similar protocol (FSTD) has been shown to increase HR approximately 20 beats per minute at rest (Throne et al., 2000).

2.4. Procedures

Three testing sessions comprised the data collection. These sessions consisted of: (1) an initial session to obtain consent to participate, familiarize participants with all

instruments and procedures, and assess maximal oxygen uptake (VO_{2max}), (2) a session that included 37 min of cycle ergometry at 60% VO_{2max}; the exercise alone condition (EAC), and (3) a session that included 37 min of cycle ergometry at 60% VO_{2max} with 20 min of the FSTD (FSC) (see Figure 8). A mental challenge alone condition was not included because of the likelihood that an adaptation to a single exposure of the FSTD could diminish its physiological effect during the dual challenge condition and because professional firefighters are most often exposed to mental and physical challenges simultaneously. The workload was chosen to limit the possibility of stimulating markers of inflammation (Langberg et al., 2002; Nybo et al., 2002) due to prolonged or high intensity exercise. In addition, this intensity is similar to the intensity experienced during fire suppression activities (Gledhill &Jamnik, 1992; Manning & Griggs, 1983; O'Connell et al., 1986). At least 48 hours were allowed to elapse between session 1 and session 2, and a minimum one week and maximum of 3 weeks transpired between session 2 and session 3. Session 2 and session 3 were counterbalanced between participants.

For session 1, participants visited the lab to participate in a graded exercise test on a cycle ergometer designed to elicit maximal exertion within 8-12 minutes. For session 2 and session 3, the participants reported to the lab at 7:00 AM, and a venous catheter was inserted by 7:30 AM. NE and EPI were obtained at -50 min, -30 min, and 0 min, 10 min, 20 min, 32 min, 37 min, and every 15 minutes for one hour post-exercise. IL-2 and IL-6

were collected at 0 min, 37 min, and at 30 and 60 min post-exercise. CD8+ and CD56+ lymphocytes were analyzed at -30 min, 37min, and 60 min post-exercise (Fig. 8).

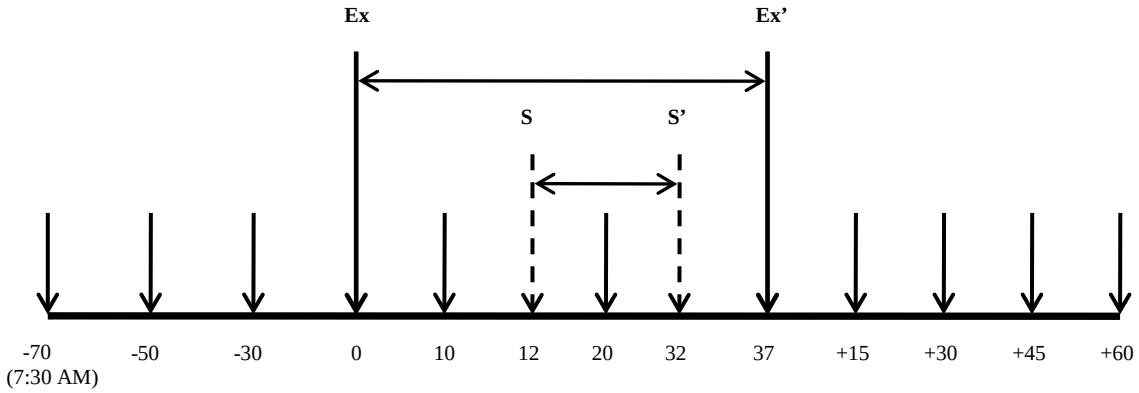


Figure 8. Time progression of the experimental protocol in minutes. The vertical solid lines extending from the ordinate represent the start of exercise (Ex), and the end of exercise (Ex'). The vertical dotted lines extending from the ordinate represent the start of the mental challenge (S), and the end of the mental challenge (S').

2.5. Blood sampling

Blood draws were performed by a physician-approved licensed allied health care professional using standard aseptic technique. An intravenous catheter (Jelco, 20g, 25 mm) was inserted into an antecubital vein, and a positive pressure adapter (CLC2000, ICU Medical, San Clemente, CA) was attached. During each blood draw, the first 1 ml of blood (with saline from the extension set) was collected into a discard syringe preceding the sample draw. Each blood draw was divided as follows: (1) 5 ml was collected into tubes containing ethylene glycol-bis-(beta-amino ethyl ether)-N, N, N', N'-tetraacetic

acid and glutathione for subsequent catecholamine analyses, (2) 6 ml of blood was collected into a tube containing EDTA for IL-2 and IL-6, and (3) 3 ml of blood was collected into tubes containing acid citrate dextrose (ACD) for CD8+ and CD56+ determination. All blood samples, except for ACD treated samples, were centrifuged for 20 minutes at 2500 RPMs at 4 degrees C, and plasma stored at –80 degrees C for future analysis.

Isolation of catecholamines from human plasma was accomplished by alumina extraction using a Chromosystems reagent kit (Alko Diagnostics, Holliston, MA). Once extracted, plasma catecholamine concentration was quantified by high performance liquid chromatography (HPLC). The Waters (Waters Corp., Milford, MA) HPLC system consisted of a pump (model 510, WISP autoinjector (Model 712) with cooling module, column, and an electrochemical detector (Model 460). Data were stored and analyzed using the Waters Millennium software package (V 2.10). The flow rate was 0.8 ml/min, samples in the autoinjector were maintained at 4 degrees C, and column oven was maintained at 40 degrees C. The column was a 15 cm reversed phase C-18 with 5 m silica particles. The sensitivity of the assay was 5 pg/ml on a column with a signal to noise ratio of 4 to 1, a between days coefficient of variation of less than 5%, and a within days variation of less than 3%. The standard curve for the range of 5 to 5000 pg/ml had a correlation coefficient of 0.998.

IL-2 and IL-6 were measured by using enzyme-linked immunosorbent assays (ELISA, BD Biosciences, San Diego, CA and Assay Designs, Ann Arbor, MI, respectively). Interassay coefficient of variation for IL-2 analysis was 4.7% and the intraassay coefficient of variation was 3.98%. Interassay coefficient of variation for IL-6 analysis was 3.61% and the intraassay coefficient of variation was 2.53%.

The CD8+ and CD56+ lymphocytes were analyzed within 48 hours of collection using a flow cytometer (EpicsXL and FC500, Beckman and Coulter, Miami, FL), with a whole blood flow cytometric assay (Mendes et al., 2000).

2.6. Statistical analyses

Data analysis was performed using the SPSS version 16.0. To assess differences between the FSC and EAC, a 2 x 6 (condition x time) repeated measures analysis of variance (ANOVA) was used to examine HR, 2 x 11 (condition x time) RMANOVAs were used to examine NE and EPI, 2 x 4 (condition x time) repeated measures ANOVAs were used to examine IL-2 and IL-6, and 2 x 3 (condition x time) repeated measures ANOVAs were used to examine CD8+ and CD56+ lymphocytes. Significant interactions were further analyzed utilizing paired t-tests.

To assess overall release of NE and EPI, IL-2, and IL-6 during the FSC and EAC, integrated trapezoidal area-under-the-curves (AUCs) were calculated. Comparisons between FSC and EAC for analyses of AUC for NE, EPI, IL-2, and IL-6 were performed

utilizing paired t-tests. Finally, Pearson product-moment correlations were utilized to examine relationships among NE AUC, EPI AUC, IL-2 AUC, IL-6 AUC, and peak CD8+ and CD56+ lymphocytes. The α-level was set at $p \leq 0.05$.

3. Results

3.1. HR, NE and EPI responses

Repeated measures ANOVA for HR revealed a significant condition by time interaction [F (5, 40) = 4.59, $p < 0.01$] with a greater increase for FSC at 20 min [t (8) = 4.15, $p < 0.003$], 32 min [t (8) = 5.32, $p < 0.001$], and 37 min [t (8) = 3.90, $P < 0.005$] (see Figure 9).

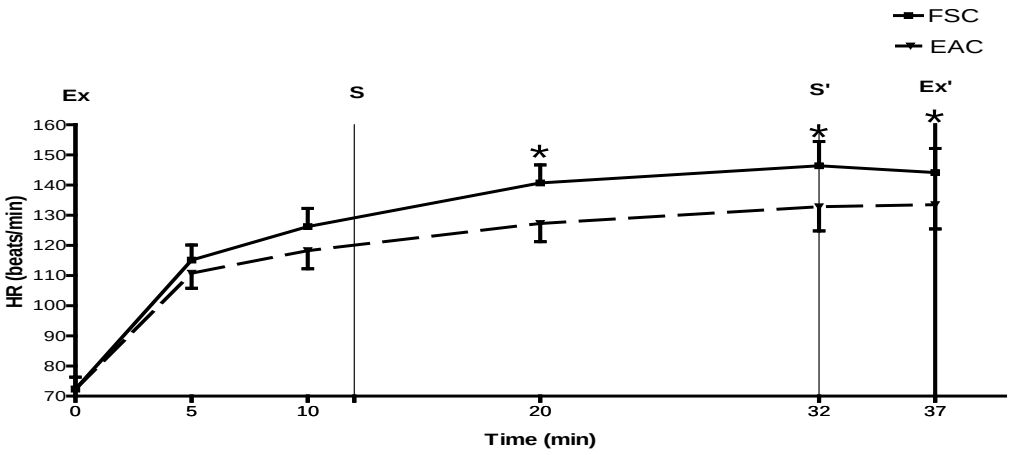


Figure 9. HR responses during exercise in FSC and EAC. A significant condition by time interaction was revealed in HR, with greater increases in FSC at 20 min, 32min, and 37 min (*$p < 0.005$). Points represent the HR values during the protocol; vertical lines depict standard errors of the means (SEM). The vertical solid lines extending from the ordinate represent the start of exercise (Ex), and the end of exercise (Ex'). The vertical dotted lines extending from the ordinate represent the start of the mental challenge (S), and the end of the mental challenge (S').

Repeated measures ANOVA for NE revealed a significant condition by time interaction [F (10, 80) = 3.01, $p < 0.001$] with a greater increase for FSC at 32 min [t (8) = 3.28, $p < 0.02$] and 37 min [t (8) = 2.45, $p < 0.05$] (see Figure. 10a). Furthermore, NE AUC revealed significantly higher concentrations in the FSC compared to the EAC [t (8) = 2.71, $p < 0.05$] (see Figure 10c).

In addition, a significant condition by time interaction was revealed for EPI [F (10, 80) = 3.34, $p < 0.001$] with a greater increase for FSC at 37 min [t (8) = 2.12, $p < 0.05$]. Moreover, EPI AUC did not reveal a significant difference between FSC and EAC (see Figure. 10b).

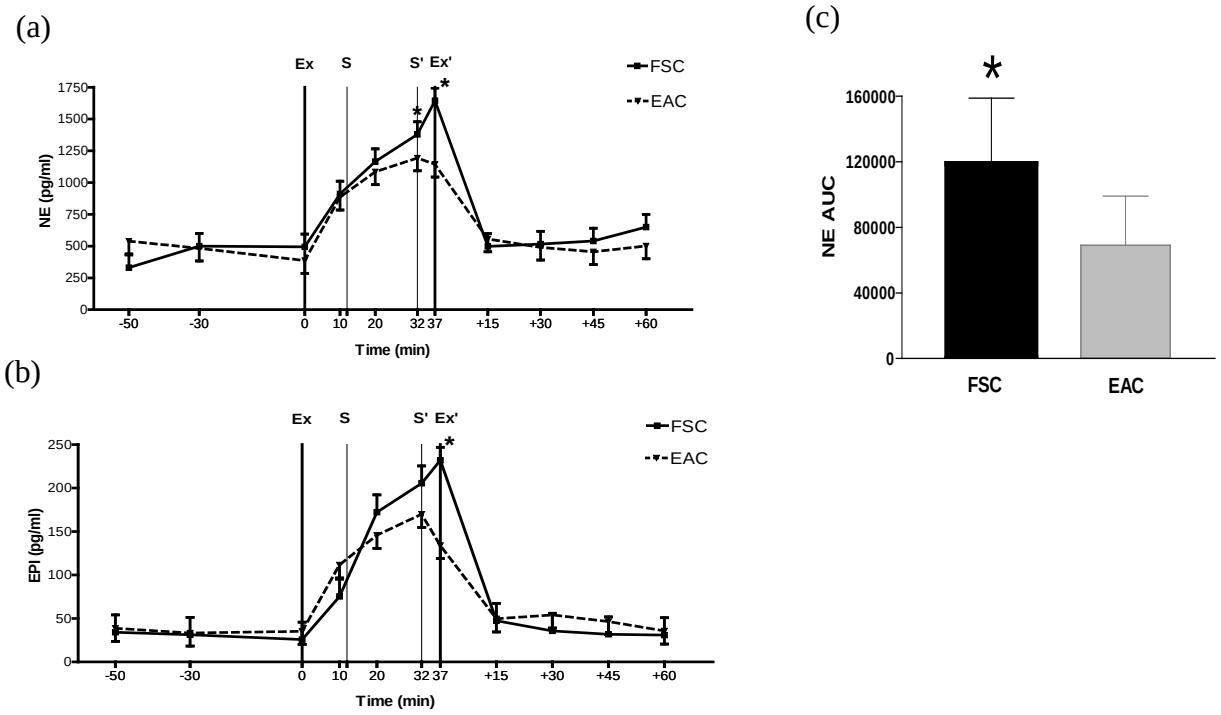


Figure 10. NE and EPI responses to dual challenge: (a) NE; (b) EPI; and (c) NE AUC. A significant condition by time interaction was revealed in NE and EPI, with greater increases in FSC at 32 min and 37 min for NE(a) and 37min for EPI (b) (*p < 0.05). NE AUC revealed significantly higher concentrations in the FSC compared to the EAC(c) (*p < 0.05). Points represent the NE and EPI values during the protocol; vertical lines depict standard errors of the means (SEM). The vertical solid lines extending from the ordinate represent the start of exercise (Ex), and the end of exercise (Ex'). The vertical dotted lines extending from the ordinate represent the start of the mental challenge (S), and the end of the mental challenge (S').

3.2. IL-2 and IL-6 cytokine responses

Repeated measures ANOVA for IL-2 revealed a significant condition by time interaction [F (3, 24) = 3.10, $p < 0.05$] with a greater increase for FSC at 37 min [t (8) = 3.17, $p < 0.02$] and 30 min post-exercise [t (8) = 3.30, $p < 0.02$] (see Fig. 11). Furthermore, IL-2 AUC did not reveal a significant difference between FSC and EAC. In addition, there were no significant differences in IL-6 between EAC and FSC and across time (see Table 2).

