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The Effect of Obesity on IL-1β, IL-1Ra, and Leptin Following Acute Mental Stress

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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List of Abbreviations

ACTH	adrenocorticotropic hormone
AMS	acute mental stress
CVD	cardiovascular disease
BP	blood pressure
EPI	epinephrine
HPA	hypothalamic-pituitary-adrenal
HR	heart rate
IL-1β	interleukin-1 beta
IL-1Ra	interleukin-1 receptor antagonist
IL-6	interleukin- 6
IL-10	interleukin- 10
LPS	lipopolysaccharide
NE	norephinephrine
SAM	sympathetic-adrenal-medullary
TNF-α	tumor necrosis factor- alpha

I. Abstract

THE EFFECT of OBESITY on IL-1β, IL-1Ra, and LEPTIN FOLLOWING ACUTE MENTAL STRESS

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Research regarding the development of cardiovascular disease (CVD) is important because CVD is the leading cause of death in the United States (US) and many countries abroad. Risk factors, such as obesity and psychological stress, should be studied in order to understand contributing factors for CVD and the cellular mechanisms which link risk factors with the development of disease. Specifically, the combined influence of multiple risk factors on inflammation is of interest because many individuals have more than one risk factor, which additively increases an individual's risk for CVD. Obesity is already characterized by disordered inflammation, which suggests that the additional burden of a psychological stressor could elicit a greater inflammatory response and a greater risk for CVD than either stressor alone.

The documents included within this thesis include the significance and specific aims of the study in addition to a review of the relevant literature related to the effects of obesity and acute mental stress (AMS) on endocrine and inflammatory markers. Specifically, this study aims to address IL-1β, IL-1Ra, and leptin following an AMS task in non-obese and obese individuals. Additionally, a manuscript is included which evaluates the change in IL-1β, IL-1Ra, and leptin following a 20 minute AMS task. Variables were examined between groups at baseline and at two time points following AMS. Additionally, the relationships among the changes in the markers following AMS were examined. Appendices include expanded methodology and all questionnaires used.

II. Review of Literature

Introduction

Cardiovascular disease (CVD) is the leading cause of death for both men and women in the United States and contributes to one third of all reported deaths (CDC 2011). Traditional independent risk factors for CVD include family history, age, hypertension, hypercholesterolemia, smoking, physical inactivity, impaired fasting blood glucose, and obesity. The prevalence of obesity has reached epidemic proportions, as reported by the most recent National Health and Nutrition Examination Survey (NHANES) data (Flegal et al. 2012). In 2008, 68.8% of US adults were categorized as overweight, while 35.7% were categorized as obese. The number of overweight and obese adults has risen annually, and the Center for Disease Control predicts that 32 million additional Americans will become obese by 2030; increasing the prevalence of adult obesity to 42%. Obesity is characterized by excess adipose tissue deposits. One mechanism in which increased adiposity contributes to an individuals's risk for CVD is by acting as a chronic physical stressor and eliciting chronic systemic inflammation (Ippoliti et al. 2013; Benson et al. 2009; Brydon 2011; Bogdanski et al. 2012).

Psychological stress has also been identified as an independent risk factor for CVD, as persistent, habitual psychological stress has the ability to elicit systemic inflammation (Rozanski, Blumenthal, and Kaplan 1999; Gu, Tang, and Yang 2012). The 2012 American Psychological Association (APA) Stress in America Survey reported that 20% of Americans experience extreme stress, with stress levels staying the same or increasing over the past year for 80% of

Americans (APA 2012). Significant sources of stress include money, work, the economy, family responsibilities, relationships, family health problems, and personal health concerns. Additionally, over the past five years, 60% of Americans attempted to reduce their stress, but few reported success (APA 2012).

While obesity and psychological stress are of interest as independent risk factors for CVD, their combined influence on the inflammatory process and CVD risk are of greater interest considering the significant prevalence of both obesity and perceived psychological stress in the United States. Previous investigators have demonstrated that a dual stress condition, combining a mental and physical stressor (exercise), elicits a greater physiological strain than either condition alone (Webb et al. 2010). This suggests that the dual load of mental stress and obesity could elicit a greater pro-inflammatory response and a greater risk for CVD than mental stress or obesity alone. Therefore, it is of interest to examine the inflammatory response to an acute mental stress (AMS) model in obese and non-obese individuals.

The Physiological Stress Response

A stressor is defined as a challenge or stimulus that threatens homeostasis and requires behavioral, psychological, or physiological adaptations (Seyle 1936; Ippoliti, Canitano, and Businaro 2013). The stress response was evolutionarily designed to initiate in response to conditions which threaten one's security, health or safety, and the availability of shelter and/or food. This defensive response was important when it was essential for early humans to protect themselves against wild animals or other humans by fighting or fleeing. Appropriately referred to as the "fight or flight" response, the physiological response to stress is a protective mechanism which allows the body to adapt to the demands of a stressor and then return quickly to

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homeostasis once the stressor has ceased (Cannon 1915). The protective systemic adaptations which occur require communication between the neural, endocrine, and immune systems.

Initiation of the neural response occurs with activation of the sympathoadrenal-medullary (SAM) axis and the hypothalamic-pituitary-adrenal (HPA) axis, inducing subsequent endocrine responses. The SAM axis provides neural input to the adrenal medulla, releasing norepinephrine (NE) and epinephrine (EPI), which are known to increase heart rate, vasoconstrict systemic vessels, dilate the bronchioles, and breakdown glycogen and triglycerides. These responses prepare an individual to fight or flee by priming the cardiovascular and respiratory systems for work and mobilizing macronutrient stores to meet eventual energy demands. Activation of the HPA axis begins with the release of corticotropin-releasing-hormone (CRH) from the hypothalamus which signals the release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH then signals the release of the glucocorticoid hormone, cortisol, from the adrenal cortex. Like the sympathetic hormones, cortisol also mobilizes energy stores through the breakdown of stored fats, glycogen, and muscle protein. However, while NE and EPI prime the body to respond to the stressor, cortisol acts to return the body to homeostasis after the stress response occurs. NE, EPI, and cortisol also act on immune cell receptors, eliciting both primary and secondary immune responses (Dragos and Tanasescu 2010).

The immune response is regulated by circulating hormones and locally released neurotransmitters, such as NE, which spill over into the blood stream from nerve endings. The body's immediate defense system, known as the innate immune system, has widespread actions but very low specificity for particular infectious agents, or antigens. White blood cells, known as leukocytes, are designed to identify and remove unknown pathogens, initiate wound healing, and recruit immune cells to the site of injury when activated. Leukocytes include macrophages,

neutrophils, and mast cells. Within this innate immune system is an acute phase response, which specifically includes the systemic inflammatory reaction caused by infection, tissue injury, trauma, or stress. Acute phase proteins are released by hepatocytes to promote inflammation, redistribute immune cells to the site of injury or infection, and enhance phagocytosis of pathogens. Another function of the innate immune system includes the production of cytokines in order to activate adaptive immune cells.

While the innate immune system works quickly to detect the presence of general antigens, the adaptive immune system acts specifically on each antigen present. Adaptive immunity consists of an acquired response by B and T lymphocytes, most commonly referred to as B and T cells, which are primed to recognize pathogens and differentially respond based on previous exposure. Functions of the adaptive immune system can be categorized into humoral and cellular immunity. Humoral immunity, mediated by B cells, involves the production of antibodies, which signal an increase in cellular immunity. Cellular immunity is mediated by T cells, macrophages, and natural killer cells which act to induce apoptosis or phagocytose infected cells. Once active, B cells, T cells, and macrophages also secrete inflammatory cytokines, which will be the focus of this review.

Inflammatory Cytokines

Cytokines are bioactive cell signaling molecules produced by virtually all nucleated cells, including immune cells, which influence the inflammatory response via autocrine, paracrine, or endocrine signaling (Elenkov et al. 2005). These proteins play a role in many signaling cascades and feedback controls of the immune system. Cytokines influence the proliferation, differentiation, and activation of immune cells, as well as synthesis and release of other

cytokines. Cytokines are categorized based on their role as pro- or anti-inflammatory messengers and work alongside hormones to augment physiological responses to various stressors. Pro-inflammatory cytokines turn on the immune response to a stressor, providing protection against pathogens, inflammation, and other physical stressors. Anti-inflammatory cytokines act as an inherent feedback control to prevent excessive pro-inflammatory cytokine damage and restore homeostasis once a stressor no longer poses a threat. Frequently studied cytokines known to contribute to the inflammatory stress response include the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β), the anti-inflammatory cytokines interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-IRa), and cytokines which can act as pro- or anti-inflammatory cytokines with various models of acute and chronic stress, such as interleukin-6 (IL-6) (Elenkov et al. 2005; Steptoe, Hamer and Chida 2007; Gouin 2011; Gabay 2006).

TNF-α, named for its ability to induce necrosis of tumor cells following acute bacterial infection, has a primary role in the acute induction of inflammation, leukocyte recruitment, and alteration of the vascular endothelium (Black 2006, Coppack 2001; Hamblin 1993; Juge-Aubry, Henrichot, and Meier 2005). TNF-α plays a major role in acute and chronic inflammation, auto-immune diseases, tumorigenesis, viral replication, septic shock, and fever. TNF-α induces the activation of many other immunoregulating hormones and cytokines, which then up-regulate TNF-α through a positive feedback loop.

IL-1β and IL-6 are further categorized as interleukins, originally named for their role in communication between leukocytes (Hamblin 1993). However, interleukins are now known to mediate responses in both immune and non-immune cells, such as endothelial cells, glial cells, hepatocytes, muscle cells, and adipocytes (Hamblin 1993). IL-1β is a particularly potent pro-

inflammatory mediator of inflammation and fever, acting locally to stimulate the production of acute phase proteins in the liver. Several roles of IL-1β include effecting the migration and activation of monocytes and lymphocytes, producing an alteration in the vascular endothelium, and inducing antibody and cytokine synthesis (Hamblin 1993; Brydon et al. 2005). In addition, IL-1β induces fibroblast, smooth muscle cell, and breast cancer cell proliferation, as well as angiogenesis and cartilage breakdown (Juge-Aubry, Henrichot, and Meier 2005). The main sources of IL-1β are peripheral blood monocytes and tissue macrophages, although IL-1β can also be secreted by epithelial cells and is present within the hypothalamus (Juge-Aubry, Henrichot, and Meier 2005). Importantly, IL-1β can stimulate IL-6 production from various cell types (Brydon et al. 2005).

IL-6, a pleotropic cytokine, is primarily responsible for the early stimulation of acute phase proteins, pro-inflammatory cytokine synthesis, and regulation of adaptive immunity following the initiation of a stress response (Gabay 2006; Tilg et al. 1993; Steptoe et al. 2001). Additionally, IL-6 helps regulate the differentiation, function, and activation of T helper cells, induces the secretion of immunoglobulins, and is the final differentiation factor for B cells, which produce antibodies (Turgeon 1996). However, IL-6 also has a subsequent protective effect by suppressing pro-inflammatory cytokine production and stimulating anti-inflammatory cytokine production in order to control the extent of both local and systemic inflammatory damage (Tilg et al. 1993; Gabay 2006). As part of this anti-inflammatory response, IL-6 has been shown to inhibit the production of pro-inflammatory cytokines TNF-α and IL-1β, while up-regulating IL-1Ra and TNF-α receptor antagonists (Tilg et al. 1993).

Together, TNF-α, IL-1β, and IL-6 are the classical pro-inflammatory cytokines, also known as the inflammatory triad, because they play a combined role in the induction of fever and

both local and systemic inflammation (Hamblin 1993; Juge-Aubry, Henrichot, and Meier 2005). These three cytokines can be measured collectively to reflect an overall state of inflammation, and often display similar responses to various models of physical and psychological stress (Hamblin 1993; Chancellor- Freedman et al. 1995; Steptoe et al. 2001; Steptoe, Hamer, and Chida 2007).

As previously mentioned, IL-10 and IL-IRa are categorized as anti-inflammatory cytokines. IL-10 can inhibit pro-inflammatory cytokine synthesis, as well as the proliferation and activation of macrophages and mast cells (Turgeon 1996). Because the synthesis and release of IL-10 is induced by the same mechanisms as pro-inflammatory cytokines, IL-10 acts as an innate control mechanism to attenuate the inflammatory processes induced by pro-inflammatory cytokines (Turgeon 1996; Charles et al. 2011). Specifically, IL-10 has been shown to attenuate the inflammatory processes induced by TNF-α, IL-1, and IL-6 and up-regulate the release of the anti-inflammatory cytokine IL-1Ra (Charles et al. 2011).

