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Obesity Related Perturbations On Cardiovascular, Immune And Endocrine Response To Active And Passive Stress

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OBESITY RELATED PERTURBATIONS ON CARDIOVASCULAR, IMMUNE AND ENDOCRINE RESPONSE TO ACTIVE AND PASSIVE STRESS

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ABSTRACT

As the obesity epidemic increases, the prevalence of obesity-related diseases grows simultaneously. The internal environment of chronic low-grade inflammation, which characterizes obesity, leaves individuals vulnerable to disease. Cardiovascular dysfunction represents a consequential effect of obesity. An early indicator of cardiovascular disease is an impaired stress response. Further, the type of stress (active versus passive) may be differentially affected representing additional distinctions in impairment. In the present study, we have used two types of stress (active and passive) to investigate obesity-instigated alterations in cardiovascular, immune and endocrine response to stress. In addition, we have evaluated how these systems may overlap to perpetuate a potentially damaging stress response.

Based on correlations used to determine relationships among the cardiovascular, immune and endocrine system we found that obese and non-obese groups responded similarly to active stress. However, the obese group exhibited blunted cardiovascular recovery following active stress. Conversely, the obese and non-obese groups responded differently to passive stress with the obese group demonstrating an overall greater stress response. This response included exaggerated reactivity of both the cardiovascular and endocrine systems. This suggests a possible link in obese individuals between the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis when activated in response to passive stress. These results indicate that the presentation of an abnormal stress response may occur at different times based on the type of stress. In response to

passive stress, obese individuals show an exaggerated stress response; whereas the impairment associated with active stress appears during recovery.

I dedicate this work to the next generation of inquisitive minds, namely:

Alexia Page

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I. INTRODUCTION

Obesity

Obesity is a rapidly spreading epidemic associated with devastating health complications including an increased risk for premature death (Katzmarzyk, Janssen, & Ardern, 2003). Approximately 2.8 million deaths are attributed to obesity each year, making obesity the "fifth leading risk for global deaths." (WHO, 2013). Worldwide more than one in 10 adults are considered obese (defined as having a body mass index ≥ 30). The United States has one of the highest concentrations of obesity in the world, with more than one-third of the adult population considered obese. According to the Centers for Disease Control and Prevention in 2008, the medical costs associated with obesity were estimated at $147 billion (Finkelstein, Trogdon, Cohen, & Dietz, 2009).

Obesity arises from an imbalance between energy consumption and expenditure. The availability of food with poor nutritional value and high caloric content coupled with the increasingly sedentary lifestyle that predominates American life serves as the impetus that propels the obesity epidemic. Although obesity is found in all nations, nationalities and economic groups, some disparities do exist. In the United States, the obesity epidemic appears concentrated in the southeastern region. This overlaps the area that has been identified as the "stroke belt" and later the "diabetes belt" due to the increased prevalence of these diseases (Borhani, 1965; Barker, Kirtland, Gregg, Geiss, & Thompson, 2011).

Excess weight, being the trademark of obesity, exerts considerable influence over many of the body's biological systems. This multi-system involvement has linked obesity to a number of debilitating and mortal disease states. This list includes the leading cause of death worldwide, heart disease, which in the United States accounted for 596,339 deaths in 2011, approximately one in four (Hoyert & Xu, 2012). The pathology of obesity has been shown to increase the likelihood of other vascular complications including stroke, hypertension, diabetes, dyslipidemia, atherosclerosis and peripheral vascular disease (Abel, Litwin, & Sweeney, 2008; Kenchaiah et al., 2002; Lakka, Lakka, Salonen, Kaplan, & Salonen, 2001; Mensah, 2004; Stapleton, James, Goodwil, & Frisbee, 2008; Wang & Nakayama, 2010; Wilson, D'Agostino, Sullivan, & Parise, 2002).

Obesity and the Cardiovascular System

Hypertension, or high blood pressure, is arguably the most recognized risk factor for cardiovascular disease. This recognition may be attributed to its prevalence, with an estimated one in three Americans suffering from hypertension. The number of individuals receiving treatment is much lower because there are no outward symptoms of hypertension, which is why it has been deemed the "silent killer" (CDC, 2013). An individual's blood pressure is represented by two values labeled systolic and diastolic. Systolic blood pressure indicates the maximum amount of pressure in the arteries corresponding to contraction of the heart, and diastolic blood pressure is measured when the heart is at rest and the pressure in the arteries is at its lowest.

Obesity has been shown to contribute to hypertension in multiple ways. One purpose of the circulatory system is to deliver oxygen rich blood, which provides nutrients to all of the tissues in the body. When an individual accumulates excess adipose

tissue the circulatory system, primarily the heart, must compensate for the additional mass by increasing the pressure in the system to ensure all tissues receive adequate blood perfusion. When deoxygenated blood returns to the heart it enters the right atrium and follows a path through the lungs to be oxygenated, the blood then returns to the heart and is pumped by the left ventricle through the aorta to reenter the vascular system. In obese individuals cardiac adaptations must be made to meet the increased metabolic demand. Increased cardiac output is achieved in obese individuals by a more forceful contraction of the left ventricle. Over time the left ventricle hypertrophies, or becomes enlarged (Levy et al., 1988). This alteration in the myocardium known as left ventricular hypertrophy is often found in combination with hypertension in obese individuals (Paunovic, Jakovljevic, & Stojanov, 2006).

The heart is not the only organ that may undergo structural changes instigated by obesity. Another mechanism through which obesity contributes to hypertension is by alteration of the vascular structure. Many obese individuals also suffer from hyperlipidemia, or high levels of lipids (fats) in the blood (Boyd, Koenigsberg, Falkner, Gidding, & Hassink, 2005; Paccaud, Schlüter-Fasmeyer, Wietlisbach, & Bovet, 2000). This condition is more commonly referred to as high triglycerides or high cholesterol. Clinically, cholesterol is quantified as high-density lipoprotein and low-density lipoprotein. When levels of low-density lipoproteins are elevated these proteins begin to migrate into the vascular tissue that makes up the inner most lining of the blood vessel, the endothelium. As the proteins accumulate, they form plaques that cause a narrowing of the lumen, or diameter of the vessel (for a review see, Moreno & Mitjavila, 2003). This process, which results in atherosclerosis, can have various detrimental outcomes defined

by the location of damage in the body. If the blood vessels supplying the heart are affected the individual may suffer from coronary heart disease, if the vessels to the brain are affected it is termed cerebrovascular disease, or if it is the vessels to the legs, peripheral vascular disease. An additional consequence of atherosclerosis is a stiffening of the vessel, which impedes vasodilatation (Cox et al., 1989). Vasodilatation and constriction are mechanisms by which blood pressure is regulated. When blood vessels are unable to dilate effectively, pressure in the circulatory system increases, leading to hypertension.

Despite the negative consequences of obesity, including tissue and vascular remodeling, an extensive amount of research has shown that these changes are not irreversible. Several studies have demonstrated a reversal of many of these alterations following weight loss (MacMahon, Wilcken, & Macdonald, 1986; Reisin et al., 1983; Ziccardi et al., 2002). To lose weight an individual must modify their lifestyle such that the energy they are expending exceeds the energy they consume as food. There are countless reasons people continue to gain or maintain excess adipose tissue. One explanation suggested by emerging research is that an individual's reaction to stress may play a role in maintaining and perhaps contributing to obesity (Björntorp, 2001). It has been well established that irregular cardiovascular functioning in response to stress is predictive of future cardiovascular disease (Obrist, 1981). Increasing evidence suggests obesity may be characterized by cardiovascular dysregulation in response to stress (Carroll, Phillips, & Der, 2008; Phillips, 2011). Further, different types of stress may elucidate distinct mechanisms for the resulting dysregulation.

A potential mediator of this dysregulation and the vascular damage that is often associated with obesity are cytokines. Cytokines are signaling molecules secreted by adipose tissue, many of which promote inflammation and contribute to abnormal endothelial functioning (Calabro, Chang, Willerson, & Yeh, 2005). Cytokines are potent effectors of the immune system and serve as regulators of the stress response. The mechanisms by which cytokines promote inflammation and their relationship with cardiovascular dysfunction will be the focus of the following sections.

The role that adipose tissue plays in chronic inflammation lends to its importance as an independent risk factor in cardiovascular dysfunction. The current study seeks to further establish the relationship between obesity and cardiovascular dysfunction and investigate the role of obesity in immune dysfunction. This will be the first study to examine immunoregulatory alterations in obese individuals following different types of laboratory stress.

Obesity Induced Inflammation

Obesity is characterized by an excess of adipose tissue. Adipose tissue was once thought to be a passive organ, used as a storage device for triglycerides that could be reabsorbed into the blood stream at times of energy depletion. It is now known that adipose tissue, composed mostly of adipocytes (i.e., fat cells), secretes a number of cytokines that promote inflammation (Berg & Scherer, 2005; Van Gaal, Mertens, & Block, 2006). This inflammation contributes to both cardiovascular and immune dysregulation.

Zhang et al. (1994) is often credited as opening the door for adipocyte endocrinology with the identification of adipose tissue as the source of the hormone

leptin. Adipose tissue has also been implicated in immunomodulation through the secretion of proinflammatory cytokines and the presence of immune cells in adipose tissue. Adipose tissue falls into two main categories: white adipose tissue and brown adipose tissue. The latter plays a role in thermogenesis, particularly in newborns, and until recently was thought to be almost non-existent in adults. Emerging evidence suggests not only the existence of brown adipose tissue in adults but also its potential role in weight loss. Although brown and white adipose tissue share a number of characteristics, brown adipose tissue does not appear to contribute to the inflammation present in obese individuals and may even provide some protection to the vascular system during inflammation (Fitzgibbons et al., 2011). Conversely, white adipose tissue, which exists in much greater prevalence, appears to be the bedrock of systemic inflammation.

White adipose tissue is composed primarily of adipocytes. As an individual consumes more calories than they expend, the adipocytes expand to accommodate the surplus of energy. In addition, more adipocytes are manufactured and infiltration by macrophages occurs. Macrophage cells are pervasive in adipose tissue and contribute significantly to the production of proinflammatory cytokines (Weisberg et al., 2003). In healthy individuals, these cytokines are released upon detection of a foreign invader. They work to promote inflammation that activates and directs the immune system to combat the threat. A recent review echoes the conclusion of numerous studies over the last two decades, “chronic tissue inflammation, particularly in adipose tissue, has been considered as a key underlying mechanism for the development of obesity-related metabolic syndrome” (Xu, 2013, p. 21).

Prior to the discovery of cytokine secretion by adipose tissue, clinicians became aware of a clustering of metabolic abnormalities that seemed to promote cardiovascular disease. These risk factors include high blood pressure, elevated blood sugar (a symptom of insulin resistance), dyslipidemia and obesity. According to the International Diabetes Federation, one in four adults worldwide suffer from metabolic syndrome. Until recently, the classification of what disorders constituted metabolic syndrome remained an issue of debate. In 2006, a workshop was held and a committee was formed to establish the worldwide definition of metabolic syndrome (Alberti, Zimmet & Shaw, 2006). According to this definition, an individual must exhibit central obesity and two of the following four symptoms to be diagnosed with metabolic syndrome: high blood pressure, elevated triglycerides, low high-density lipoprotein or elevated blood sugar. The committee also established the "platinum standard" representing the paradigm for a research diagnosis of metabolic syndrome that includes additional features. One of these additional features is confirmation of an inflammatory state, evidenced by elevated proinflammatory cytokines.

Obesity, through a path of inflammation, has been linked to all of the other features of the metabolic syndrome. As described in the previous section, obesity increases blood pressure by a number of mechanisms including a reduction in lumen diameter and hardening of the vasculature. These structural changes to the vasculature result in part from elevated blood lipids. Obesity is often accompanied by dyslipidemia, specifically elevated triglycerides and low high-density lipoproteins. Adipose cells are storages sites for excess triglycerides. When an individual consumes food, the level of circulating triglycerides increases; between meals, the stored triglycerides are released

into the blood stream through hormonal signaling. Hypertriglyceridemia is a condition often found in correlation with obesity where circulating levels of triglycerides are above a normal range. High-density lipoproteins are considered the "good" cholesterol. In contrast to low-density lipoproteins, which circulate through the body and in high concentrations can form plaques, high-density lipoproteins are thought to transport excess cholesterol from the blood to the liver where it can be recycled or broken down to be excreted from the body. Thus, a decrease in the concentration of high-density lipoproteins has obvious consequences. The perpetrator behind this reduction in high-density lipoproteins is unknown, however evidence indicates elevated cytokine production may be partially responsible (Zuliani et al., 2007).

Elevated blood glucose occurs naturally following food consumption. As glucose levels increase, the pancreas releases the hormone insulin into the blood. Insulin facilitates glucose metabolism and storage. Therefore, in the presence of insulin, levels of glucose in the blood will typically decrease. In some individuals, the tissues in the body no longer respond to insulin and as a result, blood sugar remains elevated. This inability of the body to respond to insulin is known as insulin resistance. The pancreas, unaware of the insulin resistance, though sensitive to the rise in glucose, will continue to secrete insulin leading to a state of hyperinsulinemia. The co-occurrence of hyperinsulinemia and obesity is well known, however the direction of this relationship has not been established. An analysis of data from The Bogalusa Heart Study demonstrated a temporal relationship between obesity and hyperinsulinemia (Srinivasan, Myers, & Berenson, 1999). Obesity, as measured by baseline body mass index, was able to predict subsequent hyperinsulinemia, suggesting that obesity precedes insulin resistance.

In addition to regulating glucose, insulin plays a direct role in lipid storage. Fats that are ingested are broken down by pancreatic lipase into free fatty acids and glycerol, which can be further broken down into glucose. Once out of the digestive system, some of the free fatty acids and glycerol are recombined into triglycerides. As insulin rises in response to increased glucose, it activates adipocytes to secrete lipoprotein lipase; this enzyme promotes the break down of triglycerides in the bloodstream and the absorption of free fatty acids into adipocytes, where they are stored as triglycerides. Through the stimulation of lipoprotein lipase, insulin acts as a gatekeeper. When insulin levels are high, adipocytes are able to take up and store lipids from the blood. Moreover, in an attempt to lower blood sugar, insulin also prevents the break down of lipids stored by adipose tissue thereby preventing weight loss. Thus, it is likely that insulin resistance, besides providing some impetus for obesity, also contributes to a perpetual state of adipose retention.

Many proinflammatory cytokines are elevated in obese individuals. Several of these cytokines have been linked to other symptoms of the metabolic syndrome. Two cytokines that are elevated in obese individuals and demonstrate instigation of metabolic functioning are interleukin-1β and interleukin-6. Interleukin-1β belongs to a group of cytokines know as the interleukin-1 family. This family of interleukins is able to regulate the immune response by binding to their receptor. The family contains proinflammatory cytokines such as interleukin-1α and interleukin-1β as well as an endogenous antagonist known an interleukin-1 receptor antagonist.

Interleukin-1β is elevated in obese compared to non-obese individuals (Moschen et al., 2011; Speaker, & Fleshner 2012). Adipose tissue has been shown to be a source of

interleukin-1β (Juge-Aubry et al., 2003; Lagathu et al., 2006). In a study of 21 obese patients undergoing laparoscopic adjustable gastric banding, pre- and post-weight loss levels of interleukin-1β were assessed (Moschen et al., 2011). Six months following the surgery, adipose tissue concentrations of interleukin-1β decreased significantly. This drop in interleukin-1β was accompanied by an increase in insulin sensitivity when compared to sensitivity prior to weight loss.

The effect of interleukin-1β on glucose regulation has been extensively studied. Clinical evidence substantiating this relationship revealed the effect of interleukin-1 blockade on glycemic control. Larsen and colleagues (2007) performed a double-blind, placebo-controlled study to evaluate the effect of anakinra (an interleukin-1 receptor antagonist) in patients with type 2 diabetes. Elevated levels of glucose in the blood characterize type 2 diabetes. This elevation is caused by an individual's inability to produce enough insulin or a decline in the tissues responsiveness to insulin (i.e., insulin resistance). At the end of the 13 week study, participants who had undergone treatment with anakinra exhibited an increase in glycemic control as measured by glycated hemoglobin level. The researchers attributed this outcome to an increase in pancreatic beta-cell functioning. Beta-cells are located in the islets of Langerhans within the pancreas and are responsible for the production of insulin. An in vitro study utilizing isolated rat islets of Langerhans directly assessed the effect of interleukin-1 on beta-cells (Mandrup-Poulsen et al., 1986). The investigators incubated the isolated islets for six days in multiple concentrations of interleukin-1. Following the incubation period, the culture medium was analyzed for the presence of insulin and islets were examined for decomposition. A dose-dependent reduction was found in both insulin production and

disintegration of the islets. This study provides evidence of the damaging effects of interleukin-1 on beta-cells and the ensuing deficit of insulin.

The permanence of the damage to islets caused by exposure to interleukin-1β is currently under debate. Researchers have found that the resiliency of beta-cells is dependent on the duration of exposure to interleukin-1β (Scarim, Heitmeier, & Corbett, 1997), the concentration of interleukin-1β (Spinas et al., 1986), and whether the beta-cells are active at the time of exposure to interleukin-1β, measured by concurrent exposure to glucose (Palmer et al., 1989). Owyang et al. (2010) found that the impairment resulting from exposure to interleukin-1β can be prevented and even reversed through the actions of interleukin-1β antibodies. In a mouse model of diet-induced obesity prophylactic treatment with XOMA 052, an anti-interleukin-1β antibody, was able to improve glucose tolerance, insulin secretion and sensitivity. Unlike anakinra, which inhibits the action of interleukin-1β by competitively binding to its receptor, XOMA 052 binds to interleukin-1β directly, thereby reducing the affinity for its receptor (Owyang et al., 2011). Thus, XOMA 052 does not interfere with the actions of the other interleukin-1 family members, interleukin-1α or interleukin-1 receptor antagonist. Treatment with XOMA 052 that occurs in tandem with diet-induced obesity is of limited clinical significance. Because of the initial absence of noticeable symptoms of health deterioration resulting from obesity, patients fail to seek early intervention. Noting this discrepancy, the investigators included a treatment group that "better reflects the situation of an [already] overweight patient with progressing T2DM" (Owyang et al., 2010). In this group, beginning at six weeks of age, mice were given a high fat diet to promote obesity. Prior to the initiation of treatment at 10 weeks, mice were evaluated and in addition to

obesity displayed impaired glucose tolerance, elevated glucose, impaired insulin production and reduced insulin sensitivity. Mice were then treated with XOMA 052 for nine weeks while continuing the high fat diet. At 19 weeks, mice treated with XOMA 052 demonstrated improved glucose tolerance, a decrease in circulating glucose and improved insulin production and sensitivity.

In addition to the recovery of glucose regulation in obese mice treated with XOMA 052, improvements in the levels of triglycerides, free fatty acids and cholesterol were also seen when compared to control mice (Owyang, et al., 2010). The ability of XOMA 052 to enhance lipid regulation in obesity makes it a potential candidate for the treatment of cardiovascular disease. Currently, work is being done to assess the efficacy of XOMA 052 in animal models of atherosclerosis (Bhaskar et al., 2011). Another drug that inhibits interleukin-1β, Canakinumab, is currently in phase III of clinical trials. Unlike XOMA 052, which inhibits through allosteric binding, Canakinumab prevents activation through competitive binding to interleukin-1β (Blech et al., 2012). Thus, interleukin-1β cannot be bound to Canakinumab and its receptor. This clinical trial, set to conclude in 2016, is evaluating three doses of Canakinumab in the prevention of cardiovascular events in patients who have already experienced a myocardial infarction. This study includes a number of secondary objectives. One is to quantify the progression of atherosclerosis through the monitoring of arterial plaque accumulation. Another secondary objective is to evaluate the effect of Canakinumab on glucose regulation. Patients with a preexisting diagnosis of type 2 diabetes will be monitored for changes in insulin secretion and sensitivity. An additional outcome measure of Canakinumab's effect on glucose regulation will be the time to a diagnosis of type 2 diabetes in patients

who, at the time of enrollment in the study, had a diagnosis of pre-diabetes. Successful completion of this trial may provide additional evidence for the influence of interleukin-1β in the progression of atherosclerosis and type 2 diabetes. Moreover, this study could offer insight into a potential new therapy for diseases resulting from unrestrained inflammation.

Interleukin-1β has been found to act in concert with other cytokines, even promoting their release in some instances. In vitro incubation of human adipocytes for four hours with interleukin-1β stimulated the release of interleukin-6 (Flower, Gray, Pinkney, & Mohamed-Ali, 2003). The expression of interleukin-6 by adipocytes has been found by others; additionally, in vivo evidence shows elevated levels of interleukin-6 in obese compared to non-obese individuals (Weisberg et al., 2003; Roytblat et. al, 2000).

Elevated plasma levels of interleukin-6 have demonstrated an active role in vascular dysfunction (Stapelton, James, Goodwil, & Frisbee, 2008). In a study of 508 healthy, middle-aged men elevated blood pressure predicted high levels of interleukin-6 after controlling for age and other cardiac risk factors (Chae, Lee, Rifai, & Ridker, 2001). Interestingly, the relationship between blood pressure and interleukin-6 showed a linear trend even in groups without hypertension. In other words, even in the absence of hypertension, increased levels of interleukin-6 correlated with elevated blood pressure. Another study by Bautista et al. (2004) found similar results; however, when the researchers divided the study population into quartiles based on level of interleukin-6, they found the relationship between elevated blood pressure and interleukin-6 may not be linear. After controlling for traditional cardiovascular risk factors and the presence of other cytokines, they found a three-fold increase in the likelihood of having hypertension

for participants in the second quartile when compared to the first. Participants in the third and fourth quartiles were slightly more than two times as likely to have hypertension than participants in the first quartile. These findings suggest elevated levels of interleukin-6 increase the likelihood of having hypertension, however, continued escalation of interleukin-6 may not have an additive effect on the likelihood of hypertension.