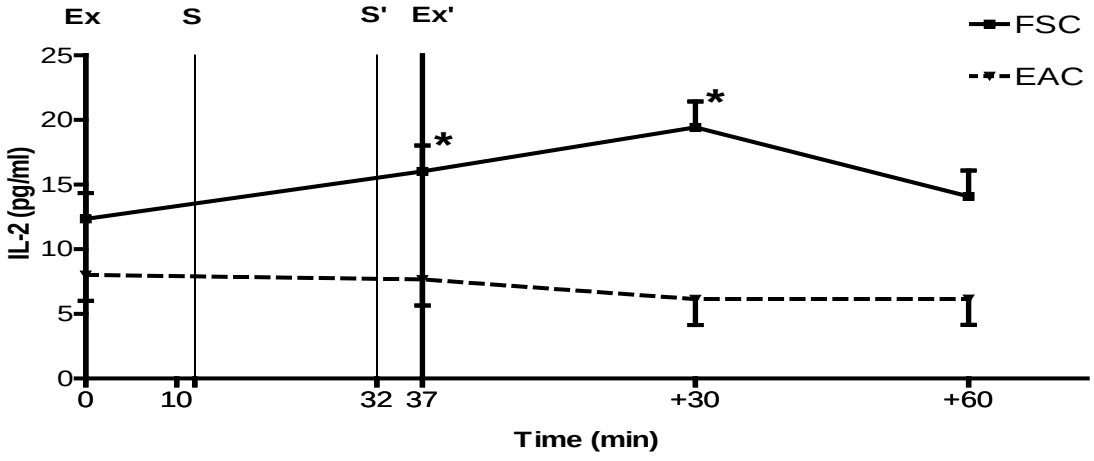


Fig. 11. IL-2 responses to dual challenge: A significant condition by time interaction was revealed in IL-2, with greater increases at 37 min and 30 min post-exercise in the FSC (*$p < 0.05$). Points represent the IL-2 values during the protocol; vertical lines depict standard errors of the means (SEM). The vertical solid lines extending from the ordinate represent the start of exercise (Ex), and the end of exercise (Ex'). The vertical dotted lines extending from the ordinate represent the start of the mental challenge (S), and the end of the mental challenge (S').

3.3. Lymphocyte changes

RMANOVA for CD56+ lymphocytes did not reveal a significant condition by time interaction between EAC and FSC. A significant increase (main effect) over time was observed in CD56+ lymphocytes [F (2, 16) = 62.63, $p < 0.001$] (see Table. 2). Additionally, there were no significant differences in CD8+ lymphocytes between EAC and FSC and across time (see Table 2).

Table 2. Effect of a dual challenge on IL-6, CD8+ and CD56+ cells (Mean ± SD)

Variable	-30 min	0 min	37 min	+ 30 min	+ 60 min	Sig.
IL-6 (pg/ml) - EAC		2.48 ± 0.02	2.48 ± 0.02	2.47 ± 0.02	2.48 ± 0.01	
IL-6 (pg/ml) - FSC		2.48 ± 0.02	2.48 ± 0.02	2.48 ± 0.01	2.48 ± 0.02	
CD 8+ cells (%) - EAC	28.17 ± 5.35		26.74 ± 5.48		26.51 ± 5.29	
CD 8+ cells (%) - FSC	28.48 ± 5.46		27.01 ± 5.22		26.91 ± 5.65	
CD 56+ cells (%) - EAC	9.26 ± 4.50		20.37 ± 8.43		7.49 ± 4.46	*
CD 56+ cells (%) - FSC	7.49 ± 3.11		19.65 ± 5.63		5.71 ± 2.51	*

There were no significant differences in IL-6 and CD 8+ cells between EAC and FSC and across time. CD56+ cells revealed an increase in % of blood mononuclear cells significantly cross time in both FSC and EAC conditions. Data are given as mean ± SD. Asterisks indicate significance cross time (*$P < 0.001$).

3.4. Correlation among variables

NE AUC was demonstrated a positive correlation with EPI AUC in both FSC and EAC ($r = 0.89$ and $r = 0.67$, $p < 0.05$, respectively). Furthermore, NE AUC was positively correlated with IL-2 AUC in FSC ($r = 0.68$, $p < 0.05$), whereas EPI AUC was not correlated with IL-2 AUC in neither FSC nor EAC. In addition, IL-6 and CD8 + and CD56+ lymphocytes were not correlated with any variables in both FSC and EAC.

4. Discussion

The purpose of this study was to examine the changes in heart rate (HR), catecholamines (NE, EPI), pro-inflammatory cytokines (IL-2, IL-6), and lymphocytes (CD8+, CD56+) in firefighters exposed to a computerized decision-making challenge (firefighting strategies and tactics drill) while participating in moderate intensity exercise. This combination of physical and psychological challenge activated the sympathoadrenal (SA) axis, eliciting the release of catecholamines (NE and EPI) and elevating HR. Furthermore, firefighters demonstrated increases in IL-2 and the number of CD 56+ lymphocytes. This response to dual challenge suggests that the addition of a mental challenge to physical stress may alter the immune response and, if exacerbated or prolonged, may play a role in the development of stress-related diseases.

Firefighters elicited a greater elevation in HR following the dual challenge. This result supports previous research by Acevedo et al. (2006) who demonstrated that the addition of a mental challenge elicited an exacerbation in HR response during exercise, suggesting that a precursor to the HR elevation was an increase in catecholamine levels (NE and EPI). NE increases in a curvilinear manner to exercise workload and has been measured in venous blood within minutes of exposure to physical stress whereas EPI increases when exercise workload exceeds 60% VO_{2max} (Frankenhaeuser, 1991). Thus, to limit the stimulation of NE and EPI release and examine the dependent or additive effect of psychological stress, a workload of 60% of VO_{2max} was used in the current study. Interestingly, firefighters elicited greater increases in NE and EPI following the dual challenge. This finding is consistent with an earlier study (Ray et al., 2006), examining firefighters from India, which observed greater NE and EPI responses in firefighters challenged with a physical and psychological demand. Furthermore, Webb and colleagues (2008) have examined healthy individuals in response to a combination of physical and psychological stress and found a greater increase for NE in dual stress condition. The NE results in this study are consistent with Webb et al. Furthermore, this study utilized a specific stressor (FSTD) with a special population (firefighters), and demonstrated significant elevations in EPI. NE AUC revealed significantly higher concentrations in the FSC compared to the EAC and demonstrated a positive correlation

with EPI AUC in both conditions. These elevations in NE and EPI suggest that the SA axis are further activated, beyond the exercise alone condition, during a dual challenge.

No studies have investigated immunological responses following a dual stress model. This study presents data demonstrating the response of immune markers to dual stress and the possible relationship to neuroendocrine activity. An exercise intensity of 60% VO_{2max} for 37 min was used to limit the release of inflammatory markers due to physical work (Langberg et al., 2002; Nybo et al., 2002). Additionally, this intensity is similar to the intensity experienced during fire suppression activities (Gledhill & Jamnik, 1992; Manning & Griggs, 1983; O'Connell et al., 1986). In this study, firefighters elicited greater IL-2 levels in response to the dual challenge, whereas no significant difference was found in IL-6. These results support previous research by Heinz et al. (2003) who have stated that acute psychological stress exposure is associated with increased IL-2 concentrations. Although other investigators (Brenner, et al., 1999) have found changes in IL-6 following exercise, the protocol in this study was quite different. More specifically, Brenner et al. utilized a protocol of two hours of cycle ergometry at 60% VO_{2max}, whereas the duration in this study was 37 min. Furthermore, although this study did not document elevations in IL-6, it is possible that changes occurred following our last measure that was taken at one hour of recovery. Steptoe et al. (2001) have demonstrated that IL-6 concentration

is elevated at two hours following acute psychological stress. Further studies are needed to investigate extended recovery periods for examining IL-6 responses.

A recent review by Matalka (2003) has summarized that acute mental stress induces the production of pro-inflammatory cytokines (e.g. IL-2) via a mild and transient increase in catecholamines (NE and EPI). In this study, IL-2 AUC was correlated with NE AUC in FSC. However, in support of the role of catecholamines in immune-regulation, Panina-Bordignon and colleagues (1997) have demonstrated that in vitro, beta-2 adrenoceptor agonist seemed to inhibit T helper cell type 1 (Th1), a major source of IL-2. It is possible that if catecholamines were consistently elevated during fire suppression activities, this could lead to an inhibition of the differentiation of naïve T helper cells toward the Th1 subtype, resulting in a decrease in IL-2.

CD56+ lymphocytes are important as a first line of defense against infection (Herbermann, 1981; Pedersen, 1985). Studies have shown increases in CD56+ lymphocytes following physical activity and acute psychological stress, independently (Biuhmi et al., 1985; Pedersen et al., 1998; Schedlowski et al., 1993). Our results demonstrate increases in CD56+ lymphocytes, but no difference was observed between FSC and EAC. Furthermore, Elenkov (2004) has shown that IL-2 has the potential to activate CD56+ and CD8+ lymphocytes. Firefighters in this study demonstrated greater IL-2 levels following a dual challenge (FSC) with a significant increase in CD56+ lymphocytes in both conditions, but no difference was found in CD8+ lymphocytes. In

addition, there was no significant relationship among IL-2 and CD56+ and CD8+ lymphocytes. It is possible that the time course for IL-2 and CD56+ lymphocytes are distinct. Also, our protocol might be ineffective at detecting peak levels for each of these following FSC and EAC, and these changes might occur at local microenvironments, such as in the tissue, before it appears in the blood. Future studies are needed to consider microenvironments and enumeration of CD4+ cells simultaneously with CD8+ and CD56+ cells in order to better explain these findings.

In conclusion, firefighters participating in a firefighting simulation task (dual challenge) responded with elevations in HR, NE, EPI, and IL-2. Relationships among catecholamines, inflammatory cytokines, and lymphocytes are dependent on the time course for each biochemical, and often can be difficult to capture. Nevertheless, these exacerbated or prolonged elevations may play a crucial role in the development of stress-related diseases such as cardiovascular diseases, perhaps through a pathway that includes elevated oxidative stress. These results are potentially important for firefighters in an actual fire scene. Furthermore, firefighters are engaged in fire suppression activities with standard work to rest ratios; approximately 15 to 20 minutes of work, then resting for approximately 10 minutes (United States Fire Administration, 2004). The repeated work-rest regimen of firefighting may further exacerbate the physiological and psychological responses, thus contributing to possible immune system dysfunction among firefighters. An important consideration for further investigation is the impact of oxidative stress and

its relationship to catecholamine release and inflammatory cytokines during repeated or prolong freighting activities. An understanding of the mechanisms may enhance our understanding of the link between physical activity, psychological stress and stress-related disease. In turn, greater understanding can facilitate efforts to address the potential negative impact of these immunosuppressive responses associated with professions exposed to dual challenges.

CHAPTER 3 MANUSCRIPT

Title: The Impact of Mental Challenge on Indicators of Endothelial Function in Obese Individuals

Abstract

A number of investigators have examined psychological stress-induced endothelial dysfunction, however, the underlying mechanisms for these responses have not been clearly elucidated. The purpose of this study was to compare the effects of mental challenge on forearm blood flow (FBF), total antioxidant capacity (a measure of oxidative stress), the release of norepinephrine (NE; stress induced neurotransmitter), and pro-inflammatory cytokine responses [both lipopolysaccharide (LPS)-stimulated TNF-α and IL-6 cytokine and mRNA] in lean and obese individuals. Twelve subjects who had a BMI above 30 kg/m^2 and were above 30% body fat were categorized as obese and twelve subjects with a BMI below 25 kg/m^2 and were below 25% body fat were categorized as lean. Blood samples were drawn and forearm blood flow was assessed prior to and following subjects' participation in a mental challenge protocol consisting of a computer-based Stroop Color-Word task and mental arithmetic task, for a total of 20 minutes. The mental challenge elicited an elevation in HR and NE in both the lean and obese groups. Furthermore, both lean and obese groups demonstrated an increase in FBF following the mental challenge, whereas no changes in total antioxidant capacity were observed. In

addition, the lean group exhibited an increase in LPS-stimulated TNF-α cytokine production from baseline to following the mental challenge, whereas the obese group demonstrated a decrease in LPS-stimulated TNF-α cytokine production. This corresponded with a decrease in LPS-stimulated TNF-α mRNA expression in the obese group, although the obese subjects maintained higher levels of both measurements (LPS-stimulated TNF-α cytokine and mRNA expression) compared with the lean group following the mental challenge. Furthermore, in the LPS-stimulated IL-6 cytokine response, the obese group demonstrated a greater increase than the lean group following the mental challenge, even though both groups showed an increase in LPS-stimulated IL-6 mRNA expression. These findings suggest that the magnitude and direction of LPS-stimulated TNF-α cytokine response and mRNA expression and LPS-stimulated IL-6 cytokine response to acute stress may be dependent upon the effects of the additional percentage of body fat seen in obese individuals.

1. Introduction

The epidemic of overweight and obesity has evolved and now includes 61.6% of American women and 70.5% of American men (National Center for Health Statistics, 2006). Obesity-related health expenses are derived from diabetes, hypertension, and cardiovascular diseases such as atherosclerosis (Quesenberry et al., 1998). One of the earliest sub-clinical stages in the atherosclerotic process is an impairment of endothelium-dependent vasodilation, also known as endothelial dysfunction (Singhai, 2005). Obesity is also associated with endothelial dysfunction. A mediator of endothelial dysfunction is shear stress (a dragging frictional force generated by blood flow in the vasculature) leading to oxidative stress. Oxidative stress is an imbalance between antioxidants and reactive oxygen species (ROS) [e.g. superoxide (O^{2-}), hydrogen peroxide (H_2O_2)] (Sies, 1997). In addition, vascular inflammation plays a critical role in endothelial dysfunction which can be induced by the production of pro-inflammatory cytokines [e.g. tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)] induced by immune cells such as T cells (Ross, 1993).

In obese individuals, the vascular response to shear stress is attenuated (Arcaro et al., 1999). This obesity-induced endothelial dysfunction may be the interaction of obesity-induced elevations in leptin and inflammation. Leptin, an adipocyte-derived hormone, is positively associated with elevated percentage body fat and plays an important role in metabolism, adiposity, and vascular inflammation, and recently has been implicated in the development of coronary heart disease (Wannamethee et al., 2007). At rest, obese individuals have demonstrated elevated plasma leptin concentrations (up to 10-fold greater

than lean individuals) (Considine et al., 1996). Obesity-related leptin levels are associated with elevated pro-inflammatory cytokines (e.g. TNF-α and IL-6) (Zumbach et al., 1997). Further evidence has shown up to three-fold increases in both TNF-α mRNA and plasma levels in obese individuals compared with non-obese individuals (Schachinger et al., 2000). This high level of TNF-α has been shown to correlate with many variables associated with metabolic syndrome (Moon et al., 2004).

Another factor that has been implicated as participating in the atherogenic process is psychological stress. It is well established that laboratory-induced psychological stress is capable of altering physiological homeostasis, and chronic stress has been demonstrated to be a determinant of cardiovascular disease (Olinski et al., 2002). Acute psychological stress has been shown to induce transient endothelial dysfunction (Ghiadoni et al., 2000). One potential mechanism that links acute psychological stress to endothelial dysfunction may be through the elevated levels of oxidative stress and pro-inflammatory cytokines. Previous investigations have demonstrated that psychological stress may contribute to the development of atherosclerosis by eliciting an elevation in oxidative stress, which can further induce oxidative DNA damage (Olinski et al., 2002). Flint and colleagues (2007) has demonstrated that catecholamines (norepinephrine and epinephrine) released during psychological stress in rats can induce DNA damage within 10 minutes.

In addition, acute psychological stress-induced endothelial function may be due to an increase in elevated circulating levels of pro-inflammatory cytokines (e.g. TNF-α and IL-6). Many studies have shown that acute psychological stress modulates immune function by altering circulating inflammatory cytokine responses (Steptoe et al., 2000).

However, the alterations in cytokine production have been inconsistent and likely relative to the nature of the stressor (e.g. laboratory-induced psychological stressors or real life stressors) (Mae et al. 1998). These different stressors either up- or down-regulate the production of pro-inflammatory cytokines via signaling from the sympathetic nervous system by activated immune cells (Elenkov et al., 2000). Recent studies have shown that people who undergo acute psychological stress demonstrate increases in leptin, and these increases are positively correlated with waist circumference (Otsuka et al., 2006). This suggests that endothelial dysfunction may be exacerbated with psychological stress in obese individuals. Furthermore, no studies have examined the cellular activation of these circulating cytokines in response to mental stress.