IL-1Ra does not directly elicit an anti-inflammatory response, but prevents the pro-inflammatory signaling of IL-1β (Charles et al. 2011). As a member of the interleukin-1 family, IL-1Ra competitively binds to IL-1 receptors, preventing IL-1β from inducing a cellular inflammatory response, including the production of IL-6 (Steptoe et al. 2001, Brydon et al. 2005). IL-1Ra is synthesized by monocytes, neutrophils, fibroblasts, epithelial cells, and microglial cells, and is secreted by hepatocytes as an acute phase protein (Juge-Aubry, Henrichot, and Meier 2005). Interestingly, IL-1Ra production is increased by IL-1β and the same intra-cellular messengers which stimulate IL-1β: TNF-α and IL-6. Additionally, an infusion of epinephrine in vivo has also produced a two to three-fold increase in plasma IL-1Ra in cells taken from both HIV+ patients and controls (Sondergaard et al. 2000). By acting as an inherent

negative feedback mechanism, IL-1Ra appears to play a significant role in the regulation of cytokine signaling.

Quantification of the catecholamines, glucocorticoids, and cytokines can provide insight into levels of inflammation at rest and the inflammatory response produced following various psychological and physical stressors. The remainder of this review will examine the immune responses to both psychological stress and obesity; and finally the dual response to both stressors by the endocrine and immune systems.

Acute Mental Stress

An AMS model is often used to elicit the neural, endocrine, and immune responses of psychological stress in the laboratory setting (Rimmele et al. 2007; Benson et al. 2009). These models last 3-20 minutes and include stressors such as mental arithmetic, the Stroop color word task, public speaking tasks, and the mirror tracing task. As the tasks begin and subject anxiety is induced, a corresponding initiation of the endocrine and inflammatory stress responses have been observed (Steptoe et al. 2001; Steptoe, Hamer, and Chida 2007; Benson et al. 2009; Carroll, Phillips, and Der 2008).

Endocrine parameters have been evaluated in a few of the AMS models previously mentioned. EPI and NE levels were shown to increase after initiation of a Trier Social Stress Test (TSST), consisting of a five minute mock job interview followed by five minutes of mental arithmetic in front of an audience (Rohleder et al. 2006). In addition, NE has been shown to increase after a 20 minute protocol of alternating the Stroop color word and mental arithmetic task (Huang et al.2010; Huang et al. 2011). Other studies have inferred an increase in catecholamine levels based on increases in heart rate, heart rate variability, blood pressure, mean

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arterial pressure, forearm vasodilation, and inflammatory cytokines (Gu, Tang, and Yang 2012; Hamer, Boutcher, and Boutcher 2006; Carroll, Phillips and Der 2008; Jones et al. 2012). Cortisol levels have also been shown to increase in response to various AMS models including a speech task, a Stroop color word task, and a public speaking task (Benson et al. 2009; Brydon et al. 2009; Brydon 2011). These AMS models have demonstrated an increase in cortisol concentrations approximately 15 to 20 minutes after the initiation of the stressor, followed by a return to baseline within 30 minutes (Benson et al. 2009). However, not all stressors have been shown to elicit cortisol reactivity (Steptoe et al. 2001). Data suggests that the HPA axis is not particularly sensitive to mental arithmetic or the Stroop color word task (Sinyor et al. 1983; Rimmele et al. 2007) because these models do not have an uncontrollable or, evaluative character, which seems to induce cortisol responses in stress tasks such as public speaking (Dickerson and Kemeny 2004; Rimmele et al. 2007; Steptoe et al 2001).

In general, an acute stressor results in the proliferation and redistribution of immune cells such as macrophages, T and B cells, and granulocytes (Steptoe, Hamer, and Chida 2007; Benson et al. 2009). Macrophages and NK cell functions are known to be particularly sensitive to psychological stress, and the activation of these cells have been shown to parallel the elevation in pro-inflammatory cytokine concentrations following AMS. AMS induced cytokine responses have been observed in both circulating plasma and serum levels and *in vitro* stimulation by lipopolysaccharide (LPS), which is used to induce an exaggerated immune response to magnify concentrations to detectable levels (Steptoe, Hamer, and Chida 2007; Huang et al. 2011). It is currently unknown whether the increases in cytokine concentrations are due to the redistribution of immune cells, an increase in cytokine release, or an increase in cytokine synthesis (Gouin 2011; Gu, Tang, and Yang 2012; Steptoe, Hamer, and Chida 2007). Interestingly, it has been

proposed that the peak cytokine responses from immediately post to 45-minutes post AMS task are mainly the result of the release of stored cytokines, whereas peak cytokine response 45-120 minutes post-stressor are the result of increased protein synthesis (Steptoe, Hamer, and Chida 2007).

Inflammatory Cytokines

A meta-analysis by Steptoe, Hamer, and Chida (2007) examined cytokine activity in various AMS models and found significant elevations in IL-1β and IL-6 across various studies. Other cytokines such as TNF-α, IL-10, and IL-1Ra displayed changes in at least one study, although significant changes were not always observed following AMS. Overall, few cytokine levels were shown to increase immediately post-stressor. Most significant increases were observed 45-, 60-, or 120-minutes post-stressor (Steptoe, Hamer, and Chida 2007). For general interpretations of the variable cytokine response to AMS, it is important to consider subject gender, time points at which cytokines are evaluated post-stressor, and the AMS models utilized.

In the meta-analysis by Steptoe, Hamer, and Chida (2007), IL-1β was significantly elevated in both stimulated *in vitro* studies and *in vivo*. IL-1β is often studied *in vitro* because the cytokine is released locally, causing plasma concentrations to be relatively low and sometimes undetectable in healthy individuals. Two studies demonstrated an *in vitro* LPS or lectin stimulated IL-1β increase following AMS (Ackerman et al. 1998; Bower et al. 2007). Ackerman and colleagues observed the increase in IL-1β following lectin stimulation and a five minute videotaped speech, in which Multiple Sclerosis patients and age- and gender-matched controls were required to defend themselves in a hypothetical scenario in which they were wrongly accused of stealing (1998). Bower and colleagues reported an increase in LPS stimulated IL-1β

following the TSST AMS task in fatigued breast cancer survivors, which was not observed in non-fatigued subjects (2006). In addition, two studies have reported significant detectable increases in plasma IL-1β following AMS (Altemas et al. 2001; Heinz et al. 2003). Altemas and colleagues suggested an increase in plasma IL-1β 60-minutes after completion of an AMS model consisting of a mock job interview and a sequential subtraction task in women (Altemas et al. 2001). It was later shown that male physicians had a significant increase in plasma IL-1β immediately after the acute stress of giving an oral presentation to colleagues at a clinical conference (Heinz et al. 2003). Furthermore, IL-1β gene expression has been shown to increase in men 30-, 75-, and 120-minutes after a five minute Stroop color word task and five minute mirror tracing task (Brydon et al. 2005). Increases in IL-1β gene expression had a significant positive association independent of age and BMI. In addition, the change in IL-1β gene expression was significantly and positively associated with a change in anxiety in response to the AMS task (Brydon et al. 2005).

IL-6 levels have consistently been shown to increase following AMS, which was statistically supported in the Steptoe meta-analysis model (2007). An increase in plasma IL-6 was shown immediately and 45-minutes post-AMS task in which women were accused of shoplifting and required to defend themselves (Brydon et al. 2008). Plasma IL-6 has also been found to significantly increase 45- and 120-minutes following a public speaking task combined with the Stroop color word task in both women and men (Benson et al 2009; Hamer and Steptoe 2007). Additionally, two studies utilizing a five minute Stroop color word task along with a five minute mirror tracing task have been shown to increase IL-6 at 30-, 75-, and 120-minutes post-task in men and women (Steptoe et al. 2001; Brydon et al. 2005). Furthermore, IL-6 mRNA increased immediately following LPS stimulation and a 20 minute alternating Stroop color word

and mental arithmetic task and decreased significantly below baseline levels one hour post-stressor (Huang et al. 2011). A similar trend was observed in plasma IL-6, as the levels increased immediately post-stressor and significantly decreased from post-stress concentrations after one hour of recovery.

The overall effects of AMS tasks on TNF-α were not significant in the Steptoe meta-analysis and individual analyses have provided equivocal results (Steptoe, Hamer, and Chida 2007). Observed differences were suggested to be dependent upon the type and length of the stressors, as well as subject gender. In 2002, Steptoe and colleagues evaluated plasma TNF-α following a five minute Stroop color word task and a subsequent five minute mirror tracing task in men and women (Steptoe et al. 2002). This AMS model elicited a significant increase in TNF-α 45-minutes post-task in men, but not women, despite similar baseline measures. These results indicate a potential influence of gender on the immune response to AMS. However, in contrast to Steptoe and colleagues (2002), a significant reduction in LPS stimulated TNF-α mRNA was shown immediately after a 20 minute alternating Stroop color word and mental arithmetic task. This change in LPS stimulated TNF-α mRNA immediately post-stressor was recovered within an hour following the AMS task (Huang et al. 2011). A similar trend was shown in the LPS-stimulated plasma TNF-α, as the levels significantly decreased immediately post-stressor and then returned closer to baseline after one hour.

Interestingly, two additional studies evaluating TNF-α in males and females following a five minute Stroop color word and five minute mirror tracing task did not observe significant changes, however the responses were not differentiated by gender (Steptoe et al. 2001; Hamer and Steptoe 2007). Interestingly, both studies found an association between TNF-α and sympathetic activity Steptoe and colleagues suggested no significant differences in plasma TNF-

α at baseline and 2-hours post-task, although a significant positive relationship was observed between TNF-α levels and heart rate 2-hours post-task (Steptoe et al. 2001). Similarly, a significant positive relationship was shown between plasma TNF-α 2-hours post-task and heart rate and blood pressure responses during 8 minutes of exercise on a cycle ergometer, after adjusting for BMI, gender, smoking, alcohol, grade of employment, and basal levels of inflammatory markers (Hamer and Steptoe 2007).

Relatively few studies have investigated the impact of AMS on IL-10, and no clear conclusions have been determined from the contradictory results. Twenty-five women participated in a psychological interview stressor preceding a sequential subtraction task (Altemas et al. 2001). This AMS task resulted in a significant increase in plasma IL-10 at 60-minutes post-stressor. A subsequent study by Buske-Kirshbaum and colleagues suggested a significant reduction in IL-10 immediately post-stressor (Buske-Kirshbaum et al. 2007). They utilized the TSST, consisting of five minutes of public speaking and a five minute mental arithmetic test. The TSST model was used again in conjunction with peripheral blood mononuclear cell (PBMC) LPS stimulation, demonstrating a reduction in IL-10 2-hours post-task (Xiang et al. 2012). There were not enough studies measuring IL-10 in response to an AMS task to be included within the Steptoe meta-analysis, and the effect of AMS on IL-10 remains unclear and warrants further investigation (Steptoe, Hamer, and Chida 2007).

IL-1Ra was also excluded from the statistical analysis by Steptoe, Hamer and Chida (2007), though levels appear to increase following various models of AMS. The effect of AMS, as demonstrated by a five minute Stroop color word task and five minute mirror tracing task, on IL-1Ra was investigated in men and women relative to age and BMI matched controls (Steptoe et al. 2001). There was a significant increase in plasma IL-1Ra 45-minutes and 2-hours following

the AMS task. Additionally, the levels of IL-1Ra at 45-minutes and 2-hours post-task both displayed significant positive correlations with the concurrent increase in diastolic pressure. The TSST model of five minutes of a fake job interview and five minutes of arithmetic was also shown to elicit a significant increase in IL-1Ra 45- and 60-minutes post-AMS as compared to baseline measures in healthy males (Rohleder et al. 2006). In 2002, Steptoe and colleagues examined the effects of gender on the IL-1Ra response following an AMS task which used the combination of the Stroop color word task and mirror tracing task (Steptoe et al. 2002). Interestingly, women had a significant increase in IL-1Ra by 6.5% at 45-minutes post-task, while men did not demonstrated a significant increase, even though IL-1Ra appeared to significantly increase post-AMS task in all subjects combined during statistical analysis.

While the effect of AMS on many hormone and cytokine responses is well understood, the effect on others is less clear and may depend on the specific AMS model induced, total length of the stressor, and timing of the post-stressor blood draws, which limits direct comparisons between studies. In addition, there is evidence to suggest that although a hormone or cytokine may behave one way in response to an acute stress, a chronic stressor may alter the response of the hormone or cytokine (Gabay 2006). A prolonged stress response is marked by immune dysregulation and an underexpression or overexpression of certain inflammatory molecules (Gouin 2011). Therefore, the evaluation of these signaling molecules is necessary in chronic stress models in addition to AMS.