One potential mechanism for the relationship between interleukin-6 and hypertension is the renin-angiotensin aldosterone system (RAAS). This system is responsible for the regulation of blood pressure and tissue perfusion through its influence on vascular resistance; disruption of this system has long been implicated in cardiovascular disorders. Renin is released primarily from the kidneys and stimulates the production of angiotensin I from angiotensinogen. Angiotensin I is then converted by angiotensin converting enzyme into angiotensin II. Angiotensin II is able to increase blood pressure through a number of mechanisms including: stimulation of the adrenal glands to release aldosterone, which increases blood volume; vasoconstriction; stimulation of the pituitary gland to secrete vasopressin; enhanced activity of the sympathetic nervous system and hypertrophy of the vascular wall and the left ventricle (for a review of the actions of angiotensin II on the cardiovascular system see Fyhrquist, Metsärinne, & Tikkanen, 1995).

The mechanisms responsible for the release of renin and thus the cascade of the RAAS are not well understood. Although the primary activation of this system likely takes place within the kidney, catecholamines have been shown to promote the release of renin through β-adrenergic activation, suggesting a mechanism of neural control. The RAAS also influences inflammation, specifically, angiotensin II promotes the synthesis

of interleukin-6 (Funakoshi, Ichiki, Ito, & Takeshita, 1999). The correlation between the presence of interleukin-6 and hypertension led researchers to examine the role of interleukin-6 in hypertension. Angiotensin II is a known promoter of hypertension; this led researchers to speculate that interleukin-6 may mediate the effect of angiotensin II in the development of hypertension. In a study using mice that lacked the gene responsible for the production of interleukin-6 and a control group, researchers administered high doses of angiotensin II (Lee et al., 2006). The investigators found that in both groups angiotensin II significantly increased blood pressure. However, this increase in blood pressure was significantly less in the interleukin-6 knockout mice. This suggests that interleukin-6 is able to exacerbate the hypertensive effect of angiotensin II.

In addition to its influence on hypertension, interleukin-6 may also instigate other features of the metabolic syndrome. Rats given increasing doses of interleukin-6 revealed a dose-dependent elevation in triglycerides (Nonogaki et al., 1995). The resulting hypertriglyceridemia suggests that interleukin-6 may have a role in lipid metabolism. In a study of 112 women, level of interleukin-6 was positively correlated with body mass index, waist circumference, blood pressure and triglycerides, and negatively correlated to level of high-density lipoprotein and insulin sensitivity (Piché et al., 2005). Consequently, the women with increased adiposity exhibited elevated levels of interleukin-6, hypertriglyceridemia and low levels of high-density lipoproteins.

Much of the research that has been conducted investigating the influence of interleukin-6 on lipid metabolism has been correlational. Not much is known about the direction of the relationship or the specific mechanisms behind the dysregulation that often accompanies elevated levels of interleukin-6. One theory to explain the rise in lipid

levels is that interleukin-6 decreases lipoprotein lipase. Lipoprotein lipase is one of the mechanisms used to clear excess triglycerides from the blood stream by promoting its up-take and storage. Evidence for this hypothesis comes from a study showing a decrease in lipoprotein lipase activity in adipose tissue samples taken from obese participants after the samples were exposed to recombinant human interleukin-6 (Trujillo et al., 2004). Additional evidence comes from a study using an animal model. In 1976, Kompiang, Bensadoun and Yang administered an anti-lipoprotein lipase serum to roosters. After only 30 minutes from the time of the injection, there was a rise in circulating triglycerides.

This inhibition of lipoprotein lipase may also be responsible for the decrease in the plaque clearing, high-density lipoproteins. Goldberg et al. (1990) designed a study to elucidate the relationship between level of circulating high-density lipoproteins and lipoprotein lipase activity. They found that the availability of an essential structural component of high-density lipoprotein, apolipoprotein A1, is decreased in response to inhibition of lipoprotein lipase. This finding lends evidence to the hypothesis that elevations in interleukin-6 may be responsible for both the low levels of high-density lipoproteins and the elevated triglycerides through a reduction in lipoprotein lipase. An epidemiological study of 1044 older adults evaluated the relationship between circulating levels of high-density lipoproteins and inflammatory markers (Zuliani et al., 2007). The researchers found that an elevated level of interleukin-6 was the strongest predictor of low high-density lipoprotein.

Mounting evidence now supports the view of obesity being a state of chronic systemic inflammation. This inflammation is considered to be at the root of many of obesity's co-morbidities. Collectively termed the metabolic syndrome, high blood

pressure, elevated blood sugar and dyslipidemia work to promote cardiovascular dysfunction often leading to debilitating disease states. A mediator in this relationship between obesity and cardiovascular dysfunction appears to emerge from an immune system gone awry. One consequence of this malfunctioning immunomodulation is the elevation in circulating cytokines originating from the excess adipose tissue. The complicit role of cytokines in the progression of cardiovascular dysfunction is still under investigation. An early indicator of cardiovascular disease is an impaired stress response. Despite the volumes of research on stress reactivity, relatively little has been done with specific attention to immune dysregulation in obese populations. The current study investigates the role that obesity plays in immune dysregulation and the impact on cardiovascular dysfunction.

Stress Response

Chronic stress is often a poorly defined construct in cardiovascular physiology research that typically involves psychosocial factors. Chronic stress is commonly characterized in the literature on cardiovascular reactivity as being social or environmental. However, chronic stress of physical origin may provide new insight into impaired cardiovascular functioning. The current study operationalizes chronic stress as obesity.

When presented with a stressor, the autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis are often activated simultaneously (Chida & Hamer, 2008). Björntorp (2001) gives a thorough description of the process of HPA activation. HPA activation in humans stimulates the production of cortisol from the adrenal glands, which then binds to glucocorticoid receptors. Stimulation of lipoprotein

lipase activity is seen with increases in cortisol (Ottosson, Vikman-Adolfsson, Enerback, Olivecrona, & Björntorp, 1994). Lipoprotein lipase, when found in adipose tissue, acts as a catalyst for fat storage. Higher concentrations of glucocorticoid receptors in visceral rather than peripheral adipose tissue leads to a rise in cortisol binding in the visceral tissue. This binding leads to an increase in lipoprotein lipase activity, resulting in accumulation of visceral adipose tissue, at the site of higher glucocorticoid receptor expression.

One consequence of the circular relationship between cortisol production and visceral adipose tissue expansion is undefined causality. It may be speculated that glucocorticoid receptor prevalence in existing adipose tissue leads to a cycle of continued increase in adiposity. Alternatively, a state of chronic stress lends evidence to a preexisting condition of impaired stress reactivity or recovery predating that of obesity. The current study will not be longitudinal and therefore cannot directly address causation between obesity and impaired stress response. Instead, this study will focus on differences in the cardiovascular response to stress in obese and non-obese individuals taking into account the influence of HPA activation by assessing baseline and reactivity concentrations of cortisol.

Cortisol, which is known as "the stress hormone," exhibits a diurnal pattern with concentrations being highest soon after waking and dropping off later in the day. This hormone serves a number of regulatory functions including: glucose metabolism, blood pressure regulation and immunoregulation. An excess of cortisol or hypercortisolism is the driving force behind Cushing's syndrome. In these patients, prolonged exposure to excess cortisol leads to a state of obesity. When treated, cortisol levels return to normal

and the obesity is often resolved (Styne, Grumbach, Kaplan, Wilson, & Conte, 1984). In obese populations, cortisol is often found to be produced and secreted in excess; however, the concentrations in circulation are normal or low in comparison to normal weight participants (Björntorp, & Rosmond, 2000). This discrepancy may arise from more rapid cortisol clearing seen in conjunction with elevated adiposity. A consequence of excess adipose tissue is elevated glucocorticoid receptor expression, with more receptors to bind to, cortisol will predictably be removed from circulation at a higher rate.

While often acting in tandem with the HPA axis following stress, study of the ANS has entertained a greater body of research. The ANS can be subdivided into the sympathetic nervous system and parasympathetic nervous system. Each of these subdivisions plays a vital role in stress response and recovery. Quick and sufficient response to stress is an integral part of survival and imperative to human health. Complications in this continuum of activation and recovery can lead to damaging consequences. The role of the stress response and cardiovascular function will be the focus of the next section.

Reactivity Hypothesis

When presented with a stressful situation, generalized arousal is often helpful in managing the stressor. In some people, the arousal or reactivity of the sympathetic nervous system is greater than what is thought to be normal. Cardiovascular reactivity is classically defined as the change from a resting or baseline state to one of psychological or physical challenge. The reactivity hypotheses as conceptualized by Obrist (1981) suggest exaggerated physiological response to acute stressors could lead to cardiovascular dysfunction such as hypertension or coronary heart disease. Several

studies confirm a large cardiovascular response to acute stress as a risk factor for cardiovascular disease (Lovallo & Gerin, 2003; Matthews, 2004; Schwartz, 2003). The reactivity hypothesis has found support in obese populations (Silva, 2009; Steptoe, 2005; Voudoukis, 1970) with some evidence pointing to central obesity as the mediating factor (Davis & Twamley, 1999; Davy & Hall, 2004).

HPA activation in response to stress is also altered by excess adiposity. Although obese participants may not show elevated HPA activity during rest, when exposed to a stressor activation of the HPA axis as measured by cortisol exceeds the reactivity of normal weight participants (Björntorp, 1997). Some researchers have found that in participants with obesity, an exaggerated sympathetic response to stress may be intertwined with a correspondingly enlarged HPA response (Pasquali et al., 1996). Extended elevations of cortisol can produce hypertension in humans (Kelly, Mangos, Williamson, & Whitworth, 1998). Not surprisingly, patients with Cushing's syndrome are at an increased risk for cardiovascular disease-related mortality (Mancini, Kola, Mantero, Boscaro, & Arnaldi, 2004). An interesting finding is that elevated cortisol may predict a cardiovascular related death even in the absence of preexisting cardiovascular disease (Vogelzangs et al., 2010).

Blunted Reactivity

More recent findings suggest that it is not only an exaggerated response that may be harmful, as suggested by the reactivity hypotheses, but that blunted reactivity to stress may also be considered dysfunctional. Blunted reactivity is characterized by a diminished cardiovascular response from baseline to change produced by an acute stressor. Blunted

reactivity has been found in obese subjects, such that as body mass index increased, heart rate response to acute stress decreased (Carroll, Phillips, & Der, 2008; Phillips, 2011).

Finding both an increase and a decrease in physiological response to acute stressors in obese subjects may seem to create a confound. However, a recent comprehensive review of the literature by Lovallo (2010) clarifies why this dichotomy may exist. Lovallo points out a common theme in the literature where, until recently, a large physiological response was thought to lead to complications whereas a smaller response is healthier. The review indicates the importance of examining exaggerated as well as diminished physiological reactions to stress as potential indicators of poor health outcomes. That is, an intermediate degree of cardiovascular response to stressors may be most adaptive in dealing with environmental events. Too much or too little response may be maladaptive and associated with negative health outcomes. Interestingly, the negative health outcomes may differ depending on which end of the continuum the reactivity falls.

As described above, an exaggerated cardiovascular response to stress is associated with subsequent hypertension and other risk factors for cardiovascular disease. Alternatively, a blunted cardiovascular response to stress appears to be an indicator of other types of negative health consequences. Bulimia nervosa is a psychological disorder characterized by extreme overeating followed by intense feelings of guilt and subsequent destructive behaviors that are meant to alleviate guilt such as crash dieting, excessive exercise and purging of food. Four criteria must be met for a diagnosis of bulimia nervosa: repeated binged eating, repeated compensatory behaviors subsequent to binge eating, persistence of above symptoms at least twice a week for three months and an undue influence of weight on self image (American Psychiatric Association, 2000). In a

study by Koo-Loeb, Pedersen and Girdler (1998), the cardiovascular reactivity of 15 women with a diagnosis of bulimia nervosa were compared to 15 controls. Baseline values of blood pressure and heart rate revealed no differences in cardiovascular function at rest. However, during stress tasks participants with bulimia nervosa exhibited a decreased blood pressure and heart rate response that appeared to be somewhat influenced by level of anxiety.

More recent investigation has linked blunted reactivity to poorer cognitive ability, depression, obesity and self-reported health (Ginty, Phillips, Roseboom, Carroll, & deRooij, 2012; Phillips, 2011). Three measures of cognitive ability were found to be related to blunted cardiovascular response to stress, such that individuals with poorer cognitive ability demonstrated a reduced cardiovascular reactivity to stress (Ginty et al., 2012). The relationship between systolic blood pressure and heart rate maintained significance for all three measures of cognitive ability even after controlling for confounders (e.g., baseline values, education, age and body mass index). Blunted diastolic reactivity remained significant for two of the three cognitive ability measures after confounders were entered; however, significance was attenuated for the third cognitive measure. Some researchers have suggested a relationship between cognitive ability and depression, such that depression is predictive of impaired cognitive functioning (Rabbitt, Donlan, Watson, McInnes, & Bent, 1995; Ravnkilde et al., 2002; Strömgren, 1977).

Emerging evidence indicates a relationship between depression and blunted cardiovascular reactivity. In 2007, data from 1608 adults were examined for an association between depressive symptoms and cardiovascular reactivity (Carroll, Phillips,

Hunt, & Der, 2007). A correlation was found between depression and blunted reactivity (systolic blood pressure and heart rate). A regression analysis that controlled for baseline values, age and body mass index found that depression was still significantly related to blunted systolic and heart rate reactivity. As a follow up to this study in 2011, researchers evaluated the predictive ability of blunted reactivity on future depression (Phillips, Hunt, Der, & Carroll, 2011). They found that blunted heart rate reactivity to stress was predictive of depression five years later. This diminished reactivity to stress is a relatively new concept and therefore lacks the longitudinal support for relationships that can be found between hyperreactivity and later disease states. Nonetheless, the studies currently available offer new insight into the paradox of cardiovascular reactivity and the negative health consequences. These findings alter some initial presumptions of a monotonic relationship between reactivity and disease that originated from the reactivity hypothesis. Instead of exaggerated or blunted reactivity, an intermediate degree of cardiovascular response to stressors may be most adaptive in dealing with environmental events. Thus, future studies should examine stress response on a continuum where response on either end of the normative distribution should be evaluated as possibly problematic.

In addition to a diminished response of the sympathetic nervous system, blunting of the HPA axis in response to stress has also been found in connection with adverse health conditions. This reduction in HPA response to stress has been linked to other conditions also characterized by blunted sympathetic activity such as eating disorders and diminished cognitive ability (Ginty, Phillips, Higgs, Heaney, & Carroll, 2012; Ginty, Phillips, Roseboom, Carroll, & deRooij, 2012). Impaired HPA response has also been found in individuals with an impaired immune response and autoimmune diseases;

personality traits such as neuroticism and a decrease in openness; and stress-related disorders such as posttraumatic stress syndrome, chronic fatigue syndrome, fibromyalgia and rheumatoid arthritis (Buske-Kirschbaum, von Auer, Krieger, Weis, Rauh, & Hellhammer, 2003; Buske-Kirschbaum, Ebrecht, & Hellhammer, 2010; Heim, Ehlert, & Hellhammer, 2000; Oswald et al., 2006; Sternberg, 1997).

In some instances, obesity appears to inhibit HPA reactivity as measured by a reduction in cortisol (Salehi, Ferenczi, & Zumoff, 2005). Prior to the release of cortisol, corticotrophin releasing hormone is secreted by the hypothalamus which then stimulates the adrenal glands to produce cortisol. Some researchers have suggested that obese individuals have a diminished response to the actions of corticotrophin releasing hormone. In an investigation of 10 obese women and seven controls, peak cortisol levels were reduced in the obese women following administration of corticotrophin releasing hormone (Kopelman et al., 1988). In addition to blunted cortisol reactivity to stress, baseline levels of cortisol may also be reduced in obese individuals due to elevated levels of leptin. As mentioned previously leptin is released from adipose tissue, further it appears in elevated concentrations in correlation with obesity (Considine et al., 1996). An in vitro analysis of the effects of Leptin on adrenocortical cells revealed inhibitory properties (Bornstein, Uhlmann, Haidan, Ehrhart-Bornstein, & Scherbaum, 1997). Following a 24-hour incubation period with leptin, cells were stimulated with corticotrophin releasing hormone. The incubated cells demonstrated a blunted response to corticotrophin manifest by reduced cortisol production. This action of leptin has been replicated (Pralong et al., 1998); however these findings must be taken with caution in regards to their application to obese populations. In human obesity, elevated leptin often

results from leptin resistance. Therefore, the actions of leptin on adrenal tissue in obese individuals may be compromised, as is the case with other tissues in the body.

Recovery from Stress

A person's reactivity to stress is undoubtedly important in creating a picture of cardiovascular health; nevertheless, it may not be telling the whole story. Although working together the HPA axis and ANS can produce helpful responses to stress that allow for quick, efficient resolve of the stressor, problems occur when these systems work in sustaining the stress response. The importance of the stress response should not overshadow the significance of effective recovery following the termination of a stressor. A review of the literature suggests that recovery from stress may help illuminate a disturbance of cardiovascular functioning, even when reactivity does not appear to yield impairments. Recovery is measured as the time it takes to return to baseline levels of cardiovascular functioning once a psychological or physical challenge has ended.

A meta-analysis by Chida and Hamer (2008) demonstrated that general life stress resulted in slowed recovery from laboratory stressors. Exposure to chronic stress has also been associated with poor recovery (Gump & Matthews, 1999; Lepore, Miles, & Levy, 1997). Impaired recovery has been positively correlated with obesity, when reactivity showed no relationship (Brydon, 2011). Further, results suggest subjects who are more obese or those with greater abdominal obesity are at an increased risk of recovery impairment (Steptoe, 2005). Although a return to individual baseline parameters will eventually occur, the inadequate recovery results in an overexposure to stress hormones, leading to adipose tissue accumulation and excessive demands on the cardiovascular system.

Immune Response

In 1975, Robert Ader coined the term psychoneuroimmunology. Prior to Ader's research, the immune system was thought to act autonomously; however, his studies demonstrated that the immune system could be trained to respond through classical conditioning. This revolutionary finding came as a surprise to Ader and Cohen (1975) as they investigated taste aversion through classical conditioning in rats. The rats were injected with Cytoxan, a drug that causes stomach upset and has immunosuppressive effects, as they drank a saccharin solution. The rats began to associate the saccharin with the aversive effects of the Cytoxan and begin to avoid the saccharin. The avoidance continued after the administration of Cytoxan had stopped. In an attempt to reverse the aversion, the rats were force-fed the saccharin solution and in theory should have "learned" that they were no longer experiencing the unpleasant effects of Cytoxan. Unexpectedly, several of the rats died. Ader and Cohen hypothesized, and later confirmed, that the immune system had been conditioned and immunosuppression could be instigated by environmental stimuli. The field that has grown out of this seminal finding encompasses interactions and relationships between an individual's perceptions and behavior and their endocrine, nervous and immune systems.

The stress response is an adaptive mechanism meant to provide support to different systems in the body when an individual is dealing with disruptions to homeostasis, perceived as a potential threat. Activation of the sympathetic nervous system allows almost instantaneous alterations in cardiac, vascular, respiratory and digestive systems, commonly referred to as the fight or flight response. These changes in response to stress occur through the release of catecholamines, namely norepinephrine

and epinephrine, which then bind to adrenergic receptors on various cells in the affected organ system. Stress induced activation of the HPA axis occurs through the release of corticotrophin releasing hormone by the hypothalamus, which then acts on the anterior pituitary to produce and release adrenocorticotropic hormone. The resulting cascade culminates in the production and secretion of glucocorticoids by the adrenal glands, the most recognized being cortisol (Björntorp, 2001). Not unlike the sympathetic response to stress, activation of the HPA axis primes the body for action. For example cortisol, by up-regulating glucose metabolism, increases blood sugar levels providing the energy required to overcome the stressor. Glucocorticoid levels are controlled through a negative-feedback loop whereby circulating levels of cortisol inhibit the release of corticotrophin releasing hormone, thereby regulating the duration and magnitude of the HPA axis stress response.

Both the sympathetic and HPA systems have bidirectional relationships with the immune system, meaning they are both influenced by and exert influence over the immune system. Further, these systems are influenced by the nature and magnitude of the immune response. Much of the communication between these systems occurs via the signaling proteins known as cytokines. In a comprehensive review of neuroimmune modulation, Elenkov and colleagues (2000) describe a number of studies confirming these relationships. The sympathetic nervous system is able to activate the immune system through sympathetic innervation of various immune organs and through activation of adrenergic receptors located on the surface of many immune cells. Sympathetic activation has heterogeneous effects on the adaptive immune system; it is generally accepted that cellular immunity is suppressed while humoral immunity may be enhanced

(Elenkov & Chrousos, 1999). These shifts in immunity reflect alterations in cytokine profiles, commonly represented as Th1 (cellular) and Th2 (humoral).

The adaptive immune system has evolved to defend against several types of invaders or pathogens including: bacteria, viruses, fungi and parasites (Sompayrac, 2003). Depending on the type of pathogen (intracellular versus extracellular) and the physiological state of the host, the immune system will be directed to produce either Th1 or Th2 cytokines. The "classical" Th1 cytokines are tumor necrosis factor – β, interferon – γ and interleukin – 2. These cytokines work together to defend against attacks by intracellular pathogens. Further, they also inhibit the production of Th2 cytokines. Th2 cytokines defend against extracellular pathogens such as certain bacteria and parasites. These cytokines, interleukins – 4, 5 and 13, antagonize Th1 cytokines (Sompayrac, 2003). Interleukin – 5 promotes the production of immunoglobulin A, an antibody that will be discussed in more detail later.

Given their mutually antagonistic properties, both Th1 and Th2 cytokines can be considered pro- and anti-inflammatory depending upon the disease process being considered. Immune dysregulation causing a persistent shift in either direction for an extended period of time could result in various immune based diseases. For example, an overactive Th1 response is associated with inflammation and autoimmune diseases such as rheumatoid arthritis. Conversely, diseases like asthma and allergies are associated with Th2 polarization. Chronic stress-mediated activation of the HPA axis has a parallel effect on the adaptive immune system, with a shift towards Th2 dominance. Thus, the previous view that stress results almost exclusively in immunosuppression has been revised to reflect the alterations that stress has on immune regulation. Short-term activation of the

stress response may be beneficial by reducing inflammation, through inhibition of Th1 cytokines. However, if the stress response is exaggerated or prolonged, the ensuing suppression of inflammatory Th1 cytokines can leave the individual vulnerable to both infection and (because of the loss of Th1/Th2 balance) development of Th2 mediated hypersensitivity diseases such as allergy, asthma and systemic lupus erythematosus. Equally as detrimental, understimulation or a blunted immune response to stress with subsequent insufficient Th2 cytokine production may result in unnecessary inflammation that could manifest as an autoimmune disease (for a review see, Marshall, 2011).