Although a number of investigators have expressed interest in examining the mechanisms underlying psychological stress-induced endothelial dysfunction, explanations for these responses in obese individuals have not been clearly elucidated. Therefore, the purpose of this study was to compare the effects of mental challenge on forearm blood flow (FBF), total antioxidant capacity (a measure of oxidative stress), the release of norepinephrine (NE; stress induced neurotransmitter), and pro-inflammatory cytokines responses [both lipopolysaccharide (LPS)-stimulated TNF-α and IL-6 cytokine and mRNA] in lean and obese individuals. It was hypothesized that obese subjects relative to lean subjects in response to an acute mental challenge would show a similar stress response (increase in HR and NE) and demonstrate an attenuated forearm blood flow response and lower levels of total antioxidant capacity. Furthermore, obese subjects would elicit higher

concentrations of LPS-stimulated TNF-α and IL-6 with corresponding elevations in mRNA expression.

2. Methods

2.1. Subjects

Studies have shown that the hormone, estrogen, contains antioxidant properties, resulting in inhibition of lipid peroxidation (Ceresini et al., 2000). Furthermore, estrogen may attenuate cardiovascular responses to mental stress (Ceresini et al., 2000). To control variability of the dependent measures of interest, male participants 18 to 40 years old were recruited to participate in the study. Subjects were placed into one of two groups based on body mass index (BMI) and body fat percentage (see Table 1 for Descriptive Characteristics). Twelve subjects who had a BMI above 30 kg/m^2 and were above 30% body fat (one subject was at 29%) were categorized as obese. Twelve subjects with a BMI below 25 kg/m^2 and body fat percentage below 25% were placed into the lean group (Vincent et al., 2004). The percentage of body fat was assessed by Dual-energy X-ray absorptiometry (DXA) (GE iDXA, Milwaukee, WI) at the Exercise Physiology Laboratory at Virginia Commonwealth University. Additionally, subjects provided an informed consent and completed a medical history questionnaire (Appendix A) prior to data collection. All experimental procedures were approved by the Virginia Commonwealth University's Institutional Review Board.

Subjects were excluded from the study if they had known or suspected cardiovascular, metabolic, rheumatologic, or other inflammatory disease/condition.

Subjects who were taking any medications that would affect cardiovascular hemodynamics, and vascular function were excluded. Subjects were also excluded from the study if they were regular users of tobacco products (cigarettes, cigars, chewing tobacco) and consumed an average of more than ten alcoholic beverages per week. Additionally, subjects who had a history of psychological disorders and/or chronic illnesses and had experienced major life events within 30 days of participation (e.g. death in family, divorce, and wedding) were excluded. Prior to each testing session, subjects were asked to fast overnight for at least eight hours and to abstain from alcohol and caffeine intake for at least 24 hours. In addition, subjects completed the Seven-Day Physical Activity Recall (Appendix B). Subjects reporting more than 150 min of physical activity per week were excluded from participation.

Table 3. Participant descriptive characteristics (mean ± SEM)

Variable	Lean (n=12)	Obese (n = 12)	Sig.
Age (years)	24.00 ± 1.24	26.73 ± 2.09	
BMI (kg/m^2)	21.65 ± 0.58	39.01 ± 1.58	*
Body fat (%)	17.26 ± 1.49	40.36 ± 1.73	*
Physical activity (mins/wk)	147.08 ± 23	126.08 ± 12	

*$p < 0.05$

2.2. Testing procedures

Subjects attended two laboratory sessions. For the first lab visit, subjects arrived at 6:20 AM. Following informed consent and completion of the medical history

questionnaire, subjects' height, weight, body fat percentage, and vascular function were assessed, and to familiarize the subjects with the mental challenge, the subjects participated in a two-minute mental challenge task [Stroop Color-Word Task (SCW) and mental arithmetic task (MA)]. During the second lab visit, subjects arrived at 6:20 AM and performed 20 minutes of mental challenge (see experimental protocol in Figure 12). Heart rate was collected at -40, 0, 20, and +60 min. Forearm blood flow was assessed at -40, 20, and +60 min. Blood samples and heart rate were collected at 0, 20, and +60 min.

Blood draws were performed by a certified phlebotomy technician using standard aseptic techniques. An intravenous catheter (BD, 20g, 25 mm) was inserted into an antecubital vein, and a positive pressure adapter (CLC2000, ICU Medical, San Clemente, CA) was attached. During each blood draw, the first 1 ml of blood (with saline from the extension set) was collected into a syringe and discarded. Immediately following, appropriate sample volumes were collected into specific collection tubes for subsequent analysis. All testing was conducted in the Exercise Physiology Laboratory in the Department of Health and Human Performance at Virginia Commonwealth University.

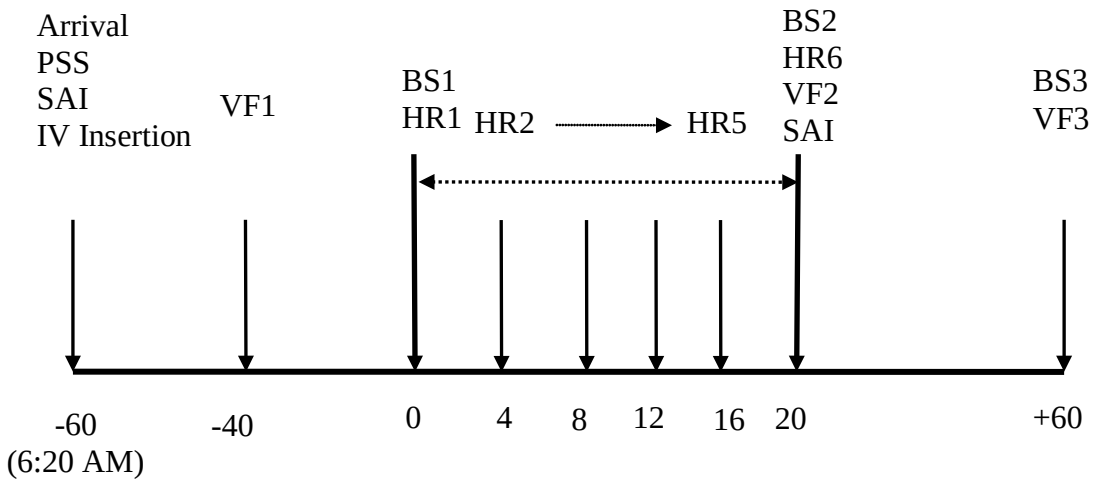


Figure 12. Time progression of the experimental protocol in minutes. PSS = Perceived Stress Scale; SAI = State Anxiety Inventory; BS = Blood Sample; HR = Heart Rate; VF=Vascular Function.

2.3. Laboratory psychological stress protocol

Subjects were instructed to participate in 5 cycles of the computer-based SCW and MA tasks (2 min SCW plus 2 min MA), for a total of 20 minutes of mental challenge. Cardiovascular responses to the mental challenge are highly correlated with responses to real life stressors (Kamarck et al., 2003). The instructions given to the subjects for the SCW task were as follows: "For this task you will be presented with a color word on this screen. You are asked to identify the font color in which the word is presented and not the conflict color word. The color word will be presented for a short period time, and you must respond quickly because a new color word will appear at one-second intervals. The colors you will identify are yellow, green, blue, and red. The number pad on the computer keyboard in front of you has designated #8 for yellow, #4 for green, #6 for blue, and #2 for red. Try to respond as quickly and accurately as possible. We will be recording the

number of colors you correctly identify." The numbers 8, 4, 6, and 2 on the number pad were also color coded appropriately. Additionally, the computer screen provided instant, continually updated feedback on number of correct and incorrect responses and number of attempts and missed attempts. An adverse sound was presented for incorrect responses.

Instructions for the MA task were as follows: "For this task you will be presented with a three-digit number from which you are asked to subtract 3, 7, 8, or 13. After typing your response on the computer keyboard in front of you, you will be informed of whether you are correct or incorrect. In either case, the correct answer will be presented, and you will be asked again to subtract either 3, 7, 8, or 13 from this three digit number. This sequence will continue for two minutes. Try to work as quickly and accurately as possible. We will be recording the number of problems you attempt and the number you answer correctly." For incorrect responses, instant feedback was provided, for correct responses, the arithmetic problem simply continued. In addition, an adverse sound was presented immediately following an incorrect response. Participant performance on these tasks is described in Table 4.

Table 4. Participant performance on mental challenge tasks (mean ± SEM)

SCW Task	# Correct	# Incorrect	# Trials	% Correct
Lean	493.25 ± 10.00	35.33 ± 7.62	546.00 ± 0.00	90.22 ± 1.87
Obese	466.77 ± 15.55	52.77 ± 8.46	546.00 ± 0.00	85.42 ± 2.83
MA Task	# Correct	# Incorrect	# Trials	% Correct
Lean	103.17 ± 9.84	25.08 ± 3.38	128.25 ± 8.80	78.90 ± 3.87
Obese	114.08 ± 10.70	24.38 ± 3.48	138.46 ± 8.38	79.92 ± 4.44

2.4. Stress and anxiety measures

In order to measure the perceptions of stress during 30 days prior to testing, the Perceived Stress Scale (PSS) were used (Appendix C). In addition, a shortened version of State Anxiety Inventory (SAI) were administered prior to and immediately post-testing (Appendix D).

2.5. Assessment of forearm blood flow

Forearm blood flow (FBF) was assessed using mercury in-Silastic strain-gauge plethysmography (MSGP; Model AI6, D.E. Hokanson, Inc., Bellevue, WA). To accomplish this, blood pressure cuffs were positioned around the subject's upper right arm and right wrist, and a mercury in-Silastic strain gauge was placed around the forearm approximately 10 cm distal to the olecranon process (Alomari et al., 2004). During each trial, the wrist cuff was inflated to a pressure of 40 mmHg for 3 minute to occlude hand circulation. Subsequently, the upper arm cuff was inflated to 250 mmHg to occlude all

forearm blood flow for a period of 5 minutes. After 5 minutes of occlusion, the cuff was released and FBF was determined during the 3 minutes period of reactive hyperemia.

2.6. Measures of plasma norepinephrine and serum total antioxidant capacity

A 5 ml blood sample was collected into a tube containing EDTA for norepinephrine analysis, and centrifuged for 15 minutes at 4000 rpms. In addition, a 5 ml blood sample was collected into serum separator tube for total antioxidant capacity and incubated at room temperature for 30 minutes and further centrifuged for 15 minutes at 1000 ×g at 4 degree Celsius. All samples were stored at -80 degree Celsius for further analyses.

The plasma norepinephrine and serum total antioxidant capacity were assayed in duplicate with commercially enzyme-linked immunosorbent assays (ELISA, BD Biosciences, San Diego, CA and Cayman Chemical Company, Ann Arbor, MI, respectively).

2.7. LPS-stimulated cytokine expression

LPS-stimulated cytokine expression for TNF-α and IL-6 in whole blood were measured following the procedures described by Wirtz et al., 2007. The LPS stock solution was prepared by dissolving LPS in pyrogene-free sterile saline (NaCl 0.9%) achieve a final concentration of 30 ng/ml LPS for the culture. Nine ml of venous blood were collected into a sterile pyrogene-free syringe containing 1ml of heparinized 0.9% saline solution. The heparinized whole blood (400 µl) was added to 50 µl of the LPS-stock

solution, or as control to 50 µl of saline in a 24-well cell culture plate. Following a 24 hours of incubation period at 37 degree Celsius in 5% CO2, the samples were centrifuged for 10 minutes at 4000 rpms at 4 degree Celsius. The supernatant was collected and stored at - 80 degree Celsius until analyzed. Both TNF-α and IL-6 stimulated plasma cytokine levels were analyzed in duplicate with enzyme-linked immunosorbent assays (ELISA, BD Biosciences, San Diego, CA).

2.8. Detection of cytokine mRNA by reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

The expression of LPS-stimulated TNF-α and IL-6 mRNAs were measured in whole blood. The LPS stock solution was prepared by dissolving LPS in pyrogene-free sterile saline (NaCl 0.9%) to achieve a final concentration of 30 ng/ml LPS for the culture. Four ml of venous blood was collected into EDTA tube. The whole blood (400 µl) was added to 50 µl of the LPS-stock solution or as control to 50 µl of saline on a 24-well cell culture plate. Following a four hour of incubation period at 37 degree Celsius in 5% CO2, total cellular RNA was extracted utilizing a QIAamp RNA blood mini kit according to manufacturer's instruction (Qiagen, Valencia, CA). The extracted RNA dissolved in diethylpyrocaebonated (DEPC)-treated water was quantified spectrophotometrically at 260-nm wavelength. The 20 µl of cDNA (20 ng/µl) was synthesized from total cellular RNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, CA). Then, a 2 µl cDNA of samples was added in a final volume of 20 µ in the premix TaqMan primer set on an ABI 7500 Fast Real-Time PCR system (Applied Biosystems, CA) when

Quantitative PCR was performed. GAPDH was amplified as a reference gene. Standard curves were produced by plotting the logarithm of the concentration of RNA standard against the cycle numbers of the logarithmic linear phase. The amount of target sequence in the samples was extrapolated from the standard curve.

2.9. Statistical analyses

Data analyses were performed using the Statistical Package for the Social Sciences (SPSS version 16.0). Comparisons of baseline levels on all variables between lean and obese groups were conducted using independent t-test. A two (groups; lean and obese) × six (time; 0, 4, 8, 12, 16, and 20 min) and two (groups; lean and obese) × two (time; 0 and 20 min) repeated measures analyses of variance (ANOVAs) were used to examine changes in HR and SAI scores to mental challenge, respectively. Two (groups; lean and obese) × three (time; 0, 20, and +60 min) repeated measures ANOVAs were utilized to evaluate the effects of mental challenge on the releases of norepinephrine, total antioxidant capacity, and levels of LPS-stimulated cytokines and mRNA expression for TNF-α and IL-6. Finally, a two (groups; lean and obese) × three (time; -40, 20, and +60 min) repeated measures analyses of variance (ANOVA) was also used to examine changes in forearm blood flow to mental challenge. Greenhouse-Geisser corrections for all repeated measures ANOVAs were utilized. Significant effects were further analyzed utilizing Bonferroni post-hoc comparisons. Statistical significance was set at $p \leq 0.05$.

Prior to statistical analysis, a number of anomalous samples were removed from the analysis. Data that represented outliers, non-responders, samples with hemolysis, unique

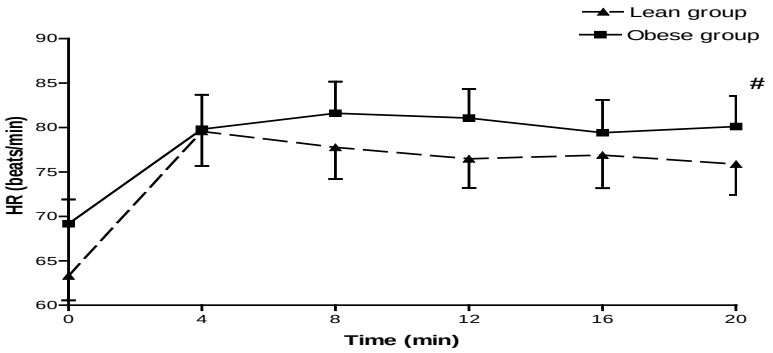
responses and trends, and subjects reporting noncompliance with the study protocol (fasting, physical activity, etc.) were excluded. Appendix E presents the total number of subject utilized for each analysis. More specifically, the total number of subjects utilized for each physiological variable is presented in appendix E. No more than two subjects were excluded from any one analysis. Coefficient of variations and normal concentration curves are presented in Appendix F.

