Responsiveness to psychological stress and food intake: The influence of adiposity in men and physical activity in women

By

Sisitha Udara Jayasinghe
BSc (Hons)

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Deakin University

October, 2014
I am the author of the thesis entitled ‘Responsiveness to psychological stress and food intake: The influence of adiposity in men and physical activity in women’ submitted for the degree of Doctor of Philosophy

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Acknowledgements

Firstly, thank you Dr Anne Turner for your continuous support, guidance and encouragement in every step of my PhD journey. Thank you for teaching me everything that you possibly could about research. Your belief in me has enabled me to reach heights that I never anticipated that I would be capable of reaching. I have grown in confidence because of your unwavering support and I am eternally thankful for it. It has been an honour and privilege to work with you. Thank you Dr Susan Torres for your support during the past four years. The feedback you provided on my written work has been invaluable. Thank you Dr Steve Fraser for your support in my experiments. Together, you all made up an excellent research team.

Thank you Sara Drew, Nina Eikelis and Sarah Phillips for your help with my biochemical analyses. You all made my life a lot easier! Thank you Emma Townsin, Maryam Delawari, Shaun Mason, Erin Brown, Evelyn Zacharewicz and Isabel Guller for helping out with the stress tests. I wouldn’t have been able to complete data collection without your help. Thank you to all my fellow PhD mates for the encouragement, advice and laughter that have been shared over the years. Thank you to all the enthusiastic volunteers who participated in my experiments. I am forever grateful for the valuable time that you all invested.

Thank you Professor Caryl Nowson and Professor Alan Tilbrook for your invaluable intellectual input. Thank you Professor Gavin Lambert for spending
your time and resources on my laboratory work. Thank you Andrew Howarth, Michael Holmes and all Deakin University laboratory staff members for providing great facilities and support.

Thank you Anton, Kassa, Shak, Sam and Pathum for your kindness and support. Thank you for putting up with me for the past few years and sticking by me when times were tough. I really value your friendship. Finally, mom, dad and my lovely sis, thank you for your unconditional love and support all the way! You guys are the best!
Publications

Journal articles


   * This paper publishes the study reported as Experimental Chapter 1 of this thesis. The cortisol data from this study were presented in the author's honours thesis entitled "Cortisol response to acute psychological stress in lean and overweight/obese men". Nevertheless, salivary alpha amylase, heart rate and heart rate variability data from this study were not presented in the author’s honours thesis and are presented in thesis format in this thesis for the first time. Cortisol data are included in this thesis (Figure 4.2) to allow the reader to gain a complete view of the physiological stress response in this study.


Conference and seminar papers


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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotrophic hormone</td>
</tr>
<tr>
<td>Adr</td>
<td>Adrenaline</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>AVP</td>
<td>Arginine vasopressin</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CBG</td>
<td>Corticosteroid binding globulin</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotrophin releasing hormone</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DHPG</td>
<td>Dihydroxybenzylamine</td>
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<tr>
<td>HPA</td>
<td>Hypothalamo-pituitary adrenal axis</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
<tr>
<td>NA</td>
<td>Noradrenaline</td>
</tr>
<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
</tr>
<tr>
<td>PVN</td>
<td>Paraventricular nucleus</td>
</tr>
<tr>
<td>SAM</td>
<td>Sympatho-adrenal medullary system</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>TSST</td>
<td>Trier Social Stress Test</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist to hip ratio</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>
Abstract
Excessive activation of the sympatho-adrenal medullary (SAM) system and hypothalamo-pituitary adrenal (HPA) axis can be detrimental and may lead to a number of chronic diseases including cardiovascular disease, type 2 diabetes and anxiety and depression. Activity of these pathways can be affected by adiposity and physical activity. Physiological reactivity of the stress pathways can also be specific to the stressor encountered. Psychological stress is commonly encountered by most people in the modern world. Food intake is also a challenge encountered by humans several times a day. Thus, investigating the effects of adiposity and physical activity on HPA axis and SAM system reactivity to psychological stress and food intake is of particular importance. This research investigated the general hypothesis that adiposity in men and physical activity in women can influence SAM system and HPA axis responsiveness to psychological stress and food intake. Catecholamines (Adrenaline, Noradrenaline and Dopamine), salivary alpha amylase, heart rate, heart rate variability and blood pressure (systolic, diastolic and mean arterial pressure) as measures of SAM system activity and both salivary as well as plasma cortisol as measures of HPA axis activity, were recorded with a sample frequency that was optimised for capturing all changes in response to a Trier Social Stress Test (TSST) and a standardised lunch. Neither men with different levels of adiposity nor women with different physical activity levels had a differential HPA axis and SAM system reactivity to psychological stress. Nevertheless, overweight/obese men had significant increases in HPA axis activity in response to food intake while lean men did not. There were no differences in HPA axis and SAM system reactivity
to food intake in low fit and high fit women. Future research could consider a less potent stressor. Different stressors and other physiological, metabolic and innate mechanisms could also be considered. Results of this research suggest that levels of adiposity in men and physical activity status in women are not significant factors in regulating responsiveness of the HPA axis and SAM system to psychological stress. This research also concludes that stress pathway activity in response to a standardised meal can be influenced by increased adiposity in men but not by increased levels of physical activity in women.
Chapter 1.0-General Introduction
The ability of stress to impair physiological processes such as growth, reproduction and immune competence and its association with diseases such as cardiovascular disease, type 2 diabetes and anxiety and depression are well-known (Chrousos, 2009). While acute responses to stress are generally considered effective in dealing with immediate threats, prolonged activation of stress processes could have significant adverse consequences for individuals (Tilbrook & Clarke, 2006). However, frequent exposure to acute activation of the stress pathways may also be detrimental. People with elevated responses to acute stress are more likely to have psychological disorders such as anxiety and depression (Miller, Rohleder, Stetler, & Kirschbaum, 2005; Throne, Bartholomew, Craig, & Farrar, 2000). They are also more likely to develop hypertension and have increased risk of developing cardiovascular disease (Carroll, Phillips, Der, Hunt, & Benzeval, 2011; Matthews et al., 2004).

Stress was originally conceptualised by Hans Selye as the sum of all non-specific systemic phenomena elicited by exposure to noxious stimuli the symptoms of which are independent of the nature of the noxious agent (Selye, 1936, 1946). Today, stress is generally defined as a “perceived or real threat to homeostasis” (Tilbrook & Clarke, 2006). The human body responds to stress via the activity of two main physiological pathways; the SAM system and the HPA axis (Elenkov & Chrousos, 2002). The former is activated rapidly and results in increases in catecholamines (adrenaline and noradrenaline) and cardiovascular parameters in
a transient response whereas the latter results in the secretion of cortisol from the adrenal cortex (Tilbrook, 2007) in a more prolonged response (Kyrou & Tsigos, 2009). Secretion of cortisol results in the mobilisation of energy stores in the body.

Stressors are mainly categorised as either physical or psychological. Physiological responses to physical stress are considered appropriate because the body is responding to an actual threat and the elevations in cardiovascular parameters and mobilisation of energy stores induced by activation of the stress systems can be used by the body during the response to the emergency. In contrast, physiological responses to psychological stress are often likely to be inappropriate since a physical response to psychological stress is often not required. In addition to psychological stress, food intake has also been found to have stimulatory effects on both the SAM system and the HPA axis. As such, this is also a stimulus that exposes the body to stress-like conditions including elevations of cardiovascular parameters and mobilisation of energy stores.