As signalers of innate immunity, interleukin-1β and interleukin-6 can be differentially affected by stress following activation of the sympathetic nervous system and HPA axis. Catecholamines that are released following sympathetic nervous system stimulation have been associated with the up-regulation of proinflammatory cytokines (Papanicolaou et al., 1996). In contrast, activation of the HPA axis has restrictive effects on the innate immune response, given that the release of glucocorticoids results in inhibition of both interleukin-1β and interleukin-6 (Kovalovsky, Refojo, Holsboer, & Arzt, 2000; Waage, Slupphaug, & Shalaby, 1990). These differential effects of stress on innate immunity again emphasize that stress is not universally immunosuppressive, but represents modulation of immune activity. Chronic disruption to immunoregulation through an impaired response to stress will likely have detrimental health outcomes.

In a reciprocal manner, there is evidence that the immune system modulates the activity of the sympathetic nervous system through a feedback mechanism utilizing cytokines. Upon detection of threats to homeostasis, interleukin-1β and interleukin-6 are released as first responders and defense signals of the immune system. These cytokines

initiate a cascade, mobilizing other participants in the immune response. Further, they are able to activate both the sympathetic nervous system and HPA axis (Besedovsky del Rey, Sorkin, & Dinarello, 1986; Chrousos, 2000). One function of the stress response is to generate the energy needed to overcome the stressor. Therefore, activation of the stress response by the immune system may represent a mechanism for the individual to produce the energy required to defend against pathogens (for a review see, Maier, 2003). This co-activation is considered adaptive in dealing with short-term stress, however this arrangement may become maladaptive when dealing with chronic stress. For instance, the cytokine-mediated activation of the HPA axis obstructs the system's internal negative feedback mechanism meant to regulate the stress response. This could result in a prolonged HPA activation and elevated levels of cortisol. Both excess cortisol and chronic stress have been linked to cardiovascular dysfunction and obesity (Björntorp, 2001; Mancini et al., 2004; Styne et al., 1984).

As will be discussed in detail below, there is a large body of evidence demonstrating that external stressors are able to alter the cardiovascular and immune system. Much of what has been discussed as the immune response to stress thus far has been what is considered a normal response. Adipose tissue, once thought to be hormonally inert, is now considered a potent endocrine organ, which can have a direct effect on an individual's physiological response to stress. Thus, it may be hypothesized that individuals with excess adipose tissue are at an increased risk for additional signaling processes that may interfere with what has evolved as the most advantageous stress response.

Indeed, a number of studies have shown that both interleukin-1β and interleukin-6 are secreted by adipose tissue. Increasing evidence suggests these cytokines may be involved in the inflammatory process that link obesity and vascular dysfunction (Calabro, Chang, Willerson, & Yeh, 2005). Thus, the relationship between obesity and impaired cardiovascular response may be mediated by elevated cytokines and prolonged, unnecessary activation of the stress response. Additional measurements are needed to test this hypothesis. Good candidates include measurement of cortisol to evaluate degree and duration of HPA activation as well as measures of specific cytokines that are members of the interleukin family and have been linked to obesity, specifically interleukin-1β and interleukin-6 (Kirschbaum & Hellhammer, 1989; Speaker & Fleshner, 2012; Yudkin, Kumari, Humphries & Mohamed-Ali, 2000).

Differences in Reaction to Active vs Passive Stress

There are many dimensions used to classify different types of stressors: sensory intake versus sensory avoidance, active versus passive, or psychological versus physical. These dimensions are not meant to be exclusive. There is considerable overlap between these distinctions; often the terms will be used interchangeably. The present study examined the active/passive and psychological/physical dimensions of two stress tasks. The differences in response and recovery to different types of laboratory stressors uncover differences in the cardiovascular pathways activated, as manifested in different hemodynamic patterns being elicited by different types of stressors. Researchers in the field of immunology have also utilized different types of stress tasks and have been successful in finding variations in immune reactivity based on task. The current study

utilized two laboratory stressors that, according to the literature, elicit different immune and hemodynamic patterns of response.

Cardiovascular Differences

Sharpley and Gordon (1999) demonstrated a difference in the cardiovascular patterns initiated by the mental arithmetic (MA) versus the cold pressor (CP) task. The results showed that the MA task elicited a sharp heart rate increase in the first thirty seconds of the task followed by a gradual decrease. The CP task showed a smaller heart rate increase that was maintained over the course of the task. These tasks have also been found to elicit different responses in blood pressure that will be described in more detail below. There is some consensus in the literature that the CP and MA tasks activate different pathways (Allen, Obrist, Sherwood, & Crowell, 1987; Andrén & Hannson, 1981, Gianaros & Sheu, 2009, Obrist, 1981; Willemsen et al., 1998). The attempts made to describe the mechanisms responsible for differences in pathways of response have not reached a consensus.

In a review of the literature, Gianaros and Sheu (2009) examine possible pathway differences that would explain the divergence of cardiovascular response. The CP task is a physical stressor that requires passive coping. The vascular resistance or vasoconstriction that occurs in response to temperature results in stimulation of α-adrenergic receptors. There appears to be agreement in the literature that α-adrenergically mediated vasoconstriction leads to an increase in total peripheral resistance (Allen et al., 1987; Andrén & Hannson, 1981; Willemsen et al., 1998).

The increase in total peripheral resistance resulting from α-adrenergic receptor activation is speculated to lead to a rise in diastolic but not systolic blood pressure

(Willemsen et al., 1998). Others have found a rise in both systolic and diastolic blood pressure following the CP task (Allen et al., 1987; Andrén & Hannson, 1981). The rise in systolic blood pressure could also be the result of an increase in heart rate (Allen et al., 1987) although some suggest there is actually a decrease in heart rate following the CP task (Willemsen et al., 1998). The possible increase in heart rate may be mediated by activation of β-adrenergic receptors (Allen et al., 1987; Sherwood, Allen, Obrist, & Langer, 1986). However, others suggest the CP task does not elicit a β-adrenergic response (Matthews, 2004). Speculation has also arisen about possible desensitization of β-adrenergic receptors in participants under chronic stress resulting in higher baseline levels of sympathetic activity (Benschop et al., 1994).

It is speculated that participants engage in active coping when presented with the MA task, which is considered a psychological stressor. During this task, increases in vascular resistance are seen in parallel with increases in heart rate. There is not complete agreement in the literature, but several studies have suggested these responses originate from both α and β-adrenergic receptor stimulation (Allen et al., 1987; Gianaros & Sheu, 2009; Willemsen et al., 1998).

Alpha-adrenergic receptors activated in response to the MA task are thought to result in vasoconstriction of peripheral vessels. This may contribute to increases seen in both systolic and diastolic blood pressure following the MA task (Phillips, 2011; Willemsen et al., 1998). However, myocardial β-adrenergic receptor activation may have a larger role in increasing heart rate due to the negligible changes in total peripheral resistance (Allen et al., 1987, Andrén & Hannson, 1981).

Relatively few studies have examined the early warning signs of impaired cardiovascular reactivity and recovery in obese populations. Results from many of the studies are mixed regarding cardiovascular measures and obesity, yet there seems to be some consistent effects. Baseline levels of blood pressure have been found to be higher in obese participants. Thus, controlling for baseline levels of cardiovascular functioning is a critical component for comparisons between tasks (Brydon, 2011; Carroll, Phillips & Der, 2008, Phillips, 2011; Steptoe, 2005).

Heart rate reactivity appears to be negatively correlated with body mass index, suggesting blunted cardiac reactivity in obese participants (Carroll, et al., 2008; Phillips, 2011). However, obesity seems to have an exaggerated effect on blood pressure reactivity. This heightened reactivity seems to be a function of an increase in diastolic blood pressure. More specifically, central adiposity may be a greater risk factor for exaggerated reactivity than is peripheral adiposity (Davis & Twamley, 1999; Steptoe, 2005). There is evidence to suggest systolic blood pressure reactivity may be blunted in obese participants (Carroll et al., 2008).

Measures of recovery have been neglected in many studies investigating obesity and cardiovascular reactivity. In studies where this information was considered, a delay in diastolic blood pressure recovery was demonstrated in obese subjects, specifically those with greater abdominal obesity (Brydon, 2011; Steptoe, 2005). The reasons for these differences in obese subjects are not fully understood.

Immune Differences

Similar to the cardiovascular response, the immune response to stress is dependent on the type of stress. Laboratory stress has been shown to alter immune

response in both animals (Moynihan, 2003) and humans (Xiang, Del Ben, Rehm, & Marshall, 2011). The MA and CP tasks have been used to find differential effects of stress on immune response. Stress induced activation of the sympathetic nervous system and HPA axis have a direct role in immunomodulation. This should not be surprising given that stress is the term used to describe the body's reaction to a threat to homeostasis and the immune system is activated in response to threat from "foreign" invaders. This illustrates one point of contact between the stress response and the immune response. When the body detects a foreign invader, it responds by promoting the release of interleukin-1β and interleukin-6. These signaling molecules then promote a stress response through activation of the sympathetic nervous system and HPA axis.

Interleukin-1β was one of the first cytokines to be discovered (Dinarello, 2010). Production of interleukin-1β is elevated by stress in healthy individuals (Ilardo, Toniolo, Aimone-Gastin, Abdelmouttaleb, & Desor, 2001) and increased by psychological stress administered in a laboratory (Heinz et al., 2003). Interleukin-1β has been proposed as a potential link between stress and heart disease. A study investigating this relationship found a positive correlation between interleukin-1β gene expression and cardiovascular reactivity (systolic blood pressure and heart rate) to a psychological stressor (Brydon, et al., 2005). Several different stress protocols have been found to cause an increase in interleukin-1β including speech tasks, mock job interviews and sleep deprivation (Ackerman, Martino, Heyman, Moyna, & Rabin, 1998; Altemus, Rao, Dhabhar, Ding, & Granstein, 2001; Heinz, et al., 2003). In a study comparing the stress response of 25 patients with psoriasis and 50 healthy controls researchers found an increase in cortisol in both groups following the stressor; however, only the control group showed an increase

in interleukin-1β (Mastrolonardo, Alicino, Zefferino, Pasquini, & Picardi, 2007). Interestingly, the patients with psoriasis had elevated baseline levels of interleukin-1β perhaps suggesting a "ceiling effect" that would explain the blunted immune response to stress.

The elevated levels of interleukin-1β found in obese individuals have led researchers to evaluate the relationship between stress and obesity. A review by Speaker and Fleshner (2012) describes the influence of interleukin-1β in advancing obesity following repeated stressors. These researchers exposed healthy, non-obese rats to a tail shock stressor and subsequently measured the concentration of interleukin-1β in adipose tissue. They found concentrations to be higher in subcutaneous compared to visceral adipose tissue. The researchers concluded that the inflammation in subcutaneous adipose tissue reduces lipogenesis and lipid uptake. This reduction in lipid accumulation by subcutaneous adipose tissue leads to a consequential shift in lipogenesis in visceral adipose tissue, thereby promoting obesity. A sustained stress response or repeated exposure to stress propels the detrimental shift and results in an unremitting increase in visceral adipose tissue. In a study of morbidly obese patients, subcutaneous expression of interleukin-1β decreased significantly following substantial weight loss (Moschen et al., 2011). The effect of obesity on alterations of interleukin-1β concentration following laboratory stressors has not been investigated.

Expression of interleukin-1β is one mechanism of activation for interleukin-6. Animal studies have shown that interleukin-6 production is increased following psychological and physical stressors (Zhou, Kusnecov, Shurin, DePaoli, & Rabin, 1993). Studies in humans have shown that experiences such as childhood maltreatment and

major depression are associated with increase in interleukin-6 response to acute stress (Carpenter et al., 2010; Pace et al., 2006). An evaluation of acute psychological stress on cardiovascular and immune response revealed a correlation between blood pressure reactivity and concentration of interleukin-6 45 minutes post task (Steptoe, Willemsen, Owen, Flower, & Mohamed-Ali, 2001).

Prolonged elevation of interleukin-6 that extends beyond the termination of a stressor suggests impairments in immune recovery from stress. This diminished ability to recover from acute stress has been found in low socioeconomic populations. Thirty-eight participants divided into high and low socioeconomic groups showed no differences in baseline measures of cardiovascular and immune variables (Brydon, Edwards, Mohamed-Ali, & Steptoe, 2004). However, the low socioeconomic group displayed a prolonged increase in interleukin-6 that extended temporally beyond the increase found in the high socioeconomic group. Further, although heart rate reactivity was the same in both groups blunted heart rate recovery was found in the low socioeconomic group compared to the high socioeconomic group.

Like interleukin-1β, interleukin-6 is elevated in individuals with obesity (Roytblat, et al., 2000). The influence of obesity on the immune response to stress and its relationship to cardiovascular response has received some consideration. Evaluation of a public speaking task on obese and non-obese females revealed differences in cardiovascular, stress and immune measures (Benson, et al., 2009). At baseline, level of interleukin-6 was elevated in obese females. Acute psychological stress caused an increase in cardiovascular measures and interleukin-6 in both groups. The rise in cortisol in response to stress was elevated in the obese group; however, baseline and reactivity

values of cortisol were higher in the control group. The obese group also exhibited blunted cardiovascular and immune recovery from the stressor. At ten minutes following termination of the stressor diastolic blood pressure and heart rate remained elevated in the obese group. At 45 minutes post-stress the obese group had increased interleukin-6.

Immunoglobulin A (IgA) has received considerably more attention in regard to the MA and CP stress tasks than interleukin-1β or interleukin-6. Immunoglobulin is another term for antibody, the protein produced by the body in response to a specific invader. Humans have five classes of immunoglobulins including IgA. Immunoglobulin A is found in the mucosal surfaces of the body as well as in tears, sweat and saliva.

Level of salivary IgA is altered by acute stress. In response to the MA task, an active stressor, salivary IgA is increased (Isowa, Ohira, & Murashima, 2004; Ring et al., 2000; Ring et al., 1999; Willemsen, Carroll, Ring, & Drayson, 2002; Willemsen et al., 1998; Willemsen, Ring, McKeever, & Carroll, 2000; Winzer et al., 1999). Conversely, a passive stressor, the CP task, results in a decrease in salivary IgA (Ring et al., 2000; Willemsen et al., 2002). As previously mentioned the CP task is largely associated with cardiovascular changes resulting from α-adrenergic activation. To determine the relationship between immunological reactivity to the CP task and the potential influence of α-adrenergic activation, doxazosin, an α-adrenergic receptor antagonist, was used. Administration of doxazosin significantly lowered blood pressure response to the CP task. Further, doxazosin blocked the significant decrease in salivary IgA during the CP task that was seen in the placebo group (Ring et al., 2000). These results suggest α-adrenergic and perhaps β-adrenergic pathways are responsible for some facet of both cardiovascular and immune response to stress. Although salivary IgA has been shown to

be differentially activated by stress tasks, these changes have not been investigated in obese individuals.

In a study evaluating the MA and CP tasks researchers found distinctive influences on immune, endocrine and cardiovascular reactivity (Isowa, Ohira, & Murashima, 2004). The MA task increased the concentration of salivary IgA compared to baseline, and secretion of cortisol was significantly lower than the level during the CP task. Cardiovascular reactivity to the MA task did not show any relationship to salivary IgA or cortisol. The change in salivary IgA during the CP task was not significantly different from baseline, though it did show a downward trend. Cortisol increased significantly from baseline during the CP task and remained elevated throughout a 15 minute rest period. A relationship did exist between cardiovascular reactivity to the CP task and endocrine measures. Blood pressure reactivity was negatively correlated to level of cortisol during the CP task. The increase in cortisol concentration to the CP task and the corresponding blunted blood pressure reactivity led the researchers to suggest, "the activity of the HPA axis might be dominant compared to that of the autonomic nervous system in passive stress but not in active" (Isowa et al., 2004, p. 117). The study outlined above was conducted on 26 female undergraduates (and one instructor) of good health with a mean body mass index of 21.22. The potential impact of obesity on immune reactivity has been largely neglected despite evidence that obesity influences the stress response and that the immune system can be altered by stress.

Appraisal of the Stressor

In 1984, Krantz and Manuck highlighted the importance of an individual's appraisal of an event on cardiovascular reactivity. In instances of chronic stress, an

individual may be more likely to perceive a new stressor as more threatening, leading to increases in cardiovascular reactivity to acute stress tasks (Baum, Davidson, Rectanus, & McArdle, 1987). When a person is subjected to a stressful situation, the situation is assessed and the individual determines whether they have the resources to overcome the stressor. It is important to gauge the participants' appraisal of a laboratory stressor to help ensure an adequate amount of stress has been evoked to elicit a response. Others have also acknowledged the importance of appraisal when evaluating responses to laboratory stress (Gump & Matthews, 1999; McEwen, 2007). It is equally important to assess an individual's existing level of perceived stress. A number of variables can influence how an individual appraises laboratory stressors. For instance, chronic stress has been shown to lead to differences in acute stress appraisal (Aldwin, Sutton, Chiara, & Spiro, 1996). Further, animal studies have shown that repeated exposure to stress that may include blunted systolic reactivity could be described as a defeat response to stress (Adams, Lins, & Blizard, 1987). In the current study, a Likert scale of appraisal was administered to evaluate the feeling of stress elicited in response to the laboratory stressors. Further, the Perceived Stress Scale was used to assess existing levels of life stress (Cohen, Kamarck & Mermelstein, 1983).

If a person decides they do not have the resources and are unable to cope with a stressful situation, a defeat reaction may ensue. The defeat reaction follows a different pattern of cardiovascular reactivity than the typical fight or flight response. Jern and colleagues (1992) found that participants exhibiting central obesity showed a vasoconstrictor response to the MA task, characterized by higher total peripheral resistance and decreased cardiac output. An explanation the researchers offer for this

finding is that obese participants respond with defeat rather than a defense reaction to this task. This interpretation of the data was based on hemodynamic similarities to rats exposed to mental stress defined as immobilization stress (Hallbäck, Magnusson, & Weiss, 1974). Performance during the MA task was not reported, thus it is unknown whether the defeat pattern of cardiovascular reactivity corresponds to a behavioral manifestation of defeat represented by lack of effort during the task (Jern, Bergbrant, Björntorp, & Hansson, 1992).

A study by Carroll and colleagues (2008) did evaluate performance on the MA task. They found performance to be positively correlated with reactivity (systolic blood pressure and heart rate). Further, obesity was associated with poorer performance on the task. Blunted reactivity in obese participants during the MA task may be a consequence of a decreased ability to cope. The resulting defeat pattern of response may include both blunted cardiovascular reactivity and poor performance on the task. In an attempt to assess performance during the MA task in the current study, responses were recorded and subsequently coded. If a participant gives up or quits responding, this may suggest a defeat response. Participants were also asked to rate how hard they tried to successfully complete the task and whether or not they felt like they gave up during the task.

Assessment of pain during the CP task was also evaluated by having participants rate their experience of pain. It should come as no surprise that obesity is associated with a diminished health-related quality of life. Interestingly, pain appears to moderate the relationship between obesity and health-related quality of life (Barofsky, Fontaine, & Cheskin, 1997; Heo, Allison, Faith, Zhu, & Fontaine, 2003). Studies have shown that pain sensitivity may be elevated in obese individuals. Different techniques have been

used to assess pain sensitivity; obese participants have reported amplified pain to a pressuring bearing device and electrophysiological methods (McKendall, & Haier, 1983; Zahorska-Markiewicz, Zych, & Kucio, 1988). The experience of pain is also related to cardiovascular reactivity, with greater reactivity being associated with increased ratings of pain (Peckerman et al., 1991). An interesting study by Caceres and Burns (1997) found that pain sensitivity could be enhanced by prior exposure to psychological stress. These researchers divided participants into two groups (high and low responders) based on blood pressure reactivity to the MA task. Participants then completed the CP task. High responders to the MA task exhibited a lower pain threshold and tolerance to the CP task.

Not all studies have confirmed the relationship between enhanced pain sensitivity and obesity. Some findings suggest obese rats are less sensitive to pain. The tail flick test is a commonly used task in the evaluation of pain perception in rats. In this thermal-pain detection task, a beam of light is concentrated on the rat's tail, the amount of time it takes for the rat to move their tail away from the light (heat) is a measure of pain threshold. Ramzan, Wong and Corcoran (1993), found that obese rats exhibited a decrease in pain avoidance behavior measured by an increase in tail flick latency. Moreover, weight of the rat was positively correlated to latency. Thus, the more the rat weighed the longer it took to move its tail away from the heat. The differences that have been found in the relationship between pain sensitivity and obesity indicate the importance of assessing pain perception in the current study. Further, controlling for the perception of pain may be important when evaluating cardiovascular reactivity given the potential relationship between pain and reactivity.

Gender

The existence of individual differences are known with respect to cardiovascular reactivity and recovery; however, there may be a gender component that adds to these differences. Thus, gender differences can complicate findings. For example, it has been demonstrated that women tend to have greater cardiovascular reactivity to some laboratory stressors than men (Matthews, 2004; Schmaus, Laubmeier, Boquiren, Herzer & Zakowski, 2008). Others have shown that women but not men have a blunted response to some laboratory stressors (Dishman & Nakamura, 2003). A gender dimorphism in cardiovascular response may also result in differences with females exhibiting a "cardiac" response and males a more "vascular" response to stress (Allen, Stoney, Owens, & Matthews, 1993).

In addition to the relationship between cardiovascular reactivity and perception of pain described previously, gender may also have an effect on pain ratings. Extensive literature reviews report that women have greater pain sensitivity, thus a lower pain threshold than men (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley III, 2009), though some suggest that this gender disparity may be task specific (Racine et al., 2012). In response to the CP task, researchers have found that women are significantly less tolerant of pain compared to men (Hellström, & Lundberg, 2000; Keogh, Bond, Hanmer, & Tilston, 2005).

Alterations in immune measures have also been found between men and women. Some researchers have found increases in salivary IgA following the MA task in men, but not in women (Willemsen et al., 2002). Numerous studies have found gender differences in stress appraisal and coping; however, these differences have not been consistent (for a review see, Davis, Matthews, & Twamley, 1999; Jick, & Mitz, 1986).