3. Results

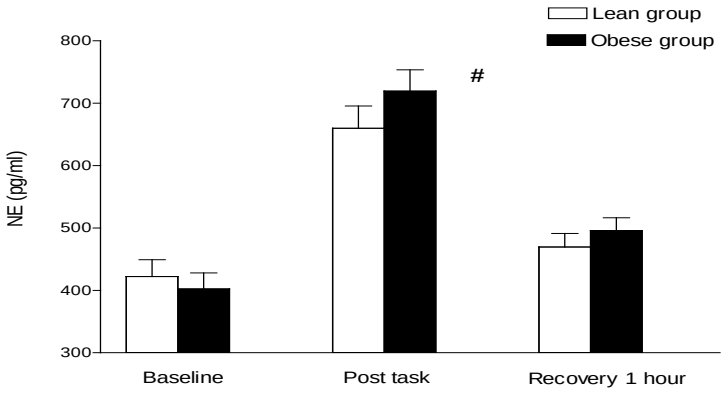
3.1. HR, NE, and SAI responses

There were no significant differences at baseline levels of HR, NE and SAI between lean and obese groups. Repeated measures ANOVA for HR, NE, and SAI revealed no significant group by time interaction. A significant increase across time (main effect for time) was observed for HR [$F(5, 100) = 41.90$, $p < 0.001$] (see Figure 13A). All HR measures during mental challenge were significantly higher than baseline measures. Similarly, NE demonstrated a significant increase across time [$F(2, 42) = 76.54$, $p < 0.001$] and tended to return baseline followed by one hour of receovery (see Figure 13B). In addition, self-reported anxiety (SAI) was significantly elevated following the mental challenge [$F(1,22) = 33.13$, $p < 0.001$] (see Figure 13C).

A.



B.



C.

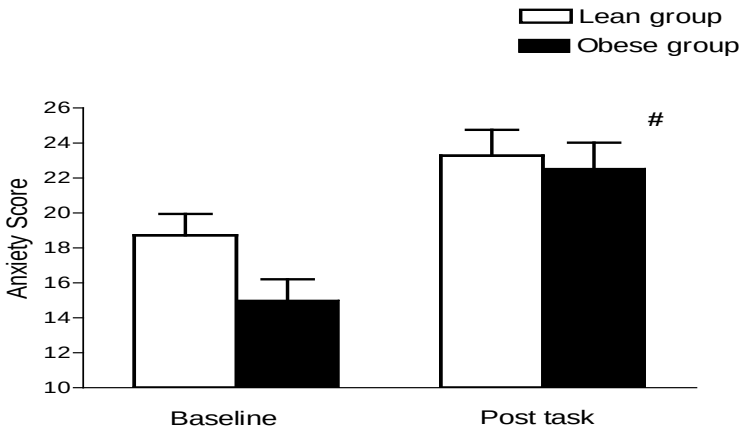


Figure 13. HR, NE, and SAI scores in response to mental challenge. A significant increase cross time was revealed in HR, NE, and SAI scores ($^{\#}p < 0.001$). Data are presented as means ± SEM.

3.2. Forearm blood flow response

At baseline, obese subjects demonstrated significantly higher FBF levels [t (20) = -3.194, $p < 0.01$]. However, repeated measures ANOVA for forearm blood flow demonstrated no significant group by time interaction although, FBF did increase significantly across time [F (2, 40) = 4.15, $p < 0.02$] (see Figure 14).

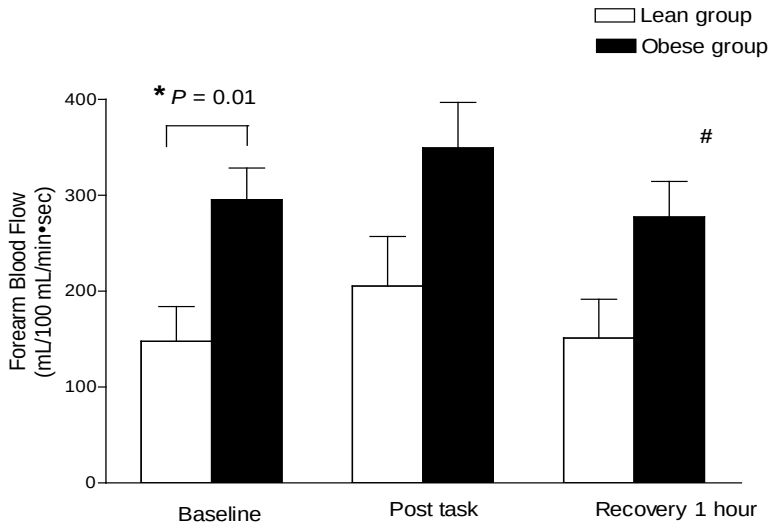


Figure 14. Forearm blood flow response to mental challenge. A greater baseline level in forearm blood flow was shown in obese group (*$p < 0.01$). A significant increase cross time was revealed in forearm blood flow ($^{\#}p < 0.02$). Data are presented as means ± SEM.

3.3. Total Antioxidant capacity

There was no significant difference at baseline level of total antioxidant capacity between both groups. An examination of total antioxidant capacity revealed no differences between group and no changes across time although Figure 15 presents a data suggesting futher examination is warranted.

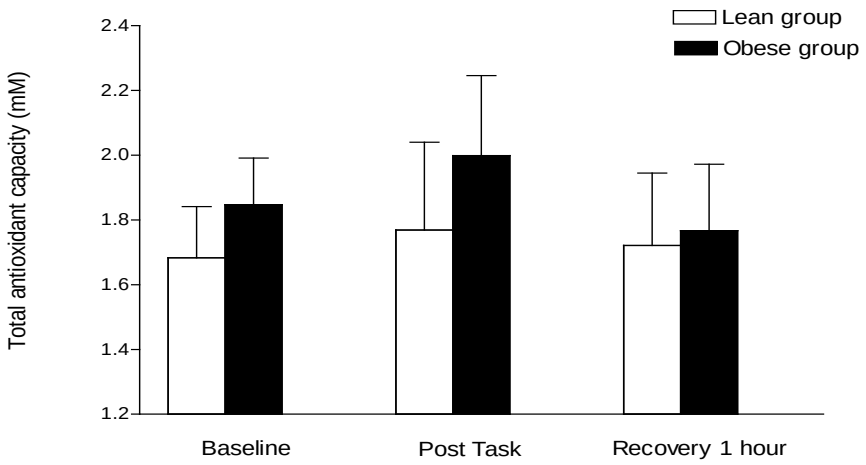


Figure 15. Total antioxidant capacity response to mental challenge. Total antioxidant capacity did not show any difference between two groups. Data are presented as means ± SEM.

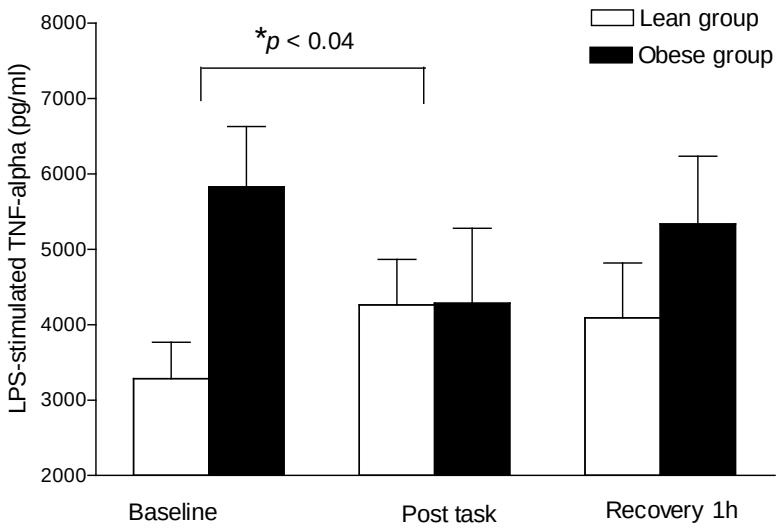
3.4. LPS-stimulated TNF-α and IL-6 cytokine response and mRNA expression

At baseline, there were no differences between groups in both LPS-stimulated TNF-α and IL-6 cytokine response and mRNA expression. To assess the viability of examining samples without LPS stimulation, eight samples without LPS stimulation were analyzed for TNF-α and IL-6 cytokine concentration and mRNA expression. These concentrations and mRNA expression were either low or undetectable in these samples. The following statistical analyses were computed for LPS stimulated TNF- α and IL-6 cytokines and mRNA expression. Repeated measures ANOVA for LPS-stimulated TNF-α cytokine revealed that the lean and obese groups responded differently to the mental challenge (group by time interaction) [F (2, 38) = 3.49, $p < 0.04$]. As presented in Figure 16A, post hoc analysis revealed that the lean group showed a significat increase in

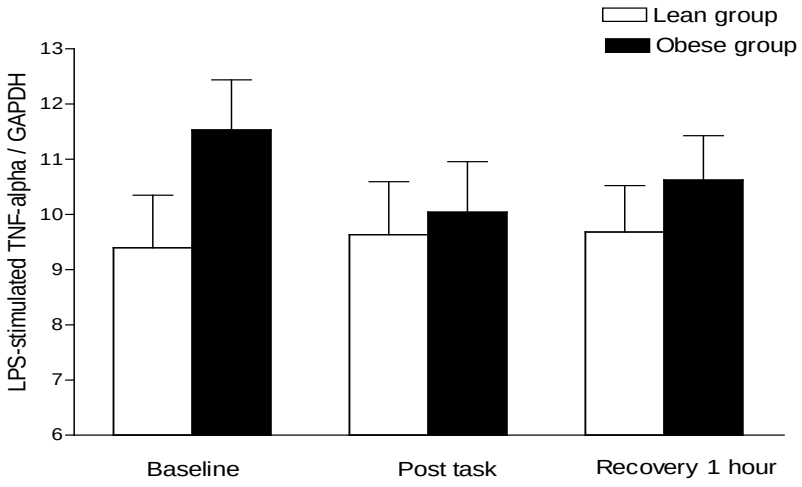
LPS-stimulated TNF-α cytokine production from baseline to immediately post task, and this increase returned to baseline following one hour of recovery. In contrast, post hoc analysis revealed a signicant decrease in LPS-stimualted TNF-α cytokine production in the obese group from baseline to post task followed by a return to baseline at one-hour of recovery (see Figure 16A).

In addition, there were no significant differences between groups and no changes across time in LPS-stimulated TNF-α mRNA expression [F (2, 38) = 2.48, $p = 0.09$] (see Figure 16B). However, the percentage change from baseline to post task was calculated, and a significant percentage change was observed with a greater decrease in the obese group [t (13.21) = 2..68, $p < 0.02$]. (see Figure 16C).

A.



B.



C.

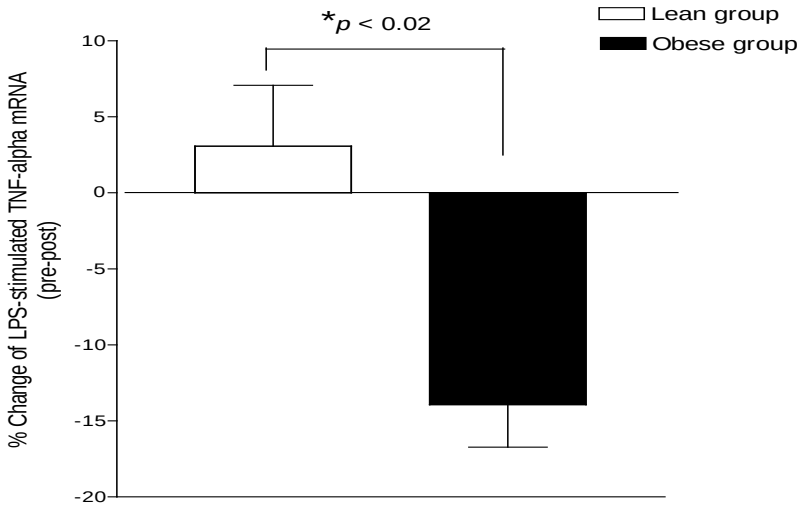
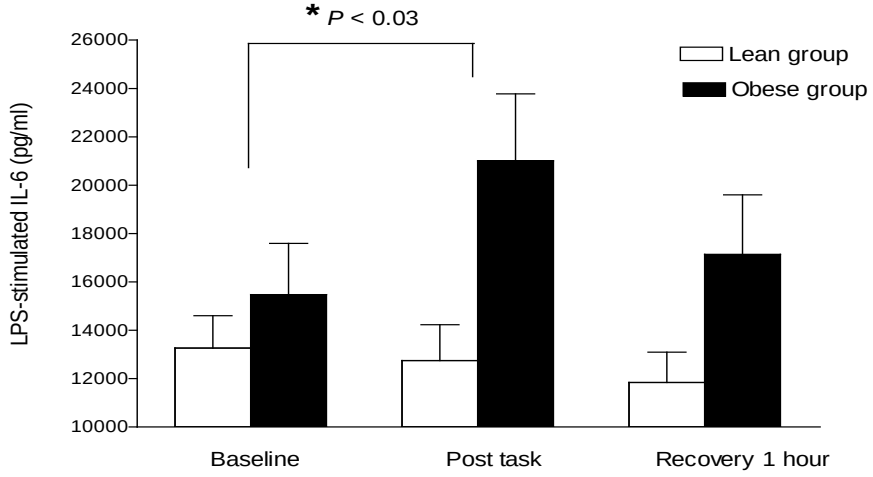


Figure 16. LPS-stimulated TNF-α cytokine response and mRNA expression to mental challenge. A significant group by time interaction was revealed as LPS-stimulated TNF-α cytokine ($^{*}p < 0.04$) decreased following mental challenge in the obese group. LPS-stimulated TNF-α mRNA expression did not show significant differences between groups and no changes across time, but a percentage change from baseline to post task was observed ($*p < 0.02$). Data are presented as means ± SEM.

A significant change between the groups (group by time interaction) [F (2, 38) = 3.68, $p < 0.03$] was observed in LPS-stimulated IL-6 cytokine with post hoc analysis revealing a signicant increase from baseline to post task in the obese group (see Figure 17A) followed by a decrease at one hour of recovery. However, no change in LPS-stimulated IL-6 production was found cross time in the lean group.

In addition, repeated measures ANOVA for LPS-stimulated IL-6 mRNA expression revealed no significant group by time interaction, although a significant increase across time was observed for both groups [F (2, 38) = 19.30, $p < 0.001$] (see Figure 17B).

A.



B.

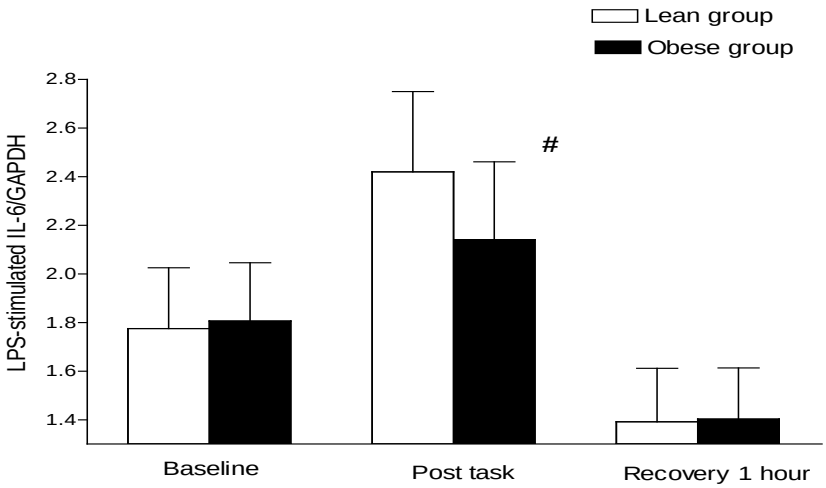


Figure 17. LPS-stimulated IL-6 cytokine response and mRNA expression to mental challenge. A significant interaction group by time was revealed in LPS-stimulated IL-6 cytokine ($^{*}p < 0.03$). Both groups demonstrated a significant increase cross time in LPS-stimulated IL-6 mRNA expression ($\#p < 0.001$). Data are presented as means ± SEM. Data are presented as means ± SEM.