Chronic stress is defined as a repeated or persistent activation of the immune system, with a chronic elevation in circulating hormones and cytokines contributing to disordered inflammation (Ippoliti, Canitano, and Businaro 2013). Chronic stressors such as depression, sleep deprivation, and marital stress appear to increase basal levels of circulating immune cells

and cytokines (Dragos and Tanasescu 2010; Gouin 2011). While an acute stressor elicits a protective inflammatory response which enhances the body's resistance to infection, a chronic stressor, characterized by systemic inflammation, may impair an individual's ability to produce a strong immune response resulting in delayed wound healing, poor response to vaccine, and increased susceptibility to disease (Dragos and Tanasescu 2010; Gouin 2011). Chronic stressors, like prolonged psychological stress and obesity, may impair the regulation of homeostasis and cause detrimental health consequences (Gouin 2011).

Obesity

One chronic stress model of significant interest is obesity, a disease reaching epidemic proportions in most of the First World and a major risk factor for CVD. Obesity is marked by relatively large adipose tissue deposits which are proposed to contribute to a low-grade inflammatory state and an increased risk for CVD (Ippoliti, Canitano, and Businaro 2013). Adipose tissue has been classified as an endocrine organ because it is capable of secreting numerous hormones and cytokines known to play a role in immune regulation, such as IL-1β, IL-6, TNF-α, and IL-1Ra (Ippoliti, Canitano, and Businaro 2013). Greater adiposity levels appear to contribute to the prolonged inflammatory response in obese individuals (Divoux et al. 2012).

There is evidence that obese individuals have an increased concentration of circulating immune cells, hormones and cytokines compared to non-obese individuals (Benson et al. 2009, Ippoliti, Canitano, and Businaro 2013; Divoux et al. 2012; Charles et al. 2011). Obese women display higher basal levels of circulating leukocytes, lymphocytes, and granulocytes compared with non-obese women (Benson et al. 2009). There are also proportionally more immune cells, such as macrophages and mast cells, per area of adipose tissue in obese individuals as compared

to lean individuals (Divoux et al. 2012). These immune cells, known to migrate into white adipose tissue deposits, may contribute to the increased release of pro-inflammatory cytokines from obese adipose tissue, in addition to adipocytes themselves.

Inflammatory Cytokines

Not only do obese individuals have more adipose tissue and immune cells within their adipose tissue deposits, but they also produce more inflammatory cytokines within the immune cells of the adipose tissue (Divoux et al. 2012). It is generally accepted that basal levels of IL-6 and TNF-α are moderately elevated in obese individuals compared with non-obese individuals, although there is evidence that in the absence of cardiovascular and metabolic diseases, an increase in inflammatory cytokines is not always observed in obese individuals (Coppack 2001, Van Guilder et al. 2006). Elevated basal IL-6 levels have consistently been shown in obese compared with non-obese populations, including adult men and women, African-Americans, diabetics, and overweight boys aged 11-12 (Benson et al. 2009; Charles et al. 2011; Fu et al. 2011; Utsal et al 2012; Marques-Vidal 2012).

Likewise, elevated basal TNF-α levels have been observed in obese women and overweight adolescent girls, compared with their non-obese counterparts (Zaccardi et al. 2002; El-Wakkad et al. 2013; and Bogdanski et al. 2012). In a cohort sample of men and women, positive associations were found between TNF-α levels and waist circumference in men and between TNF-α levels and BMI in women (Marques-Vidal 2012). Significant positive relationships have also been shown to exist between basal plasma IL-6 and TNF-α and both BMI and body fat percent in women (Brydon 2011; Bogdanski et al. 2012) In addition, obese premenopausal women with elevated IL-6 and TNF-α levels who completed a one year

intervention resulting in at least 10% weight loss through diet, exercise, and behavioral counseling demonstrated a reduction in both cytokine levels compared to baseline (Ziccardi et al. 2002). These results support the hypothesis that adipose tissue is a significant source of elevated pro-inflammatory cytokine levels, such as TNF-α and IL-6, in obesity.

The impact of obesity on IL-1β has received much less attention, but there is evidence that IL-1β may be altered with obesity. Higher levels of IL-1β were secreted from mast cells taken from obese subjects as compared to non-obese subjects in vivo (Divoux et al. 2012). Additionally, circulating levels of IL-1β were shown to be significantly greater in obese adolescent girls with a waist circumference in the 90% percentile as compared to their non-obese counterparts (El-Wakkad et al. 2013). In contrast, low IL-1β concentrations were found in obese adolescents without central obesity classified by waist circumference.

There are no studies that have shown differences between IL-10 in obese and non-obese individuals or shown any association with obesity measures (Charles et al. 2013; Utsal et al. 2012). Interestingly, other models of chronic stress have shown differences in IL-10 levels between stressed individuals and controls. Caregivers of family members with dementia have higher plasma IL-10 levels than non-caregiving controls (Gouin 2011). This increase in anti-inflammatory cytokine production is likely due to elevated circulating IL-6 levels, coinciding with a reduced regulatory function of IL-10 on IL-6 (IL-10 attenuates IL-6 production) (Gouin 2011). This can also be observed in cases of Th1 mediated septic shock, in which elevated TNF-α production increases IL-10 which cannot effectively shut down TNF-α production, thus continuing to increase the production of IL-10. Although it seems logical that a pro-inflammatory profile would be indicative of reduced anti-inflammatory cytokine production, it

seems likely that in some cases of inflammation, there is a reduced regulatory function of anti-inflammatory cytokines.

Evidence suggests that IL-1Ra is not only elevated with obesity, but displays the largest magnitude of difference in basal levels between obese and non-obese individuals compared to other cytokines (Juge-Aubry, Henrichot, and Meier 2005; Meier et al. 2002). Resting levels of IL-1Ra have been shown to be elevated 6.5 fold in obese subjects as compared with lean subjects, although there was marked variability within the obese group (Meier et al. 2002). This variability was proposed to be associated with differences in lean body mass, insulin resistance, and leptin, which will be discussed in further detail below (Meier et al. 2002). Resting levels of IL-1Ra have also been shown to have a significant positive association with BMI and waist to hip ratio after controlling for gender (Steptoe et al. 2002). Additionally, Charles et al. (2011), found associations between resting IL-1Ra and obesity, BMI, waist circumference, hip circumference, waist to hip ratio, and percent fat mass in both men and women. Furthermore, when subjects were separated between those with high and low resting plasma levels of IL-1Ra, measures of obesity were shown to explain up to 20% of the variance in IL-1Ra in the individuals with high IL-1Ra (Charles et al. 2011). Elevated levels of IL-1Ra in obese individuals have been attributed to increased macrophage, mast cell, and adipocyte numbers, as well as increased IL-1Ra expression within the adipose tissue of obese individuals (Juge-Aubry et al. 2003).

Leptin

Leptin is a hormone, secreted into the blood stream from adipose tissue, which is primarily known to regulate energy balance, reduce appetite, and stimulate SNS activity (Brydon

2011). Leptin is released in response to feeding and can act on the hypothalamus and ventral tegmental brain areas to produce satiety signals and reduce the reward value of food, thus reducing feed behavior and increasing energy expenditure (Tomiyama et al. 2012). Paradoxically, leptin is elevated in obese individuals. Produced in proportion to the amount of energy stored within adipocytes, circulating levels of leptin are markedly elevated in obese individuals and correlate with adiposity levels in non-obese individuals (Brydon et al. 2008, Brydon 2011), yet obese individuals are resistant to the inhibitory activity on food intake. In rats, a chronic infusion of leptin increases heart rate, arterial blood pressure and circulating catecholamines, which can be inhibited by α1 and β1/β2 adrenergic antagonists, although the relationship in humans is less clear (Ren 2004; da Silva et al. 2006).

Additionally, leptin is involved in immune regulation, hematopoiesis, thermogenesis, reproduction, and angiogenesis (Procaccini, Jirillo, and Matarese 2012). Within immune regulation, leptin displays both pro-inflammatory and anti-inflammatory properties. Structurally, leptin belongs to the type 1 superfamily of cytokines, with a receptor analogous to the type 1 cytokine receptor family (Li, Li, and Zhao 2006). Leptin has actions similar to those of other acute phase reactants with elevations following inflammation and infection, acting on the liver, monocytes, macrophages, dendritic cells, T and B cells, adipose tissue, and blood vessel walls (Iikuni et al. 2008). Leptin is known to stimulate the activation and proliferation of monocytes and macrophages and increase pro-inflammatory cytokine production, such as TNF-a and IL-6 from macrophages and B cells (Procaccini, Jirillo, and Matarese 2012; Speaker and Fleshner 2012; Iikuni et al 2008). Additionally, TNF-α and IL-1β increase the expression of leptin mRNA and protein in adipose tissue, further promoting an inflammatory response (Speaker and Fleshner 2012). In contrast, leptin increases the production of the anti-inflammatory hormone adiponectin,

as well as IL-1Ra, and under normal physiological conditions, reduces TNF-α production in

adipocytes (Li, Li, and Zhao 2006). While elevated levels of leptin seem to be favorable in those

fighting and recovering from HIV, bacterial infection, and sepsis, leptin appears to contribute to

the systemic inflammation in obesity and other chronic inflammatory diseases (Iikuni et al.

2008). Interestingly, higher concentrations of leptin are found in females compared with males of

the same BMI due to an inhibition of leptin production by testosterone and a proposed increase

in leptin production by estrogen and progesterone (Iikuni et al. 2008).

Leptin influences the SAM and HPA axes in order to regulate metabolic activity during

stress, and the activation of the SAM and glucocorticoids can influence the production of leptin,

although the relationships of each system with leptin are unknown following AMS (Tomiyama et

al 2012; Bjorntorp 2001; Brydon 2011; Roubos et al. 2012). Leptin has been evaluated in

relation to real life psychological stressors and a higher perception of life stress and higher

emotional peer related stress are associated with greater basal leptin concentrations (Otsuka et al

2006; Kohlboeck et al 2014). However, equivocal evidence exists regarding plasma leptin

concentrations following laboratory AMS tasks (Brydon et al. 2008; Tomiyama et al 2012).

Brydon reported a significant increase in plasma leptin 45-minutes following a Stroop color

word and public speaking task in healthy college aged females (Brydon et al. 2008).

Interestingly, waist circumference was associated with an increase in plasma leptin immediately

following the AMS task; however, this association was not observed 45-minutes post- task. In

contrast, Tomiyama and colleagues did not report a significant increase in leptin in

postmenopausal women following the TSST, with significant variation in responses ranging

from both negative and positive changes in leptin. Furthermore, the reactivity was not associated

with BMI or resting leptin levels. The impact of female sex hormones may help account for the

differences between these two studies. Additionally, it is interesting to note that cortisol

reactivity had no association with leptin (no sympathetic markers were examined).

Interestingly, many actions of leptin have known associations with IL-1β and IL-1Ra. IL-1Ra and IL-1β levels are elevated in obese individuals and leptin appears to increase the production of both cytokines in a cell specific manner. IL-1β can increase the production of leptin in adipose tissue, and leptin can induce IL-1β production in pancreatic B cells and microglial cells and IL-1Ra production in adipocytes and monocytes (Perrier, Caldefie-Chézet, and Vasson 2009). In obese individuals, plasma levels of IL-1Ra have a significant correlation with leptin levels and BMI, representative of the production of IL-1Ra by adipose tissue and the leptin-induced production by monocytes (Juge-Aubry, Henrichot, and Meier 2005). The hypothalamic effects of leptin on appetite suppression depend heavily on the presence of IL-1β (Luheshi et al. 1999). While the administration of leptin suppresses food intake, the co-administration of leptin and IL-1Ra inhibits the suppression of food intake observed in leptin-treated rats. Furthermore, knockout mice lacking the IL-1 receptors showed no reduction in food intake in response to a central injection of leptin. It was therefore proposed that the actions of leptin on food intake could be associated with and potentially mediated by the presence of central IL-1 (Luheshi et al. 1999). Additionally, it has been hypothesized that obesity-related increases in IL-1Ra may contribute to reduced central leptin sensitivity in obese patients due to the effect of IL-1Ra on IL-1 receptors, similar to the inhibition of hypothalamic signaling of leptin by IL-1Ra in rodents (Meier et al. 2002). Leptin also has important roles in lipid metabolism and adipogenesis that may be partially mediated by IL-1β and inhibited by IL-1Ra. Leptin stimulates lipolysis and fatty acid oxidation in adipocytes and also inhibits pre-adipocyte proliferation, stimulates adipocyte apoptosis, and slows insulin-dependent lipogenesis with the

inhibition of lipoprotein lipase (Houseknecht and Spurlock 2003). These peripheral effects of leptin may be partially dependent on IL-1β, as IL-1β can stimulate lipolysis and suppress lipogenesis in adipocytes in vitro (Brydon et al. 2008). Likewise, a deficiency in IL-1Ra reduces adipogenesis and IL-1Ra gene knockout mice present with reduced body fat accumulation (Juge-Aubry, Henrichot, and Meier 2005). Interestingly, while IL-1β improves peripheral glucose uptake, IL-1Ra administration induces insulin resistance in rat skeletal muscle and plasma levels of IL-1Ra correlate to insulin resistance in humans (Juge-Aubry, Henrichot, and Meier 2005). The elevation in IL-1Ra in obese individuals may contribute to a pro-obese state, inducing leptin resistance in the hypothalamus, reducing lipolysis and increasing adipocyte accumulation, and contributing to insulin resistance within muscle tissue (Brydon et al. 2008). Other support for leptin and IL-1β interaction includes the synergist promotion of breast cancer development, and in contrast, an attenuation of the cardiosuppressive effects of IL-1β by leptin in cardiac myocyte contractility (Perrier, Caldefie-Chézet, and Vasson 2009, Radin et al. 2008).