The causes for these discrepancies are not well understood. The limited sample size of the current study will not allow us to evaluate any possible effects of gender. Additionally, due to the limited amount of research that has been conducted in obese populations investigating the relationship between stress and cardiovascular and immune changes this study will be largely exploratory. For these reasons only females were recruited for the current study.

II. PROPOSED RESEARCH

As the obesity epidemic increases, the prevalence of obesity-related diseases grows simultaneously. Obesity characterizes an internal environment of low-grade chronic inflammation, which exacerbates cardiovascular dysfunction. A large body of research substantiates the pathophysiology linking obesity and cardiovascular dysfunction (Alpert, 2001; Cassidy, et al., 2005; Freedman, et al., 2004; Kenchaiah, et al., 2002). Unfortunately, immunoregulatory dysfunction has yet to receive the same attention in obese populations. However, it has been hypothesized that similar mechanisms are responsible for cardiovascular and immune response to stress.

Previous studies have shown that different stress tasks can activate different patterns of stress response. Two common tasks that are used are the MA and the CP task. Our lab has successfully demonstrated the ability of these stress tasks to elicit distinct patterns of cardiovascular stress response in obese, young adult females (Burch & Allen, in press). Participants with a higher body mass index showed greater diastolic blood pressure response to the CP task. In contrast, systolic blood pressure and heart rate response to the MA task was blunted in participants with a higher body mass index.

In the current study, we have expanded recruitment to a more diverse population of adult females, thus adding to the population to which our findings are relevant. In addition to validating earlier results from our lab and expanding their relevance, the present study allowed us to investigate obesity-instigated alterations in immune response

to different stressors (active versus passive). This was done by investigating cytokines that act as signalers of the innate immune response. We also measured changes in the adaptive immune response to stress by evaluating levels of salivary IgA.

We believe obese individuals will have a different immune response to laboratory stress compared to normal weight individuals. Many studies have shown that inflammatory cytokines, interleukin-1β and interleukin-6, are elevated in obese individuals. The potential influence this has on the acute stress response remains largely unknown. Some researchers have suggested that elevated cytokine levels at baseline may have a limiting effect on the increase that could occur in response to stress. Researchers have also found correlations between cardiovascular response to a stressor and change in cytokine concentration. This indicates there may be some link between sympathetic nervous system activation and immune response to stress. However, increases found in cortisol concentration during the CP task coupled with blunted blood pressure reactivity led researchers to propose that the stress response may be dominated by the HPA axis following passive stress.

This distinction in response to active and passive stressors is what we aim to investigate in obese individuals. Specifically, we plan to examine reactivity and recovery in the cardiovascular, immune and endocrine systems. Finding a way to stop or drastically reduce the obesity epidemic would be the paramount answer to the negative implication of excess adipose tissue on the cardiovascular and immune systems. Obesity has been on the rise for years and shows no signs of slowing. For this reason it is imperative that research efforts be made to understand the implications of obesity on vital body systems. The cardiovascular and immune systems are arguably two of the most

critical. Understanding how these systems are affected will lead to immediate but short-term solutions buying much needed time for the larger issue of obesity to be resolved.

Our hypothesis is that individual levels of obesity alter the cardiovascular, immune and endocrine stress response. These alterations are differentially based on the type of stress (e.g. passive vs. active). More specifically, we hypothesize that obese individuals will have a greater sympathetic and endocrine response to passive stress, as measured by cardiovascular variables, alpha-amylase and cortisol. Conversely, following active stress the sympathetic and endocrine response is blunted in obese individuals. Finally, although both obese and non-obese individuals exhibit an elevated immune response to active compared to passive stress as measured by interleukin-6 and IgA. However, this increase in response to active stress is blunted in obese participants.

Specific Aims

Specific Aim 1: Confirm previous cardiovascular findings from our lab, which show that obese females are more reactive to passive stress, yet exhibit blunted reactivity to active stress. Further, we expect greater adiposity will result in delayed recovery following both stress tasks.

Specific Aim 2: Evaluate obesity-related HPA axis reactivity and recovery from stressors.

Specific Aim 3: Investigate obesity-instigated alterations in interleukin-1β and interleukin-6 during reactivity and recovery from different types of stressors (passive and active).

Specific Aim 4: Explore the relationship between obesity and salivary IgA alterations to different stressors.

Specific Aim 5: Evaluate overlap between dysfunctional endocrine, immune and cardiovascular response to passive and active stress.

Aim 1: will be achieved by measuring cardiovascular activity (blood pressure and heart rate) before, during and following completion of the two stress tasks. Cardiovascular data will be compared to the results from our previous study, a population of obese and non-obese college-age females. We expect to find similar patterns of cardiovascular reactivity that will be dependent on weight as well as task type. Cardiovascular data is often used as a measure of sympathetic nervous system activation in response to stress. We are also interested in the influence of obesity on the HPA axis, which often acts in concert to promote the stress response.

Aim 2: will be completed by measuring levels of cortisol, the main effector of the stress response initiated by the HPA axis, at set time points throughout the study.

Obesity has been shown to have an adverse impact on immunoregulatory networks, which results in compromised immunity (Pallaro, et al., 2002). However, what is lacking in this body of literature is the impact of obesity on immune response to variable types of stress. Past research, evaluating the effects of obesity on the immune response to stress has focused almost exclusively on psychological stress with tasks similar to MA. Thus, researchers have failed to take advantage of the differences in stress response that task type may elicit. The present study proposes to investigate the immune response in obese participants in a novel context by utilizing the MA and CP stress tasks to uncover variations in immune reactivity based on task. Interleukin-1β and interleukin-6 may be involved in the inflammatory process that link obesity and vascular dysfunction (Calabro et al., 2005). In a comprehensive review Steptoe, Hamer and Chida (2007)

assessed the influence of acute psychological stress on circulating cytokines. The review, which included thirty studies, found robust effects for the influence of stress on elevating levels of interleukin-1β and interleukin-6.

Aim 3: will be addressed by investigating alterations in of interleukin-1β and interleukin-6 in saliva. Recent studies show that obesity may mediate the effect of stress on immune response. Obesity is positively correlated to elevated levels of interleukin-1β and interleukin-6. We predict obese participants will have elevated concentrations of interleukin-1β and interleukin-6. Further, we expect fluctuations in these cytokines to be differentially influenced by type of stressor.

Salivary IgA is a more thoroughly studied measure of immune response to acute stress. However, the lack of investigation using this measure represents another void that exists in evaluating the effect of passive stress on immune reactivity in obese participants.

Aim 4: will be achieved by quantifying baseline levels of salivary IgA and calculating change and recovery scores following the stress tasks.

Willemsen and colleagues (2002) measured immune changes in healthy, normal-weight participants. They found a decrease in immune measures following the CP task and an increase following the MA task. Moreover, α-adrenergic blockade has been shown to inhibit the decreases following the CP task (Ring, et al., 2000). This result suggests α-adrenergic activation is likely involved in the immune response to the CP task. Thus, the adrenergic pathways may be responsible for some facet of both cardiovascular and immune response to stress. Further, increases in immune response to stress that coincide with blood pressure reactivity suggests alterations in immune

measures following stress may correspond to sympathetic activation, as indicated by blood pressure reactivity (Steptoe et al., 2001).

Aim 5: will be evaluated by comparing the cardiovascular, HPA axis and immune response for each stressor. Based on previous research suggesting a link between sympathetic and HPA axis activation by stress we expect cortisol to follow a similar pattern of reactivity in obese participants. Specifically, in response to passive stress we expect a greater increase in cortisol in obese participants compared to non-obese, while active stress in obese participants will result in blunted levels of cortisol. Cardiovascular and immune dysfunction is often seen in conjunction with obesity (Ritchie, & Connell, 2007; Martin, Qasim, & Reilly, 2008; Singer, & Granger, 2007).

Elevations of interleukin-1β and interleukin-6 commonly found in obese individuals may have some effect on reactivity concentrations of these measures. The sensitivity of interleukin-1β and interleukin-6 to acute stress makes them ideal targets for evaluating immunoregulatory dysfunction, allowing us to explore our final aim.

Innovation and Significance

Although the foundation exists for the study of immune dysfunction in obese individuals, many avenues have yet to be explored. This study will be the first to examine the effects of obesity on immune and cardiovascular response to passive and active stress. The interdisciplinary nature of this study permits findings that may be of relevance to the disciplines of cardiovascular psychophysiology and psychoneuroimmunology among others. Our preliminary study demonstrated that obesity influences cardiovascular reactivity to stress that is conditionally based on type of stress.

The current study seeks to further establish the relationship between obesity and cardiovascular dysfunction and investigate the role of obesity in immune dysfunction. The current body of immune research has overlooked the importance of differential activation based on task type in obese individuals. This will be the first study to examine immunoregulatory alterations in obese individuals following passive as well as active stress.

III. METHODS

Participants

Forty-four females were recruited from Oxford, MS and the surrounding area. Participants were recruited through flyers posted in hospitals, clinics and other venues; print media (e.g., local newspaper); and through electronic recruitment (e.g., Facebook, craigslist, University mailing lists, etc.).

Mean age was 22.3 (SD = 5.9). All participants were non-smokers, with no infectious illness or history of cardiovascular dysfunction or currently taking medication for hypertension or steroids (as self-reported). Participants were classified as obese or non-obese based on the BMI; four participants had BMIs that fell outside of the group ranges and were excluded from analyses. The mean BMI of the obese group was 41.9 kg/m^2 (SD = 7.2). For the non-obese group the mean body mass index was 22.3 kg/m^2 (SD = 1.6).

Measures

Anthropometric measures.

Body mass index. – BMI was calculated for each participant by the experimenter using the standard formula of body weight in kilograms divided by the square of height measured in meters. Weight was measured using a manual balance beam scale in pounds then converted to kilograms. Height was measured using a mechanical stadiometer in inches then converted to meters.

Waist circumference. – Waist circumference was obtained using measuring tape placed horizontally across the abdomen approximately at the navel. In participants where the rib cage could be seen or palpated without discomfort, the tape was specifically placed horizontally between the twelfth rib and the ileac crest. Waist circumference was recorded in inches.

Waist to hip ratio. – Each participant's measurement obtained from the waist circumference was divided by the hip circumference in order to calculate the waist to hip ratio. Hip circumference was obtained using measuring tape placed horizontally around the body at the widest point of the gluteus maximus. The hip ratio was recorded in inches.

Physiological measures.

Blood pressure and heart rate. – A Suntech Tango automated blood pressure monitor was used to measure systolic and diastolic blood pressure. A blood pressure cuff was placed on the non-dominant upper arm of the participant. During cuff inflation, heart rate was also recorded.

Heart rate variability. - Electrocardiographic data were collected using a Biopac MP150 system (Biopac Systems Inc., CA, USA), with accompanying amplifier. The electrocardiogram (ECG) activity was sampled at 1000 Hz and filtered in real-time using a band pass (low = 0.5 Hz, high = 35 Hz) filter. This allowed high frequency noise and baseline drift to be removed from the signal. AcqKnowledge (Biopac Systems Inc., CA, USA) data acquisition software was used to display the ECG on a computer screen. This allowed viewing in real-time; in addition, a copy was stored on a Dell personal computer for later analysis. Heart rate variability was calculated for baseline, tasks and recovery periods.

A three-electrode setup was used for HRV data acquisition; disposable silver/silver chloride electrodes were used. Lead II electrode placement was as follows: negative electrode was placed right below the clavicle in the intercostal space along the right midclavicular line, ground electrode was placed right below the clavicle in the intercostal space along the left midclavicular line, positive electrode was placed on the left abdomen, just under the breast.

An event detector was used to detect R-wave peaks. The threshold for events was set manually following a visual inspection of the data. The peak was defined as the maximum value obtained within a narrow window of time. The start of this window of time was demarcated by an upward crossing of the threshold (i.e., while the slope was positive) and the end of this period of time was demarcated by a downward crossing of the threshold. Once all the R-waves had been detected, a visual inspection was performed and manual corrections were carried out to exclude abnormal beats and identify artifacts.

Time domain analysis was chosen to assess HRV. Markers placed at the peak of the R waves were used to extract the time at which the beat occurred and to calculate the time periods between adjacent R-waves (R-R interval). Both the time data and the corresponding R-R intervals were exported to excel. The data were divided into segments and HRV was calculated for each segment.

The mean successive difference statistic was computed for baseline, tasks and recovery periods; these values are reported in milliseconds (ms). This method has been used in a number of studies (Allen, Matthews, & Kenyon, 2000; Christie & Friedman, 2004). This statistic represents the average of the difference between successive heartbeats for a given period of time and is used as a measure of heart rate variability. As

a differencing technique, the measure removes slow changes due to drift in the R-R interval series (Weinberg & Pfeifer, 1984), and it has accurately tracked both pharmacologically manipulated changes in cardiac vagal control (Hayano et al., 1991) and changes in vagal activation with passive head-up tilt (Vybiral, Bryg, Maddens, & Boden, 1989). Indeed, Hayano and colleagues (1991) found that the mean successive difference showed the highest correlation (r = 0.92) among eleven other potential HRV measures with change in mean R-R interval as a result of complete vagal blockade with atropine and sympathetic blockade with propranolol.

Endocrine and Immune measures.

Cortisol, Interleukin-6, Immunoglobulin A and Alpha-amylase. – Salivary samples were collected using the passive drool technique. The collection date, start time and time completed were recorded. Flow rates were calculated and recorded as part of the conversion factor needed for the final concentration analysis.

Saliva was collected from participants at 7 different time points; approximately two milliliters of saliva was collected at each time point. Immediately following collection, samples were stored in a cooler on ice. At the end of the study session (approximately 2.5 hours) samples were transferred to a freezer set to -80 degrees Celsius for storage. Once all saliva samples were collected, they were transported to Jackson, MS to the lab of Dr. Gailen Marshall for analysis. Samples were transported on ice and remained frozen for the duration of the trip.

Once at the lab, all samples were thawed and weighed. Sample preparation for analysis began by centrifuging samples at 1500 g (@3000 rpm) for 15 minutes. This allowed mucins and other particulates to be removed from the sample, which may

otherwise disrupt antibody binding. The supernatant was then aliquoted into separate microcentrifuge tubes (~500 ul) to be used in separate assays and stored at -20 degrees Celsius. On the day the assay was to be performed, the appropriate samples were removed from the freezer, thawed and vortexed.

It was our desire to measure interleukin-1β, due to its role in obesity related inflammation. However, in consideration of its partial overlap with interleukin-6 it was decided that only interleukin-6 would be measured at this time. In addition, the assessment of alpha-amylase was added. This salivary analyte demonstrates a close relationship to catecholamines, which typically require a blood sample for analysis; further, alpha-amylase has been shown to be a sensitive measure of sympathetic activation (Thoma, Kirschbaum, Wolf & Rohleder, 2012; van Stegeren, Rohleder, Everaerd, & Wolf, 2006).

Saliva samples were analyzed using commercially available assay kits (Salmetrics, State College, PA). Cortisol, interleukin-6 and IgA were analyzed using enzyme-linked immunosorbent assay (ELISA). Alpha-amylase was analyzed using a kinetic reaction assay kit. Manufacturer supplied protocols were followed for all salivary assays. All samples were assayed in duplicate and mean scores were used in statistical analysis.

Questionnaires.

Perceived stress scale – 10 (PSS). – The PSS-10 consists of 10 items with responses on a five-point Likert scale. The PSS is a self-report measure used to obtain how stressful participants perceive events in their life to be. The PSS has demonstrated

reliability with an alpha coefficient ranging from .84 to .86 depending on the sample population (Cohen, Kamarck, & Mermelstein, 1983).

Brief COPE. – The brief COPE consists of 28 items with responses on a four-point Likert-type scale. The Brief COPE is an abbreviated version of the COPE. This self-report measure is designed to measure coping strategies based on 14 subscales: self-distraction, active coping, denial, substance use, use of emotional support, use of instrumental support, behavioral disengagement, venting, positive reframing, planning, humor, acceptance, religion and self-blame. Reliability analysis performed on repeated administrations of the subscales has produced alpha coefficients averaging at or above .50 (Carver, 1997).

Likert scale of perceived stress specifically from laboratory stressors. – A 10 point Likert-type scale ranging from 0 (Not Stressful) to 9 (Extremely Stressful) was given to participants to assess the self-reported stress produced by each laboratory stressor administered. During the CP task (minutes 1 and 4) participants were asked to rate their experience of pain from 0 (Not Painful) to 9 (Extremely Painful). Immediately following the conclusion of the CP task they were asked to give a final pain rating. Following the MA task participants were asked to rate how hard they tried to successfully complete the task 0 (Not Hard) to 9 (Extremely Hard) and whether or not they felt like they gave up during the task.

Laboratory Stressors

Cold pressor.

The CP task lasted for a total of 8 minutes. Participants were asked to place their right hand in a container filled with ice and water. The participant was instructed to place

their hand palm down, with fingers spread apart to allow adequate water coverage, in the container. Using the pisiform bone as a landmark, the participant was asked to keep the water at a horizontal level across the wrist just above this bone. Water temperature was maintained near 10 degrees Celsius; a pump was used to circulate water from a chamber containing ice and water into the chamber with the participant's hand. Water temperature was measured using a digital thermometer just prior to the task and temperature was recorded on the data sheet.

Mental arithmetic.

The MA task lasted for a total of 8 minutes during which the participant was asked to subtract verbally by 7s from 921 as quickly and accurately as possible. Answers were monitored and if the participant gave an incorrect answer, the experimenter corrected the participant, and then asked them to continue from the last correct response. The MA task was audio recorded and subsequently coded for number of answers attempted and number of correct answers.

Procedure

A waiver of signed consent was requested from the Institutional Review Board in order to obtain screening information from potential participants. Participants' age, history of smoking, current illness, history of cardiovascular dysfunction and current medications (hypertension, depression, steroids or beta-blockers) was used to assess inclusion and exclusion criteria. Further, self-reported weight and height was used to screen potential participants.

Prior to the study visit, potential participants were given written instructions asking that they: do not consume alcohol or caffeine or exercising vigorously for at least

12 hours before the study session; they should eat a light breakfast at least 1 hour before their session; be aware of their dental hygiene, on the night before the session be careful not to make their gums bleed, and do not brush their teeth on the morning of the session. Participants were contacted the night before the study session to remind them of the restrictions. To control for the circadian rhythm of cortisol and cardiovascular activity, all study visits were scheduled in the morning, beginning between 8:30am - 9am and concluding no later than 11:30am.

Upon entering the laboratory the participant was invited to sit in a lounge chair, all potential participants were given an informed consent form to read over and sign. After the consent form was signed, the participant was assigned an identification number and asked a series of questions to collect demographic information and determine any contraindications to participation in the study (e.g., age, cardiovascular risk factors, oral contraceptive use, adherence to pre-session instructions). The participant was then asked to complete two questionnaires, the PSS and the Brief COPE. Next, the passive drool method of saliva collection was explained to the participant.

The participant was instructed to turn off or silence their cell phone. A blood pressure cuff was placed on the left upper arm. Following a brief explanation of electrode placement electrodes were placed on the participant. A three-leadwire system was used. Immediately prior to placing the electrodes, the area was cleansed with an alcohol wipe. The wipe was also used to gently abrade the area to encourage conductivity of electrical signal. The participant was then instructed to rest quietly while reading through a selection of magazines for ten minutes. The magazines were used to encourage relaxation while giving participants some stimuli to prevent sleeping. Magazines were screened for

distressing or offensive content. During this initial rest period heart rate was measured continuously for the ten minutes. A baseline value of heart rate variability was calculated by averaging the mean successive difference at minute two through just before minute four, and minute four through just before minute six. These two mean successive difference values were then averaged for a baseline value of heart rate variability.

Blood pressure and heart rate were taken at the beginning of the first, third, fifth, seventh and ninth minute during the baseline rest period. The first reading was used to acclimate the participant to the feeling of the blood pressure cuff inflating and give the participant time to relax. Thus, this reading was discarded. The second and third readings were averaged and the single number was used as the baseline blood pressure and heart rate reading. The fourth and fifth readings were also discarded due to elevations during saliva collection. The first saliva collection occurred at minute seven of the baseline rest period. The collection start time and completion time were recorded on the data sheet.

Once the initial rest period was complete, the participant performed either the MA task or the CP task; the order was counterbalanced. The MA task lasted for a total of eight minutes. Performance of the MA task was recorded for later evaluation. During the MA task, blood pressure and heart rate readings were recorded at minutes zero, one, three, five and seven. The first three measurements were averaged to determine a mean blood pressure and heart rate score for the task.

Heart rate was recorded throughout the task. A mean heart rate variability score was calculated for each task by averaging the mean successive difference at minute zero through just before minute two, and minute two through just before minute four. These two mean successive difference values were then averaged for a task value of heart rate

variability. A saliva sample was taken immediately following the task. Following the MA task the participant was given the MA Stress Questionnaire, where they rated their perception of the degree of stress produced by the MA task, their perceived effort and whether or not they gave up during the task.

The CP task lasted for a total of eight minutes. During the CP task, blood pressure and heart rate readings were recorded at minutes zero, one, three, five and seven. The first three measurements were averaged to determine a mean blood pressure and heart rate score for the task. A saliva sample was taken immediately following the task. Following the CP task the participant was given the CP Stress Questionnaire, where they rated their perception of the degree of stress produced by the CP task and the amount of pain experienced during the task. Following the first task, be it the MA task or the CP task, the participant was asked to sit quietly and read the magazines provided for a second rest period that lasted for forty minutes. Heart rate variability was recorded continuously until minute twelve of the rest period. Blood pressure and heart rate readings were taken at the beginning of minutes: one, three, five, seven, nine, twelve, fifteen, twenty, twenty-five, thirty, thirty-five and forty. Saliva samples were taken at minute 20 and minute 40. Subsequent to administration of the final laboratory stressor, the participant had a third and final rest period. During this time blood pressure, heart rate, heart rate variability and saliva was taken as described for the second rest period. The blood pressure cuff and electrodes were then removed.

Next, weight and height was measured. In the interest of protecting female participants' privacy only female experimenters were in the room at this time. The participant was asked to remove their shoes and any outerwear that could affect their

weight. Waist circumference was measured using the method described above. The participant was asked to raise their shirt to their belly button to ensure accurate measurement. The participant was instructed to stand up straight and breathe normally being sure not to hold their breath. The hip circumference measurement was then taken using the method described above. The participant was debriefed and any questions they had regarding the study was answered.