4. Discussion

The purpose of this study was to compare the effects of mental challenge on forearm blood flow (FBF), total antioxidant capacity (a measure of oxidative stress), the release of norepinephrine (NE; stress induced neurotransmitter), and pro-inflammatory cytokines (both LPS-stimulated TNF-α and IL-6 cytokine and mRNA) in lean and obese individuals. The mental challenge activated the sympathoadrenal (SA) axis, eliciting an elevation in HR and the release of NE in both lean and obese groups. These elevations were supported by an increase in self-reported anxiety (SAI) scores. Furthermore, both

lean and obese groups demonstrated an increase in FBF following the mental challenge, whereas an increase in total antioxidant capacity was not observed in both groups. These responses suggest that FBF may not be associated with the release of total antioxidant capacity in response to acute mental stress. The main findings of this study are that the lean group exhibited an increase in LPS-stimulated TNF-α cytokine production from baseline to following the mental challenge, whereas the obese group demonstrated a decrease in LPS-stimulated TNF-α cytokines. This corresponded with a decrease in LPS-stimulated TNF-α mRNA expression in the obese group, although the obese subjects maintained higher levels of both measurements (LPS-stimulated TNF-α cytokine and mRNA expression) compared with the lean group following the mental challenge. Furthermore, in the LPS-stimulated IL-6 cytokine response, the obese group demonstrated a greater increase than the lean group following the mental challenge, even though both groups showed an increase in LPS-stimulated IL-6 mRNA expression. These findings suggest that the magnitude and direction of LPS-stimulated TNF-α cytokine response and mRNA expression and LPS-stimulated IL-6 cytokine response to acute stress may be dependent upon the effects of the additional percentage of body fat seen in obese individuals. These results suggest that obese individuals may experience an exacerbated response, or even be more susceptible to, stress related inflammatory responses.

HR responses to an acute mental stressor differ relative to the timing and duration of the stress. In this study, there were no HR differences between lean and obese individuals during and following the mental challenge. Elevations in HR are usually in correspondence with increases in catechalamines release. Wirtz and colleagues (2007)

have demonstrated that levels of catecholamines (norepinrprhine and epinephrine) can be elevated during and following a 10-min of laboratory-induced stressor. In this study, NE increased following a mental stressor and there were no observed differences between lean and obese subjects. These HR and NE responses in conjunction with the self-reported increase in perceived stress (SAI increases) demonstrate the effectiveness of the mental challenge in eliciting a similar physiological stress response in both groups. Interestingly, a recent review by Matalka (2003) has summarized that acute mental stress induces the production of pro-inflammatory cytokines (e.g., TNF-α and IL-6) via a mild and transient increase in catecholamines (NE and EPI).

FBF and total antioxidant capacity were measured in this study. A mediator of endothelial dysfunction is shear stress (a dragging frictional force generated by blood flow in the vasculature) leading to oxidative stress. A previous study has shown that blood flow response to shear stress at rest is attenuated in obese individuals (Arcaro et al., 1999). In this study, at baseline obese subjects demonstrated significantly higher FBF levels. Kaplan (1995) has demonstrated that a higher forearm blood flow in obese individuals is related to vascular response to adrenergic stimuli (an increased alpha-adrenergic tone in the vasculature) (Kaplan, 1995). Although no difference was found in FBF between the lean and obese groups, both groups demonstrated an increase in FBF following the mental challenge. Agapitov et al. (2002) has demonstrated that obesity is associated with a greater increase in blood pressure in response to acute mental stress. Thus, most previous studies have focused on forearm vascular resistance data (not FBF data) when comparing lean to obese individuals. Because blood pressure was not measured in this study, this undermines the

ability to understand the changes in FBF that may occur in lean and obese individuals following mental challenge. Future studies must consider blood pressure when investigating vascular function in obese individuals.

Moreover, a recent study by Hamer et al. (2007) suggests that vasodilatation responses in response to acute mental stress in individuals with higher fatness are blunted. Therefore, to examine whether or not antioxidant responses to mental challenge help to explain changes in FBF, this study assessed total antioxidant capacity in lean and obese individuals. Total antioxidant capacity demonstrated no differences between groups and across time. This suggests that laboratory-induced psychological stress may not elevate the level of antioxidants. However, it is well documented that the ratio of ROS to antioxidant capacity provided a greater understanding of oxidative stress. Thus, the measure of ROS and total antioxidant capacity in acute and chronic stress models is warranted. Furthermore, an examination of the different oxidative stress responses to mental stress in lean and obese may provide additional information that links psychological stress and endothelial function.

No studies have investigated the inflammatory cytokine responses to mental challenge in lean and obese individuals. This study demonstrates LPS-stimulated TNF-α and IL-6 cytokine responses and corresponding mRNA expressions. To date, the studies examining the production of LPS-stimulated TNF-α and IL-6 cytokines to acute psychological stress have demonstrated inconsistent results. In non-obese individuals, some studies have shown an increase in LPS-stimulated TNF-α production (Maes et al. 1998), whereas others have shown the opposite response (Wirtz et al., 2007). These conflicting results have also been observed in LPS-stimulated IL-6 cytokines (Gobel et al.,

2000; Rohleder et al., 2001). The inconsistent findings in LPS-stimulated TNF-α and IL-6 production is likely related to variations in timing of measure, varying laboratory techniques, and the nature of the stressor (e.g. laboratory-induced psychological stressors or real life stressors) (Maes et al. 1998).

Different stressors, via signaling from the sympathetic nervous system to activate immune cells, up- and down-regulate the production of pro-inflammatory cytokines (Elenkov et al., 2000; Nance and Sanders, 2007). For examples, the beta-adrenoreceptor has been shown to have an inhibitory effect on TNF-α cytokine release (Hasko and Szabo, 1998), whereas alpha-adrenoreceptor is involved in TNF-α production (Izeboud et al., 1999). Recent studies have demonstrated that stress-induced catecholamines up-regulate the activation of NF-kB, further enhancing production of TNF-α cytokines (Barnes and Karin, 1997; Pavlov and Tracey, 2005; Bierhaus et al., 2003). In contrast, catecholamines have been shown to decrease TNF-α production in LPS-stimulated cells (Izeboud et al., 1999). In this study, the lean group elicited an increase in LPS-stimulated TNF-α cytokine production from baseline to following the mental challenge, whereas a decrease was found in the obese group corresponding with a decrease in LPS-stimulated TNF-α mRNA expression. These results suggest that stress-induced NE plays a critical role in inhibiting LPS-stimulated TNF-α production in obese individuals via activation of beta-adrenoreceptor. Although the NF-kB following LPS stimulation is involved in the up-regulation of TNF-α mRNA (Zuckerman et al., 1991), the decreased TNF-α mRNA in obese individuals could be via NF-kB inhibition by stress-induced catacholamines. However, NE in this study was demonstrated a similar response in both groups. This may

indicate that the sensitivity and/or ratio of beta and alpha-receptor response to mental challenge may differ between lean and obese individuals. An understanding of the possible differences in sensitivity of beta and alpha-adrenergic receptors in obese individuals is unclear in the literature.

Interestingly, lean individuals showed no change in the LPS-stimulated IL-6 cytokine response to mental challenge, whereas a greater elevation in the LPS-stimulated IL-6 cytokine was observed in obese individuals, and both groups showed an increase in LPS-stimulated IL-6 mRNA expression. Although beta-adrenoreceptor may have an anti-inflammatory effect, the action of beta-adrenoreceptor stimulation on IL-6 production is still contentious. For example, Van Der Poll et al. (1996) infused epinephrine into human subjects and did not affect IL-6 cytokine production following an LPS challenge. Similarly, in this study the increase in NE resulted in no change in LPS-stimulated IL-6 cytokines in lean subjects, however, the obese subjects did demonstrate an increase, thus, suggesting an attenuation of beta-adrenoreceptor activation in obese individuals.

In addition, although the production of IL-6 can be up-regulated by TNF-a, the results of this study did not support this expectation. It may be the case that the magnitude of this regulation in response to psychological stress is time-course specific and our protocol was not able to observe any differences. The pattern in responses for IL-6 and TNF-α suggests that obese and lean subjects differ in the regulatory mechanisms responsible for the inflammatory responses to mental stress. The high production of LPS-stimulated IL-6 cytokine in obese group may indicate that obese individuals are more susceptible to stress-related inflammatory responses. Further investigation should attempt

to expand the understanding of the mechanisms that link obesity, psychological stress and inflammation.

Although the results of this study provide support for the interaction of obesity and psychological stress in potentially exacerbating pro inflammatory responses, it is evident that more in depth investigation is warranted. For example, a number of variables in this study approached significance and it is possible that a greater number of subjects could have influenced significant findings. In addition, the techniques and procedures utilized for LPS-stimulated whole blood samples should be critically examined to specifically address time of incubation and cell isolation protocols which have not been standardized. The importance of cell isolation has been presented by De Groote et al. (1992). They have demonstrated that the stimulation of isolated mononuclear cells resulted in greater levels of IL-1 when compared to the stimulation of whole blood cultures. Proteins in whole blood cultures seem to down-regulate the cytokine response. In addition, examination of additional stress hormones (e.g cortisol and epinephrine) and neurotransmitters will provide a greater understanding of the relationship between psychological stress, obesity, and varying patterns of pro-inflammatory responses. Finally, a greater understanding of these relationships may provide information that could facilitate our effort to address the incidence of cardiovascular disease and stress-related disorders in lean and obese populations.

References

Acevedo, E.O., Webb, H.E., Weldy, M.L., Fabianke, E.C., Orndorff, G.R., Starks, M.A., 2006. Cardiorespiratory responses of hi fit and low fit subjects to mental challenge during exercise. Int J Sports Med 27, 1013-1022.

Adachi, S., Kawamura, K., Takemoto, K., 1993. Oxidative damage of nuclear DNA in liver of rats exposed to psychological stress. Cancer Res. 53, 4153-4155.

Agapitov A.V., Correia M.L.G., Sinkey C.A., Dopp J.M., Haynes W.G. Impaired skeletal muscle and skin microcirculatory function in human obesity. Journal of Hypertension 2002, 20:1401-1405.

Akimoto, T., Akama, T., Tatsuno, M., Saito, M., Kono, I., 2000. Effect of brief maximal exercise on circulating levels of interleukin-12. Eur. J. Appl. Physiol. 81, 510-512.

Alomari M, Solomito A, Reyes R, et al. Measurements of vascular function using strain-gauge plethysmography: technical considerations, standardization, and physiological findings. Am J Physiol Heart Circ Physiol. 2004;286:H99-H107.

Arcaro G, Zamboni M, Rossi L, et al. Body fat distribution predicts the degree of endothelial dysfunction in uncomplicated obesity. Int J Obes Relat Metab Disord. 1999; 23: 936-942.

Barnes, P.J., Karin, M., 1997. Nuclear factor-kappaB: A pivotal transcription factor in chronic inflammatory diseases. N. Engl. J. Med. 226, 1066-1071.

Beckman JS, Beckman TW, Chen JC et al. Apparent hydroxyl radical production by

peroxynitrite: Implication of endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci. 1990;87:1620-1624.

Belviranli M and Gokbel H. Acute exercise induced oxidative stress and antioxidant changes. Eur J Gen Med. 2006;3:126-131.

Berry C, Hamilton CA, Brosnan MJ et al. Investigation into the sources of superoxide in human blood vessel. Angiotensin II increases superoxide production in human internal mammary arteries. Circulation. 2000;101:2206-2212.

Bierhaus, A., Wolf, J., Andrassy, M., et al. 2003. A mechanism converting psychosocial stress into mononuclear cell activation. Proc. Natl. Acad. Sci. USA 100, 1920-1925.

Biuhmi, Z., Thomas, J.E., Park, M., Park, M., Dowdeswell, I.R., 1985. The effect of acute exercise on natural killer-cell activity of trained and sedentary human subjects. J. Clin. Immunol. 5, 321-328.

Bouloumıe A, Marumo T, Lafontan M, et al. Leptin induces oxidative stress in human endothelial cells. FASEB J. 1999;13:1231–1238.

Brenner, I.K., Natale, V.M., Vasiliou, P, Moldoveanu, A.I., Shek, P.N., Shephard., R.J., 1999. Impact of three different types of exercise on components of the inflammatory response. Eur J Appl Physiol Occup Physiol 80, 452-460.

Cai H and Harrison DG (2000). Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 87, 840-844.

Calver A, Collier J, Moncada S et al. Effect of local intra-arterial NF-monomethyl-L-argomome om patients with hypertension: The nitric oxide dilator mechanism appears abnormal. J Hypertens. 1992;10:1025-1031.

Ceresini G, Freddi M, Morganti S, et al. The effects of transdermal estradiol on the response to menta stress in postmenopausal women: a randomized trail. Am. J. Med. 2000;109:463-468.

Chudek J and Wiecek A. Adipose tissue, inflammation and endothelial dysfunction. Pharmacol Rep. 2006;58:Suppl 81-88.

Clines DB, Pollak ES, Buck CA et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood.1998;91:3527-3561.

Considine RV, Sinha MK, Heiman ML, et al. Serum immuno-reactive-leptin concentration in normal-weigh and obese humans. New Engl J Med. 1996;334:292-295.

Cooper, D.M., Radom-Aizik, S., Schwindt, C., Zaldivar F Jr., 2007. Dangerous exercise: lessons learned from dysregulated inflammatory responses to physical activity. J Appl Physiol 103, 700-709.

Cosentino, M., Rasini, E., Colombo, C., Marino, F., Blandini, F., Ferrari, M., Samuele, A., Lecchini, S., Nappi, G., Frigo, G., 2004. Dopaminergic modulation of oxidative stress and apoptosis in human peripheral blood lymphocytes: evidence for a D1-like receptor-dependent protective effect. Free Radic. Bio. Med. 36, 1233-1240.

Cotran RS, Kumar V, and Collins T. (1999). Pathologic Basis of Disease, 6th edition. W.B. Saunders Co., Philadelphia, PA.

Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science. 1991;251:788-791.

Dallaire P and Marette A. Obesity-linked insulin resistance: is nitric oxide the missing link? Canadian Journal of Diabetes. 2004;28:59-66.

Dawes M, Chowienczyk PJ, and Ritter JM. Effects of inhibition of the L-arginine/nitric oxide pathway on vasodilation caused by β-adrenergic agonists in human forearm. Circulation. 1997;95:2293-2297.

DeanWeld, J.E., 2000. Mental stress induces transient endothelial dysfunction in humans. Circulation 102, 2473–2478.

De Caterina R, Libby P, Peng HB et al. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. J Clin Invest. 1995;96:60-68.

De Groote, D., Zangerle, P.F., Gevaert, Y., et al., 1992. Direct stimulation of cytokines (IL-1β, TNF-α, IL-6, IL-2, IFN-y and GM-CSF) in whole blood. Cytokine 4, 239-248.