It appears evident that there are specific neural, endocrine, and immune responses to acute stressors, as the effects of AMS models on catecholamine, glucocorticoid, cytokine, and cytokine mediators have been examined, and discussed above. Chronic stress seems to elicit similar activation of each system; however, the prolonged responses contribute to low-grade inflammation often observed in stressors such as obesity. Therefore, it is of interest to examine if there are different endocrine and immune responses to AMS models in healthy compared with chronically stressed individuals who already exhibit marked basal inflammation.

The Effect of Obesity on Acute Mental Stress

Obesity and psychological stress are major independent risk factors for CVD, but little is known about the potential inflammatory response of the combined stressors. Increased adiposity has been associated with a blunted cardiovascular response to AMS, as heart rate, blood pressure, cardiac output, stroke volume, and total peripheral resistance have all shown reduced reactivity (Carroll, Phillips, and Der 2008; Jones et al. 2012; Phillips 2011). On the other hand, a few studies have supported an association between increased adiposity and an exaggerated response of the HPA axis. A larger cortisol responses to AMS has been shown in women with a BMI>30 compared with women with a BMI<30 (Benson et al. 2009). ACTH levels also increased following AMS, but this response was not significantly different between groups. Epel and colleagues reported increased cortisol responses in individuals with larger waist hip ratios (Epel et al. 2000). Interestingly, although cortisol levels increased in response to AMS in obese individuals, those same participants demonstrated both an increase (Epel et al. 2000) and decrease (Benson et al. 2009) in AMS-induced anxiety. Epel and colleagues also repeated the AMS model over four days to measure any change in response due to stressor habituation and found that women with a higher waist to hip ratio lacked habituation and continued to secrete significantly more cortisol in response to familiar challenges. Moreover, no significant differences in EPI or NE responses between obese and lean individuals have been reported following an AMS task (Sothmann, Hart, and Horn 1995, Huang et al. 2014), although associations have been observed between changes pre- to post in NE and both BMI and percent BF (Huang et al. 2014).

Very few studies have been published on the immune responses to AMS in obese individuals. Only one known study has examined immune cell redistribution. No differences

were observed in the redistribution of leukocyte subpopulations between obese and non-obese women after public speaking (Benson et al. 2009). Additionally, there is little evidence of the impact of obesity on cytokine responses to AMS. The same public speaking stressor by Benson and colleagues induced a significant increase in IL-6 45-minutes post-task in all subjects combined. Obese subjects displayed significantly higher IL-6 levels both pre- and post-task compared to the non-obese subjects, however, the change in IL-6 from baseline to 45-minutes post-task was not significantly different between groups (Benson et al. 2009). The authors noted that IL-6 changes are often significantly different from baseline at later time points and evaluation of IL-6 at 2-hours post-task may have revealed significant differences between groups.

LPS-stimulated mRNA and plasma levels of IL-6 and TNF-α have also been analyzed in males after twenty minutes of alternating Stroop color word and mental arithmetic tasks (Huang et al. 2011). The percent change in LPS stimulated plasma IL-6 from pre- to immediate post-stress demonstrated a significant positive relationship to BMI. No significant association was observed between LPS stimulated IL-6 mRNA and BMI. The percent change in LPS stimulated TNF-α mRNA levels (from pre- to immediate post-stress) displayed a significant negative correlation with BMI. It should be noted that both LPS-stimulated IL-6 mRNA and plasma TNF-α did not exhibit significant correlations with BMI, although overall trends following AMS were similar between mRNA and protein secretion. These results may indicate different patterns of cytokine behavior in the presence of a dual stressor. Additionally, these results indicate that adiposity may affect the synthesis and release of cytokines following AMS in a time specific manner.

There has been no known evaluation of IL-1β, IL-10, and IL-1Ra in obese individuals in response to AMS, although IL-1Ra responses have indicated an interesting association with waist circumference in healthy individuals. Plasma IL-1Ra was analyzed in college-aged females after completing a five minute Stroop color word task and five minute public speaking task (Brydon et al. 2008; Brydon 2011). Increases in plasma IL-1Ra levels 45-minutes post-AMS were positively associated with waist circumference independent of age, ethnicity, smoking, and basal cytokine levels. Additionally, when subjects were divided into tertiles by waist circumference, those with a high waist circumference had a 15% greater increase in IL-1Ra at 45-minutes post-task. However, these individuals were categorized with a waist circumference greater than 70 and were not able to be categorized as obese. Waist circumference was also positively related to changes in leptin levels immediately post-stress (Brydon et al. 2008; Brydon 2011). Plasma concentrations of IL-1Ra and leptin were significantly and positively correlated immediately and 45-minutes post-stress, and a positive association was observed between basal leptin levels and the stress-induced increase in IL-6 45-minutes post-task. This suggests that leptin may have a significant impact on the cytokine responses seen with differing levels of adiposity in healthy non-obese individuals, but should be examined within obese individuals.

The effect of obesity on the inflammatory response to AMS is still vastly unknown, and further examination is warranted to facilitate a greater understanding of the combined influence of obesity and psychological stress on CVD risk. The dual stress of AMS and obesity is hypothesized to elicit a greater pro-inflammatory response and a greater risk for CVD than either stressor alone. Therefore, it is of interest to examine the differences in the inflammatory response, specifically IL-1β, IL-1Ra, and leptin following an AMS task in obese and non-obese males. There has been no known concomitant evaluation of IL-1β, IL-1Ra, and leptin following

AMS in obese and non-obese individuals, and the evaluation of all three cytokines is warranted to provide a complete picture of the pro- and anti-inflammatory interactions between these molecules. In addition, evaluation of these inflammatory mediators in males will provide an additional contribution to the literature because males typically display lower plasma leptin and urinary IL-1β levels when compared to females (Iikuni et al. 2008; Lynch, Dinarello, and Cannon 1994), monocytes from males secrete less IL-β (Lynch, Dinarello, and Cannon 1994), and a gender difference in the IL-1Ra gene polymorphism is known to exist (Bessler 2007), suggesting that gender differences may affect the responses of leptin and IL-1Ra to AMS. Additionally, data interpretation is more difficult in females because female sex hormones and the menstrual phases impact cytokine secretion and must be controlled for (Konecna et al. 2000). Therefore, the purpose of this study is to examine IL-1β, IL-1Ra, and leptin following an AMS task in non-obese and obese males in order to provide a greater understanding of the contribution of inflammation and obesity to CVD risk.

III. Research Significance and Aims

Significance

Cardiovascular disease (CVD) is the leading cause of death for both men and women in the United States (US) and contributes to one third of all reported deaths (CDC 2011). Traditional independent risk factors for CVD include family history, age, hypertension, hyper-cholesterolemia, smoking, physical inactivity, impaired fasting blood glucose, and obesity. The prevalence of obesity has reached epidemic proportions in the US, with 68.8% of US adults categorized as overweight and 35.7% categorized as obese (Flegal et al. 2012). Since the 1960's, the prevalence of obesity has risen annually and the Center for Disease Control predicts that 32 million additional Americans will become obese by 2030; increasing the prevalence of adult obesity to 42% (CDC 2011). Substantial evidence has also suggested that psychological stress can all be considered an independent risk factor for CVD (Rozanski, Blumenthal, and Kaplan 1999). A majority of Americans experience psychological stress, with 20% currently reporting extreme stress and 80% reporting their stress levels increasing over the past year (APA 2012).

Both obesity and psychological stress may contribute to one's risk for CVD by operating as chronic stressors and eliciting systemic inflammation (Ippoliti et al. 2013; Brydon 2011; Rozanski, Blumenthal, and Kaplan 1999). The immune response is regulated by circulating hormones and locally released neurotransmitters which spill over into the blood stream from nerve endings. Once a pathogen, trauma, or stressor is detected, the innate, or immediate response system, reacts by inducing local or systemic inflammation. Immune cell signaling

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messengers, known as cytokines, mediate this response by initiating the signaling cascades of the immune system (Elenkov et al. 2005). Cytokines influence the proliferation, differentiation, and activation of immune cells, as well as the synthesis and release of other cytokines. Cytokines are categorized based on their role as pro- or anti-inflammatory messengers, working alongside hormones to turn on or off a specific response to a stressor to provide protection and later to restore homeostasis once a stressor no longer poses a threat.

An acute mental stress (AMS) model is often used to replicate the neural, endocrine, and immune responses of psychological stress (Rimmele et al. 2007; Benson et al. 2009). As the tasks begin and subject anxiety is induced, a corresponding initiation of an inflammatory response has been observed (Steptoe et al 2001; Steptoe, Hamer, and Chida 2007; Benson et al. 2009; Carroll et al. 2008). In general, an acute stressor results in the proliferation and redistribution of immune cells and an increase in both pro- and anti-inflammatory cytokine levels. The increase in cytokines may result from an increase in the release of either stored or synthesized cytokines from immune cells (Steptoe, Hamer, and Chida 2007; Benson et al 2009). AMS models appear to increase IL-6 and IL-1β fairly consistently, while other cytokines are suggested to have more variable results (Steptoe, Hamer, and Chida 2007). Further examination, specifically investigating the effects of gender, time points at which cytokines are evaluated, and different AMS models, may be warranted to help explain the variations reported (Steptoe, Hamer, and Chida 2007).

While an acute stressor elicits a protective inflammatory response which enhances the body's resistance to infection, a chronic stressor impairs the regulation of homeostasis and disrupts the body's ability to defend itself (Gouin 2011). Chronic stressors such as obesity, depression, sleep deprivation and marital stress appear to increase the basal levels of circulating

immune cells and cytokines (Dragos and Tanasescu 2010; Gouin 2011). As such with obesity, adiposity levels have been positively correlated with the concentration of circulating immune cells and cytokines (Ippoliti et al. 2013; Altintas et al. 2012; Divoux et al. 2012; Charles et al. 2011; Benson et al. 2009). Not only do obese individuals have more adipose tissue, but they have more immune cells, such as macrophages and mast cells, within their adipose tissue that contribute to the production of more inflammatory cytokines than adipose tissue of non-obese individuals (Altintas et al. 2012; Divoux et al. 2012).

While obesity and psychological stress are of interest as independent risk factors for CVD, their combined influence on inflammation and CVD risk are also of interest considering the significant prevalence of both obesity and perceived psychological stress in the United States. Previous investigators have demonstrated that a dual stress condition, combining a mental and physical stressor (exercise), elicits a greater physiological strain than either condition alone (Webb et al. 2010). Because obesity is already characterized by disordered inflammatory basal activation, this suggests that the additional burden of a psychological stressor could elicit a greater inflammatory response and a greater risk for CVD than either stressor alone. While cardiovascular variables, such as HR and BP, and hormones, such as cortisol and catecholamines, have been examined in an obese population following AMS, much less research has been reported on the role of cytokines within these combined models of stress (Carroll, Phillips, and Der 2008; Jones et al. 2012; Phillips 2011; Benson et al. 2009). Plasma IL-6 concentrations have been shown to be greater at baseline and 45-min post AMS in obese women as compared to non-obese women, however, the change in IL-6 from baseline to 45-minutes post was not significant. The authors noted that IL-6 changes are often seen at later time points following AMS tasks and evaluation at 2 hours post-AMS task may have revealed significant

differences in the IL-6 response (Benson et al. 2009). Additionally, LPS stimulated plasma concentrations and mRNA for IL-6 and TNF-α have been analyzed in males following AMS, with the change in plasma IL-6 from baseline to post AMS task having a positive correlation with BMI and the change in TNF-α mRNA having a negative correlation (Huang et al. 2011). These results indicate that adiposity may affect the synthesis and release of cytokines following AMS.