Men and women differ in their stress responses (Kudielka & Kirschbaum, 2005). The sex steroids in the body are thought to be responsible for these differences (Turner, Keating, & Tilbrook, 2012). Thus, to prevent any influence by the sex steroids on the stress responsiveness, researchers need to consider men and women in different experimental groups or in different studies. In women, whose sex steroid milieu changes during the different phases of the menstrual cycle,
stage of the menstrual cycle and steroidal contraceptive use needs to be considered during the experimental design stage of a study (Kajantie & Phillips, 2006). Since aging also affects the stress-induced activity of the stress pathways, stress experiments should also consider the age of participants when designing experimental inclusion and exclusion criteria (Kudielka, Hellhammer, & Wust, 2009). Age ranges of the participants in all experiments are kept to 20 years and only men or women from the same phase of the menstrual cycle are considered for specific hypotheses in this research in order to control for the effects of age and sex steroids.

Diseases related to excessive stress pathway activity are on the rise in the modern society. Thus, it is important to study factors that may influence the responsiveness of the stress pathways since this may provide some insight into the causes of these diseases. Adiposity and physical activity status are two such physiological conditions that have the ability to influence the activity of the stress pathways. Stress responsiveness is thought to be elevated with increased levels of adiposity (Tilbrook, Rivalland, Turner, Lambert, & Clarke, 2008) and decreased/attenuated with increasing levels of physical fitness (Rimmele et al., 2009). This PhD program will test the general hypothesis that adiposity in men and physical activity in women can influence SAM system and HPA axis responsiveness to psychological stress and food intake.
Chapter 2.0 - Review of Literature

2.1 Stress

2.1.1 Defining stress
Our working definition of stress is “a complex physiological state that embodies a range of physiological and behavioural processes that occur when there is a real or perceived threat to homeostasis” (Tilbrook, 2007; Tilbrook & Clarke, 2006). Homeostasis is a stable internal environment (Cannon, 1935). Not all stress is negative or detrimental. Mild, controllable challenges (which is also known as eustress) are an integral part of emotional and intellectual development (Selye, 1975).

A stressor is defined as any stimulus, either external or internal, that has the potential to disrupt homeostatic balance (Maier & Watkins, 1998; Turner et al., 2002a). Stress that lasts for a brief period (seconds, minutes or up to a few hours) is known as acute stress and stress that is continuous and lasts for longer periods (days, weeks or months) is known as prolonged or chronic stress (Turner, Hemsworth, & Tilbrook, 2002b).

2.1.2 Physiological responses to stress
There are two main stress pathways that are activated in response to a stressor, the SAM system and the HPA axis (Elenkov & Chrousos, 2002). Both of these
Chapter 2- Literature

systems work together to respond to the disruption of homeostasis or possible threats to homeostasis.

2.1.3 Sympatho-adrenal medullary (SAM) system
An immediate response to stress comes from the activation of the SAM system (Dalin, Magnusson, Haggendal, & Nyberg, 1993). A schematic representation of this system is shown in Figure 2.1. The prominent controller within the SAM system is the locus coeruleus–noradrenaline system/central sympathetic system (Chrousos & Gold, 1992; Kyrou & Tsigos, 2009). Central components of the SAM system contain reciprocal neuronal connections with the paraventricular nucleus at the level of the hypothalamus. The locus coeruleus is located in the upper dorsolateral pontine tegmentum and has neurons with extensively branched axons that project throughout the central nervous system and supply noradrenaline to several brain regions such as neocortex, hippocampus, cerebellum, and most of the thalamus (Benarroch, 2009).

This system also contains pre-ganglionic neurons whose cell bodies lie in the inter-mediolateral component of the spinal cord and postganglionic nerves which receive synaptic information from pre-ganglionic nerves (Kyrou, Chrousos, & Tsigos, 2006). Post ganglionic neurons of the sympathetic nervous system mostly release noradrenaline (Figure 2.1), whereas the pre ganglionic neurons are cholinergic (Kyrou et al., 2006). Some preganglionic neurons of the sympathetic nervous system project to the adrenal medulla where they stimulate
the release of adrenaline and noradrenaline into the bloodstream in response to stress (Figure 2.1). Out of all SAM system secretions from the adrenal medulla, 75-80% is adrenaline (Carrasco & de Kar, 2003).

This SAM system responds to various kinds of stressors and controls a wide range of functions (Charmandari, Tsigos, & Chrousos, 2005). Activation of the SAM system results in increased activity of the cardiovascular and respiratory systems and decreased activity of the digestive system. Smooth muscles of the vasculature, skeletal muscle, heart, kidney, gut, adipose tissue and many other organs are innervated by post-ganglionic sympathetic nerves (Kyrou et al., 2006). Activation of the SAM system is an essential, rapid and transient response to stress. Nevertheless, excessive activity of this pathway may have detrimental consequences.

There are many ways of measuring SAM system activity although no method can be considered perfect (Grassi & Esler, 1999). Activation of the SAM system results in marked changes in a number of cardiovascular parameters including heart rate, blood pressure (Turner et al., 2002b) and heart rate variability (Acharya, Joseph, Kannathal, Lim, & Suri, 2006). Thus, changes in cardiovascular parameters have been used as proxy measures of the activity of the SAM system. Power spectral analysis of heart rate variability (HRV) is a comparatively newer measure of sympathetic activity compared to heart rate and blood pressure (Acharya et al., 2006). This is performed via sophisticated
mathematical partitioning and there are two main rhythmic influences (high frequency and low frequency) (Task Force Of The European Society Of, The North, Society Of, & Electrophysiology, 1996).

The main drawbacks of these haemodynamic measures include the limited within-subject reproducibility and the lack of correlation between hemodynamic responses to different stimuli (Grassi & Esler, 1999). Reasons for the common use of these measures include non-invasiveness and minimal requirement of specific skill, expertise or equipment. The fact that these measures can be implemented in a non-laboratory setting has also aided their popularity. Measuring venous plasma concentrations of adrenaline and noradrenaline has also been frequently used to measure SAM system activity in humans (Grassi & Esler, 1999). Nevertheless, while measuring plasma catecholamines gives a more direct measure of SAM system activity, it is invasive and requires specialist skills and equipment to perform.

Salivary alpha amylase is also used as an indirect marker of SAM system activity (Ali & Pruessner, 2012; Nater & Rohleder, 2009; Rohleder, Wolf, Maldonado, & Kirschbaum, 2006). Salivary alpha amylase is a digestive enzyme that plays a major role in complex carbohydrate digestion (Zakowski & Bruns, 1985) and it has recently gained popularity as a marker of SAM system activity. Both animal studies and human studies have reported that the autonomic nervous system
(particularly the sympathetic nervous system) coupled with β adrenergic mechanisms are heavily involved in the production and release of salivary alpha amylase (Nater & Rohleder, 2009). Most of these studies have used either α or β adrenergic receptor antagonists to investigate the involvement of the sympathetic nervous system activity on the production of salivary alpha amylase. The results of these investigations indicate a positive association between the release of salivary alpha amylase and sympathetic nervous system activity. Salivary alpha amylase is sensitive to a number of different stressors (e.g. exercise, cold exposure) including psychological stress (Bosch et al., 1996; Bosch, De Geus, Veerman, Hoogstraten, & Amerongen, 2003; Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Nater et al., 2005).

Early literature indicates that salivary alpha amylase is highly predictive of plasma catecholamine levels (particularly noradrenaline) in humans (Chatterton et al., 1996). While early studies were concerned that salivary alpha amylase would be influenced by saliva flow rate (Nater et al., 2005), more recent research has demonstrated no influence of saliva flow rate on salivary alpha amylase concentrations (Rohleder et al., 2006). While this method appears to be accurate and non-invasive, it must be acknowledged that this is an indirect measure of the activity of the SAM system. Despite providing logistical advantages in experiments involving large sample sizes, precision of these indirect measures of the SAM system may not be as good as some of the direct measures. Furthermore, it should be acknowledged that there are advantages and
disadvantages to each method of measuring the activity of the SAM system. Using a number of measures adds the advantage of giving a more holistic picture of the response of the SAM system.