DiCarlo, SE, Collins HL, Howard MG et al. Postexertional hypotension: A brief review. Sports Medicine, Training, and Rehabilitation. 1994;5:17-27.

Dimsdale, J.E., 2008. Psychological stress and cardiovascular disease. J. AM. Coll. Cardiol. 51, 1237-1246.

Elenkov, I.J. Chrousos G.P. Stress Hormone, Proinflammatory and antiinflammatory cytokines, and autoimmunity. Annals of the New York Academy Sciences. 2002;336:290-303.

Elenkov, I.J. Glucocorticoids and the Th1/Th2 balance. Annals of the NewYork Academy Sciences. 2004;1024:138–146.

Epel ES, McEwen B, Seeman T, et al. Stress and body shape: stress-induced cortisol secretion is consistently greater among women with central fat. Psychosom Med.

2000;62:623-632.

Fernandez-Garcia, B., Lucia, A., Hoyos, J., Chicharro, J.L., Rodriguez-Alonso, M., Bandres, F., Terrados, N., 2002. The response of sexual and stress hormones of male pro-cyclists during continuous intense competition. Int J Sports Med 23, 555-560.

Flint MS., Baum A, Chambers WH, et al. Induction of DNA damage, alteration of DNA repair and transcriptional activation by stress hormones Psychoneuroendocrinology, 2007;32,470-479.

Frankenhaeuser, M., 1991. The psychophysiology of workload, stress, and health: Comparison between the sexes. Ann Behav Med 13, 197-204.

Freeman BA. (1993) in Free Radicals in MolecularBiologyAging and Disease, ed. Armstrong, D. (Raven, New York), pp. 43-52.

Furchgott RF. The 1989 Ulf von Euler lecture; studies on endothelium-dependent vasodilation and the endothelium-derived relaxing factor. Acta Physiol Scand. 1990; 139:257-270.

Garcia CE, Kilcoyne CM, Cardillo C, et al. Evidence that endotelial function in patients with hypercholesterolemia is not due to increased extracellular nitric oxide breakdown by superoxide anions. Am K Cardiol.1995;76:1157-1161.

Gelderman KA, Hultqvist M, Pizzola A et al. Macrophages suppress T cell response and arthritis development in mice by producing reactive oxygen species. J Clin Invest. 2007;117:3020-3028.

Ghiadoni, L., Donald, A.E., Cropley, M., Mullen, M.J., Oakley, G., Taylor, M., O'Connor,

G., Betteridge, J., Klein, N., Steptoe, A.,

Gledhill, N., Jamnik, V.K., 1992. Characterization of the physical demands of firefighting. Can J Appl Sport Sci 17, 207-213.

Glover WE. Greenfield ADM, and Shanks RG. The contribution made by adrenaline to the vasodilatation in the human forearm during emotional stress. J Physiol. 1962;164:1607-1610.

Goebel, M.U., Mills, P.J., Irwin, M.R>, Ziegler, M.G., 2000. Interleukin-6 and tumor necrosis factor-alpha production after acute psychological stress, exercise, and infused isoproterenol: didderential effects and pathways. Psychosom. Med. 62, 591-598.

Griendling KK and Ushio-Fukai M. NADH/NADPH oxidase and vascular function. Trends Cardiovasc. Med. 1997;7:301-307.

Halliwill JR, Lawler LA, Eickhoff TJ et al. Forarm sympatheric withdrawal and vasodilatation during mental stress in humans. J Physiol. 1997;504:211-220.

Hamer M, Boutcher YN, and Boutcher SH. The role of cardiopulmonary baroreceptors during the forearm vasodilatation response to mental stress. Psychophysiology. 2003;40:249-253.

Hamer M., Boutcher Y.N., Boutcher S.H. Fatness is related to blunted vascular stress responsivity, independent of cardiorespiratory fitness in normal and overweight men. International Journal of Psychophysiology 63; 251-257, 2007.

Hamilton CA, Miller WH, Al-Benna S et al. Strategies to reduce oxidative stress in cardiovascular disease. Clin Sci (Lond). 2004;106:219-234.

Harris CW. Edwards JL, Baruch A et al. Effect of mental stress on brachial artery flow-mediated vasodilation in healthy normal individuals. Am Heart J. 2000;139:405-411.

Hasko´ G, Szabo´ C. Regulation of cytokine and chemokine production by transmitters and co-transmitters of the autonomic nervous sytem. Biochem Pharmacol 1998;56:1079–87.

Haynes WG, Ferro CJ, O'Kane KPJ et al. Systemic endothelin receptor blockade decreases peripheral vascular resistances and blood pressure in man, Circulation. 1996;93:1860-1870.

Herbermann, R.B., 1981. Natural Killer (NK) cells and their possible roles in resistance against disease. Clin Immunol Rev 1, 1-65.

Heinz, A., Hermann, D., Smolka, M.N., Rieks, M., Graf, K.J., Pohlau, D., Kuhn, W., Bauer, M., 2003. Effects of acute psychological stress on adhesion molecules, interleukins and sex hormones: implications for coronary heart disease. Psychopharmacology 165, 111-117.

Izeboud CA, Monshouwer M, van Miert AS, Witkamp RF. The β-adrenoceptor agonist clenbuterol is a potent inhibitor of the LPS-induced production of TNF-a and IL-6 in vitro and in vivo. Inflamm Res 1999;48:497–502.

Ji LL, Gomez-Cabrera MC, and Vina J. Exercise and Hormesis. Ann N.Y. Acad. Sci. 2006;1067:425-435.

Jovanovic DV, Di Battista JA, Martel-Pelletier J, et al. IL-17 stimulates the production and

expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J Immunol. 1998;160:3513-3521.

Kamarck TW, et al. Correspondence between laboratory and ambulatory measures of cardiovascular reactivity: a multilevel modeling approach. Psychophysiology. 2003; 40, 675-683.

Kang, D.H., Fox, C., 2001. Th1 and Th2 Cytokine Responses to Academic Stress. Res Nurs health 24, 245-257.

Kaplan N.M. Individualized therapy of hypertension. Marcel Dekker Inc.; New York, 1995.

Kazuhiro S and Michel T. Expression and regulation of endothelial nitric oxide synthase. Treads in cardiovascular medicine. 1997;7:28-37.

Keaney JF, Larson MG, Vasan RS, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol. 2003;23:434-439.

Kearney MT, Duncan ER, Kahn M et al. Insulin resistance and endothelial cell dysfunction: studies in mammalian models. Exp Physiol. 2007;93:158-163.

Khan BV, Harrison DG, Olbrych MT et al. Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. Proc Natl Acad Sci USA. 1996;93:9114-9119.

Kidd, P. Th1/Th2 Balance: the hypothesis, its limitations and implications for health and disease. Alternative Medicine Reviews. 2003;8:223-246.

Kobayashi, M., Ritz, L., Ryan, M., Hewick, R.M., Clark, S.C., Chan, S., Loudon, R.,

Sherman, F., Perussia, B., Trinchieri, G., 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocyte. J. Exp. Med. 170, 827-845.

Komatsu J, Koyama H, Maeda N et al. Earlier onset of neutrophil-mediated inflammation in the ultraviolet-exposed skin of mice deficient in myeloperoxidase and NADPH oxidase. Inflamm. Res. 2006;55:200-206.

Langberg, H., Olesen, J.L, Genner, C., Kjaer, M., 2002. Substantial elevation of interleukin-6 concetration in peritendinous tissue, in contrast to muscle, following prolonged exercise in human. J. Physiol. 542, 985-990.

Li LL. Modulation of skeletal muscle antioxidant defense by exercise: Role of redox signaling. Free Radical Biology and Medicine. 2008;44:142-152.

Loffreda S, Yang SQ, Lin HZ, et al. Leptin regulates proinflammatory immune responses. FASEB Journal. 1998;12:57-65.

Lopes HF, Martin JL, Nashlar K, et al. DASH diet lowers blood pressure and lipid-induced oxidative stress in obesity. Hypertension. 2003; 41:422-430.

Lucia, A., Diaz, B., Hoyos, J., Fernandez, C., Villa, G., Bandres, F., Chicharro, J.L., 2001. Hormone levels of world class cyclists during the Tour of Spain stage race. Br J Sports Med 35, 424-430.

Luscher TF, Barton M. Biology of the endothelium. Clin Cardiol. 1997;20:113-11.

Luscher TF., Yang Z, Tschudi M et al. Interaction between endothelin-1 and enothelium-derived relaxing factors in human arteries and veins. Cir Res. 1990;66:1088-1094.

MacLean A, Wei XQ, Huang FP et al. Mice lacking inducible nitric-oxide synthase are

more susceptible to herpes simplex virus infection despite enhanced Th1 cell responses. J Gen Virol. 1998;79:825-30.

Maes M., Song C., Lin A., et al., 1998. The effects of psychological stress on humans: Increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. Cytokine 10, 313-318.

Manning, J.E., Griggs, T.R., 1983. Heart rate in fire fighters using light and heavy breathing equipment: similar near-maximal exertion in response to multiple work load conditions. J Occup Med 25, 215-218.

Matalka, K.Z., 2003. Neuroendocrine and cytokines-induced responses to minutes, hours, and days of mental stress. Neuro Endocrinol. Lett. 24, 283-292.

Marui N, Offermann MK, Swerlick R et al. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human. J Clin Invest. 1993;92:1866-1872.

McInnes IB, Leung B, Wei XQ, Gemmell CC, Liew FY. Septic arthritis following Staphylococcus aureus infection in mice lacking inducible nitric oxide synthase. J Immunol. 1998;160:308-15.

Mendes, R., Bromelow, K.V., Westby M., Galea-Lauri, J., Smith, I.E., O'Brien, M.E., Souberbielle, B.E., 2000. Flow cytometry visualization of cytokine production by natural killer cells and CD56+CD3+ NK-T cells in whole blood. Cytometry 39, 72-78.

Miesel R, Kroger H, Zuber M et al. Elevated levels of oxygen- and nitrogen-centered radicals in patients with rheumatic diseases. Zeitschr. Rheumatol.1994;53:6.

Miesel R, Hartung R, Zuber et al. Total radical trapping antioxidative capacity of plasma and whole blood chemiluminescence in patients with inflammatory and autoimmune rheumatic diseases. Redox Report. 1995A;1:323.

Miesel R, Ulbrich N, Krtger N et al. Assessment of collagen type II induced arthritis in mice by whole blood chemiluminescence. Autoimmunity. 1995B;19:153-159.

Miljkovic DJ, Cvetkovic I, Vcukovic O, et al. The role of interleukin-17 in inducible nitric oxide synthase-mediated nitric oxide production in endothelial cells. Cell Mol Life Sci. 2003;60:518-525.

Moncada S. and Higgs EA. Endogenous nitric oxide: physiology, pathology and clinical relevance. Eur. J. Clin. Invest. 1991;21:361-374.

Moon YS, Kim DH, Song DK. Serum tumor necrosis factor-alpha levels and components of metabolic syndrome in obese adolescents. Metabolism. 2004;53:863-867.

Moran JM, Gonzalez-Polo RA, Soler G et al. Th1/Th2 cytokines: an easy model to study gene expression in immune cells. CBE Life Sci Educ. 2006;5:287-295.

Morel, P.A., Oriss, T.B., 1998. Crossregulation between Th1 and Th2 cells. Crit. Rev. Immunol. 18, 275-303.

Murr C, Schroecksnadel K, Winkler C et al. Antioxidant may increase the probability of development allergic diseases an asthma. Medical hypotheses. 2005;64:973-977.

Nakamura A., Johns E.J, Imaizumi A., Yanagawa Y., Kohsaka T. Modulation of interleukin-6 by beta2-adrenoceptor in endotoxin-stimulated renal macrophage cells. Kidney International 56, 839-849, 1999.

Nathan C. Inducible nitric oxide synthases: what difference does it make? J. Clin. Invest.

1997;100:2417-2423.

Nathan, C.F., Murray, H.W., Wiebe, M.E., Rubin, B.Y., 1983. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J. Exp. Med. 158, 670-689.

National Center for Health Statistics. Chartbook on Trends in the Health of Americans. Health, United States, 2006. Hyattsville, MD: Public Health Service. 2006. 7.

Niedbala W, Wei XQ, Piedrafita D et al. Effects of nitric oxide on the induction and differentiation of Th1 cells. Eur J Immunol. 1999;29:2498-505.

Niedbala W, Cai B, Liew FY. Role of nitric oxide in the regulation of T cell functions. Ann Rheum Dis. 2006; 65 Suppl 3:iii37-40.

Nieman, D.C., 1994. Exercise, upper respiratory tract infection, and the immune system. Med Sci Sports Exerc 26, 128-139.

Nussler AK, Billiar TR. Inflammation, immunoregulation, and inducible nitric oxide synthase. J Leukocyte Biol. 1993;54:171-178.

Nybo, L., Moller, K., Pedersen, B.K., Nielsen, B., Secher, N.H., 2002. Association between fatigue and failure to preserve cerebral energy turnver during prolonged exercise. Acta Physiol. Scand. 179, 67-74.

Olinski R, Gackowski D, Foksinski M, et al. Oxidative DNA damage: Assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. Free Radic. Biol. Med. 2002;33:192-200.

Olusi SO. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotectic enzymes in humans. Int. J. Obes. 2002;26:1159-1164.

Oswald IP, James SL. Nitrogen oxide in host defense against parasites. Meth: A Comp Meth Enzymol. 1996; 10:8-14.

Otsuka R, Yatsuya H, Tamakoshil K, et al. Perceived psychological stress and serum leptin concentrations in Japanese men. Obesity. 2006;14:1832-1838.

Pavlov V.A., Tracey, K.J., 2005. The cholinergic anti-inflammatory pathways. Brain Behav. Immun. 19, 493-499.

Paulose M, Bennett BL, Manning AM et al. Selective inhibition of TNF-alpha induced cell adhesion molecule. Micro Pathog. 1998;25:33-41.

Peng HB, Rajavashisth TB, Libby P et al. Nitric oxide inhibits macrophage-colony stimulating factor gene transcription in vascular endothelial cells. J Bio Chem. 1995;270:G562-G568.

Plumpton C, Haynes WG, Webb DJ et al. Phosphoramidon inhibition of the in vivo conversion of big endothelin to endothelin-1 in the human forearm. Br J Pharmacol, 1995;116:1821-1828.

Pober JS, Slowik MR, De Luca, LG et al. Elevated cyclic AMP inhibits endothelial cell synthesis and expression of TNF-induced endothelial leukocyte adhesion molecule-1, and vascular cell adhesion molecule-1, but not intercellular adhesion molecule-1. J. Immunol. 1993;150: 5114-5123.

O'Connell, E.R., Thomas, P.C., Cady, L.D., Karwasky, R.J., 1986. Energy costs of simulated their climbing as a job-related task in fire fighting. J Occup Med 28, 282-284.

Olinski, R., Gackowski, D., Foksinski, M., Rozalski R., Roszkowski, K., Jaruga, P., 2002.

Oxidative DNA damage: Assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. Free Radic. Biol. Med. 33, 192-200.

Otsuka R, Yatsuya H, Tamakoshil K, et al. Perceived psychological stress and serum leptin concentrations in Japanese men. Obesity. 2006;14:1832-1838.

Panina-Bordignon, P., Mazzeo, D., Lucia, P.D., D'Ambrosio, D., Lang, R., Fabbri, L., Self, C., Sinigaglia, F., 1997. Beta2-agonists prevent Th1 development by selective inhibition of interleukin-12. J. Clin. Invest. 100, 1513-1519.