In order to further understand the impact of obesity on AMS, examination of interleukin 1-beta (IL-1β), interleukin 1-receptor antagonist (IL-1Ra), and leptin is warranted. Alongside the classical mediators of inflammation, IL-6 and TNF-α, IL-1β is a potent pro-inflammatory mediator of inflammation and fever. IL-1β contributes to the migration and activation of monocytes and lymphocytes, alteration of the vascular endothelium, and the induction of further cytokine synthesis, such as the production of IL-6 (Hamblin 1993; Brydon et al. 2005). Plasma IL-1β has been shown to increase following AMS in vivo and following LPS stimulation in vitro, and significant increases in IL-1β gene expression have been reported (Steptoe, Hamer, and Chida 2007; Altemas et al. 2001; Heinz et al. 2003; Brydon et al. 2005). There is evidence that IL-1β may be elevated in obese individuals at rest (El-Wakkad et al. 2013), however, there have been no known studies examining IL-1β following AMS in relation to measures of adiposity. Evaluation of IL-1β may provide a more complete understanding of the classical inflammatory markers following this dual model of chronic and acute stress.

IL-1Ra, an anti-inflammatory cytokine, competitively binds to IL-1 receptors, preventing its potent pro-inflammatory counterpart, IL-1β, from inducing further inflammatory cytokine production, immune cell migration, and vascular modifications (Charles et al. 2011; Steptoe et al. 2001, Brydon et al. 2005). IL-1Ra is activated by the same intra-cellular messengers which

stimulate IL-1β, such as epinephrine, TNF-α and IL-6, acting as negative feedback and attenuating further pro-inflammatory cytokine production. Resting IL-1Ra levels in obese individuals have been shown to be elevated 6.5 times that of non-obese subjects and significant associations have been shown with BMI, waist and hip circumferences, and percent fat mass in subjects including non-obese men and women (Meier et al. 2002; Juge-Aubry, Henriochet, and Meier 2005; Charles et al. 2011). Interestingly, Meier and colleagues observed marked variability of IL-1Ra concentrations in the obese group, which were suggested to be attributed to the differences in lean body mass, insulin resistance, and leptin (Meier et al. 2002). IL-1Ra has been shown to increase following AMS tasks in both men and women, though gender differences have been suggested (Steptoe et al. 2001; Steptoe et al. 2002; Rohleder et al. 2006). Differences in IL-1Ra production following AMS have not been compared in lean and obese individuals. Concurrent evaluation of IL-1β and IL-1Ra may provide a greater understanding of the balance, or potential imbalance, between pro- and anti-inflammatory cytokine production following AMS in obese individuals.

Lastly, it is of interest to examine leptin due to its functions as an immunoregulator. Leptin, released from adipocytes, is a hormone primarily associated with the regulation of energy balance, appetite, and sympathetic nerve activity (Brydon 2011). Leptin can act on the hypothalamus and ventral tegmental brain areas to produce satiety signals and reduce the reward value of food in order to reduce feeding behavior and increase energy expenditure (Tomiyama et al 2012). Paradoxically, leptin is elevated in obese individuals suggesting a state of leptin resistance. Additionally, leptin is involved in immune regulation, hematopoiesis, thermogenesis, reproduction, and angiogenesis (Procaccini, Jirillo, and Matarese 2012). Within immune regulation, leptin displays both pro-inflammatory and anti-inflammatory properties. Leptin has

actions similar to those of other acute phase reactants with elevations following inflammation and infection, acting on the liver, monocytes, macrophages, dendritic cells, T and B cells. Leptin is known to stimulate the activation and proliferation of moncocytes/macrophages and increase pro-inflammatory cytokine production, such as TNF-a and IL-6 from macrophages and B cells (Procaccini, Jirillo, and Matarese 2012, Speaker and Fleshner 2012, Iikuni et al 2008). Conversely, TNF-α and IL-1β increase the expression of leptin mRNA in adipose tissue, promoting inflammation (Speaker and Fleshner 2012). While elevated levels of leptin seem to be favorable in those fighting and recovering from HIV, bacterial infection, and sepsis, leptin appears to contribute to the systemic inflammation in obesity and other chronic inflammatory diseases (Iikuni et al. 2008). Additionally, activation of the sympathoadrenal-medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes can influence the production of leptin (Tomiyama et al 2012; Bjorntorp 2001). A higher perception of life stress and higher emotional peer related stress are associated with greater basal leptin concentrations (Otsuka et al 2006; Kholboeck et al 2014), however, equivocal results exist regarding plasma leptin concentrations following AMS (Brydon 2011; Tomiyama et al 2012). Brydon reported a significant increase in plasma leptin 45-minutes following a Stroop color word and public speaking task in women in healthy college aged females (Brydon 2011). Interestingly, waist circumference was associated with increase plasma leptin immediately following the task, but not 45-minutes post. In contrast, Tomiyama and colleagues did not report a significant increase in leptin reactivity in postmenopausal women following the TSST, with significant variation in responses ranging from an increase to a decrease in leptin (Tomiyama et al. 2012).

Leptin, IL-1β and IL-1Ra have associated physiological roles impacting the regulation of appetite, energy balance, breast cancer development and cardiac myocyte contractility (Luheshi

et al. 1999; Houseknecht and Spurlock 2003; Brydon et al. 2008; Perrier et al. 2009; Radin et al. 2011). Leptin plays a cell specific role in the production of IL-1β and IL-1Ra, while IL-1β increases the production of leptin in adipocytes. Additionally, IL-1Ra has been proposed to contribute to the central resistance to leptin in obese patients (Meier et al. 2002; Brydon et al. 2008; Brydon 2011). IL-1Ra and leptin have previously demonstrated associations with waist to hip ratio in healthy females following an AMS task (Brydon et al. 2008), although there has been no known concomitant evaluation of IL-1β, IL-1Ra, and leptin following AMS in obese and non-obese individuals. The evaluation of all three immunomodulators is warranted to provide a complete picture of the pro- and anti-inflammatory interactions between these molecules. In addition, evaluation in males is warranted because males typically display lower leptin levels when compared to females (Iikuni et al. 2008) and a gender difference in the IL-1Ra gene polymorphism is known to exist (Bessler 2007), suggesting that gender differences may affect the responses of leptin and IL-1Ra to AMS. Therefore, the purpose of this study will be to examine IL-1β, IL-1Ra, and leptin following an AMS task in non-obese and obese males.

Specific Aims

While the pathophysiologies of obesity and psychological stress are of interest as independent risk factors for CVD, their combined influence on the inflammatory process and CVD risk should be examined due to the significant prevalence of both obesity and perceived psychological stress in the United States. It is therefore of interest to examine the differences in the inflammatory response to an AMS model in obese compared to non-obese individuals. Previous investigators have demonstrated that a dual stress condition elicits a greater physiological strain than either condition alone (Webb et al. 2010), which suggests that the

combined burden of mental stress and obesity could elicit a greater inflammatory response and a greater risk for CVD than either stressor alone.

Of specific interest is the concurrent evaluation of IL-1β, IL-1Ra, and leptin following AMS in obese and non-obese males. Evaluation of IL-1β and IL-1Ra may provide a greater understanding of the balance, or potential imbalance, between pro- and anti-inflammatory cytokine production following AMS in obese individuals, with IL-1β acting to increase inflammation and IL-1Ra acting as an inherent anti-inflammatory feedback control. Additionally, leptin may serve to indicate one mechanism by which AMS may induce hormone and cytokine production, demonstrating relationships with IL-1β and IL-1Ra. This proposal includes two specific aims.

Specific Aim 1 will explore the response of plasma IL-1β, IL-1Ra, and serum leptin concentrations 30 and 120-minutes following an AMS task in obese and non-obese males. No previous studies have reported significant differences in IL-1β, IL-1Ra, or leptin immediately following an AMS task, and therefore these time points were chosen in order to examine the concentration changes following the secretion and signaling of other messengers. Epinephrine and norepinephrine are often released in response to an acute stress and initiate a cascade of cellular reactions, including the release of other cytokines, that are known to influence the synthesis and release of the molecules of interest. Significant changes have been observed in IL-1β and IL-1Ra up to 120-minutes following the task and in leptin up to 45 minutes following the task (Heinz et al. 2003; Brydon et al. 2005; Steptoe et al. 2001; Brydon et al. 2008).

Hypothesis: It is hypothesized that the obese group will demonstrate elevations in resting IL-1β, IL-1Ra, and leptin. Additionally, while no known studies have previously reported IL-1β,

IL-1Ra, and leptin following AMS in relation to measures of adiposity, it is therefore

hypothesized that there will be no significant differences in the change in IL-1β, IL-1Ra, and

leptin from baseline following AMS between obese and non-obese groups.

Specific Aim 2 will examine the relationship of IL-1β, IL-1Ra, and leptin to one another, as well

as to measures of adiposity, in response to an acute psychological stress.

       Hypothesis: Based upon previous investigations demonstrating relationships within cell-

signaling pathways, it is hypothesized that concentrations of IL-1β, IL-1Ra, and leptin will be

significantly associated with one another. Obesity and acute psychological stress will not affect

these relationships.

IV. Manuscript

The Impact of Obesity on IL-1β, IL-1Ra, and Leptin Following

Acute Mental Stress

Abstract

Introduction: Obesity is an independent risk factor for cardiovascular disease (CVD), eliciting chronic systemic inflammation. Previous research within our laboratory has suggested that obesity may impact the production of mRNA and cytokine protein synthesis following acute psychological stress. Therefore, it was of interest to examine additional immune signaling messengers, specifically interleukin 1-beta (IL-1β), interleukin-1 receptor antagonist (IL-1Ra), and leptin, following an acute mental stress (AMS) task in non-obese and obese males. *Methods:* Non-obese (N=10, 21.2±2.6 yrs, 21.8±1.7 kg/m2, 16.7±4.1 %FAT) and obese (N=10, 21.4±2.5 yrs, 37.2±4.5 kg/m2, 40.0±3.5 %FAT) males volunteered to participate in a 20 minute acute mental challenge (Stroop Color-Word Task and Mental Arithmetic Task). Blood was collected prior to the mental challenge and again 30-min and 120-min following the mental challenge. IL-1β, IL-1Ra, and leptin were later quantified via enzyme linked immunosorbent assays. *Results:* There was a significant main effect for time for IL-1β (*P*= 0.027), with an increase observed from 30 to 120-minutes post in the obese group alone (*P*= 0.043). There was a significant main effect for group for IL-1Ra and leptin due to basal elevations (*P*= 0.001 and *P*<0.001, respectively). Leptin displayed a significant main effect for time (*P*= 0.0014), which was not observed with IL-1Ra (*P*= 0.482). Additionally, leptin was significantly decreased at 30-minutes

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post-task in only the obese group (*P*= 0.017). A significant relationship was shown between the percent change in leptin and IL-1Ra 120-minutes following AMS in the non-obese group (r= 0.756, *P*= 0.011), and remained significant when controlling for body mass index (r= 0.725, *P*= 0.027), percent body fat (r= 0.783 *P*= 0.013), resting leptin (r= 0.736, *P*= 0.024), and resting IL-1Ra levels (r= 0.682, *P*= 0.043). This association was not significant in the obese group (r=0.378, *P*=0.281). *Conclusion:* Changes in IL-1β and leptin were only observed within the obese group. Interestingly, the relationship between the change in leptin and IL-1Ra in response to an acute mental challenge was only observed in non-obese individuals and may be impacted by adiposity levels. No association in obese subjects may be attributed to the elevated basal levels of leptin and IL-1Ra observed with increased fat mass.

Introduction

Cardiovascular disease (CVD) is the leading global cause of death, accounting for approximately 30% of all reported deaths (WHO 2011). Among traditional CVD risk factors, obesity is a major target of research and clinical importance due to its epidemic prevalence, with approximately 35.7% of adults in the United States currently categorized as obese (BMI≥ 30) and an additional 32 million additional Americans predicted to become obese by 2030 (Flegal et al 2012, CDC 2011). Obesity contributes to the risk for CVD by acting as a chronic systemic stressor and eliciting inflammation, which includes elevations in basal levels of hormones, immune cells, and cytokines (Ippoliti, Canitano, and Businaro 2013). While an acute stressor may elicit a protective inflammatory response, chronic stressors such as obesity are maladaptive and impair an individual's ability to produce a beneficial immune response to an additional stressor (Dragos and Tanasescu 2010). Previous research within our laboratory has suggested

that obesity may impact the production of mRNA and cytokine protein synthesis following acute psychological stress (Huang et al. 2011), and therefore, it was of interest to examine the effect of obesity on an acute mental stress (AMS) task in the context of other immune signaling messengers.