**Figure 2.1**: Schematic representation of the SAM system
2.1.4 Hypothalamo-pituitary adrenal (HPA) axis

The other main physiological system activated in response to stress is the HPA axis (Figure 2.2). Activation of this system has a longer time course than that of the SAM system.

![HPA axis diagram]

**Figure 2.2**: A schematic representation of the HPA axis; CRH: corticotrophin–releasing hormone; AVP: Arginine vasopressin; ACTH: Adrenocorticotrophic hormone.

Corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are the major controllers of the HPA axis (Charmandari et al., 2005). The major source of CRH and AVP are neurons located in the parvocellular division of the paraventricular nucleus of the hypothalamus which project to the median eminence. The median eminence is located at the base of the hypothalamus and
is the place at which hormones are released into the hypophyseal portal vessels which are specialised blood vessels that transport neuropeptides produced by the hypothalamus to target cells in the anterior pituitary gland (Tilbrook, 2007). After being synthesised, CRH and AVP are stored in nerve terminals and are then released into the hypophyseal portal blood (Tilbrook, Turner, & Clarke, 2000). The secretion of CRH and AVP normally increases during stress (Johnson, Kamilaris, Chrousos, & Gold, 1992). During acute stress, the amplitude and synchronisation of paraventricular CRH and AVP secretions into the hypophyseal portal system is higher than when non-stressed (Charmandari et al., 2005). The combined action of CRH and AVP on corticotroph cells of the anterior pituitary gland stimulates the secretion of peptides derived from pro-opiomelanocortin which include adrenocorticotropic hormone (ACTH), the opioid peptide beta-endorphin and alpha-melanocyte-stimulating hormone (Tilbrook & Clarke, 2006). CRH acts as the primary secretagogue of ACTH. AVP plays a synergistic role. Stressors of different types stimulate the release of CRH and AVP into the hypophyseal portal circulation in a pulsatile fashion (Tsigos & Chrousos, 2002). Once secreted, ACTH travels via the systemic circulation to the adrenal cortex to stimulate the synthesis and secretion of glucocorticoids (Johnson et al., 1992). ACTH is the primary regulator for the production and secretion of glucocorticoids and the principle glucocorticoid in humans is cortisol (Tsigos & Chrousos, 2002).

As a response to ACTH stimulation, cortisol is secreted from the zona fasciculata region of the adrenal cortex. Cortisol is the end product and principal effector
hormone of the HPA axis. Most cortisol circulates in the blood bound to a binding protein, corticosteroid binding globulin (CBG). It is the unbound fraction that is free to have the biological actions of cortisol (Kudielka et al., 2009). Cortisol acts in its target tissues by binding to intra-cellular receptors known as glucocorticoid and mineralocorticoid receptors. Cortisol also has negative feedback actions in the hypothalamus and anterior pituitary gland to inhibit the activity of the HPA axis (Jessop, Dallman, Fleming, & Lightman, 2001). This negative feedback is exerted via glucocorticoid receptors and mineralocorticoid receptors (Tilbrook & Clarke, 2006). Mineralocorticoid receptors are found in the hippocampus and limbic brain and is involved in maintaining the basal activity of the HPA axis (Reul & Dekloet, 1985). Glucocorticoid receptors which are abundant in the hypothalamus involved in feedback of both stress induced and basal cortisol levels (De Kloet, Vreugdenhil, Oitzl, & Joels, 1998; Kim, Cole, Kalman, & Spencer, 1998). Cortisol is a glucocorticoid that can have a significant impact on most central and peripheral body systems (Levine, Zagoory-Sharon, Feldman, Lewis, & Weller, 2007).

Cortisol is normally secreted into the circulation in a pulsatile fashion that displays a strictly regulated circadian rhythm with highest levels of cortisol secreted in the mornings and the lowest levels in the evenings (de Weerth, Zijl, & Buitelaar, 2003; Spiga, Walker, Terry, & Lightman, 2014). These secretory pulses usually occur approximately once every 60–90 min (Windle, Wood, Kershaw, Lightman, & Ingram, 2013). This pulsatile secretion of the stress system hormones are often
affected by changes in lighting, feeding schedules and activity as well as following stress and pathophysiological demand (Charmandari et al., 2005; Chrousos, 1998b; Shanks et al., 2000; Windle et al., 2001). Cortisol levels drop significantly throughout the day even without a substantial external stimulus (Stone et al., 2001). In stress experiments, it is important to standardise the time of the day at which a stressor is to be imposed when planning the experimental design of the study.

There are several physical and behavioural adaptations induced by activation of the HPA axis (Chrousos & Gold, 1992; Kyrou et al., 2006). Glucocorticoids target most body cells and are involved in releasing glucose from glycogen stores, releasing amino acids from skeletal muscle, releasing lipids from adipose tissue (lipolysis), promoting glucose production in the liver, promoting peripheral utilisation of lipids and inhibiting the activities of white blood cells (Tsigos & Chrousos, 2002). Some of the physical adaptations to stress include redirection of oxygen and nutrients to the skeletal muscles, altered cardiovascular tone, increased heart rate, increased gluconeogenesis and lipolysis, inhibition of growth, inhibition of digestion and containment of immune response (Chrousos & Gold, 1992). Examples of behavioural adaptation include increased arousal and alertness, increased cognition and vigilance and suppression of appetite (Chrousos & Gold, 1992). All these adaptations help in dealing with a stressful situation. In most cases, these changes prepare the body for emergencies.
Nevertheless, if stress is severe and/or prolonged, these physiological responses can have detrimental consequences.

Cortisol both in its free form and bound form has been used as a direct measure of HPA axis activity in psychobiological research for decades (Dickerson & Kemeny, 2004). Measurement of cortisol in plasma is a common way to determine the levels of cortisol to which the body is exposed. Nevertheless, plasma cortisol measurement requires blood sampling via venepuncture or cannulation which can activate the stress pathways (Kirschbaum & Hellhammer, 1994). Nevertheless, this can be minimised by timing the cannulation well in advance of when the experiment is to take place. An advantage of measuring plasma cortisol is that a consistent volume of sample can be collected at each collection to ensure sufficient sample for analysis.

Salivary cortisol measurement is relatively easy to undertake. Saliva sample collection can be done with minimal assistance of trained professionals and can be done outside laboratory environments, if necessary (Gozansky, Lynn, Laudenslager, & Kohrt, 2005). CBG bound cortisol does not pass through the acinar cell layer that line the salivary glands and therefore salivary cortisol is an indicator of the levels of unbound/ free cortisol in blood (Kirschbaum & Hellhammer, 1994). Saliva flow rates can be influenced by stress and therefore measuring cortisol can be problematic if there is insufficient volume generated (Beltzer et al., 2010). As with the SAM system, there are advantages and
disadvantages of each available method of measuring activity of the HPA axis and the method choice for a particular study needs to be determined by the experimental requirements. Nevertheless, measurement of cortisol in plasma and saliva are both commonly used and widely accepted methods.

### 2.2 Different types of stressors
Stressors can generally be classified into two main categories; physical stress and psychological stress.

#### 2.2.1 Physical stress
Physical stressors are real threats to homeostasis and generate a “reactive” glucocorticoid response to stress. As asserted by Herman and colleagues, reactive glucocorticoid responses are induced by genuine challenges to physiological homeostasis that are identified by sensory pathways (Herman et al., 2003). These genuine challenges use direct neuronal pathways via the brain stem to stimulate the paraventricular nucleus to activate the HPA axis.

There are different kinds of physical stressors that can elicit a stress response. Some of the most common physical stressors imposed in human research include exercise, exposure to cold (cold pressor test), medical procedures, occupational tasks and sporting tasks (Michaud, Matheson, Kelly, & Anisman, 2009). The extent of the stress pathway activation depends on the magnitude and the duration of the physical stress. For example, maximal exhaustive exercise elicited significant salivary cortisol responses (Kraemer, Blair, Kraemer, & Castracane,
1989; Luger et al., 1987) and exercise exceeding 70% of maximal aerobic capacity (VO₂ max) elicited significant saliva cortisol responses when sustained for more than 70 minutes (Lovallo, Farag, Vincent, Thomas, & Wilson, 2006).