Pedersen, B.K., 1985. Natural killer cells in relation to disease. Allergy 40, 547-557.

Pedersen, B.K., Tvede, N., Hansen, F.R., Andersen, V., Bendix, T., Bendixen, G., Bendtzen, K., Galbo, H., Haahr, P.M., Klarlund, K., et al., 1998. Modulation of natural killer cell activity in peripheral blood by physical exercise. Scand. J. Immunol. 27, 673-678.

Quesenberry CP, Caan B, Jacobson A. Obesity, health services, and health care costs among members of a health maintenance organization. Arch Intern Med. 1998;158:46-472.

Rajagopalan S, Kurz S, Munzel T et al. Angiotensin II-mediated hypertension in the rat increased vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alternation of vasomotor tone. J. Clin. Invest. 1996;97:1916-1923.

Ray, M.R., Basu, C., Roychoudhury, S., Banik, S., Lahiri, T., 2006. Plasma catecholamine levels and neurobehavioral problems in Indian firefighters. J Occup Health 48, 210-215.

Rea RF and Wallin BG. Sympathetic nerve activity in arm and leg muscles during lower body negative pressure in humans. J. Appl. Physiol. 1989;66:2778-2781.

Rohleder, N., Schommer, N.C., Hellhammer, D.H., Engel, R., Kirschbaum, C., 2001. Sex differences in glucocorticoid sensitivity of pro-inflammaotry cytokine production after psychological stress. Psychoneuroendocrinology 28, 261-273.

Rosen, O., Haug, E., Pedersen, B.K., Bahr, R., 2001. Increased neuroendocrine response to a repeated bout of endurance exercise. Med Sci Sports Exerc 33, 568-575.

Ross R. The pathogenesis of atherosclerosis: A perspective for the 1990s. Nature. 1993;362:801-809.

Ross R. Atherosclerosis – an inflammatory disease. New Engl J Med. 1999;340:115-126.

Rowell, L. B., and D. R. Seals. Sympathetic activity during graded central hypovolemia in hypoxemic humans. Am. J. Physiol. 1990;259:H1197-H1206.

Schafer A, Bauersachs J. Endothelial dysfunction, impaired endogenous platelet inhibition and platelet activation in diabetes and atherosclerosis. Curr Vasc Pharmacol. 2008;6:52-60.

Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation. 2000;101: 1899-1906.

Schedlowski, M., Jacobs, R., Stratmann, G., Richter, S., Hädicke, A., Tewes, U., Wagner, T.O., Schmidt, R.E., 1993. Changes of natural killer cells during acute psychological stress. J. Clin. Immunol. 13, 119-126.

Schreck R, Albermann K, Baeuerle PA. Nuclear factor KB: An oxidative stress-responsive

transcription factor of eukaryotic cells (a review). Free Radic. Res. Commun. 1992;17:221-237.

Shepherd JT. Interactions of neurotransmitters and endothelial cells in determining vascular tone. Adv Exp Med Biol. 1995;381:1-13.

Sies H. Oxidative stress: oxidants and anitoxidants. Exp. Phyiol. 1997;82:291-295.

Singhai A. Endothelial dysfunction: Role in obesity-related disorders and the early original of CVD. The Proceedings of the Nutrition Society. 2005;64:15-22.

Sinisalo J, Paronen J, Mattila KJ, et al. Relation of inflammation to vascular function inpatient with coronary heart disease. Atherosclerosis. 2000;149:403-411.

Sivonova, M., Zitnanova, I., Hlincikova, L, et al., 2004. Oxidative stress in university students during examinations. Stress, 7(3), 183-188.

Snelgrove RJ, Edwards L, Rae AJ et al. An absence of reactive oxygen species improves the resolution of lung influenza infection. Eur. J. Immunol. 2006A; 36:1364-1373.

Snelgrove RJ, Edwards L, Williams AE et al. In the absence of reactive oxygen species, T cells default to a Th1 phenotype and mediate protection against pulmonary Cryptococcus neoformans infection. J. Immunol. 2006B;177:5509-5516.

Spiecker M, Darius H, Kaboth K et al. Differential regualtion of endothelial cell adhesion molecule expression by nitric oxide donors and antioxidants. J Leukoc Biol. 1998;63:732-739.

Steensberg, A., Toft, A.D., Bruunsgaard, H., Sandmand, M., Halkjaer-Kristensen, J., Pedersen, B.K., 2001. Strenuous Exercise Decreases the Percentage of Type 1 T Cells in the Circulation. J Appl Physiol 9, 1708-1712.

Steptoe, A., Willemsen, G., Owen, N., Flower, L., Mohamed-Ali, V., 2001. Acute mental stress elicits delayed increases in circulating inflammatory cytokine levels. Clin Sci (Lond) 1, 185–192.

Stern, A.S., Podlaski, F.J., Hulmes, J.D., Pan, Y.C., Quinn, P.M., Wolitzky, A.G., Familletti, P.C., Stremlo, D.L., Truitt, T., Chizzonite, R., et al., 1990. Purification to homogeneity and partial characterization of cytoxic lymphocyte maturation factor from human B-lymphoblastoid cells. Proc. Natl. Acad. Sci. U.S.A. 87, 6808-6812.

Taylor-Robinson AW, Liew FY, Severn A et al. Regulation of the immune response by nitric oxide differentially produced by T helper type 1 and T helper type 2 cells. Eur. J. Immunol. 1990;24: 980-984.

Throne, L.C., Bartholomew, J.B., Craig, J., Farrar, R.P., 2000. Stress reactivity in firefighters: An exercise intervention. Int J Stress Manag 7, 235-246.

Tifenbacher CP. Tetrahydrobiopterin: a critical cofactor for eNOS and a strategy in the treatment of endothelial dysfunction? Am. J. Physiol. Heart Circ. Physiol. 2001;280:H2484-H2488.

Timimi FK, Ting HH, Haley EA, et al. Vitamin C improves endothelium-dependent vasodilation in patients with insulindependent diabetes mellitus. J Am Coll Cardiol. 1998;31:552-557.

Toledano M and Leonhard W. Modulation of transcription factor NF-KB by oxidation-reduction in vitro. Proc. Natl. Acad. Sci. USA. 1991;88:4328-4332.

Tomioka, H, Saito H. Characterization of immunosuppressive functions of murine

peritoneal macrophages induced with various agents. J. Leukocyte Biol. 1991;51, 24-31.

Tschudi MR, Mesaros S, Luscher TF et al. Direct in situ measurement of nitric oxide in mesenteric resistance arteries. Hypertension.1996;27;32-35.

Tudor KS, Hess KL, Cook-Mills JM. Cytokines modulate endothelial cell intracellular transduction required for VCAM-1-dependent lymphocyte transendothelial migration. Cytokine. 2001;15:196-211.

Uchakin, P.N., Tobin, B, Cubbage, M., Marshall, Jr., Sams, C., 2001. Immune responsiveness following academic stress in first-year medical stress. J. Interferon Cytokine Res. 21, 687-694.

Umeki, S. Activation factors of neutrophil NADPH oxidase complex. Life Sci. 1994;55:1-13.

United States Fire Administration, 2004. Health and wellness guide. Emmitsburg: Federal Emergency Management Agency; USFA, Publication No. FA-267.

Van Der Poll T, Coyle SM, Barbosa K, Braxton CC, Lowry SF: Epinephrine inhibits tumor necrosis factor-alpha and poteniates interleukin-10 production during human endotoxemia. J Clin Invest 97; 713-719, 1996.

Van der Veen RC, Dietlin TA, Dixon Gray J et al. Macrophage-derived nitric oxide inhibits the proliferation of activated T helper cells and is induced during antigenic stimulation of resting T cells. Cell Immunol. 2000;199:43-49.

Verma S, Anderson TJ. The ten most commonly asked questions about endothelial function in cardiology. Cardiol Rev. 2001;9:250-252.

Verma S, Anderson TJ. Fundamentals of endothelial function for the clinical cardiologist. Circulation. 2002;105:546-549.

Vincent HK, Morgan JW, Vincent KR et al. Obesity exacerbates oxidative stress levels after acute exercise. Med Sci Sports Exerc. 2004;36:772-779.

Vider J, Laaksonen DE, Kilk A et al. Physical exercise induces activation of NF-kB in human peripheral blood lymphocytes. Antiox Redox Signaling. 2001;3:1131-1137.

Wannamethee SG, Tchernova J, Whincup P, et al. Plasma leptin: associations with metabolic, inflammatory, haemostatic risk factors for cardiovascular disease. Atherosclerosis. 2007;191:418-426.

Wassmann S., Stumpf M., Strehlow K., Schmid A., Schieffer B., Böhm M., Nickenig G., 2004. Interleukin-6 induces oxidative stress and endothelial dysfunction by overexpression of the angiotensin II type 1 receptor. Circ. Res. 94, 534-541.

Webb D and Vallance P. Endothelial function in hypertension. 1997. Germany, Springer-Verlag Berlin Heidelberg.

Webb, H.E., Weldy, M.L., Fabianke-Kadue, E.C., Orndorff, G.R., Kamimori, G.H., Acevedo, E.O., 2008. Psychological stress during exercise: cardiorespiratory and hormonal response. European Journal of Applied Physiology 104, 973-98.

Wei XQ, Charles IG, Smith A et al. Altered immune responses in mice lacking inducible nitric oxide synthase. Nature.1995;375:408-11.

Xiao BG, Ma CG, Xu LY et al. IL-12/IFN-γ/NO axis plays critical role in development of Th1-mediated experimental autoimmune encephalomyelitis. Molecular immunology. 2008;45:1191-1196.

Xie, Q, Cho H, Calaycay J et al. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. Science. 1992; 256:225.

Wirtz PH, von Känel R, Emini L, et al. Variations in anticipatory cognitive stress appraisal and differential proinflammatory cytokine expression in responses to acute stress. Brain Behav Immum. 2007;21:851-859.

Yao Z, Painter SL, Fanslow WC, et al. Human IL-17: a novel cytokine derivedfrom T cells. J Immunol. 1995;155:5483-5486.

Yu HJ, Kim CS, Yoo DG et al. NADPH oxidase and mitochondrial ROS are involved in the TNF-a-induced vascular celll adhesion molecule-1 and monocyte adhesion in cultured endothelial cells. Korean J Physiol Pharmacol. 2006;10:217-222.

Ziolkowska M, Koc A, Luszczykiewicz G, et al. High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism. J Immunol. 2000;164:2832-38.

Zumbach MS, Boehme MW, Wahl P, et al. Tumor necrosis factor increases serum leptin levels in humans. J Clin Endocrinol Metab. 1997;82:4080-4082.

Zuckerman SH, Evans GF, Guthrie L. Transcriptional and post-transcriptional mechanism involved in the differential expression of LPS-induced IL-1 and TNF-mRNA. Immunology, 1991; 73: 460-465.

APPENDIX A

Medical History Questionnaire

Complete each question accurately. All information provided is strictly confidential.

Part I: Subject Information

Name (Print) _____ Home Phone _____

Current Mailing Address _____ Work Phone _____

Personal Physician _____ Email Address _____

Person to Contact in Case of Emergency _____ Phone _____

Gender: _____ Female _____ Male Date of Birth: _____

Height _____ Weight _____

Part II. Medical History

List any physical injuries or limitations that you have at this time: _____

_____

Have you ever been diagnosed as having any cardiovascular abnormalities?
Yes ____ No ____

If yes, what was diagnosed and when was the diagnosis conducted? _____

_____

Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

Heart Attack	Bypass surgery	Sickle-Cell Anemia
Heart Palpitations	Arrhythmia	Chest pain
Shortness of breath	Stroke	Anemia
Heart valve problem/Murmur		

Have you been diagnosed with an autoimmune disease? If yes, please circle the appropriate disease.

Rheumatoid arthritis	Lupus	Crohn's Disease
Multiple Sclerosis	Psoriasis	Type I/Type II Diabetes

APPENDIX B

Seven-Day Physical Activity Recall

Instructions:
This questionnaire is called the Seven-Day Physical Activity Recall. The information from it will be used to estimate the number of calories you burn up through physical activity.

1: On the average, how many hours did you sleep each night during the last five weekday nights, Sunday through Thursday?

Enter a numeric value (0 if not applicable) ___ ___. ___

2: On the average, how many hours did you sleep each night last Friday and Saturday nights?

Enter a numeric value (0 if not applicable) ___ ___. ___

3: How many hours did you spend during the last five weekdays doing these moderate activities or others like them?

Enter a numeric value (0 if not applicable) ___ ___. ___

4: How many hours did you spend last Saturday and Sunday doing these moderate activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

5: How many hours did you spend during the last five weekdays doing these hard activities or others like them?

Enter a numeric value (0 if not applicable) ___ ___. ___

6: How many hours did you spend last Saturday and Sunday doing these hard activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

7: How many hours did you spend the last five weekdays doing these very hard activities, or others like them?

Enter a numeric value (0 if not applicable) ___ ___. ___

8: How many hours did you spend last Saturday and Sunday doing these very hard activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

9: Were you employed outside the home during the last seven days? If no, put zeros for questions 9-13. If yes, how many days?

Enter a numeric value (0 if not applicable) ___ ___. ___

10: How many hours per day?

Enter a numeric value (0 if not applicable) ___ ___. ___

11: How many of these hours per day were spent doing moderate activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

12: How many of these hours per day were spent doing hard activities?
Enter a numeric value (0 if not applicable) ___ ___. ___

13: How many of these hours per day were spent doing very hard activities?

Enter a numeric value (0 if not applicable) ___ ___. ___

14: Compared to your physical activity over the past three months, was last week's physical activity more, less, or about the same?

1-More 2-Less 3-About the same

Very Hard Activities (>7.0 METs)

These include strenuous sports involving a lot of movement and running. Very few household or occupational tasks are included, except carrying heavy loads, digging or chopping with heavy tools, or other similar hard physical labor.

- Boxing—in ring, sparring
- Circuit training
- Climbing hills with 5-20 kg load
- Cycling, racing (intensive)
- Digging ditches
- Farming—barn cleaning
- Field hockey
- Football
- Forestry—fast ax chopping, barking trees, carrying logs, sawing by hand
- Gardening, digging
- Marching, rapid
- Racquetball
- Rope jumping
- Running, jogging—cross country, 6-10 min/mile
- Skiing, cross country
- Skindiving as frogman, moderate motion
- Soccer
- Squash
- Swimming, continuous- Intensive
- Tennis, singles

Moderate Activities (3-5 METs)

These activities involve modest increases in heart rate & breathing—e.g., many household & home repair tasks.

- Bowing
- Calisthenics without weights
- Carpentry
- Childcare
- Cleaning, heavy (such as vacuuming, sweeping)
- Croquet
- Cycling—leisure, 5.5 mph mild
- Electrical work
- Feeding farm animals, manual milking
- Fencing
- Forestry—slow ax chopping, power sawing, stacking firewood, weeding
- Frisbee playing
- Gardening—hedging, raking, planting, mowing
- Golf—no power cart
- Grocery shopping
- Gymnastics
- Heavy cooking
- Horse shoes
- Horseback riding
- Laundry – heavy
- Locksmith
- Machine tooling—lath, punch press, tapping & drilling, welding
- Mopping floor
- Motor-cross
- Mowing lawn—push & power mower
- Music—playing drums
- Painting—outside
- Planting seedlings
- Plastering
- Sailing & board sailing
- Scraping Paint
- Stock clerking
- Surfing
- Sweeping
- Swimming – mild
- Table tennis
- Tai-chi
- Walking on firm level surface, 3-4 mph – Average to fairly brisk
- Window cleaning
- Yoga

Hard Activities (5.1-6.9 METs)

Most people will have noticeable increases in breathing and will likely perspire—e.g., vigorous household, home repair and gardening tasks, heavy industrial work, and some construction and vigorous sports.