Interleukin 1-beta (IL-1β) is a potent pro-inflammatory mediator of inflammation, contributing to the activation and migration of immune cells, production of cytokines, and alteration of the vascular endothelium. Interestingly, the same cellular messengers which stimulate IL-1β also stimulate interleukin 1-receptor antagonist (IL-1Ra), a competitive inhibitor of IL-1β. IL-1Ra is categorized as an anti-inflammatory cytokine because it acts as a control to attenuate further pro-inflammatory cytokine production. Basal concentrations of IL-1β have been shown to be elevated in obese adolescent girls with a waist: hip ratio greater than 0.8 (El-Wakkad et al. 2013), Additionally, there is substantial evidence that IL-1Ra is elevated in obese individuals at rest (Meier et al. 2002; Juge-Aubry et al. 2005; Charles et al. 2011). Moreover, both cytokines have been shown to increase following AMS tasks (Steptoe et al. 2001; Steptoe et al. 2002; Rohleder et al. 2006; Heinz et al. 2003; Brydon et al. 2005). Concurrent evaluation of IL-1β and IL-1Ra may provide a greater understanding of the relationship between pro- and anti-inflammatory cytokine production following AMS in obese individuals.

Leptin is a hormone released from adipocytes which is primarily associated with the regulation of energy balance, appetite, and sympathetic nerve activity. The hypothalamic effects of leptin on appetite suppression depend heavily on the presence of IL-1β because mice born without IL-1 receptors have not shown a reduction in food intake in response to a central injection of leptin (Luheshi et al. 1999). Additionally, while the administration of leptin suppresses food intake, the co-administration of leptin and IL-1Ra inhibited the suppression of

food intake observed in leptin treated rats. Within the immune system, leptin exhibits both pro- and anti-inflammatory effects. Cortisol, IL-1β, and TNF-α are known to increase the secretion of leptin, and leptin plays a cell specific role in the production of IL-1β in pancreatic B cells and microglia and IL-1Ra in monocytes and macrophages (Procaccini, Jirillo, and Matarese 2012; Speaker and Fleshner 2012; Iikuni et al 2008; Perrier, Caldefie-Chézet, and Vasson 2009). The production of IL-1Ra following leptin secretion appears to act as a negative feedback mechanism to attenuate the shared pro-inflammatory actions of IL-1β and leptin and may also reduce further leptin production by IL-1β. Leptin levels are considerably elevated in obese individuals and increases in leptin have been reported following AMS tasks (Brydon 2011). Leptin may therefore impact the release of IL-1β and IL-1Ra following AMS and provide evidence for potential differences observed between groups.

To date, there has been no known examination of IL-1β, IL-1Ra, and leptin following AMS in obese and non-obese individuals, and the investigation of all three cytokines is warranted to provide a complete picture of the pro- and anti-inflammatory interactions between these molecules. Concomitant examination of IL-1β and IL-1Ra may provide a greater understanding of the balance between pro- and anti-inflammatory cytokine production following AMS in obese individuals. Additionally, leptin may provide an understanding of a mechanism associated with the production of both cytokines following AMS. Therefore, the purpose of this study was to examine the effect of obesity on IL-1β, IL-1Ra, and leptin concentration changes at 30 minutes and 120 minutes following an AMS task. No previous studies have reported significant differences in IL-1β, IL-1Ra, or leptin immediately following an AMS task, and therefore these time points were chosen in order to examine the concentration changes following the secretion and signaling of other messengers. Epinephrine and norepinephrine are often

released in response to an acute stress and initiate a cascade of cellular reactions, including the

release of other cytokines, that are known to influence the synthesis and release of the molecules

of interest (Dragos and Tanasescu 2010; Rohleder et al. 2006). Significant changes have been

observed in IL-1β and IL-1Ra up to 120-minutes following the task and in leptin up to 45-

minutes following the task (Heinz et al. 2003; Brydon et al. 2005; Steptoe et al. 2001; Brydon et

al. 2008). To our knowledge, no previous studies have reported IL-1β, IL-1Ra, and leptin

concentrations following AMS in obese and non-obese males. Therefore, it was difficult to

predict whether groups would show significant differences following AMS. However, it was

hypothesized that psychological stress in addition to obesity would elicit a greater pro-

inflammatory response than psychological stress alone.

Methods

Subjects

Twenty apparently healthy male subjects between the ages 18-28 from the general population

volunteered to participate in this study. Subjects were categorized by their BMI into either the

non-obese (BMI ≤25) or obese (BMI ≥30) group. Subjects were excluded from the study if they

had known or suspected cardiovascular, metabolic, rheumatologic, or other inflammatory

diseases/conditions or if they were taking medication, using tobacco products, or consuming an

average of more than ten alcoholic beverages per week. Subjects were also excluded if they had

a history of psychological disorders or if they had experienced a major life event (e.g. death in

family, divorce, or wedding) within 30 days of participation. All subjects completed a Seven-

Day Physical Activity Recall questionnaire (Blair et al. 1985), and those who reported more than

150 minutes of moderate or higher physical activities per week were excluded from participation

to limit the impact of physical activity. Subjects were instructed to refrain from exercise for at least 48 hours prior and caffeine and alcohol 24 hours prior to testing. Written informed consent was obtained from each subject before participation in the study. All procedures were approved by Virginia Commonwealth University's Institutional Review Board.

Testing procedures

Subjects were initially screened by completing a medical history questionnaire and a physical activity readiness questionnaire (PAR-Q) prior to the start of testing. Body composition was assessed with dual-energy X-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare, Madison, WI) in order to confirm subject grouping (Non-obese: %BF<25, Obese: %BF≥30). Subjects who met all criteria were instructed to report to the Exercise Physiology Research Laboratory on two different occasions. The first day, participants arrived at 07:00AM. Subjects participated in a four-minute mental stress task to familiarize themselves with the psychological stressor.

Within 48 hours, participants returned to the laboratory at 07:00AM following an overnight fast (at least 9 hours) and an intravenous catheter was inserted by certified phlebotomy technician. Subjects rested for one hour and blood was drawn immediately before the initiation of the AMS task (REST). Blood was drawn 30 (POST30) and 120 (POST120) minutes following the stress task.

Acute Mental Stress Task

A computer-based mental task was used, alternating between two-minute cycles of a Stroop-color word and mental arithmetic tasks for a total of twenty minutes (Huang et al. 2010). Subjects were instructed to do their best and that their scores would be recorded. Auditory

feedback was given by the program when participants entered an incorrect answer. In addition, a research investigator stayed in the room, inducing a socio-evaluative component by providing bogus critical feedback to the subject regarding the number of incorrect answers, the speed of their reactions, and their performance compared to their peers.

Quantification of IL-1β, IL-1Ra, and leptin

Blood samples for analysis of plasma IL-1β and IL-1Ra were collected into EDTA tubes and centrifuged for 15 minutes at 2000×g at 4 °C. Blood samples for the analysis of serum leptin were collected in serum separator tubes, allowed to clot for 30 minutes, and centrifuged for 15 minutes at 2000×g at 4 °C for the analysis of cortisol. All samples immediately aliquoted into microtubes and were stored at −80 °C until analyzed. Concentrations of IL-1Ra and leptin were determined through enzyme-linked immunosorbent assays according to manufacturer's specifications (R&D Systems, Minneapolis, MN). All samples were analyzed in duplicate and the mean concentration of each sample was used for statistical analysis. Inter- and intra-assay coefficients of variation (CVs) for IL-β were 14.0% and 6.5%, respectively. Inter- and intra-assay CVs for IL-1Ra were 10.6% and 3.4%, respectively. Inter- and intra-assay CVs for leptin were 10.4% and 5.5%, respectively.

Statistical Analysis

Subject demographics were compared using descriptive statistics and independent-samples *t*-tests. A mixed between- within repeated measures (RM) ANOVA was used to examine concentrations of IL-1β, IL-1Ra, and leptin across time and between groups. If a significant main effect for time was observed, a RM ANOVA was used to examine differences across time in all

subjects and a Bonferroni post-hoc analysis was used when the F-statistic was significant. Furthermore, paired sample t-tests were used at each time point indicated within the post-hoc analysis to compare the differences in each group. Log transformations were performed on IL-1β, IL-1Ra, and leptin concentrations to approximate normal distributions for all subsequent correlation analyses. Pearson product-moment correlations were utilized to evaluate relationships among demographic characteristics, IL-1β, IL-1Ra, and leptin at rest, and percent changes following the AMS task. All analyses were run using SPSS (V21, Chicago, IL). Data is expressed as mean ± SEM unless otherwise noted with statistical significance set at $P < 0.05$.

Results

Subject Characteristics

Demographic characteristics for both non-obese and obese groups are presented in Table 1. Body fat, weight, BMI, REST IL-1Ra, and REST leptin were significantly different between the groups.

Mental Stress Task

To examine the effect of the AMS task on IL-1β, IL-1Ra, and leptin, concentrations were evaluated at REST, POST30, and POST120. The between-within RM ANOVA for IL-1β produced a significant effect for time ($P= 0.027$) but not group ($P= 0.849$). A subsequent RM ANOVA was used to examine the changes in IL-1β following AMS ($P= 0.022$). Bonferroni pairwise comparisons showed that concentrations at POST120 were significantly different from POST30 ($P= 0.017$). Importantly, there was not a significant difference in POST30 IL-1β concentrations between the two groups ($P= 0.948$). The change from POST30 to POST120 was

examined in each group and was only significant in the obese group ($P= 0.043$), although the relationship approached significance in the non-obese group ($P= 0.070$). There were no significant differences between the concentrations at POST30 or POST120 from PRE ($P= 1.00$, $P= 0.221$ respectively). These results are presented in Table 2 and Figure 1.

The mixed between-within RM ANOVA for IL-1Ra did not produce a significant effect for time ($P= 0.482$) but revealed a significant effect for group ($P< 0.001$), with significantly greater levels of IL-1Ra levels at all time points. These results are presented in Table 2 and Figure 2.

The between-within RM ANOVA for leptin produced a significant effect for time ($P= 0.0014$) and group ($P< 0.001$). A subsequent RM ANOVA was used to examine the changes in leptin following AMS ($P= 0.028$). Bonferroni pairwise comparisons showed that concentrations at POST30 were significantly different from PRE ($P= 0.033$). Additionally, the concentrations at POST120 approached significance from the concentrations PRE ($P= 0.063$). Paired samples t-tests were used to examine where differences lie within each group individually. These results are presented in Table 2 and Figure 3. There was a significant decrease in leptin at POST30 in the obese group ($P= 0.017$) but no significance observed in the non-obese group ($P= 0.240$).

Relationships at Rest

Relationships between basal concentrations of IL-1β, IL-1Ra, and leptin were examined. Table 3 provides the correlation matrix between demographic characteristics and values at REST. IL-1Ra and leptin were significantly related to BMI, percent BF, LBM, IL-1β, and each other. IL-1β was significantly related to percent BF.

Furthermore, these associations were examined in each group individually. Tables 4 and 5 show the correlation matrices in the non-obese and obese groups, respectively. There was a significant association between %BF and leptin with IL-1Ra in the non-obese group alone, and IL-1β and IL-1Ra showed a trend towards an association between baseline concentrations. (r=0.611, *P*=0.061).

Relationship between Leptin and IL-1Ra Following AMS

To examine a pathway by which leptin may influence the release of IL-1Ra, the significant changes in leptin from REST to POST120 were used for all correlation analyses. The percent change in leptin POST 120 was significantly related to the percent change in IL-1Ra POST120 (r=0.584, *p*=0.007). When each group was examined individually, the relationship remained significant in the non-obese group (r= 0.756, *P*= 0.011), shown in Figure 4a, and remained significant when controlling for BMI (r= 0.725, *P*= 0.027), percent BF (r= 0.783 *P*= 0.013), REST leptin (r= 0.736, *P*= 0.024), and REST IL-1Ra levels (r= 0.682, *P*= 0.043). This relationship was not significant in the obese group (r=0.378, *P*=0.281), shown in Figure 4b.

Discussion

The purpose of this study was to examine IL-1β, IL-1Ra, and leptin following an AMS task in non-obese and obese males. Results demonstrated a significant increase in IL-1β from 30-minutes to 120-minutes post-task in the obese group alone and a significant decrease in leptin 30-minutes post-task in the obese group alone. Interestingly, a significant association was observed between basal concentrations of IL-1Ra and leptin in the non-obese group and between the percent change in IL-1Ra and leptin at 120-minutes following an AMS task in non-obese group alone. Leptin has previously been shown to regulate the secretion of IL-1Ra (Gabay et al.