IV. RESULTS

Anthropometric measures

Independent groups t-tests were performed in order to verify differences between BMI groups on anthropometric measures. All statistical analyses were performed using SPSS with a type I error level set at $p = 0.05$, unless otherwise noted. Between-group differences were evaluated for BMI, WC (measured in inches), WHR and weight (measured in pounds). As expected, there were significant group differences with the obese group having significantly higher WC (obese mean = 46.7, SD = 6.5; non-obese mean = 31.8, SD = 2.4; $t(38) = -9.61$, $p < 0.000$), WHR (obese mean = 0.9, SD = 0.1, non-obese mean = 0.8, SD = 0.05, $t(38) = -3.95$, $p = 0.001$) and weight (obese mean = 255.2, SD = 54.5, non-obese mean = 133.3, SD = 11.3, $t(38) = -9.80$, $p < 0.000$) (Table 1).

Analysis of questionnaires

To evaluate group differences in questionnaire scores a series of t-tests were performed. Table 2 presents the means and standard deviations for the obese and non-obese groups on PSS, stress experienced during the CP and MA tasks, pain experienced during minute one and minute four of the CP task and perceived effort during the MA task. PSS scores were within the normal range for both groups, scores of 20 or higher are typically seen in high stress populations. There were no significant differences between the obese and non-obese group on any of the questionnaire scores.

The Brief COPE was administered to uncover differences in coping styles between the two groups. Of the 14 subscales on the Brief COPE, there were two that showed significant group differences, denial and behavioral disengagement. Questions used to assess denial included: “I've been saying to myself "this isn't real” and “I've been refusing to believe that it has happened.” Behavioral disengagement was appraised using the following statements: “I've been giving up trying to deal with it” and “I've been giving up the attempt to cope.”

Due to violations of Levene’s test, showing an unequal distribution of variance between the groups, adjustments were made to p-values. Even after adjustment, the obese group showed greater use of denial (obese mean = 3.1, SD = 1.7, non-obese mean = 2.1, SD = 0.3, $t(38) = -2.57$, $p = 0.018$) and behavioral disengagement (obese mean = 3.3, SD = 1.5, non-obese mean = 2.5, SD = 0.9, $t(38) = -2.07$, $p = 0.047$) than the non-obese group.

Mental arithmetic performance

Participants’ responses during the MA task were recorded for later analysis. Following the study session the recordings were coded for number of responses attempted and number of incorrect responses. These values were used to calculate number of correct responses as well as an overall percent correct. The means and standard deviations for the obese and non-obese groups on total number of responses attempted and percent of correct responses are presented in Table 3.

Performance on the MA task was evaluated between groups. There were no significant differences between the two groups on number of responses attempted ($p = 0.335$) or percent of correct responses ($p = 0.450$).

Group differences in baseline levels of cardiovascular and salivary measures

In order to determine group differences present at baseline, t-tests were used to compare baseline levels of all cardiovascular (SPB, DBP, HR and HRV) and salivary (cortisol, interleukin-6, IgA and alpha-amylase) measures. As expected, the obese group had significantly higher SBP, DBP and HR levels at baseline, although all values were within an acceptable clinical range. Baseline level of HRV was not significantly different between groups; however, the obese group did have a trend towards a lower level of HRV as would be expected (Table 4).

Salivary data were incomplete for a subset of the measures. To analyze saliva, manufacturer recommended dilutions were used when assaying the samples. This resulted in an over dilution of a portion of the samples and thus there were insufficient analytes present for detection. Sample size for each of the salivary measures is presented in Table 5. Inspection of the salivary measures revealed subsets that were not normally distributed. Based on outcomes from visual inspection of the data and the Shapiro-Wilk test (used due to sample size less than 50) for normality, IgA and alpha-amylase were found not to be normally distributed. To approximate a normal distribution a base 10 logarithmic transformation was used for analysis of baseline difference and all other analysis of IgA and alpha-amylase.

Analysis of group difference between baseline level of salivary measures revealed significant differences in levels of cortisol and alpha-amylase, $t(37) = 2.87$, $p = 0.007$ and $t(38) = 2.29$, $p = 0.027$, respectively. The obese group had lower levels of both cortisol and alpha-amylase at baseline. There were no significant group differences found in baseline levels of interleukin-6 or IgA (Table 6).

Cardiovascular manipulation check

Prior to evaluating difference in cardiovascular reactivity, we first wanted to establish that significant differences were present between baseline levels and task levels on the cardiovascular measures; that is, that our tasks did indeed produce significant levels of cardiovascular response compared to baseline. Each physiological variable was analyzed individually utilizing a two-factor (2 group x 3 periods) repeated measures analysis of variance (ANOVA). The grouping factor was used to separate participants into the obese and non-obese groups; analyses were then performed to compare baseline, CP, and MA levels of SBP, DBP, HR and HRV (Figure 1). Greenhouse-Geisser corrections were used for two of the ANOVAs (DBP and HR) due to violations of sphericity. There were no significant interactions between period and group. There was a significant main effect of period for all cardiovascular variables with the exception of HRV: SBP ($F(2, 76) = 34.179$, $p < 0.000$), DBP ($F(1.681, 76) = 42.263$, $p < 0.000$) and HR ($F(1.623, 76) = 5.912$, $p = 0.007$).

Follow up pairwise comparisons were evaluated to confirm both stressors (CP and MA) produced effects that were significantly different from baseline for cardiovascular measures (SBP, DBP and HR) averaging across both groups. Adjustments to alpha levels for multiple comparisons were done using Bonferroni corrections. For SBP, the level during the CP (mean = 130) and MA (mean = 128) task were significantly higher than the level at baseline (mean = 119, both ps < 0.000). Level of DBP during the CP (mean = 82) and MA (mean = 80) tasks were also significantly higher than at baseline (mean = 72, both ps < 0.000). For HR, both tasks (CP, mean = 80, $p = 0.020$ and MA, mean = 82, $p = 0.007$) showed elevated levels compared to baseline (mean = 78). Although the analysis

of HRV was not significant, the data showed a non-responsive trend by the obese group across periods (baseline mean = 29.5, CP mean = 29.1, MA mean = 29.3) in comparison to the non-obese group (baseline mean = 37, CP mean = 38.4, MA mean = 30.2).

There was also a significant main effect of group (obese and non-obese) for SBP ($F(1,38) = 25.390$, $p < 0.000$) and DBP ($F(1, 38) = 13.023$, $p = 0.001$). No other main effects were present. Follow up pairwise comparisons revealed that the obese group had higher levels of SBP (obese = 133, non-obese = 119, $p < 0.000$) and DBP (obese = 81, non-obese = 75, $p = 0.001$) when averaged across the three periods.

Based on these findings, we conclude that our stress tasks did produce a significantly elevated cardiovascular (SBP, DBP and HR) response; further, although not significant, there was a different pattern of HRV response to the stress tasks between groups. In addition, the elevated levels of basal blood pressure present in the obese group did not hinder their reactivity to the stress tasks, demonstrated by the significant main effect of group across all three periods.

Analysis of cardiovascular reactivity

Aim one was divided into two parts; the first part was to evaluate cardiovascular reactivity differences between groups to the two stress tasks. This was achieved by utilizing a mixed-model, repeated measures analysis of covariance (ANCOVA). In these (2 group x 2 period) ANCOVAs, group (obese and non-obese) was used as a between-subjects factor and differences between reactivity values (calculated as the difference between task average and baseline) for the CP and MA task were the within-subjects factor; baseline values were entered as a covariate.

There was no significant interaction for SBP. However, there was a significant main effect of group ($F(1, 37) = 5.775$, $p = 0.021$) (Table 7). The average SBP reactivity value across both tasks was 12.5 for the obese group and 6.8 for the non-obese. For DBP, the interaction was also not significant; but again a main effect of group was present ($F(1,37) = 9.308$, $p = 0.004$). The average DBP reactivity value was 11.3 for the obese and 6.6 for the non-obese. There was a significant interaction between group and task for HRV ($F(1,37) = 4.381$, $p = 0.043$). Follow up analysis did not reveal any significant differences between groups for either task (CP, $t(38) = 0.427$, $p = 0.672$; MA, $t(38) = -1.389$, $p = 0.173$).

Figure 2 displays change scores for each of the cardiovascular variables separated by group. On average, the obese group experienced greater blood pressure reactivity in response to stress. Based on visual inspection of Figure 2, the exaggerated reactivity appears to be similar across tasks for DBP; however, for SBP reactivity the obese group appears to have greater reactivity to the CP task. HRV reactivity is illustrated in Figure 2d, the obese group appears to have a similar level of HRV across the two stress task. In contrast, the non-obese group shows greater HRV during the CP task in comparison to level during the MA task. There were no significant main effects, period effects or interactions for HR during the tasks.

Analysis of cardiovascular recovery

The second part of aim one was to evaluate cardiovascular recovery differences between groups following termination of the two stress tasks. Recovery was investigated for each physiological measure using a (2 group x 3 period) mixed-model, repeated measures ANCOVA. Recovery was divided into early, middle and late recovery. For

blood pressure and HR, early recovery was calculated as the difference between task average, and an average of minutes one and three of the recovery period (see Figure 3). Likewise, middle recovery was calculated as the difference between task average, and an average of minutes five and seven of the recovery period, and late recovery as the difference between task average, and an average of minutes nine and twelve of the recovery period. For HRV, recovery was also divided into early, middle and late periods. Due to the continuous measurement of this variable the twelve minutes of recorded HRV were divided into six even segments and mean successive difference scores were computed for each of the six segments. The six scores were then divided into three pairs; the pairs were then averaged to form early, middle and late periods (see Figure 4). HRV recovery scores were calculated similarly to the other cardiovascular variables by subtracting the early, middle and late recovery period values from the task average.

Group (obese and non-obese) was used as a between-subjects factor; baseline values and reactivity scores were entered as covariates. Recovery from the CP task was analyzed first (Table 8). There was a significant main effect of period for HRV recovery from the CP task, due to violations of sphericity Greenhouse-Geisser corrections were used, $F(2, 68) = 6.376$, $p = 0.009$. Both groups showed an increase in HRV following the termination of the CP task. Collapsed across group, pairwise comparisons revealed HRV increased significantly from the early (mean = 0.799) to middle (mean = -7.644, $p < 0.000$) as well as the middle to late (mean = -10.639, $p = 0.036$) recovery period. Recovery from the MA task was analyzed next. A significant main effect of group was found for SBP recovery, $F(1, 36) = 4.230$, $p = .047$) (Table 9). The average SBP recovery following the MA task was 5.0 for the obese group and 9.1 for the non-obese group.

Therefore, on average across the three recovery periods the obese group showed less SBP recovery from the MA task than the non-obese group. A significant interaction was present for HRV recovery from the MA task, $F(2, 72) = 3.934$, $p = 0.024$. The data revealed a similar increase in HRV in both groups from early to middle recovery. However, at the late recovery period obese group showed a decrease in HRV compared to the non-obese group.

There were no other significant main effects or interactions for the recovery analysis on DBP or HR from either task.

Salivary manipulation check

Prior to evaluating reactivity difference between groups on salivary measures we first wanted to assess differences across time. Four repeated measures ANOVAs were used to investigate period differences on the salivary measures. All participants were entered into the analysis at once to provide an overall picture of response to the stress tasks. Level at baseline, tasks and recovery periods were evaluated for changes in levels of cortisol, interleukin-6, IgA and alpha-amylase (Figures 5).

There was a significant effect of period for cortisol ($F(1.948, 74.031) = 15.991$, $p < 0.000$) and IgA ($F(2.837, 102.117) = 6.147$, $p = 0.001$). Follow up testing was done using t-tests to determine if the levels of cortisol and IgA during the stress tasks were significantly different from levels at their respective rest periods. For cortisol, participants had significantly elevated levels present during both of the stress tasks when compared to their respective rest periods (CP: $t(38) = 4.295$, $p < 0.000$; MA: $t(38) = 3.689$, $p = 0.001$). For IgA, participants had significantly elevated levels during the MA task when compared to the subsequent rest period ($t(39) = 3.987$, $p < 0.000$); however the

level of IgA present during the CP task was not significantly different from the following rest period ($t(30) = 1.912$, $p = 0.064$).

A significant period effect was not present for alpha-amylase ($p = 0.075$); however, the change over time was similar to that of cortisol and IgA, with both task levels being elevated relative to their respective rest periods. Period effect for interleukin-6 was non-significant ($p = 0.593$) and did not show the pattern of response present among the other salivary measures.

Analysis of salivary reactivity

Aims two through four were achieved through analysis of the salivary measures. We wanted to know if there were group differences or interactions between obesity and reactivity to the two stress tasks. To assess reactivity in the salivary measures, repeated measures ANOVAs were performed. Reactivity scores for the two tasks were calculated for each participant by subtracting the baseline value from the task value. A grouping factor was use to separate participants into obese and non-obese groups. Salivary reactivity is presented in Figure 6.

There was a significant interaction between group and task for cortisol ($F(1, 37) = 5.047$, $p = 0.030$) (Table 10). Follow up analysis revealed a significant difference in cortisol reactivity between groups for the CP task ($t(37) = -2.170$, $p = 0.010$), but not the MA task ($t(37) = .338$, $p = 0.737$). The obese group had an exaggerated cortisol response to the CP task compared to the non-obese group.

The interaction between group and task was also significant for alpha-amylase ($F(1, 38) = 6.923$, $p = 0.012$). Follow up analysis demonstrated marginally significant group differences in alpha-amylase reactivity to the MA task ($t(30.356) = -2.034$, $p =$

0.051), but not to the CP task (t(38) = 0.487), p = 0.629). The obese group responded to the MA task with greater alpha-amylase reactivity compared to the non-obese group. There were no significant main effects or interactions for task reactivity comparison by group for interleukin-6 or immunoglobulin-A.

Analysis of salivary recovery

In addition to reactivity differences among the salivary measures, we also wanted to know if there were group differences or interactions between obesity and tasks for recovery scores. Recovery differences between groups in salivary measures were investigated using repeated measures ANCOVAs. Separate analyses were performed for each task. Group (obese and non-obese) was used as a between-subjects factor and task value and the corresponding recovery value comprised the within-subjects factor; baseline values were entered as a covariate.

None of the ANCOVAs revealed significant period (task and recovery) by group interactions. For the MA task, there was a significant main effect of period for both cortisol and IgA, F(1, 36) = 6.921, p = 0.012 and F(1, 37) = 6.957, p = 0.012, respectively (Table 12). As expected, cortisol and IgA task levels were higher than subsequent levels during the rest period. The interaction between period and group for IgA approached significance (p = 0.072). As shown in Figure 7f, the obese group shows a trend towards blunted reactivity.

None of the other recovery analyses for salivary measures were significant (Table 11). However, with few exceptions (cortisol recovery from the CP task and interleukin-6 recovery from both tasks) a visual inspection of the graphs reveals a trend by both groups

in the expected direction. That is, that task levels were higher than levels during the rest period (Figure 7).

Correlational analysis

Aim five of the current study was accomplished through correlational analysis. This analysis was used to investigate relationships among cardiovascular, endocrine, and immune reactivity to the stress tasks. Changes scores were used for both the cardiovascular and salivary measures (e.g., task average minus baseline) allowing us to control for differences in baseline values. Correlations were computed separately for the obese and non-obese groups to determine differences in the relationships among cardiovascular and salivary measures; these correlations are displayed in Table 13.

For the CP task there was a significant correlation for the obese group between SBP reactivity and cortisol ($r = 0.495$, $p = 0.031$). In the non-obese group there was a significant correlation between blood pressure reactivity and IgA (SBP, $r = 0.448$, $p = 0.048$; DBP, $r = 0.550$, $p = 0.012$). These results suggest that for the CP task greater SPB reactivity was related to increased cortisol for the obese group. Although this relationship between cardiovascular reactivity and cortisol was not found in the non-obese group during the CP task, there was an association between blood pressure and IgA. Blood pressure reactivity to the CP task increased in non-obese participants, as did IgA.

For the MA task the obese group showed a significant correlation between HR reactivity and cortisol ($r = 0.615$, $p = 0.005$). There was also a significant correlation between HR reactivity to the MA task and IgA ($r = 0.504$, $p = 0.024$) in the obese group. In the non-obese group, HR reactivity to the MA task was significantly correlated with cortisol (HR, $r = 0.525$, $p = 0.018$). Although the relationships between cardiovascular

reactivity and salivary response to the CP task were distinct between the obese and non-obese groups, a different pattern emerged for the MA task. Both the obese and non-obese groups showed an increase in HR reactivity in response to the MA task that was significantly correlated to an increase in cortisol during the task. In addition, there was a significant relationship between HR reactivity and increased IgA during the MA task in the obese group.

V. DISCUSSION

The present study was designed to examine differences between obese and non-obese individuals in their cardiovascular, immune and endocrine response to two acute stress tasks. The CP and MA tasks were chosen because they represent different categories of stress (e.g., physical and psychological) and require distinct methods of coping (e.g., passive and active). The distinction between these two tasks comes about in part due to the different patterns of physiological arousal associated with each task.

Prior to conducting our main analyses, group differences were assessed on a number of factors. As expected, participants in the obese group had significantly higher anthropometric measurements than the non-obese group. Several questionnaires were administered throughout the study to assess life stress, stress experienced during the tasks, pain experienced during the CP task and perceived effort on the MA task. There were no group differences present on any of these measures. The Brief COPE, a measure of coping strategy, was administered at the beginning of the study session. Group differences were found for two of the coping mechanisms: denial and behavioral disengagement. The obese group reported greater use of denial and behavioral disengagement in dealing with recent stressful events in their life. There were no differences found between groups for performance on the MA task, measured as total number of answers attempted or percent of correct responses. As expected, the obese

group had significantly higher baseline levels of SBP, DBP and HR compared to the non-obese group; as well as reduced HRV, though this was not significant. The obese group exhibited lower levels of cortisol and alpha-amylase at baseline.

The first aim of this project was to confirm previous findings from our lab in which obese participants had greater cardiovascular reactivity to passive stress, yet exhibit blunted reactivity to active stress. Further, we anticipated greater adiposity would result in delayed cardiovascular recovery following both of the stress tasks. Analysis revealed significant reactivity differences between groups. The obese group demonstrated greater blood pressure reactivity when averaged across tasks. We were able to confirm our hypothesis that obese participants would have exaggerated cardiovascular reactivity to passive stress. However, we failed to detect blunted reactivity in response to active stress.

Our hypothesis of blunted reactivity was based on findings from our previous study (Burch & Allen, 2014). In that study, participants were instructed to subtract a two-digit number from a four-digit number for the MA task. In the present study participants began with a three-digit number and subtracted by a one-digit number. This change may have resulted in the task being perceived as easier. If the task was perceived as achievable then the participant may have been actively engaged in an attempt to complete the task. This would suggest that the defeat reaction that may have resulted in the previously found blunted reactivity might not have been triggered in the current study. It is possible that there is a threshold of task stressfulness, and a perceived lack of resources to overcome the stressor, that must be met to cause blunted reactivity.

Changes in alpha-amylase were also assessed as a second measure of autonomic activation. As with blood pressure and HR, alpha-amylase increases as a result of sympathetic activation (Nater & Rohleder, 2009). A study conducted by Leslie, Putney and Sherman (1976) attempted to elucidate the mechanisms behind alpha-amylase secretion. By removing the parotid glands of rats they were able to quantify the effects of in vitro stimulation of alpha and beta-adrenergic receptors. Although the cells were responsive to both types of adrenergic stimulation (suggesting the presence of both types of receptors), they found that isoprenaline, a non-selective beta-adrenergic agonist, resulted in a significantly greater increase in alpha-amylase from the parotid tissue. Pharmacological studies in humans have corroborated these early findings in animals suggesting that alpha-amylase secretion is primarily stimulated by the activation of beta-adrenergic receptors (van Stegeren et al., 2006).

In the current study we found that in response to the MA task, the obese group had elevated levels of alpha-amylase compared to the non-obese group. This suggests that the obese group may be more sensitive to the beta-adrenergic activation that occurs as a result of the MA task. This finding is in opposition to our hypothesis of blunted sympathetic reactivity by the obese group in response to active stress. A rise in alpha-amylase in response to psychological stress in the absence of a corresponding increase in cardiovascular measures have led some to posit that alpha-amylase is an acutely sensitive measure of the sympathetic stress response (van Stegeren et al., 2006).

A review of the literature returned a single study evaluating the impact of obesity on alpha-amylase response to an acute stress task. In this study, the authors found no difference in alpha-amylase reactivity to an active stress task between an obese and non-

obese group (Jayasinghe, Torres, Nowson, Tilbrook & Turner, 2014). However, the sample consisted entirely of older men and the mean BMI of the obese group was 30.6, compared to a mean BMI of 23.5 in the non-obese group. These findings together with the result of the current study suggest that there may be a gender difference in alpha-amylase response to stress in obese individuals, with only obese females showing an exaggerated alpha-amylase stress response. Alternatively, a greater difference between BMI groups may be required for distinctions in the stress response to surface.

Cardiovascular recovery from the two stress tasks was also evaluated as part of our first aim. We hypothesized that the obese group would have a delay in recovery following both of the stress tasks. Three time points during the recovery period were used to assess early, middle and late recovery for each of our cardiovascular measures. Two recovery analyses revealed significant group differences. Following the MA task, the obese group demonstrated blunted SBP and HRV recovery. When collapsed across early, middle and late recovery the obese group exhibited blunted SBP recovery compared to the non-obese group. Visual inspection of the data revealed that the non-obese group had fallen below their baseline SBP by the late recovery period. The obese group however failed to reach their baseline level of SBP, even by the late recovery period. For HRV, recovery was similar between the two groups for early and middle recovery; HRV was increasing in both groups following termination of the MA task. However, during the late recovery period the obese group did not continue to increase in HRV, but instead showed a decreasing trend. Thus we were able to affirm our hypothesis of a delayed cardiovascular recovery from the MA task although we were unable to identify differences in CP recovery.