- Aerobic dance
- Badminton
- Climbing hills with no load
- Coal shoveling
- Cycling—leisure, 9.4 mph (moderate)
- Farming—shoveling grain
- Fast walking
- Folk dancing
- Forestry—hoeing, planting by hand
- Karate or Judo
- Roller skating
- Scrubbing floors
- Skiing, water or downhill
- Tennis, doubles
- Walking on level brisk or striding, firm surface @ 4.5 mph
- Weight lifting or training (count only lifting time)
- Swimming moderate

APPENDIX C

Perceived Stress Scale (PSS)

Instructions: The questions in this scale ask you about your feelings and thoughts during the last month. In each case, please indicate with a check how often you felt or thought a certain way.

1. In the last month, how often have you been upset because of something that happened unexpectedly?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

2. In the last month, how often have you felt that you were unable to control the important things in your life?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

3. In the last month, how often have you felt nervous and "stressed"?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

4. In the last month, how often have you felt confident about your ability to handle your personal problems?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

5. In the last month, how often have you felt that things were going your way?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

6. In the last month, how often have you found that you could not cope with all the things that you had to do?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

7. In the last month, how often have you been able to control irritations in your life?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

8. In the last month, how often have you felt that you were on top of things?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

9. In the last month, how often have you been angered because of things that were outside of your control?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

10. In the last month, how often have you felt difficulties wer piling up so high that you could not overcome them?
___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

APPENDIX D

State Anxiety Inventory (SAI)

Instructions: A number of statements which people have used to describe themselves are given below. Read each statement and then point to the most appropriate number to the right of the statement to indicate how you feel *right now, at this moment*. There is no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	Not at All	Somewhat	Moderately	Very Much
I feel comfortable	1	2	3	4
I feel secure	1	2	3	4
I feel regretful	1	2	3	4
I am presently worrying over possible misfortunes	1	2	3	4
I feel pleasant	1	2	3	4
I feel anxious	1	2	3	4
I feel over-excited	1	2	3	4
I feel relaxed	1	2	3	4
I feel tense	1	2	3	4
I feel content	1	2	3	4

APPENDIX E

Total Number of Subjects Utilized for Each Variable

Variable	Lean	Obese
HR	12	12
NE	11[a]	12
SAI Score	12	12
Forearm blood flow	10[b]	12
Total antioxidant capacity	10[a]	12
LPS-stimulated TNF-alpha	11[c]	10[d]
LPS-stimulated IL-6 cytokine	11[c]	10[d]
LPS-stimulated TNF-α RNA	10[e]	11[f]
LPS-stimulated IL-6 RNA	10[e]	11[f]

a = 1 hemolyzed samples
b = 2 no data presented
c = 1 hemolyzed samples; subject noncompliance
d = 1 hemolyzed samples, and 1subject's samples without LPS challenge
e = 2 subjects' RNA samples were not analyzed.
f = 1subject's samples without LPS challenge

APPENDIX F

Coefficient of Variation and Normal Standard Curves

	Standard Curve (R Square)	Intra-coefficient	Inter-coefficient
Total Antioxidant Capacity ELISA Kits	1. 98.53% 2. 98.21	None	None
Norepinephrine ELISA Kits	1. 99.45% 2. 98.45%	None	None
TNF-α ELISA Kits	1. 99.56% 2. 100% 3. 99.62	4.3%	None
TNF-α mRNA (qPCR)	1. 99.68% 2. 98.86%	None	None
IL-6 ELISA Kits	1. 99.58% 2. 99.24% 3. 99.89%	5.5%	None
IL-6 mRNA (qPCR)	1. 99.23% 2. 99.11%	None	None
GADPH (qPCR)	1. 98.82% 2. 99.60%	None	None

APPENDIX G

Human Research Committee Approval Letter

VCU Memo

Virginia Commonwealth University

Office of Research
Office of Research Subjects Protection

BioTechnology Research Park
800 East Leigh Street, Suite 114
P.O. Box 980568
Richmond, Virginia 23298-0568

(804) 827-0868
Fax: (804) 827-1448

DATE: January 23, 2009

TO: Edmund Acevedo, PhD
Health and Human Performance
Box 842020

FROM: Lea Ann Hansen, Pharm D Lea Ann Hansen, Pharm D 1/15
Chairperson, VCU IRB Panel D
Box 980568

RE: **VCU IRB #: HM11964**
Title: The Impact of Mental Challenge on Indicators of Endothelial Function in Obese Individuals

The following study involving the research use of human subjects was ***approved*** by the VCU IRB on January 15, 2009 according to 45 CFR 46.108(b). The changes requested by the Panel received in the Office of Research Subjects Protection on January 22, 2009 satisfactorily meet the stipulations set forth in the January 15, 2009. This approval includes the following items reviewed by this Panel:

PROTOCOL: The Impact of Mental Challenge on Indicators of Endothelial Function in Obese Individuals
- Research Plan (Version 2, dated 1/5/09; received in ORSP 1/5/09)

CONSENT/ASSENT:
- Research Subject Information and Consent Form (Version 3, dated 1/22/09; 5 pages; received in ORSP 1/23/09)

ADDITIONAL DOCUMENTS:
- Advertisement entitled "Research Participants Needed" (Version 2, dated 1/5/09; received in ORSP 1/5/09)

This approval expires on January 14, 2010. Federal Regulations/VCU Policy and Procedures require continuing review prior to continuation of approval past that date. Continuing Review report forms will be mailed to you prior to the scheduled review.

If you have any questions, please contact Dr. Lea Ann Hansen, Chairperson, VCU IRB Panel D, at leaann.hansen@gmail.com or 804-994-2444; or you may contact Aleksandra Baldwin, IRB Coordinator, VCU Office of Research Subjects Protection, at akbaldwin@vcu.edu or 827-1445.

Attachment – Conditions of Approval

VITA

PERSONAL

Citizenship: Taiwan
Birth Date: 19 September, 1977
Marital Status: Married; Tomoko Okada
Children: None

EDUCATION

2006-Present **Doctoral Candidate**
Virginia Commonwealth University, Richmond, Virginia
Rehabilitation and Movement Science (Exercise Physiology)
Advisor: Edmund O. Acevedo, Ph.D., FACSM, FAPA
Dissertation Title: The Impact of Mental Challenge on Indicators of Endothelial Function in Obese Individuals

The University of Mississippi, Oxford, Mississippi
Doctoral Program (Exercise Physiology), 24 credit hours
Advisor: Edmund O. Acevedo, Ph.D., FACSM, FAPA

2003-2005 **Master of Science**
Indiana State University, Terre Haute, Indiana
Exercise Science
Advisor: Thomas W. Nesser, Ph.D., CSCS

1996-2000 **Bachelor of Science**
Fu-Jen Catholic University, Taipei, Taiwan
Physical Education

PROFESSIONAL EXPERIENCE

2006-Present **Graduate Teaching & Research Assistant**
Department of Health and Human Performance

Virginia Commonwealth University
Richmond, Virginia

2005-2006 **Graduate Teaching & Research Assistant**
Department of Health, Exercise Science, and Recreation Management
The University of Mississippi
Oxford, Mississippi

2004-2005 **Computer Consultant**
Computer Lab
Indiana State University
Terre Haute, Indiana

HONORS/AWARDS

- **Summer Dissertation Fellowship**, Graduate School, Virginia Commonwealth University, 2009
- **Schumacher Dissertation Research Award**, School of Education, Virginia Commonwealth University, 2009
- **Academic Scholarship** (Tuition Waiver), Department of Physical Education, Indiana State University, 2003-2004
- **Outstanding Academic Achievement Award**, Department of Physical Education, Fu-Jen Catholic University, 1997
- **Fourth Place Winner**, Taiwan Intercollegiate Rowing Competition (4×), Fu-Jen Catholic University, 1998
- **Bronze Medal Winner**, Taiwan Intercollegiate Rowing Competition (2×), Fu-Jen Catholic University, 1997

CERTIFICATIONS

- Certified as a Phlebotomist by National Health Career Association (Certification number 0311-9693).

COURSES TAUGHT

Virginia Commonwealth University

HPEX 200 – Strength, Endurance, and Flexibility
HPEX 250 – Medical Terminology
HPEZ 334 – Measurement and Analysis Lab
HPEZ 375 – Exercise Physiology Lab
HEMS 601 – Movement Physiology (with Dr. Acevedo)

The University of Mississippi

ES 147 – Tennis
ES 151 – Weight Lifting
ES 153 – Sports Conditioning
ES 156 – Jogging
ES 475 – Exercise Testing and Prescription Lab

PEER REVIEWED PUBLICATIONS

Huang, C.J., Garten, R.S., Wade, L.R., Webb, H.E., & Acevedo, E.O. (in press). Physiological Responses to Stair Climbing in Professional Firefighters Wearing Rubber and Leather Boots. European Journal of Applied Physiology.

Warnick, J.E., **Huang, C.J.**, Acevedo, E.O., & Sufka, K.J. Modeling the Anxiety-Depression Continuum in Chicks. Journal of Psychopharmacology. 2009: 23(2): 143-156.

Huang , C.J, Nesser, T.W., & Edwards, J.E. Physiological Determinants of Rowing Performance. Journal of Exercise Physiology Online, 2007: 10(4): 43-50.

MANUSCRIPTS IN REVIEW

Huang, C.J., Webb, H.E., Garten, R.S., Kamimori, G.H., Evans, R.K., & Acevedo, E.O. Stress Hormones and Immunological Responses to a Dual Challenge in Professional Firefighters. International Journal of Psychophysiology.

MANUSCRIPTS IN PREPARATION

Huang, C.J., Webb, H.E., Ronald, R.K., McCleod K.A., Tangsilsat, S.E., Kamimori, G.H., & Acevedo, E.O. An Examination of Immunoendocrine and Oxidative Responses Utilizing a Dual Stress Model.

NATIONAL SCIENTIFIC PRESENTATIONS AS PUBLISHED ABSTRACTS

Huang, C.J., Webb, H.E., Ronald, R.K., McCleod K.A., Tangsilsat, S.E., Kamimori, G.H., & Acevedo, E.O. (2009, May). An Examination of Immunoendocrine and Oxidative Responses Utilizing a Dual Stress Model. Paper presented at the Annual American College of Sports Medicine Conference, Seattle, WA.

Huang, C.J., Webb, H.E., Evans, R.K., & Acevedo, E.O. (2008, May). Effects of a Dual Challenge on Stress Hormones, Th1/Th2 Cytokines, and Lymphocyte Responses in Firefighters. Paper presented at the Annual American College of Sports Medicine Conference, Indianapolis, IN. [Abstract] Medicine & Science in Sports & Exercise. 2008;45(5): S431.

NATIONAL SCIENTIFIC PRESENTATIONS (not included in abstracts)

Huang, C.J., Garten, R.S., Wade L.R., Acevedo, E.O. (2008, April). Physiological Responses to Stair Climbing In Professional Firefighters Wearing Rubber and Leather Boots. Paper presented at the Annual American College of Occupational and Environmental Medicine Conference, New York, NY.

Huang , C.J, Nesser, T.W., & Edwards, J.E. (2005). Physiological Determinants of Rowing Performance. Paper presented at the Annual National Strength Conditioning Association Conference, Las Vegas, NV.

REGIONAL SCIENTIFIC PRESENTATIONS

Huang, C.J., Webb, H.E., Ronald, R.K., McCleod K.A., Tangsilsat, S.E., Kamimori, G.H., & Acevedo, E.O. (2009, February). An Examination of Immunoendocrine and Oxidative Responses Utilizing a Dual Stress Model. Paper presented at the Annual Southeast American College of Sports Medicine Conference, Birmingham, AL.

Huang, C.J., Webb, H.E., Evans, R.K., & Acevedo, E.O. (2008, February). Effects of a Dual Challenge on Stress Hormones, Th1/Th2 Cytokines, and Lymphocyte Responses in Firefighters. Oral Presentation at the Annual Southeast American College of Sports Medicine Conference, Birmingham, AL.

UNIVERSITY/DEPARTMENT PRESENTATIONS

Huang, C.J. Garten, R.S., Wade L.R., Acevedo, E.O. (2009, April). An Examination of Immunoendocrine and Oxidative Responses Utilizing a Dual Stress Model. Poster Presented at the 12th Annual Graduate Student Research Symposium and Exhibit, Virginia Commonwealth University, Richmond, VA.

Huang, C.J. Garten, R.S., Wade L.R., Acevedo, E.O. (2008, April). Physiological Responses to Stair Climbing In Professional Firefighters Wearing Rubber and Leather Boots. Poster Presented at the 11th Annual Graduate Student Research Symposium and Exhibit, Virginia Commonwealth University, Richmond, VA.

Huang, C.J. (2008, November). The Measurement of Gene Expression with RT-qPCR Techniques to Examine Adaptations to Physical Activity. Doctoral Seminar Series. Department of Health and Human Performance. Virginia Commonwealth University, Richmond, VA.

Huang, C.J. (2008, February). Effects of a Dual Challenge on Stress Hormones, Th1/Th2 Cytokines, and Lymphocyte Responses in Firefighters. Doctoral Seminar Series. Department of Health and Human Performance. Virginia Commonwealth University, Richmond, VA.

Huang, C.J. (2007, March). The Stress Response and Immune system. Doctoral Seminar Series. Department of Health and Human Performance. Virginia Commonwealth University, Richmond, VA.

Huang, C.J. (2006, October). The Effects of Immolina Supplementation on Exercise-Induced Muscle Soreness and Indicators of Inflammation and Tissue Damage. Doctoral Seminar Series. Department of Health and Human Performance. Virginia Commonwealth University, Richmond, VA.

GRANTS

Huang, C.J.

Graduate Student Research Fund. Indiana State University, Fall 2004
"Physiological Determinants of Rowing Performance"
$500

GRANT PROPOSALS PREVIOUSLY SUBMITTED

Huang, C.J.

American College of Sports Medicine Doctoral Research Grants, "The Impact of Mental Challenge on Indicators of Endothelial Function in Obese Individuals" $5,000 (Not Funded), 2009.

Evans, R.K., **Huang, C.J.**, & Acevedo, E.O.

Jeffress Memorial Trust, "The effects of repeated bouts of intense exercise on the Th-1/Th-2 cytokines balance and vascular function in obese individuals" $29,565 (Not Funded), 2007.

Acevedo, E.O., Taylor, K.F., & **Huang, C.J**.

Alzheimer's and Related Diseases Research Award,
"Effects of Chronic and Acute Stress on the Th-1/Th-2 Cytokine Balance in Family Caregivers of Dementia Patients" $39,884 (Not Funded), 2007.

UNIVERSITY SERVICE

The University of Mississippi

- Instructor Search Committee, Exercise Science, 2006.
- President of Taiwanese Student Association, 2005

Fu-Jen Catholic University

- President of Taitung City Student Association, Taiwan, 1997.

UNIVERSITY ACTIVITIES

Indiana State University

- Representative of University's Integrated Marketing Program, 2004.

Fu-Jen Catholic University

- Student Leader Camp, 1998.
- Rowing Team, 1996-1999

PROFESSIONAL ORGANIZATIONS

- Psychoneuroimmunology Research Society, 2007 – present
- The American Physiological Society, 2005-2006
- American College of Sports Medicine, 2004 - present
- Southeast American College of Sports Medicine, 2005 - present