2001). Therefore, the findings within the current study suggest that obesity may impact IL-1β and leptin responses following AMS and may influence the interaction between leptin and IL-1Ra.

The concentrations of IL-β, IL-1Ra, and leptin were examined at rest and following AMS. At rest, IL-1Ra and leptin were significantly elevated in obese males as compared to their non-obese counterparts, which is supported in previous research findings (Juge-Aubry, Henrichot, and Meier 2005; Meier et al. 2002; Brydon et al. 2008). Following the AMS task, there was a significant increase in IL-1β at 120 minutes compared with 30 minutes following AMS in the obese group, suggesting that obese individuals may have a greater inflammatory response following psychological stress. Previous research has supported changes in IL-1β following an AMS task. Results have demonstrated a significant increase in IL-1β in healthy male physicians following a two hour oral presentation at a medical conference (Heinz et al. 2003), and a significant increase 40-minutes following a 15 minute Trier Social Stress Test (TSST) in healthy females (Altemas et al. 2001). Although differences in obese and non-obese individuals have not been previously examined following AMS, one study reported that rats exposed to an acute tail shock stressor exhibited a significant 5-fold increase in subcutaneous IL-1β, but not in visceral IL-1β production (Speaker and Fleshner 2012). Obese individuals have more subcutaneous adipose tissue than non-obese individuals which may contribute to the increase in IL-1β seen within the obese group of the current study. Our results suggest a greater pro-inflammatory response in obese individuals which warrants further examination due to the role of pro-inflammatory cytokines and the development of CVD.

There was no significant change in IL-1Ra over time. Many previous findings have indicated an increase in IL-1Ra at 45, 60, and 120 minutes following different AMS tasks in

women (Steptoe et al. 2001; Steptoe et al. 2002; Rohleder et al. 2006). However, one study reported no significant increase following AMS in healthy young females (Brydon 2011). In males, various results have been reported, with an increase in IL-1Ra 45- and 60-minutes following the TSST in healthy males (Rohleder et al. 2006) and no significant increase 45-minutes following a combination of the Stroop color word and mirror tracing task (Steptoe et al. 2002). A gender difference in the IL-1Ra gene polymorphism is known to exist, which could potentially influence the release and signaling of IL-1Ra between males and females and provide support for the differences shown between our results and others (Bessler 2007). Additionally, there is evidence that different physiological responses occur with different AMS tasks, which may further justify observed differences between studies in male populations (Rimmelle et al. 2007). Our results did not indicate an effect of obesity on the IL-1Ra response to an AMS task in males. One study has reported a significant association between the change in IL-1Ra following AMS and waist hip circumference, however subjects were primarily healthy non-obese females with a mean BMI of 23.3 (*SD*=3.1) and a mean percent BF of 25% (*SD*=5.4) (Brydon 2011). The effects of gender and adiposity on IL-1Ra secretion following AMS should be further examined.

Leptin significantly decreased from resting values at 30-minutes post-AMS task in the obese group, but was not significantly different at any time point in the non-obese group. These findings contrast earlier results which reported an increase in leptin within healthy college aged females at 45 minutes post AMS task (Brydon 2011). However, no significant increases were reported in leptin immediately and 30 minutes following a modified TSST in post-menopausal women, which may indicate a potential involvement in sex hormones influencing the regulation of leptin (Tomiyama et al. 2012). Similar to the current study, the previously mentioned studies reported substantial variation in leptin responses, suggesting both decreases and increases in

leptin concentrations following an AMS task. Observed leptin variations within subjects and between studies may be due to gender and hormonal differences, considering estradiol and dexamethasone have been shown to increase leptin secretion *in vitro* from adipocytes taken from females but not males, who tend to have greater levels of testosterone, a known inhibitor of leptin secretion (Casabiell et al. 1998; Wabitisch et al. 1997). To date, the only known study to examine gender differences in leptin concentrations following an acute stressor reported no significant differences between male and female rats following an acute swimming task (Zarian, Karimi, and Dorneyani 2011). However, no known studies evaluating gender differences in leptin concentrations following an acute stressor have been reported in humans, warranting future studies that examine leptin production in males and females. Additionally, catecholamine secretion, which has been shown to increase following AMS, is known to decrease leptin concentrations (Huang et al. 2014; Rayner and Trayhurn 2001), which may support our reduction in leptin. Catecholamine secretion should be examined in non-obese and obese subjects following AMS in relation to leptin secretion.

In the present study, the significant concentration difference between leptin at rest and 30 minutes post-task occurred only in obese individuals. While Tomiyama and colleagues reported no significant association between leptin responses and BMI (2012), subjects were healthy post-menopausal women with a BMI range of 17.7- 37.5. Examination of the relationship between leptin and BMI in all subjects may have blunted any relationship that may have been seen in the obese subjects alone. In addition, differential timing of leptin quantification may have contributed to differences in results, as blood draws were taken 60 and 90 minutes post-task.

Previously, Meier and colleagues proposed that variability within IL-1Ra levels in obese individuals at rest could be associated with leptin and lean body mass (Meier et al. 2002).

Therefore, associations between adiposity levels, LBM, and blood marker concentrations were examined. Our results suggested that IL-1Ra and leptin demonstrated significant relationships with BMI, percent BF, LBM, IL-1β, and one another in all individuals, but few relationships remained significant when each group was examined individually. This may be attributed to the small variation in adiposity within in each group.

Interestingly, basal IL-1Ra concentrations were significantly associated with percent BF and basal leptin levels in non-obese subjects only, suggesting that obesity may impact this relationship. Monocyte and adipocyte *in vitro* stimulation by leptin has previously been shown to increase IL-1Ra secretion, although this has not been examined in cells taken from obese individuals (Gabay et al. 2001; Faggioni et al. 1999; Perrier, Caldefie- Chezet, and Vasson 2009). Obese individuals display greater leptin concentrations, which appear to contribute to reduced central leptin sensitivity in obesity (Zhang and Scarpace 2006). Impaired leptin signaling, due to reduced leptin receptor sensitivity or saturated leptin signaling, may impact the relationship between leptin and IL-1Ra at rest in obese individuals. Moreover, a significant relationship was found between the percent change in leptin and IL-1Ra 120 minutes following an AMS task in non-obese subjects. This relationship existed while controlling for percent BF and baseline concentrations of leptin and IL-1Ra, but was not significant in the obese group. It is plausible that impaired leptin signaling may also be responsible for altering the relationship between leptin and IL-1Ra in obese individuals following AMS. An additional hypothesis is that IL-1Ra may be primarily regulated by different hormones and cytokines following AMS in obese compared with non-obese individuals. Examination of leptin receptor sensitivity could provide additional information about the effects of leptin on anti-inflammatory cytokine production following acute stress.

These results must be viewed within the context of this study's limitations. There was no group of individuals who did not perform the AMS task to differentiate physiological leptin responses due to diurnal variability and fasting. However, the diurnal variation in leptin, linked to food intake, has been shown to be completely abolished in mice following a period of fasting (Ahren 2000). Our subjects reported to the Exercise Physiology Research Lab in a fasted state (~9 hours postprandial). Additionally, while fasting reduces leptin concentrations over time, heart rate increased in all subjects following AMS (data not reported), indicating sympathetic stimulation, which has been shown to reduce leptin secretion from adipocytes (Gabay et al. 2001; Faggioni et al. 1999; Perrier, Caldefie- Chezet, and Vasson 2009). Additionally, the exact time course for the secretion of hormones and cytokines is unknown, and our values were limited to 30 and 120 minutes following our 20 minute stressor. The time points at which these responses peak and return to baseline are unknown. Therefore our findings should only be taken in view of the specific time points examined.

In conclusion, there was a significant change in IL-1β and leptin following AMS in the obese group. This finding suggests that obesity may influence the physiological response to an AMS task. Additionally, there was a significant relationship between the percent change in leptin and IL-1Ra following the AMS task in non-obese individuals. This relationship was not observed in the obese subjects. Furthermore, the release of leptin and IL-1Ra following an AMS task may be different in males compared with previously observed findings in females. Additional research is warranted that further examines the effects of obesity and gender on AMS responses and their association to CVD risk. The results of this study suggest that while leptin and leptin resistance are often examined in relation to hunger and satiety in obese individuals, consideration

should be given to other physiological consequences related to leptin, including relationships to cytokine activity following acute stressors.

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List of Tables and Figures

Table 1: Subject Characteristics

Variable	Non-obese	Obese	*P* value
Age (yrs)	21.2 ± 0.83	21.4 ± 0.79	0.863
Height (cm)	176.4 ± 2.06	180.7 ± 2.63	0.220
Weight (kg)	67.2 ± 2.05	122.1 ± 6.86	<0.001*
BF (%)	16.7 ± 1.30	40.0 ± 3.54	<0.001*
BMI (kg/m^2)	21.8 ± 0.55	37.2 ± 4.57	<0.001*
REST IL-1β (pg/mL)	0.185 ± 0.022	0.227 ± 0.017	0.145
REST IL-1Ra (pg/mL)	158.7 ± 12.99	409.47 ± 40.17	<0.001*
REST Leptin (ng/mL)	1.74 ± 0.33	26.20 ± 0.047	<0.001*

Data are shown as mean ± SEM.

BF, body fat; BMI, body mass index; REST, resting values taken before the mental stressor; IL-1β, interleukin 1-beta; IL-1Ra, interleukin 1-receptor antagonist

* *P*<0.05

Table 2: IL-1β, IL-1Ra, and Leptin in Non-Obese and Obese Subjects Following AMS

Variable	Measure	REST	POST30	POST120
Non-obese	IL-1Ra (pg/ml)	158.7 ± 13.0	158.9 ± 11.5	146.0 ± 7.31
	IL-1β (pg/ml)	0.19 ± 0.022	0.20 ± 0.030	0.30 ± 0.070
	Leptin (ng/ml)	1.7 ± 0.33	1.4 ± 0.28	1.3 ± 0.21
Obese	IL-1Ra (pg/ml)	409.5 ± 40.17	398.9 ± 35.43	393.7 ± 35.75
	IL-β (pg/ml)	0.23 ± 0.017	0.19 ± 0.033	0.28 ± 0.036**
	Leptin (ng/ml)	26.2 ± 4.71	23.8 ± 4.12*	24.1 ± 4.49

Data are shown as mean ± SEM.

*$P < 0.05$ different from REST

**$P < 0.05$ different from POST30

Table 3: Correlations between Blood Markers and Demographic Characteristics at REST in All Subjects

Variable	BMI	%BF	LBM	IL-1β	IL-1Ra	Leptin
BMI	1.0	0.914*	0.837*	0.351	0.830*	0.824*
%BF	-	1.0	0.613*	0.475*	0.868*	0.928*
LBM	-	-	1.0	0.151	0.575*	0.538*
IL-1β	-	-	-	1.0	0.573*	0.509*
IL-1Ra	-	-	-		1.0	0.868*
Leptin	-	-	-	-	-	1.0

* $P < 0.05$

Table 4: Correlations between Blood Markers and Demographic Characteristics at REST in the Non-obese Group

Variable	BMI	%BF	LBM	IL-1β	IL-1Ra	Leptin
BMI	1.0	0.557	0.219	-0.043	0.203	0.380
%BF	-	1.0	-0.071	0.564	0.414	0.727*
LBM	-	-	1.0	-0.434	-0.025	-0.233
IL-1β	-	-	-	1.0	0.611	0.391
IL-1Ra	-	-	-		1.0	0.649*
Leptin	-	-	-	-	-	1.0

* $P < 0.05$

Table 5: Correlations between Blood Markers and Demographic Characteristics at REST in the Obese Group

Variable	BMI	%BF	LBM	IL-1β	IL-1Ra	Leptin
BMI	1.0	0.256	0.787*	0.026	0.534	0.533
%BF	-	1.0	-0.238	0.095	0.610	0.201
LBM	-	-	1.0	-0.068	-0.430	0.055
IL-β	-	-	-	1.0	0.555	0.487
IL-1Ra	-	-	-	-	1.0	0.313
Leptin	-	-	-	-	-	1.0