2.2.2 Psychological stress
Psychological stressors need processing by higher brain centres (MacLullich et al., 2006). Physiological responses to psychological stressors are generated due to anticipation of a possible disruption of physiological homeostasis, rather than to a genuine threat. Therefore, the stress response generated in such instances is “anticipatory” (Herman et al., 2003). Commonly used psychological stressors in human research include mental arithmetic (Reims et al., 2004), public speaking (Benson et al., 2009) and colour-word matching tasks (Waldstein, Burns, Toth, & Poehlman, 1999). A psychosocial stressor that has become very commonly used in human research is the Trier Social Stress Test (TSST), which involves an anticipatory period, public speaking task and a mental arithmetic challenge which has been found to be reliable and potent in activating the SAM system and the HPA axis (Kirschbaum, Pirke, & Hellhammer, 1993).

While physiological responses to physical stress are appropriate in a biological sense, because they prepare the body for a physical response, the physiological stress systems are also activated in response to psychological stress, which may not be appropriate since there is often no physical response needed. Thus physiological responses to psychological stress may often be unnecessary, and
if individuals encounter frequent psychological stressors, this may be particularly detrimental. It is thought that frequent, prolonged psychological stress can have debilitating long-term consequences (McEwen, 1998; Phillips, Roseboom, Carroll, & de Rooij, 2012; Picard, Juster, & McEwen, 2014b). Because of the potential health benefits to be gained by reducing its impact, there is much to be gained by investigating responses to psychological stress.

2.2.3 Food intake
Food intake can also have marked influences on stress physiology. It has been found that food intake can elicit both SAM system (Chang, Ko, Lien, & Chou, 2010; Cozzolino et al., 2010) as well as HPA axis responses (Harthoorn & DransWeld, 2008; Lemmens, Born, Martens, Martens, & Westerterp-Plantenga, 2011). It appears that the degree of stress pathway activation in response to food intake depends on a multitude of factors including nutrient content of the ingested food, sex, and physiological factors such as the distribution of body fat (Lemmens et al., 2011; Martens, Lemmens, Adam, & Westerterp-Plantenga, 2012; Martens, Rutters, Lemmens, Born, & Westerterp-Plantenga, 2010).

Available evidence in humans in this area is inconclusive. Gibson and colleagues reported that a high protein (32% of energy in protein) mid-day meal can elicit greater (1.5 -2 fold increase) HPA axis responses compared with a low protein (5% of energy in protein) mid-day meal in young healthy females (Gibson et al., 1999). They also showed that a high protein meal (39% energy in protein) can
elicit a significant HPA axis response in young men (Gibson et al., 1999). Nevertheless, Martens and colleagues measured cortisol responses in men to three different types of shakes (high protein shake, high carbohydrate shake and high fat shake) and reported that only the high carbohydrate shake resulted in an increase in cortisol secretion (Martens et al., 2010). Each of the shakes used contained 2306±77 kJ. Furthermore, Vicennati and colleagues reported that only high carbohydrate meals (89% carbohydrate, 11% protein, 0% fat) but not high protein/fat meals (53% lipids, 43% protein and 4% carbohydrate) resulted in a significant HPA axis response in women who predominantly had a visceral body fat distribution compared with women with peripheral body fat distribution and normal weight healthy controls (Vicennati, Ceroni, Gagliardi, Gamberi, & Pasquali, 2002).

The stimulatory effects of food are not surprising given the links between the brain-gut axis and the stress pathways (Hussain & Bloom, 2013; Ishizuka, Quigley, & Yen, 1983; Schwartz, Woods, Porte, Seeley, & Baskin, 2000). Mechanisms that regulate stress system activity in response to food intake are still unclear. Since, food intake is a challenge experienced by the human body several times per day, a greater understanding of the physiological response to food intake is of fundamental importance.
2.3 Sex differences in stress responsiveness and the influence of sex steroids

Males and females have different risk levels for a number of diseases. For example, women are more likely to suffer from autoimmune illnesses, anxiety depression, phobia and panic disorders and men are more prone to develop coronary heart disease, infectious disease, anti-social behaviour and substance abuse (Bebbington, 1996; Cleary, 1987; Kudielka, Hellhammer, & Kirschbaum, 2000; McCarty et al., 1995; Weich, Sloggett, & Lewis, 2001). Stress pathway activation also differs between the sexes of various age groups (Lu et al., 2014). Sex differences exist across both the SAM system as well as the HPA axis (Kajantie & Phillips, 2006). Nevertheless, sex differences in response to psychosocial stress remains inconclusive (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Kudielka & Kirschbaum, 2005; Lu et al., 2014). Both men and women are included in this research and appropriate measures are taken (especially when including women) to minimise the effects of sex on the results.

In most instances, men have shown accentuated activation of the stress pathways in response to external stimuli compared with women. For example, men have shown higher HPA axis responses to potent stimuli such as the TSST (Kirschbaum et al., 1999; Kirschbaum, Wust, & Hellhammer, 1992; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004b; Kudielka et al., 1998) and other less stressful stimuli such as speech tasks (Nicolson, Storms, Ponds, & Sulon, 1997), visual problem solving/mirror drawing.
(Steptoe, Fieldman, Evans, & Perry, 1996) and mathematical tasks (Tersman, Collins, & Eneroth, 1991; Traustadottir, Bosch, & Matt, 2003). Men have also shown greater activity of the SAM system in response to external stimuli (Traustadottir et al., 2003; Ward et al., 2004), whereas others suggest that SAM system responses to psychological stress are comparable between the sexes (Earle, Linden, & Wenberg, 1999; Larson, Ader, & Moynihan, 2001; Owens, Stoney, & Matthews, 1993).

Sex steroids play a major role in determining stress responsiveness (see Kajantie & Phillips, 2006) for an extensive review). While the sex steroid milieu is reasonably constant in men, it changes throughout the menstrual cycle in women. Activity of the stress pathways has been shown to be influenced by the stage of the menstrual cycle. For instance, the increment in cortisol levels in the first 20-30 minutes after waking up (cortisol awakening response) is higher in women during ovulation compared with women in either the follicular phase or the luteal phase of the menstrual cycle (Wolfram, Bellingrath, & Kudielka, 2011). It has also been found that women in the luteal phase of the menstrual cycle have higher cortisol reactivity to both physical (cold pressor) as well as psychological (TSST) stress compared with women in the follicular phase (Kirschbaum et al., 1999; Tersman et al., 1991). In sex comparisons, it has been found that women in the luteal phase of the menstrual cycle have salivary cortisol responses to psychological stress that are comparable to those of men (Rohleder, Schommer, Hellhammer, Engel, & Kirschbaum, 2001).
Because the sex of an individual and the sex steroids present can influence the activity of the SAM system and the HPA axis, findings from either sex cannot be extrapolated to the other. As such, it is important that researchers consider sex and sex steroid status when designing stress studies. Ideally, males and females should be included as different groups within a study or in different studies. If the participants include women who are post-menarche but pre-menopausal, the experimental design of the study needs to take into consideration stage of menstrual cycle and steroidal contraceptive use as well.