The second aim of the current study was to evaluate HPA reactivity and recovery from the stress tasks, with a specific interest in difference between the obese and non-obese groups. We hypothesized that the obese group would exhibit exaggerated HPA reactivity to the CP task and blunted reactivity to the MA task. Stimulation of the HPA axis, a system often activated concurrently with the sympathetic nervous system, results in the release of cortisol. Cortisol level at baseline was higher in both groups than at any subsequent period, including the samples taken immediately following the stress tasks. We are not sure why participants demonstrated elevated levels of cortisol at baseline. It is possible that the ten-minute rest period was not a sufficient amount of time to allow participants to reach a baseline level of cortisol. The obese group had lower basal levels of cortisol. Reactivity differences in cortisol, that took baseline differences into account, were also present. As anticipated the obese group had an exaggerated cortisol response to the CP task compared to the non-obese group. However, we were unable to identify group differences in cortisol response to the MA task.

A diminished level of basal cortisol in obese individuals, or those with an elevated waist-to-hip ratio has been found by others (Salehi et al., 2005; Soros, Zadik, & Chalew, 2008; Ljung, Andersson, Bengtsson, Björntorp, & Mårin, 1996). In response to stress, the effect of obesity on cortisol secretion has been mixed with findings of both exaggerated (Epel et al., 2000; Moyer et al., 1994) and blunted (Miller et al., 2013; Phillips, Roseboom, Carroll & de Rooij, 2012) cortisol reactivity in obese participants. Yet others have failed to find a relationship between cortisol reactivity to laboratory stress tasks and obesity (Brydon, 2011). All of these studies used stress tasks that would be classified as active stressors. We predicted a blunted cortisol response to active stress in the obese

compared to the non-obese group. Similar to the results of our cardiovascular variables we failed to detect blunted cortisol reactivity. It is possible that the same explanation is responsible for both results. That is, blunted reactivity will only manifest in situations where a threshold of perceived stressfulness is met.

In response to stress we would expect some activation of the HPA axis and subsequent release of cortisol. The release of cortisol results in the inhibition of insulin and thus an increase in the level of glucose availability (Lambillotte & Henquin, 1997). The availability of glucose is important when the stress response requires action (e.g., fight or flight). However, in instances of stress activation that is best resolved by passive submission the exaggerated rise in cortisol and influx of unnecessary glucose can contribute to insulin resistance, a hallmark of the metabolic syndrome. The pattern of HPA response seen in the non-obese group, where the level of cortisol following the CP was lower than the level following the MA task, represents a more advantageous model of HPA reactivity.

HPA recovery from the tasks was also evaluated. There was not a significant change in cortisol level following the CP task. In contrast, following the MA task cortisol dropped significantly from task level to level during the subsequent rest period. Based on these findings it appears that obesity is associated with increased exposure to cortisol through inappropriate reactivity to passive stress.

Our third aim was to investigate changes in the level of interleukin-6 in response to different stressors. More specifically, we wanted to know if differences would be present between obese and non-obese individuals. Unfortunately, interleukin-6 data were

missing for several participants, resulting in a considerable decrease in our ability to detect group differences. None of the interleukin-6 analyses produced significant results.

Examining group differences in the immune response to stress tasks was our fourth aim. Due to the presence of stress hormone receptors on immune cells, elements of the immune system are particularly responsive to stress. The non-invasive collection of saliva allowed us to quantify the immune response to stress by assessing changes in the level of IgA. In healthy individuals IgA level typically increases in response to active stress (Isowa, Ohira, & Murashima, 2004; Ring et al., 2000; Ring et al., 1999; Willemsen, Carroll, Ring, & Drayson, 2002; Willemsen et al., 1998; Willemsen, Ring, McKeever, & Carroll, 2000; Winzer et al., 1999) and decreases in response to passive stress (Ring et al., 2000; Willemsen et al., 2002). Although not significant, visual inspection of the graph (Figure 6c) suggests that on average both groups were more responsive to the active stressor (MA).

Analysis of immune reactivity was not significant. However, as predicted, both groups show a trend towards greater immune reactivity to the MA task compared to the CP task. Marginally significant group differences were found for immune recovery following the stress tasks. When averaged across group, the level of IgA dropped significantly during the rest period following the MA task. However, the obese group demonstrated blunted immune recovery compared to the decrease seen in the non-obese group. A significant decrease was not present for IgA recovery from the CP task, though both groups showed a decreasing trend. In addition, the obese group again showed a trend towards blunted recovery.

Although there has been some research into baseline differences of IgA in obese and non-obese groups, the investigation of IgA stress response differences are severely lacking. Confirmed by the current study, there do not appear to be differences, based on adiposity, in basal levels of IgA (Cieslak, Frost & Klentrou, 2003). Mounting evidence suggests that the immune system is compromised in obese individuals (for a review see Milner & Beck, 2012). Although immunological differences at baseline were not found between the obese and non-obese groups, recovery differences were present. It appears that the obese group showed less movement in immune response throughout the study when compared to the non-obese group. This lack of change may represent an unfavorable immune response. Blunted IgA response to stress in obese individuals warrants further investigation. The relationship between obesity and compromised immunity may be better demonstrated by an overall blunted immune response to challenge rather than differences in baseline parameters.

The final and overarching aim of our study was to investigate relationships among cardiovascular, endocrine and immune responses to stress, with the intention of characterizing differences between obese and non-obese groups. In response to the CP task, the obese and non-obese group showed different patterns of associations between measures. For the non-obese group, blood pressure reactivity to the CP task was related to IgA reactivity. However, for the obese group cortisol was related to increased SPB activity in response to the CP task.

Our hypothesis of exaggerated cardiovascular and HPA activity in response to passive stress in the obese group was confirmed. Further, we observed an association between the HPA and sympathetic nervous system response to passive stress in the obese

group. The damaging effects of stress hyper-reactivity, both of the cardiovascular and HPA axis have been described above. Here we conclude that for obese individuals the relationship between the sympathetic nervous system and the HPA axis may be a synergistic one that works together to contribute to a disproportionate response to a passive stress task. Indeed, a comprehensive review by Chrousos and Gold (1992) describes a "positive, reverberatory feedback loop so that activation of one system tends to activate the other as well" (p. 1246). Confirmation and investigation into the implications of this finding should be examined further.

The immunological response to the CP task was similar between the obese and non-obese group. However, the rise in IgA in the non-obese group was related to the increase in blood pressure reactivity to the task. In response to passive stress the non-obese group demonstrated significantly less cardiovascular (SBP) reactivity; yet, they exhibit a relationship between blood pressure and immune response to the task.

Although the response to passive stress shows dissimilar patterns in the relationships among the variables, response to the active task shows a more congruent pattern between the groups. Both the obese and non-obese groups responded to the MA task with an increase in HR that was significantly related to level of cortisol. Additionally, the obese group exhibited an increase in IgA that was positively related to HR reactivity during the MA task.

The similar HPA activation by active stress in both the obese and non-obese group suggests that this response may not be impaired in obese individuals. Further, level of cortisol was related to sympathetic activation in both groups during the task. It is important to consider that although the groups showed similar patterns of reactivity in

response to active stress the obese group demonstrated blunted cardiovascular recovery following the MA task. Additionally, although the obese group failed to show a relationship between immune and cardiovascular reactivity to passive stress, the relationship was present in response to active stress.

The current study is not without limitations. Males were excluded despite a considerable amount of research indicating gender differences in stress reactivity (Allen, Stoney, Owens, & Matthews, 1993; Girdler, Turner, Sherwood, & Light, 1990; Lawler, Wilcox, & Anderson, 1995). Due to limited resources it was not feasible to obtain an adequate sample to evaluate gender differences. Additionally, a previous study from our laboratory found cardiovascular reactivity difference using a female population. We hoped to replicate these findings and therefore we chose to recruit only female participants. Future studies should investigate similar impairments of obesity on stress response in males.

Saliva collection presented a number of challenges. Saliva was collected using the passive drool technique; to achieve collection participants would drool into a collection vial while investigators were present to ensure adequate collection. Based on cardiovascular measures taken during saliva collection, it appears that most participants experienced some sympathetic arousal, at least during the initial collection. Although the cardiovascular data recorded during this period was not used in the analysis, this response to saliva collection could have impacted some of the salivary measures. Participants also demonstrated considerable variation in the amount of time necessary to produce the required saliva volume. The quantity of salivary analytes present in saliva sample is influenced by collection duration (Beltzer et al., 2010). In an attempt to correct for these

variations, collection time was recorded and flow rates were calculated for each sample and used as a conversion factor in the appropriate analyses.

We did not control for the potential effects of menstrual cycle. It is possible that menstrual phase may have an impact on some of the salivary measures, particularly cortisol. A final limitation of the current study was the lack of an assessment of physical fitness. In a study of 906 women, researchers found that self-reported fitness was related to less coronary artery disease and lower risk of other adverse cardiovascular events; despite obesity not being independently related to these outcomes (Wessel et al., 2004). There are a number of fitness measures that could be used, ranging from maximal oxygen uptake tests to self-report questionnaires used to quantify physical activity. Future studies examining obesity and cardiovascular function should consider including a measure of physical fitness.

In conclusion, we found that in response to active stress, the relationship between HPA and sympathetic activation was similar for obese and non-obese groups. However, the obese group appears to have blunted sympathetic and parasympathetic recovery following active stress. Conversely, the response to passive stress was different between groups with the obese group demonstrating an overall greater stress response to the CP task. This response included exaggerated reactivity of both the sympathetic and HPA systems as measured by blood pressure and cortisol. Moreover, the increases in SBP and cortisol in response to passive stress were positively correlated in the obese participants. This suggests a possible link in obese individuals between the sympathetic and HPA systems when activated in response to passive stress. These results indicate that the presentation of an abnormal stress response may occur at different times based on the

type of stress. In response to passive stress, obese individuals show an exaggerated stress response; whereas the impairment associated with active stress appears during recovery.

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VII. APPENDIX

Table 1. Group means and standard deviations (SD) for anthropometric measures

	Obese		Non-Obese	
	Mean	SD	Mean	SD
BMI**	41.9	7.2	22.3	1.6
WC (in)**	46.7	6.5	31.8	2.4
WHR**	0.9	0.1	0.8	0.05
Weight (lbs)**	225.2	54.5	133.3	11.3

Notes: BMI = body mass index, WC = waist circumference, WHR = waist to hip ratio.
* $p < .05$
** $p < .01$

Table 2. Group means and standard deviations (SD) for questionnaires

	Obese		Non-Obese	
	Mean	SD	Mean	SD
PSS	17.7	5.4	15.8	5.6
CP Stress	3.7	2.0	3.9	2.0
MA Stress	5.3	2.0	4.6	1.9
CP Pain Minute 1	4.1	2.6	4.3	2.2
CP Pain Minute 4	3.9	2.6	3.5	1.5
MA Effort	7.5	1.5	6.9	1.3

Notes: PSS = Perceived Stress Scale, CP = cold pressor, MA = mental arithmetic.

Table 3. Group means and standard deviations (SD) on MA performance

	Obese		Non-obese	
	Mean	SD	Mean	SD
Total Responses Attempted	64.95	49.1	52.3	30.8
Percent of Correct Responses	85	13	81	17

Notes: MA = mental arithmetic.

Table 4. Group means and standard deviations (SD) for baseline cardiovascular measures

	Obese		Non-Obese	
	Mean	SD	Mean	SD
SBP**	125.3	9.1	113.6	8.7
DBP*	74.1	5.8	70.1	5.9
HR*	82.2	10.8	74.1	11.3
HRV (ms)	29.5	23.1	37.0	29.3

Notes: SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, HRV = heart rate variability.
* $p < .05$
** $p < .01$

Table 5. Number of participants with complete data for each salivary measure

	Obese	Non-Obese
Cortisol (μg/dL)	19	20
Interlukin-6 (pg/mL)	11	11
Log_{10} IgA (mL/min)	19	18
Log_{10} Alpha-amylase (U/min)	20	20

Notes: IgA = immunoglobulin A.

Table 6. Group means and standard deviations (SD) for baseline salivary measures

	Obese		Non-obese	
	Mean	SD	Mean	SD
Cortisol (μg/dL)**	.28	.16	.45	.20
Interlukin-6 (pg/mL)	7.4	5.6	5.5	4.3
Log_{10} IgA (mL/min)	2.1	.50	2.1	.42
Log_{10} Alpha-amylase (U/min)*	1.6	.58	2.0	.42

Notes: IgA = immunoglobulin A.
* $p < .05$
** $p < .01$

Table 7. ANCOVA Comparing Cardiovascular Task Reactivity, by Task and Group (covarying baseline)

	Task (CP or MA)			Group (Obese or Non-obese)			Task*Group	
	F	P		F	P		F	P
SBP	0.137	0.713		5.775	**0.021**		1.126	0.295
DBP	3.125	0.085		9.308	**0.004**		0.102	0.751
HR	0.725	0.400		0.773	0.385		2.640	0.113
HRV	1.021	0.319		4.381	**0.043**		0.000	1.000

Notes: CP = cold pressor, MA = mental arithmetic, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, HRV = heart rate variability.

Table 8. ANCOVA Comparing Cardiovascular CP Recovery, by Recovery Period (early, middle and late) and Group (covarying baseline and reactivity)

	Recovery Period			Group (Obese or Non-obese)			Task*Group	
	F	P		F	P		F	P
SBP	0.128	0.814		0.551	0.463		0.207	0.743
DBP	0.051	0.915		1.740	0.196		0.588	0.558
HR	1.757	0.189		0.062	0.804		1.128	0.316
HRV	6.376	**0.009**		0.610	0.440		1.902	0.172

Notes: CP = cold pressor, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, HRV = heart rate variability.

Table 9. ANCOVA Comparing Cardiovascular MA Recovery, by Recovery Period (early, middle and late) and Group (covarying baseline and reactivity)

	Recovery Period			Group (Obese or Non-obese)			Task*Group	
	F	P		F	P		F	P
SBP	0.364	0.696		4.230	**0.047**		0.832	0.440
DBP	0.974	0.383		0.971	0.331		1.187	0.311
HR	0.703	0.498		0.342	0.562		0.151	0.860
HRV	0.307	0.736		0.826	0.369		3.934	**0.024**

Notes: MA = mental arithmetic, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, HRV = heart rate variability.

Table 10. ANOVA Comparing salivary reactivity by task and Group

	Task (CP or MA)			Group (Obese or Non-obese)			Task*Group	
	F	P		F	P		F	P
Cortisol (μg/dL)	0.217	0.644		2.491	0.123		5.071	**0.030**
Interlukin-6 (pg/mL)	0.886	0.361		2.276	0.151		0.816	0.380
Log_{10} IgA (mL/min)	1.056	0.311		0.035	0.853		0.148	0.703
Log_{10} Alpha-amylase (U/min)	0.007	0.932		0.926	0.342		6.923	**0.012**

Notes: CP = cold pressor, MA = mental arithmetic, IL-6 = interleukin – 6, IgA = immunoglobulin A, AA = alpha amylase.

Table 11. ANCOVA Comparing salivary recovery from the CP task and Group (covarying baseline)

	Period			Group (Obese or Non-obese)			Task*Group	
	F	P		F	P		F	P
Cortisol (μg/dL)	1.720	0.198		1.077	0.306		10876	0.179
Interlukin-6 (pg/mL)	3.733	0.069		1.081	0.312		0.439	0.516
Log_{10} IgA (mL/min)	3.077	0.088		0.734	0.397		2.116	0.115
Log_{10} Alpha-amylase (U/min)	1.791	0.189		0.278	0.601		1.316	0.259

Notes: CP = cold pressor, IgA = immunoglobulin A.

Table 12. ANCOVA Comparing salivary recovery from the MA task and Group (covarying baseline)

	Period			Group (Obese or Non-obese)			Task*Group	
	F	P		F	P		F	P
Cortisol (μg/dL)	6.921	**0.012**		2.550	0.119		0.435	0.514
Interlukin-6 (pg/mL)	3.340	0.086		1.020	0.328		1.659	0.216
Log_{10} IgA (mL/min)	6.957	**0.012**		0.675	0.417		3.439	0.072
Log_{10} Alpha-amylase (U/min)	1.851	0.182		0.696	0.410		0.780	0.383

Notes: MA = mental arithmetic, IgA = immunoglobulin A.

Table 13. Correlations between cardiovascular and salivary reactivity scores to the CP and MA tasks, separated by group.

Non-obese CP task

	Cortisol Reactivity	Interleukin-6 Reactivity	IgA Reactivity	Alpha-amylase Reactivity
SBP Reactivity	0.367	-0.193	0.448*	0.132
DBP Reactivity	0.128	-0.061	0.550*	0.347
HR Reactivity	0.223	-0.127	0.294	0.221
HRV	0.143	-0.475	0.311	-0.199

Obese CP task

	Cortisol Reactivity	Interleukin-6 Reactivity	IgA Reactivity	Alpha-amylase Reactivity
SBP Reactivity	0.495*	-0.099	0.326	0.099
DBP Reactivity	0.148	0.405	0.431	0.171
HR Reactivity	0.389	-0.016	0.151	-0.287
HRV	-0.200	0.028	0.001	0.138

Non-obese MA task

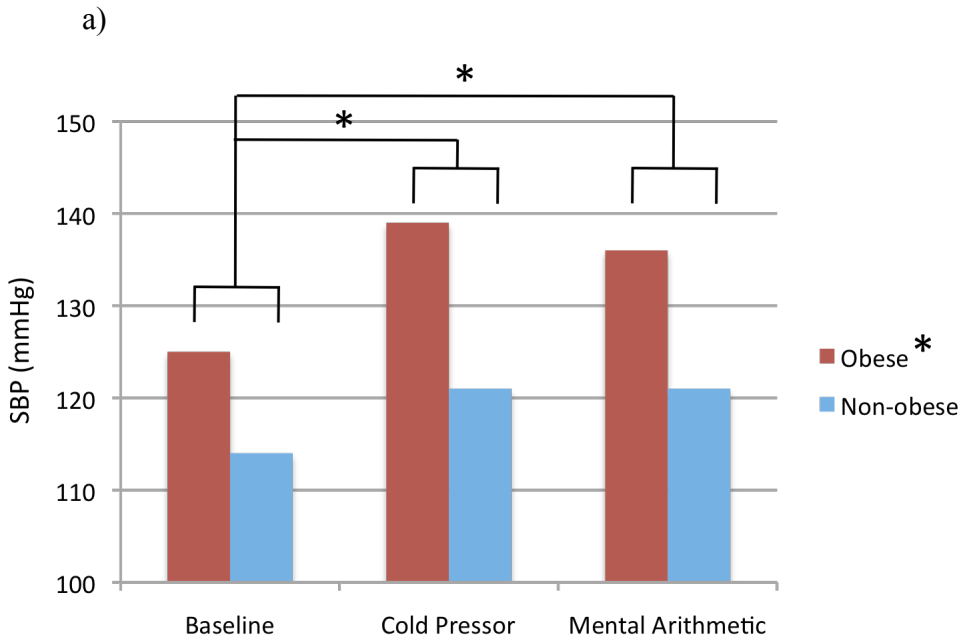
	Cortisol Reactivity	Interleukin-6 Reactivity	IgA Reactivity	Alpha-amylase Reactivity
SBP Reactivity	0.436	-0.035	-0.302	0.261
DBP Reactivity	0.188	0.451	-0.107	0.152
HR Reactivity	0.525*	0.204	-0.397	0.081
HRV	-0.404	-0.010	0.159	0.079

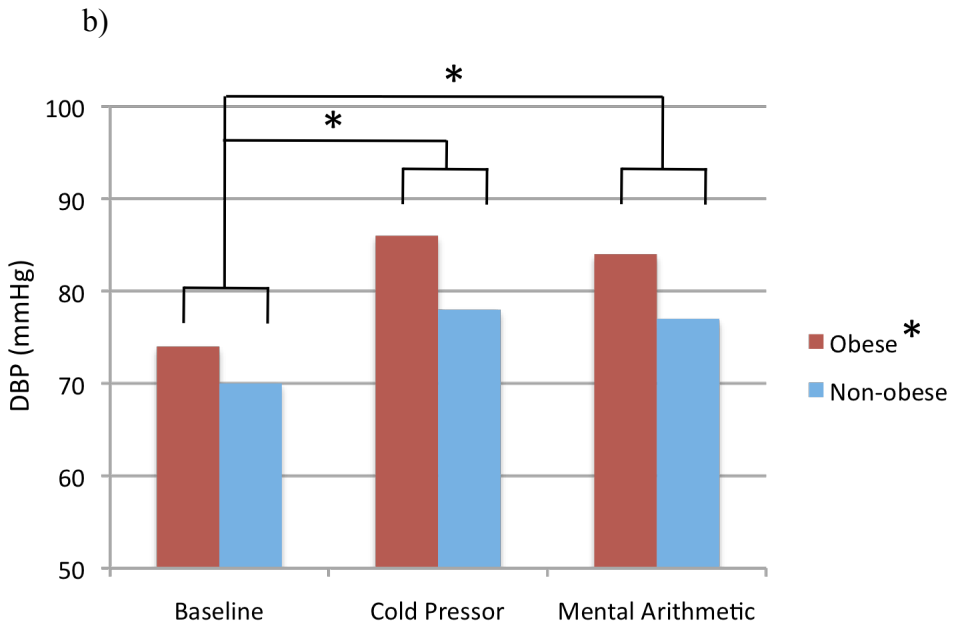
Obese MA task

	Cortisol Reactivity	Interleukin-6 Reactivity	IgA Reactivity	Alpha-amylase Reactivity
SBP Reactivity	0.389	-0.300	0.180	0.050
DBP Reactivity	0.164	-0.326	-0.029	-0.118
HR Reactivity	0.615**	-0.058	0.504*	0.201
HRV	-0.322	-0.123	-0.022	-0.011