*$P<0.05$

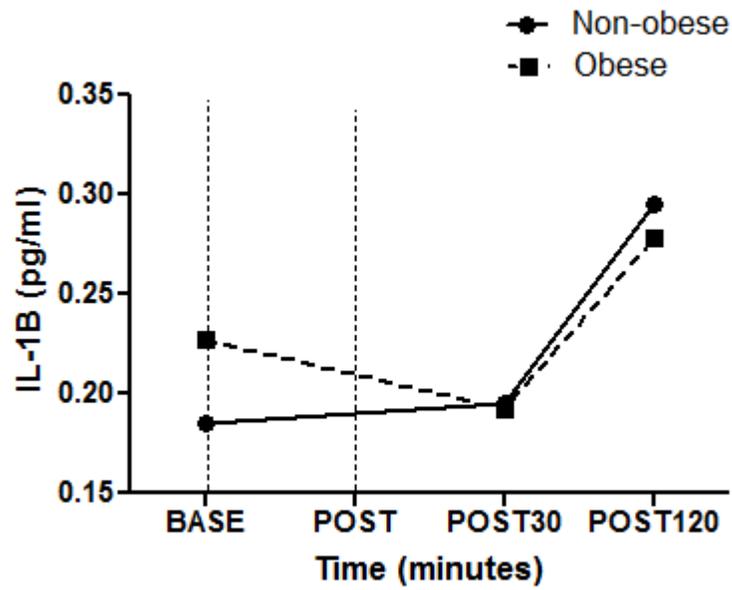**Figure 1: Effect of AMS on IL-1β Concentrations in the Non-obese and Obese Group**

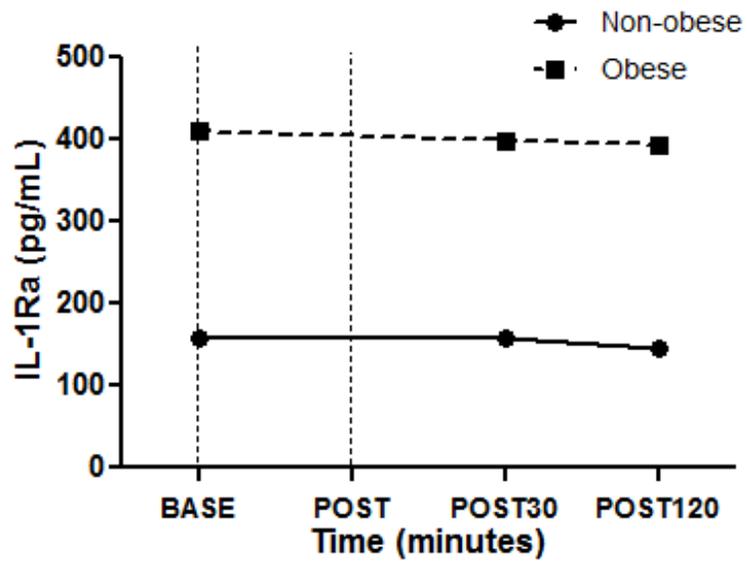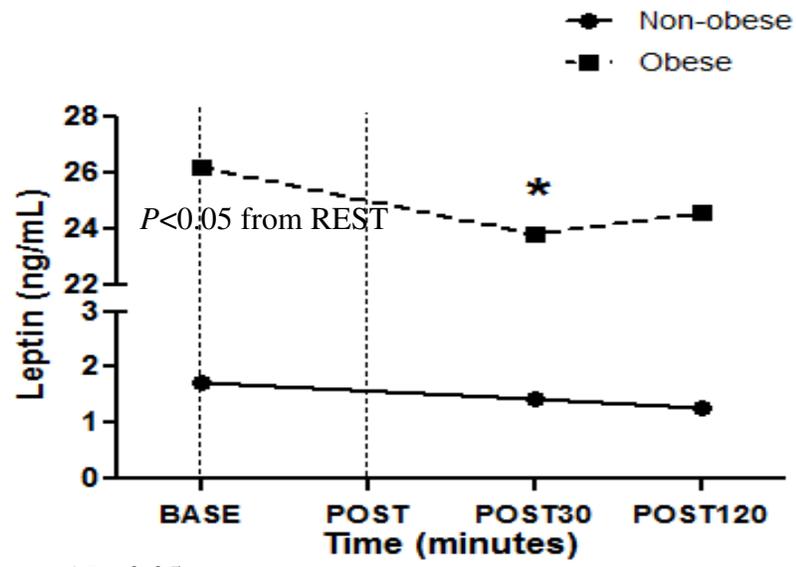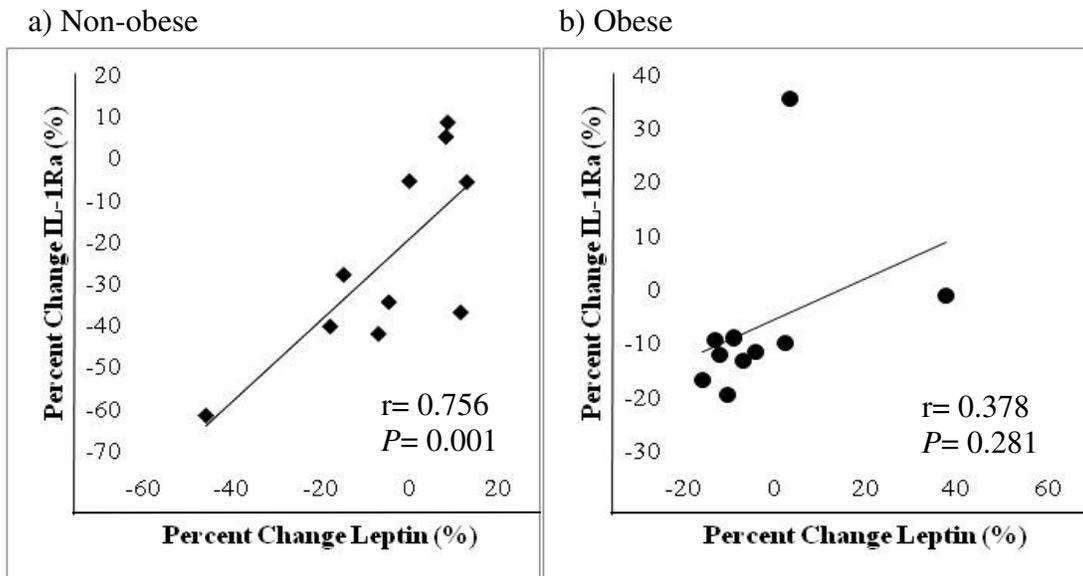**Figure 2: Effect of AMS on IL-1Ra Concentrations in the Non-obese and Obese Group**

Figure 3: Effect of AMS on Leptin Concentrations in the Non-obese and Obese Group

*P< 0.05

Figure 4: Relationship between the Percent Changes in Leptin and IL-1Ra POST120 in the Non-obese (a) and Obese (b) Groups.

a) Non-obese   b) Obese

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Appendix I.

Expanded Methods

Subjects

20 apparently healthy male subjects between the ages 18-28 were recruited from the general

population near VCU and volunteered to participate in this study. Subjects were categorized by

their BMI into either the non-obese (BMI ≤25) or obese (BMI ≥30) group. Body mass index

(BMI) was calculated as weight (kg)/height (m2). Height was measured with a standing

stadiometer (Mobile Stadiometer model 213, Seca, Hanover, MD) to the nearest centimeter and

weight was measured to the nearest kilogram with a scale (Mini Platform Stand on Scale,

Scaletronix, White Plains, NY). Subjects were excluded from the study if they had known or

suspected cardiovascular, metabolic, rheumatologic, or other inflammatory diseases/conditions

or if they were taking medication, using tobacco products, or consuming an average of more than

ten alcoholic beverages per week. Subjects were also excluded if they had a history of

psychological disorders or if they had experienced a major life event (e.g. death in family,

divorce, or wedding) within 30 days of participation. To limit the impact of physical activity, all

subjects completed a Seven-Day Physical Activity Recall questionnaire (Blair et al., 1985) and

those who reported more than 150 minutes of moderate and higher physical activities per week

were excluded from participation (See appendix II). Subjects were instructed to refrain from

exercise for at least 48 hours prior and caffeine and alcohol 24 hours prior to testing. Written

informed consent was obtained from each subject before participation in the study. All

procedures were approved by Virginia Commonwealth University's Institutional Review Board.

Testing procedures

Subjects were initially screened by completing a medical history questionnaire and a physical activity readiness questionnaire (PAR-Q) prior to the start of testing (See appendix III and IV). Body composition was assessed with dual-energy X-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare, Madison, WI). Subjects who met inclusion criteria were instructed to report to the Exercise Physiology Research Laboratory on two different occasions. The first day, participants arrived at 07:00AM following an overnight fast. Subjects participated in a four-minute mental stress task to familiarize themselves with the psychological stressor.

Within 48 hours, participants returned to the laboratory at 07:00AM and an intravenous catheter was inserted by certified phlebotomy technician. Subjects rested for one hour and blood was drawn immediately before the initiation of the AMS task (REST). Blood was drawn immediately following the stress task (POST), and 30 (POST30), 60 (POST60), and 120 (POST120) minutes after the stress task.

Acute Mental Stress Task

A computer-based mental task was used, alternating between two-minute cycles of the Stroop-color word and mental arithmetic tasks for a total of twenty minutes (Acevedo et al. 2006, Webb et al. 2011). Participants were presented with a color word for 0.5 s, displayed in a conflicting font color with simultaneous audible presentation of a word color. Participants were continuously provided with on-screen feedback regarding the number of answers correct, wrong, and un-answered during the Stroop-color word task. The mental arithmetic task involved subtracting a single- or double- digit number from a triple-digit number presented

on the screen. When a response was incorrect, a buzzer went off and ''WRONG'' was displayed on-screen. An example of computer screenshots during the test can be found in Appendix V. Subjects were instructed to do their best and that their scores would be recorded. In addition, a research investigator stayed within the room, recording HR each minute and inducing a socio-evaluative component by providing negative feedback to the subject regarding the number of incorrect answers, the speed of their reactions, and their performance compared to their peers. The script of evaluative comments included, "you're responding too slowly'' and ''most people do much better than this."

Quantification of IL-1β, IL-1Ra, and leptin

Blood samples for analysis of plasma IL-1β and IL-1Ra were collected into EDTA tubes and centrifuged for 15 minutes at 2000×g at 4 °C. Blood samples for the analysis of serum leptin were collected in serum separator tubes, allowed to clot for 30 minutes, and centrifuged for 15 minutes at 2000×g at 4 °C for the analysis of cortisol. All samples immediately aliquoted into microtubes and were stored at −80 °C until analyzed. Concentrations of IL-1β, IL-1Ra, and leptin were determined through enzyme-linked immunosorbent assays according to manufacturers specifications (R&D Systems, Minneapolis, MN). All samples were analyzed in duplicate with the standard zero OD subtracted from all standards and samples to reduce background noise. The standard curves were generated using a line of best fit through the log concentrations versus the log ODs with Microplate Reader Software (Biorad Laboratories, Inc, Hercules, CA). The mean concentration of each sample was used during the statistical analysis and the mean CV for all plates are reported for each analyte. All washes were performed with an Immunowash 1575 automatic plate washer (Biorad Laboratories, Inc, Hercules, CA).

Appendix II.

Seven-Day Physical Activity Recall Questionnaire

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

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

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

Appendix III.

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		Asthma

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

      Other _____ (please specify)

Have you been diagnosed with any of the following? If yes, please circle the appropriate ailment.

      Rheumatic fever   High blood pressure   Kidney/Liver disease

      Diabetes   High Cholesterol   Color Blindness

Have you ever been diagnosed with a psychological disorder? _____ Yes _____ No

Part III. Health Related Behavior

Do you smoke? _____ Yes _____ No

Do you drink alcohol? _____ Yes _____ No

      If yes, indicate number of alcoholic beverages per week?

      _____ Less than 10   _____ 10   _____ Greater that 10

Do you exercise regularly (30 minutes, 3 times per week)? _____ Yes _____ No

      If so, what exercises do you participate in regularly? _____

Have you ever been diagnosed with asthma? _____ Yes _____ No

      If so, are you currently using an inhaler? _____ Yes _____ No

Have you ever been diagnosed with allergies? _____ Yes _____ No

      If yes, please list medications (prescription or over-the-counter) you are currently taking

Have you been diagnosed with hearing problems? _____ Yes _____ No

Have you been diagnosed with vision problems? _____ Yes _____ No

Have you recently (within 1 month) experienced a major negative life event (i.e., death in family, divorce)? _____ Yes _____ No

Are you taking any medications (prescription/nonprescription) or supplements? ____Yes____No

If yes, please list: _____

_____

Appendix IV.

Physical Activity Readiness Questionnaire

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	Has your doctor ever said that you have a heart condition **and** that you should only do physical activity recommended by a doctor?
☐	☐	2.	Do you feel pain in your chest when you do physical activity?
☐	☐	3.	In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
☐	☐	6.	Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7.	Do you know of **any other reason** why you should not do physical activity?

If

you

answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to **all** PAR-Q questions, you can be reasonably sure that you can:
- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:
- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE_____

SIGNATURE OF PARENT _____ WITNESS _____
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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Appendix V.

Stroop Color Word and Mental Arithmetic Examples