### 2.4 Stress and age

Aging results in changes in stress responsiveness (Pardon, 2007). The effects of aging on HPA axis responsiveness has been studied far more extensively than effects of aging on SAM system responsiveness to stress (for extensive reviews see (Kudielka et al., 2009; Otte et al., 2005)). Available evidence on the effects of aging on both HPA axis and SAM system activity remains inconclusive. In regards to HPA axis activity, most studies have found increases in activity with aging (Kudielka et al., 2009; Otte et al., 2005; Seeman, Singer, Wilkinson, & McEwen, 2001; Strahler, Mueller, Rosenloecher, Kirschbaum, & Rohleder, 2010). Nevertheless, there have been a couple of instances where no influence of age on HPA axis activity in response to stress were found (Nicolson et al., 1997; Rohleder et al., 2001)

In regards to the effects of aging on SAM system responsiveness, studies have reported increases (Pascualy et al., 1999; Uchino, Uno, Holt-Lunstad, &
Flinders, 1999), decreases (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004a; Strahler et al., 2010) as well as comparable responses between young and old individuals (Esler et al., 1995; Wood, Maraj, Lee, & Reyes, 2002) in SAM system activity in response to various types of stress. Because age may influence SAM system and HPA axis responses to stress, age should be considered when defining inclusion and exclusion criteria in stress research.

2.5 Modifiable factors that can influence the activity of the stress pathways

Level of adiposity (obesity) and the level of physical activity may have significant influences on the activity of the SAM system and the HPA axis. The following section of this review will discuss the effects of obesity and physical activity on SAM system and HPA axis activity in response to external challenges.
2.5.1 Adiposity and stress responsiveness

Obesity is a condition defined by excess adiposity. There are different categories based on the level of adiposity (Table 2.1).

Table 2.1: Classifications of underweight, lean, overweight and obese

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.50</td>
</tr>
<tr>
<td>Severe thinness</td>
<td>&lt;16.00</td>
</tr>
<tr>
<td>Moderate thinness</td>
<td>16.00-16.99</td>
</tr>
<tr>
<td>Mild thinness</td>
<td>17.00-18.49</td>
</tr>
<tr>
<td>Normal range</td>
<td>18.50-24.99</td>
</tr>
<tr>
<td>Overweight</td>
<td>≥ 25.00</td>
</tr>
<tr>
<td>Pre-obese</td>
<td>25.00-29.99</td>
</tr>
<tr>
<td>Obese</td>
<td>≥ 30.00</td>
</tr>
<tr>
<td>Obese class I</td>
<td>30.00-34.99</td>
</tr>
<tr>
<td>Obese class II</td>
<td>35.00-39.99</td>
</tr>
<tr>
<td>Obese class III</td>
<td>≥ 40.00</td>
</tr>
</tbody>
</table>

WHO 2000 & WHO 2014

Since obesity has reached epidemic proportions in most parts of the world and is associated with numerous stress related chronic conditions such as type 2 diabetes and cardiovascular disease (Allison et al., 2008), a better understanding of how obesity can influence other physiological processes such as stress responsiveness is of significant importance. Adiposity is currently viewed as a chronic inflammatory status due to the ability of adipose
tissues to secrete various inflammatory cytokines such as IL-6 and TNF-α (Nishimura, Manabe, & Nagai, 2009; Tilg & Moschen, 2008). Inflammation is believed to be a key player in the development of the aforementioned chronic conditions (Hotamisligil, 2006). Nevertheless, it is not a focus of this research to investigate the inflammatory responses associated with adiposity and stress pathway activation.

Both human and animal studies have looked at the effects of adiposity on the activity of SAM system and the HPA axis. The findings from fourteen such human studies and one animal study are summarised in Table 2.2. It is apparent that these investigations have yielded varying outcomes. Most of the research done in this area has been in women of different age groups (Benson, Arck, Blois, Schedlowski, & Elsenbruch, 2011; Epel et al., 2000; Hillman, Dorn, Loucks, & Berga, 2012). Therefore a paucity of evidence exist in this area for men. Given that there are sex differences in stress pathway responsiveness to stress (Fu, Wang, Weng, & Tao, 2012; Katz et al., 2000), findings in females cannot be extrapolated to men. Previous research in obesity and stress has reported both increased (Benson et al., 2009; Waldstein et al., 1999), decreased (Carroll, Phillips, & Der, 2008; Phillips et al., 2012) as well as comparable (Therrien et al., 2010) stress pathway activation in individuals with increased levels of adiposity compared with lean individuals in response to external stimulation. Not only BMI and total body fat, level of abdominal body fat has also been found to have significant implications for stress responsiveness. For instance Epel et al. reported significant increases of cortisol in women with high levels of abdominal body fat (measured via waist
to hip ratio) compared with women with low levels of abdominal body fat (Epel et al., 2000). Increased adiposity can impact stress pathway activation in other species as well. In our lab, we have shown that sheep with excess adiposity have increased SAM system as well as HPA axis responsiveness to isolation/restraint stress (Tilbrook et al., 2008).

While it is interesting to see such variation in results, it should be acknowledged that these investigations had considerable differences in methodology. Research in this area have focused on multiple age groups (Table 2.2) and different sexes (Table 2.2) when selecting the participant groups. Inter individual variability in stress responsiveness make generalisation of findings between different cohorts untenable. It is also notable that a variety of psychological stressors such as puzzle solving, colour-word stroop tasks, mental arithmetic and mirror tracing have been utilised (Table 2.2). It has been suggested that the aforementioned mental challenges do not reflect real world stress and are therefore unable to sufficiently activate the stress pathways (Jackson & Dishman, 2006). This could be circumvented by using a potent stressor such as the Trier Social Stress Test which carries both a mental challenge as well as an uncontrollable and social evaluative threat and is representative of real world stress (Dickerson & Kemeny, 2004). It is notable that most research has not used the TSST (Barnes, Treiber, Davis, Kelley, & Strong, 1998; Benson et al., 2009; Epel et al., 2000; Ljung et al., 2000; Pasquali et al., 1996; Reims et al., 2004; Waldstein et al., 1999) or modified versions of it (Therrien et al., 2010) have been implemented. This
might explain why there was no difference in cortisol responsiveness seen between lean and obese individuals in some studies.

Lack of substantial separation of obese and lean individuals in experimental groups of human stress experiments is another limitation noteworthy of mentioning that may have contributed towards insignificant findings. For instance, Ljung et al found no differences in stress responsiveness (ACTH responsiveness) between men who had a high waist to hip ratio (WHR) and men who had a low WHR (Ljung et al., 2000). Nevertheless, this result could be due to the fact that there were no distinctively different groups based on BMI. Animal experiments on the other hand have used distinctively different groups when obese and lean animals were compared. For example, in our sheep study where we found significant differences, we had a 23% difference in percentage body fat between lean and obese sheep (Tilbrook et al., 2008).

Ideally, a carefully designed, robust stress experiment will use a protocol which entails a potent stressor with frequent sampling before, during and after stress. Experimental Chapter 1 of this thesis considers the influence of adiposity on SAM system and HPA axis responsiveness to psychosocial stress (Trier Social Stress Test) in men aged 50-70 years.

As described above (Section 2.2.4.3) food intake can activate both SAM system as well as the HPA axis. Food intake may result in differential outcomes in the activity of HPA axis and SAM system in lean and overweight/obese individuals but this has not previously been investigated.
Experimental Chapter 2 in this thesis considers the effects of adiposity on SAM system and HPA axis responses to food intake in lean and overweight/obese men.
Table 2.2: Cross sectional studies that investigated the effects of obesity on stress responsiveness.