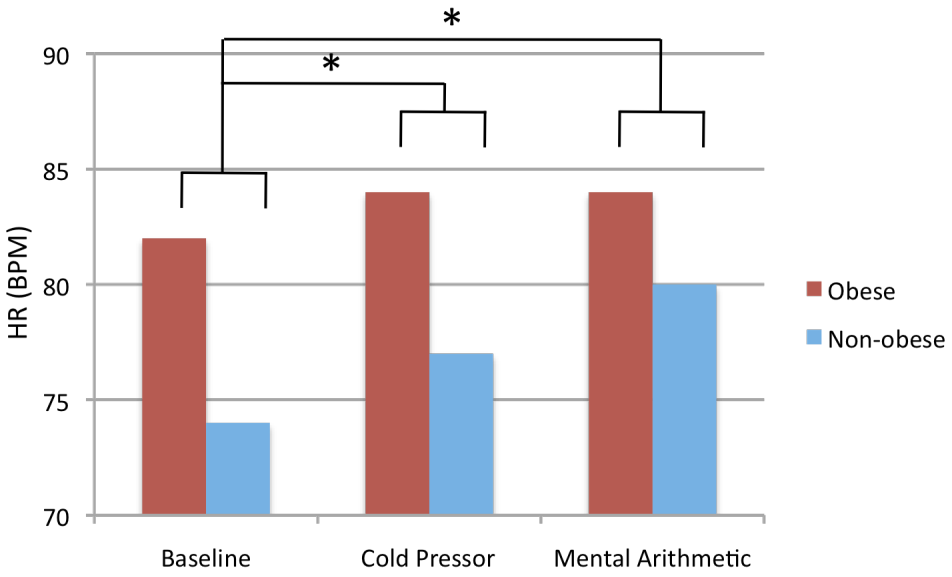
Notes: CP = cold pressor, MA = mental arithmetic, IgA = immunoglobulin A, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, HRV = heart rate variability.
* $p < .05$
** $p < .01$

Figure 1. Cardiovascular levels at baseline, CP and MA separated by group. a) systolic blood pressure (SBP), b) diastolic blood pressure (DBP), c) heart rate (HR) and d) heart rate variability (HRV). Notes: * = $p < 0.05$





c)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85
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70
HR (BPM)
Baseline
Cold Pressor
Mental Arithmetic
Obese
Non-obese

d)

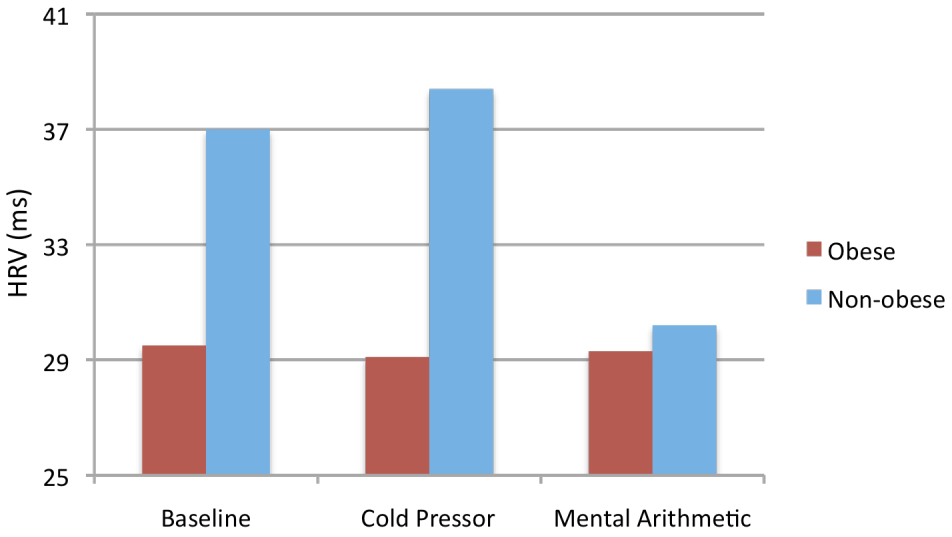
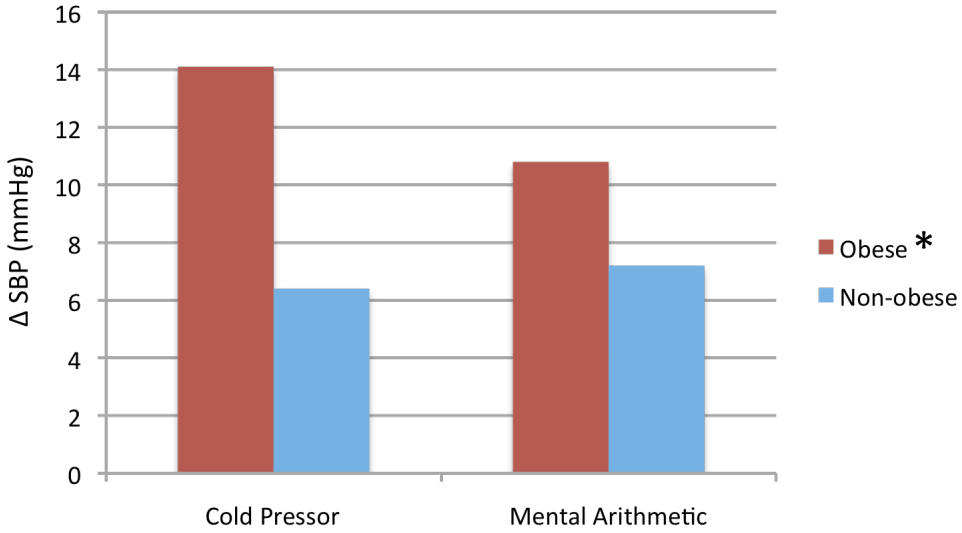
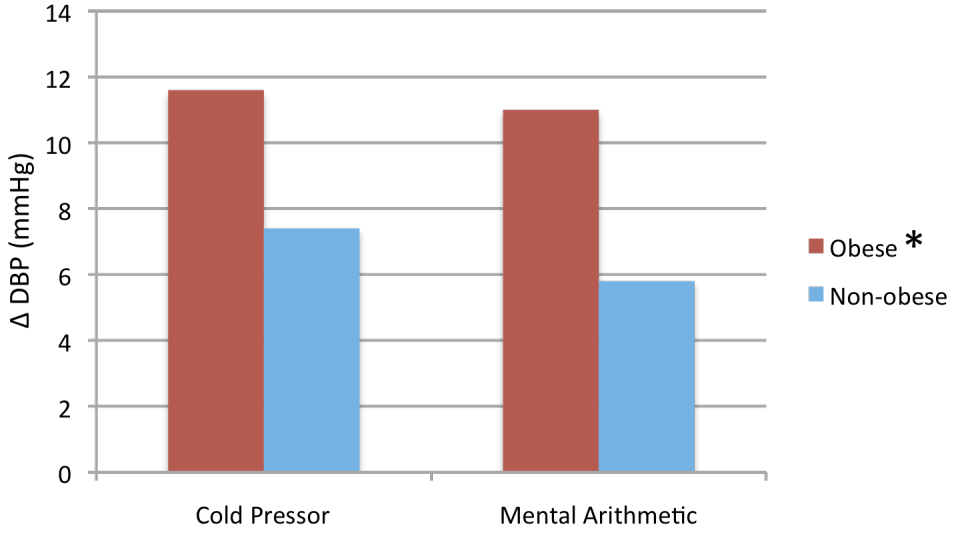

41
37
33
29
25
HRV (ms)
Baseline
Cold Pressor
Mental Arithmetic
Obese
Non-obese

Figure 2. Cardiovascular reactivity to the CP and MA task, separated by group. a) systolic blood pressure (SBP), b) diastolic blood pressure (DBP), c) heart rate (HR) and d) heart rate variability (HRV). Notes: * = $p < 0.05$

a)



b)



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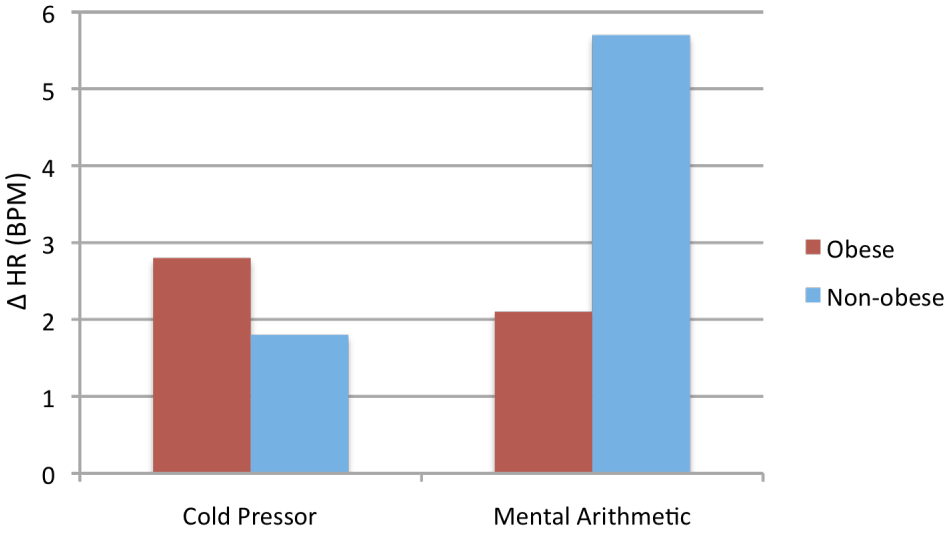
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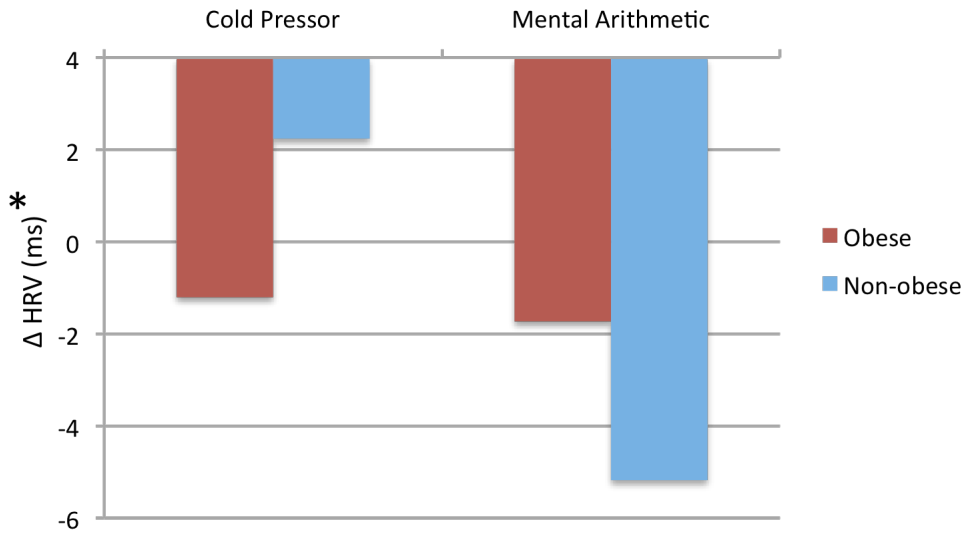
0
Δ HR (BPM)
Cold Pressor
Mental Arithmetic
Obese
Non-obese

d)


Cold Pressor
Mental Arithmetic
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-2
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-6
Δ HRV (ms) *
Obese
Non-obese

Figure 3. Calculation of blood pressure (BP) and heart rate (HR) recovery.

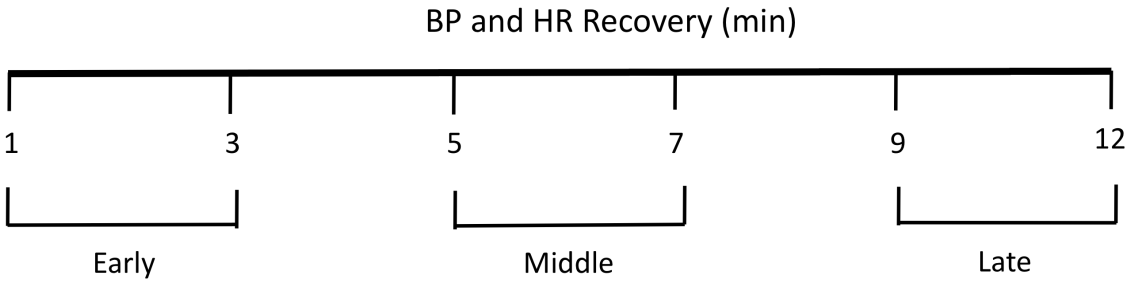


Figure 4. Calculation of heart rate variability (HRV) recovery.

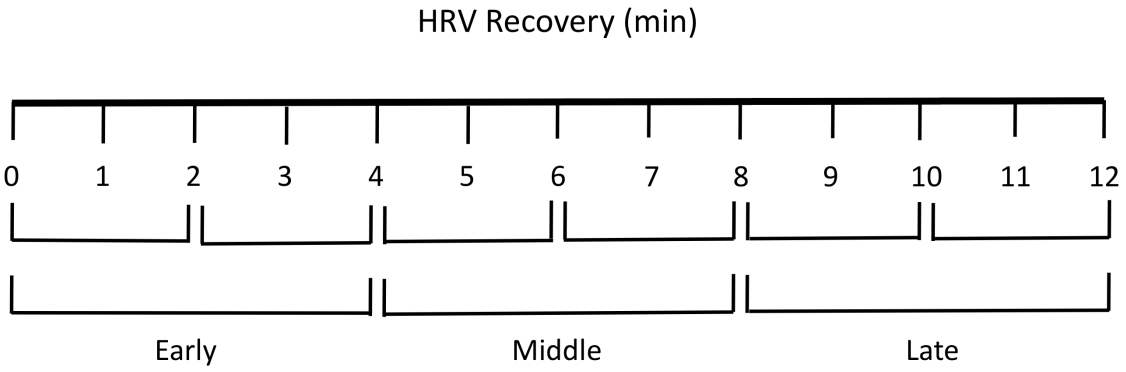
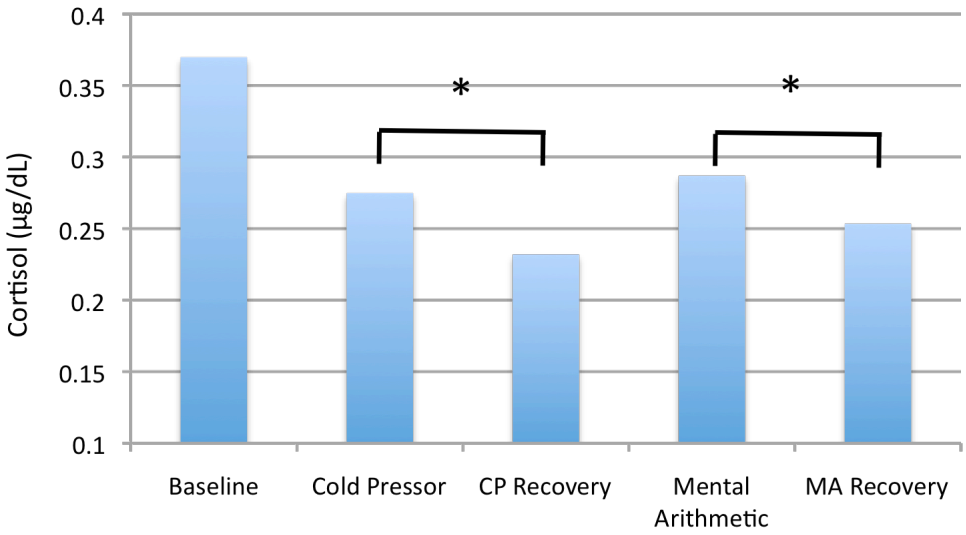
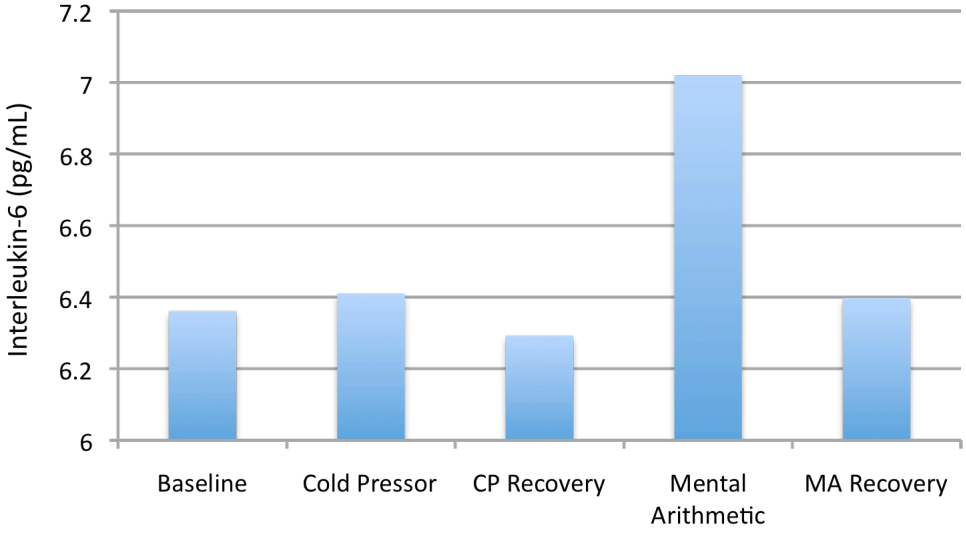


Figure 5. Salivary levels at baseline, cold pressor (CP), CP recovery, mental arithmetic (MA) and MA recovery. a) cortisol, b) interleukin-6, c) immunoglobulin A, d) alpha amylase. Notes: * = $p < 0.05$

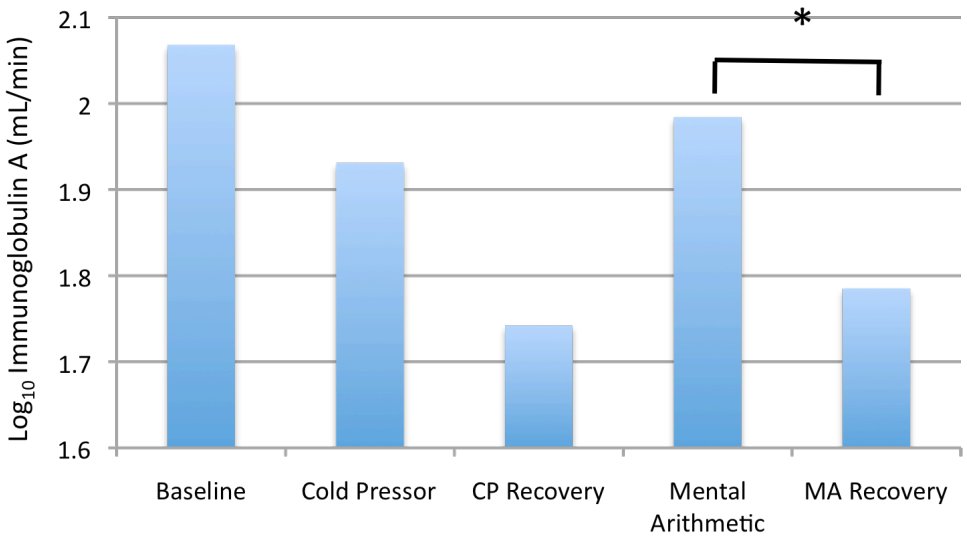
a)



b)



c)


2.1
2
1.9
1.8
1.7
1.6
Log10 Immunoglobulin A (mL/min)
*
Baseline
Cold Pressor
CP Recovery
Mental Arithmetic
MA Recovery

d)

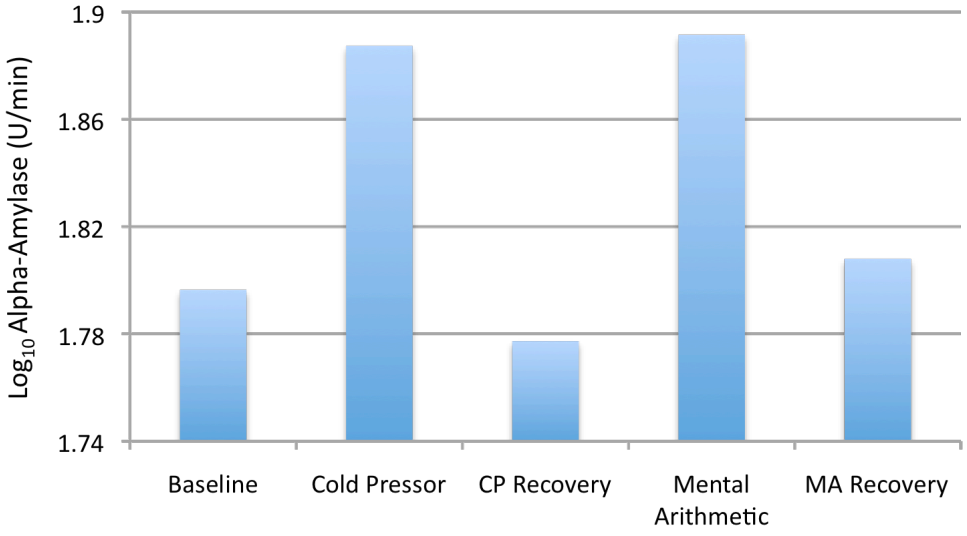
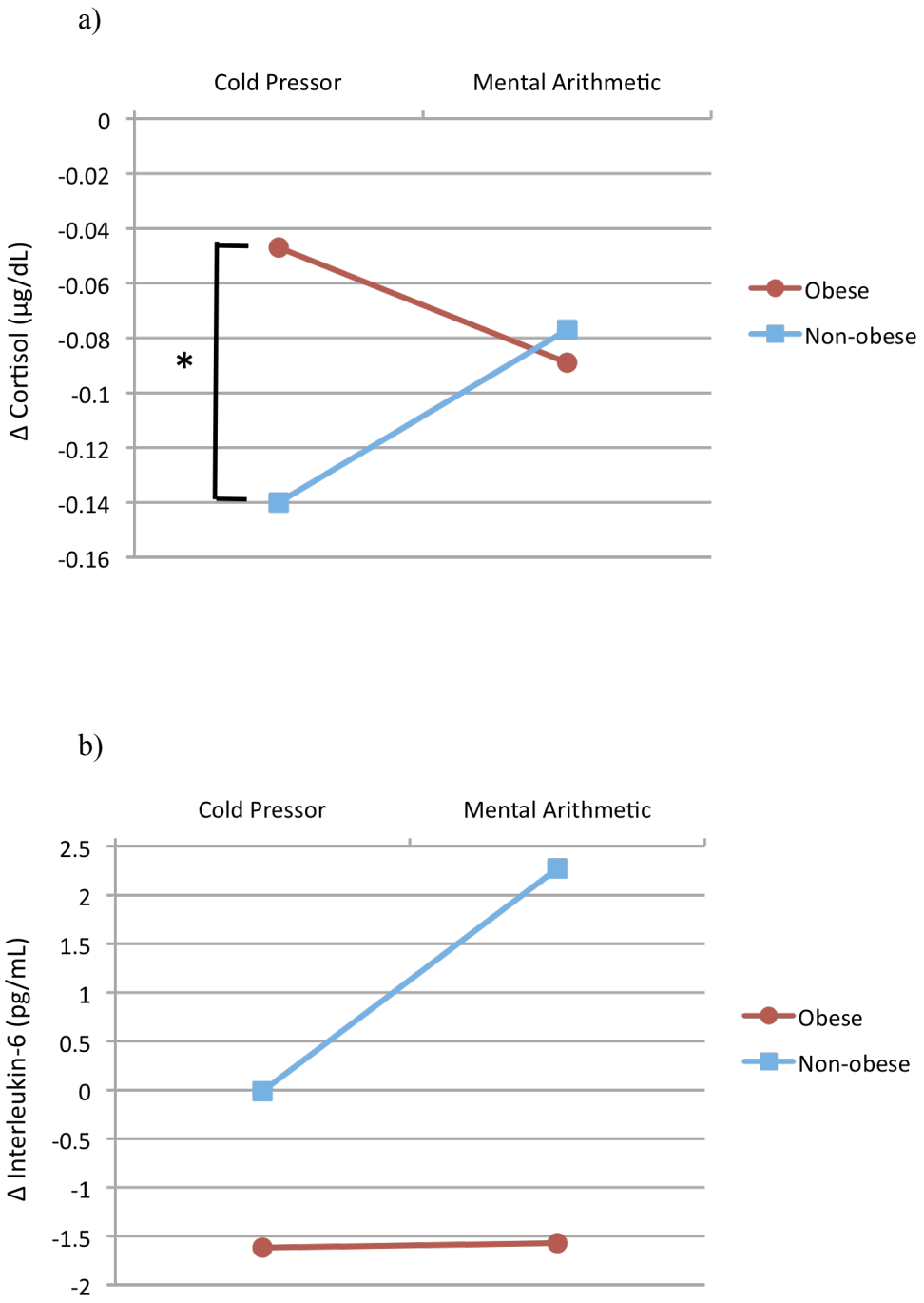
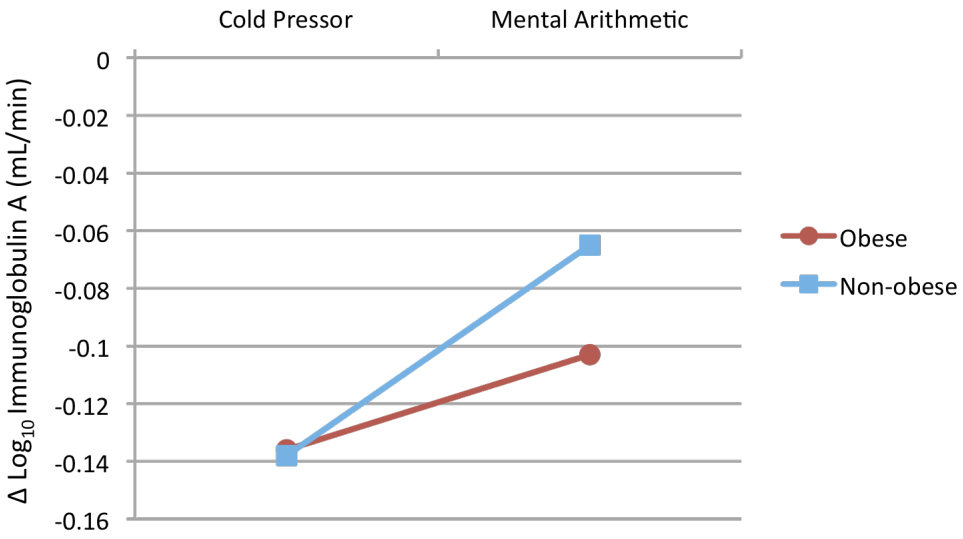

1.9
1.86
1.82
1.78
1.74
Log10 Alpha-Amylase (U/min)
Baseline
Cold Pressor
CP Recovery
Mental Arithmetic
MA Recovery

Figure 6. Reactivity of salivary measures. a) cortisol reactivity to the cold pressor and mental arithmetic task, b) interleukin-6 reactivity to the cold pressor and mental arithmetic task, c) immunoglobulin A reactivity to the cold pressor and mental arithmetic task, d) alpha-amylase reactivity to the cold pressor and mental arithmetic task. Notes: # = indicates marginally significant differences. Notes: * = $p < 0.05$, # = $p = 0.051$



c)


Cold Pressor
Mental Arithmetic
0
-0.02
-0.04
-0.06
-0.08
-0.1
-0.12
-0.14
-0.16
Δ Log10 Immunoglobulin A (mL/min)
Obese
Non-obese

d)

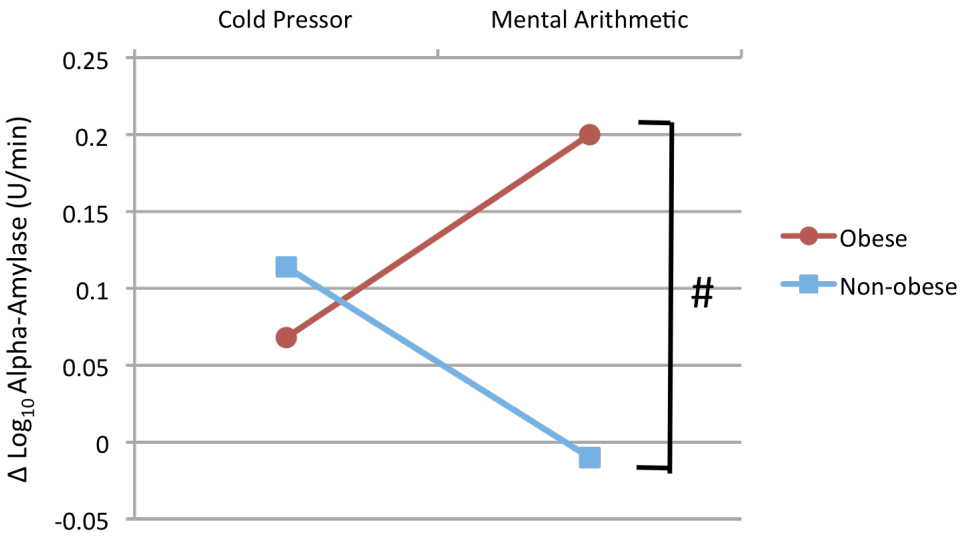
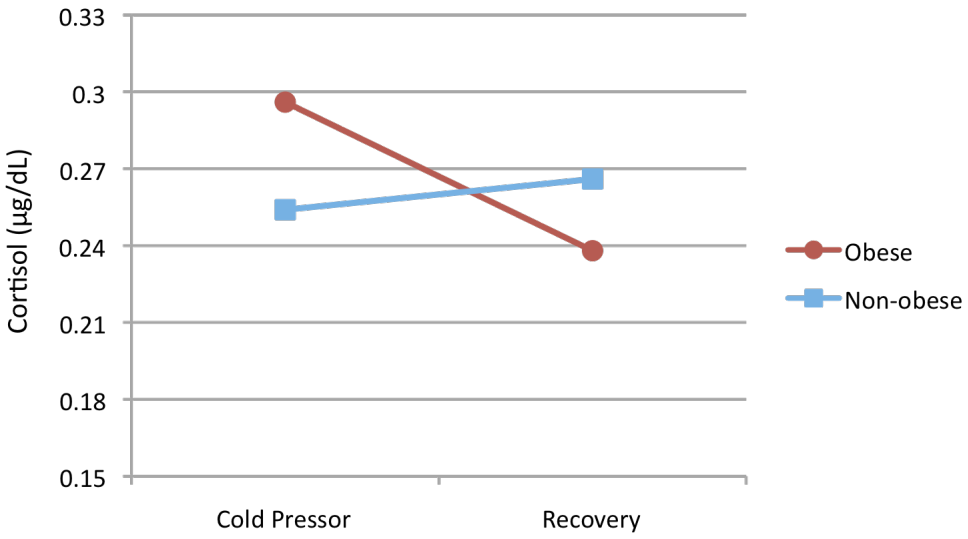
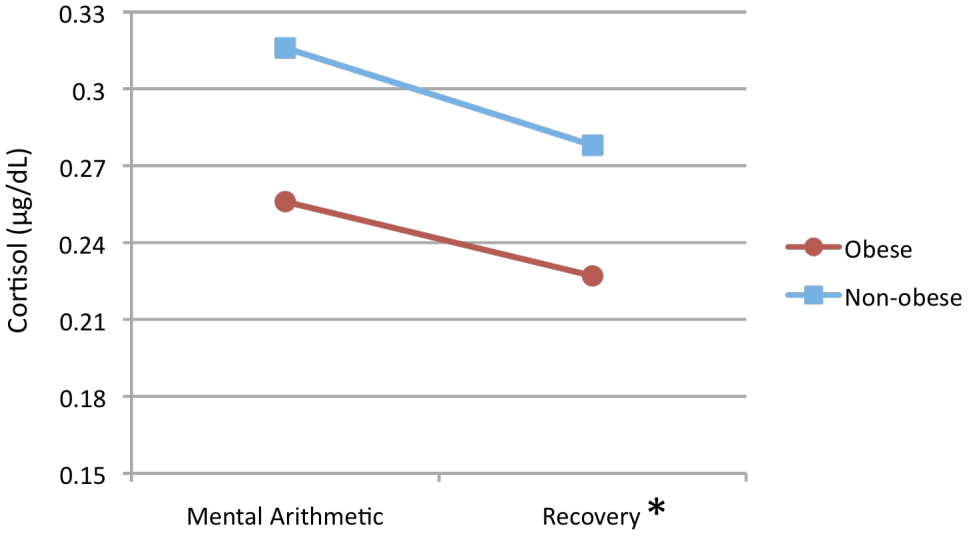

Cold Pressor
Mental Arithmetic
0.25
0.2
0.15
0.1
0.05
0
-0.05
Δ Log10 Alpha-Amylase (U/min)
#
Obese
Non-obese

Figure 7. Recovery of salivary measures. a) cortisol recovery from cold pressor, b) cortisol recovery from mental arithmetic, c) interleukin-6 recovery from cold pressor, d) interleukin-6 recovery from mental arithmetic, f) immunoglobulin A recovery from cold pressor, g) immunoglobulin A recovery from mental arithmetic, h) alpha-amylase recovery from cold pressor, i) alpha-amylase recovery from mental arithmetic. Notes: * = $p < 0.05$

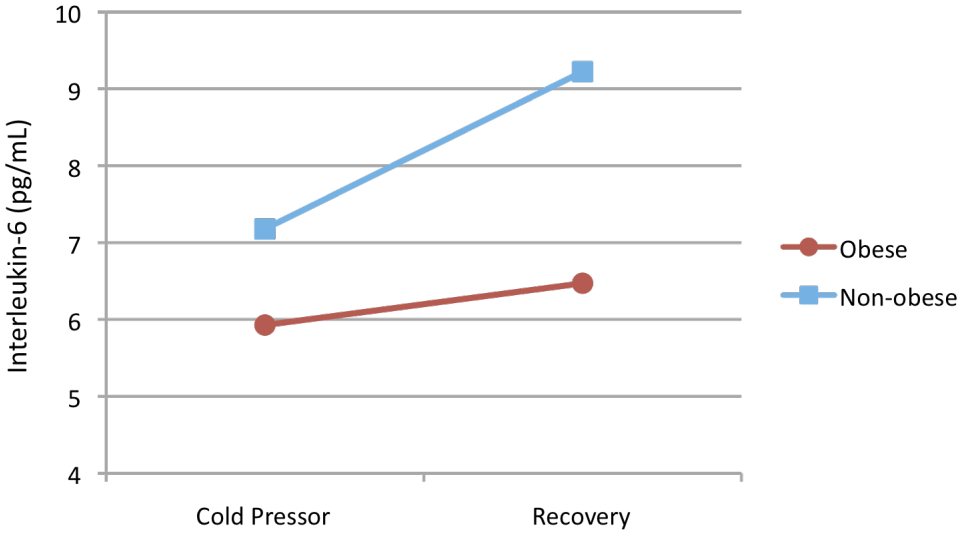
a)



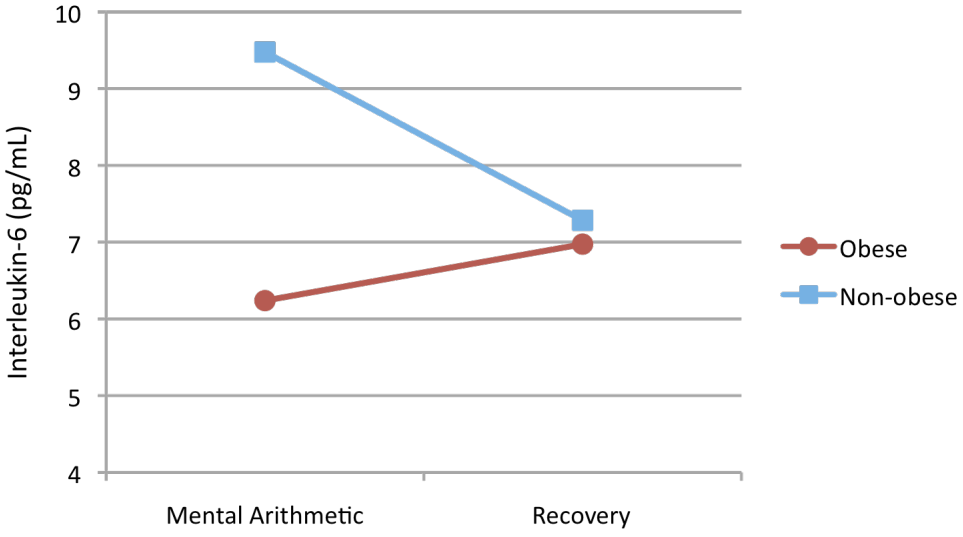
b)



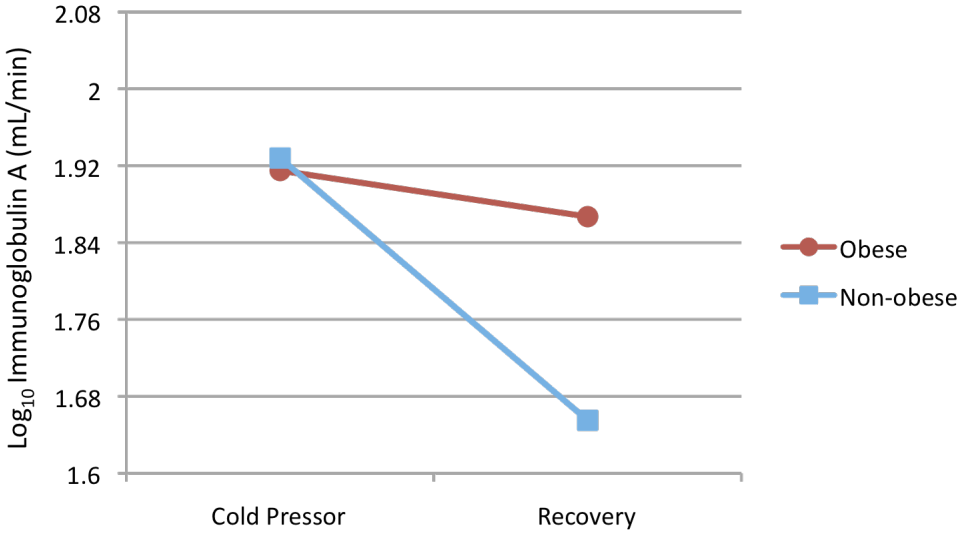
c)


10
9
8
7
6
5
4
Interleukin-6 (pg/mL)
Cold Pressor
Recovery
Obese
Non-obese

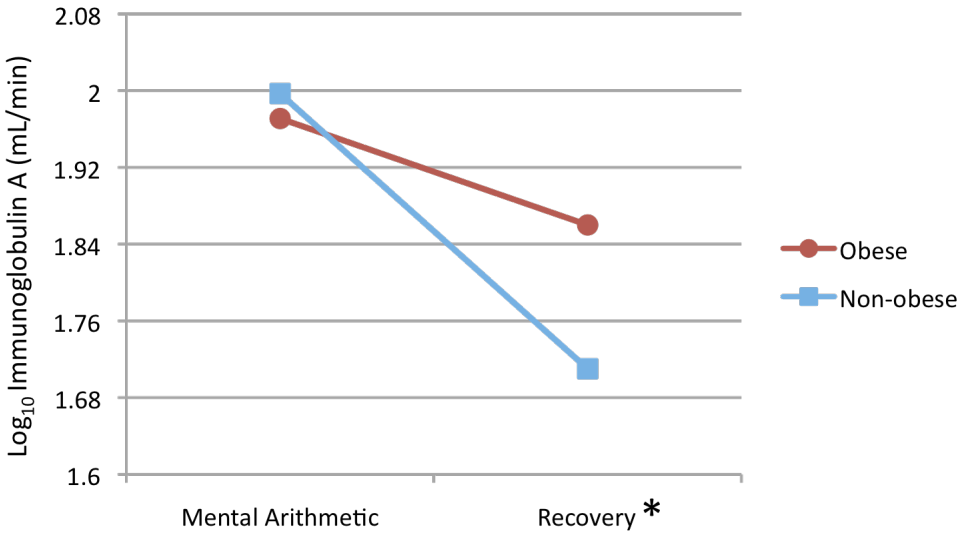
d)


10
9
8
7
6
5
4
Interleukin-6 (pg/mL)
Mental Arithmetic
Recovery
Obese
Non-obese

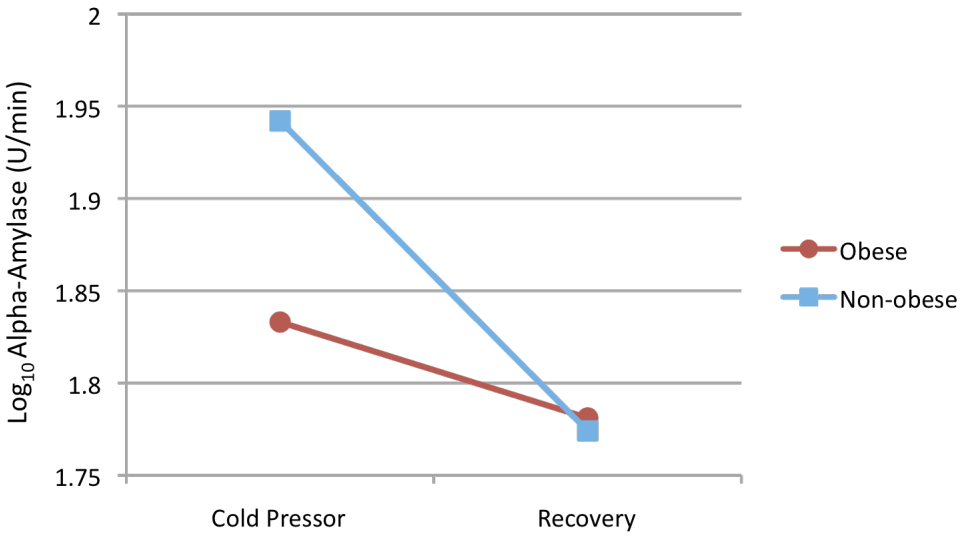
e)


2.08
2
1.92
1.84
1.76
1.68
1.6
Log10 Immunoglobulin A (mL/min)
Cold Pressor
Recovery
Obese
Non-obese

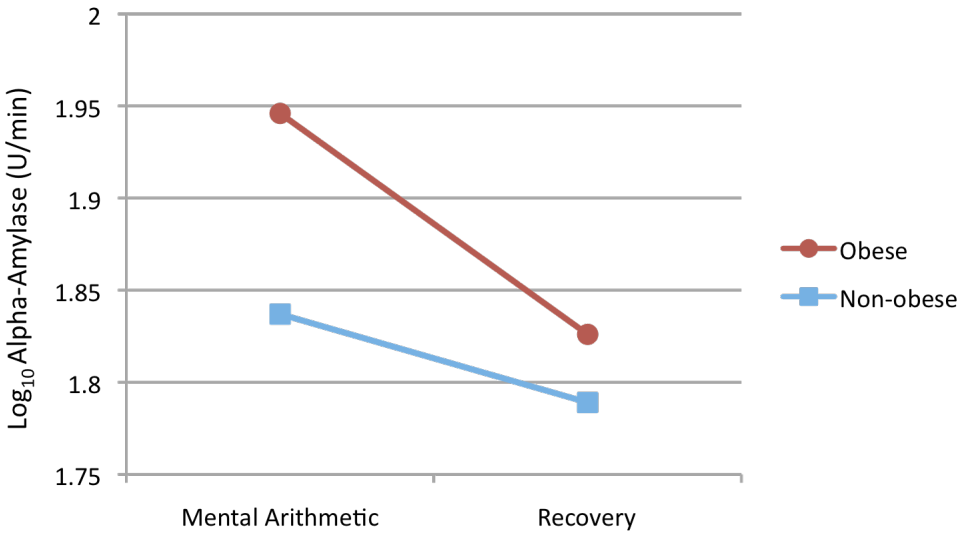
f)


2.08
2
1.92
1.84
1.76
1.68
1.6
Log10 Immunoglobulin A (mL/min)
Mental Arithmetic
Recovery *
Obese
Non-obese

g)


2
1.95
1.9
1.85
1.8
1.75
Log10 Alpha-Amylase (U/min)
Cold Pressor
Recovery
Obese
Non-obese

h)


2
1.95
1.9
1.85
1.8
1.75
Log10 Alpha-Amylase (U/min)
Mental Arithmetic
Recovery
Obese
Non-obese

VITA

Ashley Elizabeth Burch was born August 27, 1982 in Salt Lake City, Utah. She attended Augusta State University graduating with honors in 2006 with degrees in psychology, social work and criminal justice. Ashley was accepted to the experimental psychology graduate program at the University of Mississippi, where she pursued research in cardiovascular psychophysiology under the direction of Dr. Michael Allen. Ashley also began researching aspects of psychoneuroimmunology with the guidance of Dr. Gailen Marshall, a physician and researcher at the University of Mississippi Medical Center. Upon the completion of her doctorate Ashley will pursue postdoctoral training.