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Stressor</th>
<th>HPA axis outcomes</th>
<th>SAM system outcomes</th>
</tr>
</thead>
</table>
| (Pasquale et al., 1996) | Women (25-35 years)  
N=7 V-BFD obese  
N=9 S-BFD obese  
N=6 Lean | CRH/AVP test  
Puzzle solving  
Mental arithmetic  
Control saline test | Greater increase in cortisol and ACTH in obese compared with lean individuals in the CRH/AVP test | Greater increase in SBP in V-BFD women compared with lean and S-BFD women during puzzle solving and mental arithmetic |
| (Barnes et al., 1998) | Normotensive male and female adolescents (mean age 14.8 years ± 1.4)  
N=21 Low WHR black  
N=11 Low WHR white  
N=15 High WHR white  
n=17 High WHR black  
Mean BMI high WHR=29.0±6.3 kg/m²  
Mean BMI low WHR=21.4±4.5 kg/m² | Postural change  
Forehead cold stimulation  
Video game challenge | No measurement | Greater SBP and DBP responses in high WHR group compared with low WHR group |
| (Waldstein et al., 1999) | African American men and women (52-79 years)  
N=12 lower BMI (mean BMI=25.8) | Mental arithmetic  
Speech task | No measurement taken | Greater SBP, DBP and HR reactivity in obese compared to overweight participants |
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Task</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Epel et al., 1999)</td>
<td>Healthy men with high WHR (they compared these men to a group of women from a previous study which had the exact same study design)</td>
<td>Stroop colour-word</td>
<td>High WHR men showed greater increases in cortisol compared with high and low WHR women</td>
</tr>
<tr>
<td>(Epel et al., 2000)</td>
<td>Premenopausal women (mid follicular phase) N=30 high WHR (mean=0.83) N=29 low WHR (mean=0.72)</td>
<td>Mental arithmetic, Solving anagrams, Logic puzzles, Performing arithmetic aloud, Forming designs with blocks, Speech</td>
<td>Greater cortisol response in high WHR women compared with low WHR women</td>
</tr>
<tr>
<td>(Ljung et al., 2000)</td>
<td>Overweight and obese men (50-55 years) N=23 high WHR (mean=1.06) N=27 low WHR (mean=0.94)</td>
<td>OGTT with CRH stimulation, Mental arithmetic</td>
<td>No difference in cortisol and ACTH responses between high and low WHR. Greater 24 hour ACTH levels in high WHR compared with low WHR</td>
</tr>
<tr>
<td>Study</td>
<td>Group Description</td>
<td>Task</td>
<td>Stressor</td>
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<tr>
<td>----------------------------</td>
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<tr>
<td>Reims et al., 2004</td>
<td>Men (18-19 years)</td>
<td>Insulin induced hypoglycaemia</td>
<td>Mental arithmetic</td>
</tr>
<tr>
<td></td>
<td>N=36 Lean normotensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=62 lean borderline hypertensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=7 overweight normotensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=29 overweight borderline hypertensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tilbrook et al., 2008</td>
<td>Gonadectomised female sheep</td>
<td>Isolation and restraint</td>
<td>Greater increase in cortisol in obese compared with lean sheep</td>
</tr>
<tr>
<td></td>
<td>N=7 lean (mean body fat=8.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=7 fat (mean body fat=31.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carroll et al., 2008</td>
<td>Adult males and females (n=1647)</td>
<td>Time-pressured mental arithmetic</td>
<td>Individuals with greater BMI and waist-hip ratios and those categorized as obese displayed smaller HR reactions to stress</td>
</tr>
<tr>
<td>Benson et al., 2009</td>
<td>Pre-menopausal women (mean age 31.9 years)</td>
<td>Public speaking</td>
<td>Greater cortisol response in obese compared with lean women</td>
</tr>
<tr>
<td></td>
<td>n=15 Obese</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=24 lean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Design</td>
<td>Results</td>
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<td>----------------------</td>
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<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>(Therrien et al., 2010)</td>
<td>Men and women (age range 30-41 years)</td>
<td>TSST</td>
<td>No difference in cortisol levels in response to stress between lean and obese men</td>
</tr>
<tr>
<td></td>
<td>N=20 obese males</td>
<td></td>
<td>No difference in cortisol levels in response to stress between lean and obese men</td>
</tr>
<tr>
<td></td>
<td>N=17 lean males</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>N=10 obese females</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=12 non-obese females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Fu et al., 2012)</td>
<td>Boys, n=16 (obese=10, lean=6); girls, n=25 (obese 13, lean=12)</td>
<td>TSST</td>
<td>Weight status is associated with cortisol responses to stress in girls but not boys.</td>
</tr>
<tr>
<td></td>
<td>Age range= 12-14 years</td>
<td></td>
<td>Cortisol reactivity was higher in the lean girls compared with the obese girls.</td>
</tr>
<tr>
<td>(Hillman et al., 2012)</td>
<td>Girls (n=262)</td>
<td>Venepuncture</td>
<td>Lower reactivity in girls with higher adiposity levels</td>
</tr>
<tr>
<td></td>
<td>Age range= 11-17 years</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No difference in SBP in obese compared with lean women.
<table>
<thead>
<tr>
<th>Study</th>
<th>Participant Details</th>
<th>Procedure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phillip et al., 2012</td>
<td>Adult males and females (n=725)</td>
<td>Stroop task, mirror tracing task and speech</td>
<td>BMI and skinfold measures were negatively associated with salivary cortisol reactivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BMI and skinfold measures were negatively associated with heart rate reactivity</td>
</tr>
<tr>
<td>Lu et al., 2014</td>
<td>Boys and girls (n=87)</td>
<td>TSST for children</td>
<td>Greater cortisol responses associated with higher percentage body fat levels in girls but not boys.</td>
</tr>
<tr>
<td></td>
<td>Mean age = 12.7 years</td>
<td></td>
<td>No measures taken</td>
</tr>
</tbody>
</table>

2.5.2 Physical activity and stress responsiveness

The levels of physical activity that an individual undertakes may also influence their stress responsiveness. Exercise has the ability to improve the efficiency of the mechanisms that can oxidise mobilised energy which can be beneficial in better managing the unnecessarily mobilised energy often resulting from psychological stress (Achten & Jeukendrup, 2004). Physical activity can have influences on a number of different physiological processes such as neuroendocrine, inflammatory, dopaminergic and haemodynamic systems. Any number of these processes could be responsible for the beneficial effects of physical activity and its ability to moderate the stress response. The focus in this research was the effect of physical activity on the neuroendocrine and cardiovascular responses to acute psychological stress and to food intake.

The ability of regular exercise to moderate some of the debilitating effects of stress related diseases such as cardiovascular diseases (Barlow et al., 2006; KETELHUT, FRANZ, & SCHOLZE, 2004) and depression (Motl, Birnbaum, Kubik, & Dishman, 2004; Nabkasorn et al., 2006) are widely reported. Protective effects of exercise on metabolic diseases are also well documented (Stewart et al., 2005; Tsatsoulis & Fountoulakis, 2006). Given the ability of excessive stress responsiveness to contribute to the development of chronic disease (Chrousos, 2009), it is possible that physical activity may bring about at least some of its beneficial effects by modifying stress responsiveness. Nevertheless, the role of physical activity in influencing stress responsiveness is not fully understood (Klaperski, von Dawans, Heinrichs, & Fuchs, 2013).
Evidence from eight cross sectional studies is summarised in Table 2.3. It is evident that these investigations had considerable variations in study methodology such as different testing cohorts, various stressors, different sampling frequencies and various measures of SAM system activity. It is also evident that a majority of the studies have been done in males (Table 2.3). Evidence from investigations considering the effect of physical activity status on stress responsiveness in females is scant. Furthermore, many studies in the area have not investigated the HPA axis and the SAM system activity, simultaneously, using direct measures (Table 2.3). It may be argued that the HPA axis is less prominent in acute stress responsiveness studies in laboratory settings compared with SAM system given that the HPA axis is harder to measure and has a longer time course of activation. Nevertheless, given the intricate interconnection between the HPA axis and the SAM system and because of the role it plays in the physiological stress response and in the progression to chronic disease, it is important to investigate both pathways simultaneously in research of this nature.

The ability of the psychological stressor to generate a substantial physiological response is paramount to the outcomes of a stress experiment. The TSST has the capability of sufficiently activating both the SAM system and HPA axis. Some of the studies described in Table 2.3 have implemented less potent stressors instead.
Another limitation in most previous experiments is the infrequent sampling during the stress procedure. With HPA axis measurements (i.e. cortisol), frequent sampling during stress might not be as crucial compared with SAM system measurements. The reason being that HPA axis has a longer time course of activation which means that the peak response can be delayed until after the end of the stress procedure (usually 30 minutes maximum with a potent stressor). Nevertheless, a sufficient sampling frequency is required to properly characterise the response. Conversely, SAM system activity tends to peak relatively quickly and may often fall within the stress period. Therefore infrequent sampling may not capture valuable information about the true peak of SAM system response.

Ideally, a carefully designed, robust stress experiment will use a protocol which entails a potent stressor with frequent sampling during stress which also entails simultaneous direct measurements of HPA axis and SAM system. Experimental Chapter 3 of this thesis considers the influence of physical activity on SAM system and HPA axis responsiveness to psychosocial stress (Trier Social Stress Test) in women aged 30-50 years.

Table 2.3 shows that the level of physical activity may differentially affect SAM system and HPA axis responses to psychological stress. Not only in response to psychological stress, but physical activity status may also have differential influences on the stress pathway activation in response to food intake. Given that exercise has the ability to modify energy mobilisation kinetics within the
body, it is also possible that food intake will result in different physiological responses in individuals who undertake high and low levels of physical activity. This has not been investigated previously. Experimental Chapter 4 of this research will investigate if responses of SAM system and HPA axis to food intake will differ in women who participate in high and low levels of physical activity.
<table>
<thead>
<tr>
<th>Study/authors</th>
<th>Participants</th>
<th>Stressor</th>
<th>HPA axis outcomes</th>
<th>SAM system outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(de Geus, Van Doornen, &amp; Orlebeke, 1993)</td>
<td>Male academic workers (25-40 years of age)</td>
<td>Reaction time task, memory search task and cold pressor test</td>
<td>No measurements taken</td>
<td>Higher HR, SBP and DBP reactivity in high fit individuals</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No effect on fitness of urinary catecholamine levels in response to challenge</td>
</tr>
<tr>
<td>(Traustadottir, Bosch, &amp; Matt, 2005)</td>
<td>Younger and older women</td>
<td>MSRP: A test battery that combines a psychological stressor, mental challenges and a physical challenge</td>
<td>Greater cortisol response in older unfit compared with other groups</td>
<td>Greater HR response in young unfit compared with other groups</td>
</tr>
<tr>
<td></td>
<td>N=9 young unfit (19-36 years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=11 older fit (59-81 years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=13 older unfit</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(Rimmele et al., 2007)</td>
<td>Men (age range 19-24 years)</td>
<td>TSST</td>
<td>Significantly lower cortisol response in elite sportsmen compared with untrained men</td>
<td>Significantly lower HR response in elite sportsmen compared with untrained men</td>
</tr>
<tr>
<td>Study (Year)</td>
<td>Design</td>
<td>Stressor</td>
<td>Outcomes</td>
<td></td>
</tr>
<tr>
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<td>--------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>(Rimmele et al., 2009)</td>
<td>Men (age range = 23-25 years)</td>
<td>TSST</td>
<td>Significantly lower cortisol response in elite sportsmen compared with untrained men</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=18 Elite</td>
<td></td>
<td>Significantly lower HR response in elite and amateur sportsmen compared with untrained men</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=50 amateur</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=24 untrained</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Palatini et al., 2010)</td>
<td>Stage one, physically active and inactive hypertensive patients (sedentary, n=75; active n=44) and normotensive controls (n=63)</td>
<td>Public speaking</td>
<td>No measures taken</td>
<td>Higher blood pressure responses in the sedentary hypertensive individuals compared with both active hypertensive individuals and controls</td>
</tr>
<tr>
<td>Authors</td>
<td>Group Description</td>
<td>Intervention/Task</td>
<td>Findings</td>
<td>Control Measure</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------------------------------------</td>
<td>--------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>(Martikainen et al., 2013)</td>
<td>Children (n=258), Age 8 years</td>
<td>TSST for children</td>
<td>Higher cortisol responses to stress in the lowest active children</td>
<td>No measures taken</td>
</tr>
<tr>
<td>(Klaperski et al., 2013)</td>
<td>Young women (inactive, n=17; moderately active, n=15; vigorously active, n=15), Age range (18-28 years)</td>
<td>TSST for groups</td>
<td>Significantly high salivary cortisol levels in the inactive group in response to stress as opposed to the other two groups.</td>
<td>Significantly high heart rate response in the inactive group to stress as opposed to the other two groups.</td>
</tr>
<tr>
<td>(Webb et al., 2013)</td>
<td>Healthy men (high fit, n=8; low fit, n=8)</td>
<td>Exercise alone or exercise and stroop colour word task</td>
<td>Increased cortisol responses in the low fit group in response to combined, exercise and stroop colour word task condition</td>
<td>No measures taken</td>
</tr>
</tbody>
</table>

MSRP: Matt stress reactivity protocol, ACTH: adrenocorticotropic hormone, HR: heart rate, BP: blood pressure, SBP: systolic blood pressure, DBP: diastolic blood pressure, HPA axis: hypothalmo-pituitary adrenal axis, SAM: sympatho-adrenal medullary system
2.6 Stress and chronic disease

2.6.1 Stress and cardiovascular disease
According to the World Health Organisation (WHO), an estimated 17.3 million people died from cardiovascular disease in 2008 and this number is expected to rise to 23.3 million by 2030 (Mendis, Puska, & Norrving, 2011). From the National Health Survey (NHS) conducted 2007-08, it was found that an estimated 3.4 million Australians (17% of the population) had one or more long term cardiovascular diseases (Australian Institute of Health and Welfare, 2011). In 2007, it was reported that cardiovascular disease (CVD) was responsible for the deaths of 46,670 Australians (Australian Institute of Health and Welfare, 2011).

Stress, in particular psychological stress, has been shown to have significant contributions to cardiovascular disease development. Stress results in acute elevations in blood pressure and there is evidence suggesting that elevated blood pressure reactivity to psychological stress is associated with carotid atherosclerosis (Spence, Barnett, Manuck, & Jennings, 1996), carotid intima-media thickness (Lynch, Everson, Kaplan, Salonen, & Salonen, 1998) and increased left ventricular mass (Allen, Matthews, & Sherman, 1997). There is also evidence to suggest that elevated blood pressure during stress is a risk factor for the development of future hypertension (Carroll et al., 2011). In the Whitehall II study, in 1003 men, it was found that systolic blood pressure (SBP) response but not diastolic blood pressure (DBP) response was predictive of blood pressure status in five years (Carroll, Smith, Sheffield, Shipley, &
Diagnosis of cardiovascular disease is performed through an array of measures ranging from blood pressure checks to measurement of various biomarkers in blood. Measurement of blood pressure (both pulsatile and steady components) is one of the most commonly used diagnostic tools (Darne, Girerd, Safar, Cambien, & Guize, 1989). An individual’s lipid profile is often used in the process of assessing cardiovascular health. Low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol are commonly used in cardiovascular disease screenings (Cleeman et al., 2001). Markers of inflammation such as C-reactive protein are also considered as reliable measures of cardiovascular disease (Ross, 1999). All processes of the atherosclerotic plaque formation (initiation, growth and complication) are believed to be influenced by the inflammatory system (Plutzky, 2001). Therefore measuring the ongoing inflammatory processes is important in determining vascular health.

2.6.2 Stress and type 2 diabetes
According to the WHO, in 2004, an estimated 3.4 million people died from consequences of high fasting blood glucose (Alwan, 2011). In 2008, 3.8% of the Australian population had type 2 diabetes. Furthermore, it was reported that 275 Australians develop diabetes every day and at the current rate, it is
estimated that 3.3 million Australians will have type 2 diabetes by 2031 (Australian Institute of Health and Welfare, 2009).

Hyperglycaemia and insulin resistance are major risk factors for type 2 diabetes (Surwit, Schneider, & Feinglos, 1992). Both stress pathways act as instigators of these risk factors. Activation of the HPA axis has been shown to be a major cause for the development of insulin resistance (Bjorntorp, Holm, & Rosmond, 1999; Rosmond & Bjorntorp, 2000). Cortisol influences total insulin secretion and therefore has a marked effect on progression to type 2 diabetes (Agardh et al., 2003). Activation of the sympathetic nervous system has also been shown to have associations with the development of type 2 diabetes. Prospective studies with 10-20 years of follow up have found that elevated sympathetic reactivity is associated with subsequent development of insulin resistance (Flaa, Aksnes, Kjeldsen, Eide, & Rostrup, 2008; Masuo, Kawaguchi, Mikami, Ogihara, & Tuck, 2003). Recently, it has been suggested that the glucose imbalance created by stress, especially via affecting mitochondrial regulation of energy metabolism and cellular signalling is a key player in the development of type 2 diabetes (Picard, Juster, & McEwen, 2014a).

Fasting plasma glucose is one of the most common diagnostic measures of type 2 diabetes with a fasting plasma glucose level of greater than 126 mg/dL (7 mmol/L) on more than one occasion (Mahler & Adler, 1999; Rendell, 2006) forming the basis of a diagnosis. Another measure that is commonly used to
investigate anomalies in blood glucose regulation is Homeostatic model assessment (HOMA) insulin resistance (Wallace, Levy, & Matthews, 2004).

2.6.3 Stress and depression and anxiety
Globally, more than 350 million people of all ages suffer from depression (Alwan, 2011). According to ‘Beyond Blue’, in Australia, nearly one in seven people will experience some type of anxiety disorder in any one year including around one in six women and one in 10 men. One in four people will suffer from an anxiety disorder sometime during their life. Depression also has a high prevalence in Australia. It is reported that around one million adults and 100,000 young people live with depression each year (Beyond Blue, 2011). The neurobiology of the relationship between stress system activation and psychological disorders is still not well understood.

Excessive amounts of cortisol in individuals with depressive illness provides the best evidence for the link between stress and psychological disorders (Brown, Barton, & Lambert, 2009; Tafet & Bernardini, 2003). A chronic elevation in levels of CRH and ACTH has been reported in patients with major depressive disorder. It has been reported that depression is associated with higher basal activity of the HPA axis (indicated by plasma cortisol) compared with non-depressed controls and that depressed individuals show less suppression activity when subjected to a standard dexamethasone test (Arborelius, Owens, Plotsky, & Nemeroff, 1999). The finding that individuals with depression have hypercortisolism also strengthens the argument that
stress may lead to depression (Pearson Murphy, 1997). Stress (particularly psychological stress) is implicated in many facets of major depressive disorder (Burke, Davis, Otte, & Mohr, 2005). These include onset (Daley, Hammen, & Rao, 2000), symptom severity (Hammen, Davila, Brown, Ellicott, & Gitlin, 1992) and the entire course of the illness (Kendler, Karkowski, & Prescott, 1999).

The involvement of stress in anxiety is somewhat similar to that of the involvement of stress in depression. For instance, it has been shown that anxiety related disorders such as post-traumatic stress disorder (PTSD) is associated with hyperactivity of the HPA axis. Bremner et al (1997) found that there is increased concentrations of CRH in the cerebrospinal fluid of Vietnam combat veterans with PTSD (Bremner et al., 1997).

Depression and anxiety are mainly measured via questionnaires which entail questions about an individuals’ feelings, emotions and mood. Some of the most common questionnaires that are used to diagnose depression and anxiety are Beck’s Depression Inventory (Smarr & Keefer, 2011) and Speilberger’s state/trait anxiety questionnaire, respectively (Julian, 2011). State anxiety refers to the levels of anxiety at present, whereas trait anxiety refers to the levels of pervasive anxiety (Julian, 2011).
The Center for Cognitive therapy has stipulated following guidelines for the diagnosis of depression using the Beck Depression Inventory (Beck, Steer, & Carbin, 1988)

1. None or minimal depression <10
2. Mild to moderate depression 10-18
3. Moderate to severe depression 19-29
4. Severe depression 30-63

The link between stress and chronic diseases highlights the importance of gaining a better understanding of factors that can influence stress responsiveness.

2.7 Significance of this research
Chronic diseases including cardiovascular disease, type 2 diabetes and depression and anxiety impact negatively on human health. Large responses to acute stress have been shown to be associated with the development of the aforementioned diseases. Therefore, understanding the physiology of stress is of utmost importance, in order to better understand the ramifications or slow progression to these diseases, and will lead to significant improvements in the health and well-being of individuals.

Different physiological conditions such as adiposity and physical activity status can influence stress responsiveness. Basic human needs such as food intake can also affect the activity levels of the stress pathways. Sex differences in these relationships should also be considered. This thesis will investigate how
adiposity in men and physical activity status in women may affect responsiveness to psychological stress. It will also investigate the influence of food intake on stress pathway activation in both men with different levels of adiposity and women who engage in different levels of physical activity. This will provide a better understanding of how the stress systems work under the influence of different types of external stimulation, in relation to different physiological conditions and allow inferences to be drawn as to how it will affect disease progression. This has the potential to be hugely beneficial in the implementation of public health strategies in different sexes and different age groups.

### 2.8 Summary/conclusion

As more light is shed on the physiology of stress, it has become increasingly apparent that unnecessary stress responsiveness (particularly to psychological stress) is impacting negatively on human health. While having optimally functioning stress systems is adaptive, it is well known that chronic stress is associated with physical, behavioural and/or neuropsychiatric manifestations (Chrousos, 2009). Repeated exposure to acute stress may also be detrimental to health. In modern society, some of the most common negative health consequences related to excessive stress responsiveness, include cardiovascular disease, type 2 diabetes, anxiety and depression. Apart from being detrimental to health, these diseases are also amongst the most costly conditions to be treated (Chrousos, 2000a; Licinio & Wong, 2005). Thus, a better understanding of stress physiology (especially related to these conditions) is of utmost importance.
Stress activates a number of different physiological systems out of which the SAM system and the HPA axis are the most prominent (Elenkov & Chrousos, 2002). Since excessive activity of these systems can lead to chronic diseases, it is important to understand factors that influence these systems and to take steps to reduce stress responsiveness where possible. In particular, psychological stress may be a good target since responses to psychological stress are often unnecessary (i.e. there is no physical response required). Routine tasks such as food intake can also have a significant impact on stress pathway activation and requires further investigation.
2.9 Hypotheses

2.9.1 General hypothesis
This thesis will test the general hypothesis that adiposity in men and physical activity in women can influence SAM system and HPA axis responsiveness to psychological stress and food intake.

2.9.2 Specific hypotheses:

1. Men with high levels of adiposity will have greater SAM system and HPA axis responses to psychological stress (TSST) compared to those with low levels of adiposity.

2. Men with high levels of adiposity will have greater SAM system and HPA axis responses to food intake compared to those with low levels of adiposity.

3a. Women who undertake high levels of physical activity will have lower SAM system and HPA axis responses to psychological stress (TSST) compared with those who undertake low levels of physical activity.

3b. Low levels of stress reactivity will be associated with a more desirable profile of cardio metabolic risk markers.
4. Women who undertake higher levels of physical activity will have lower SAM system and HPA axis responses to food intake compared with those who undertake low levels of physical activity.
Chapter 3.0 General methods
In this program of research, one study was conducted in men to consider the influence of adiposity on stress system responsiveness to psychological stress (Experimental Chapter 1) and food intake (Experimental Chapter 2). A separate study was conducted in women to consider the influence of physical activity on stress system responsiveness to psychological stress (Experimental Chapter 3) and food intake (Experimental Chapter 4). To avoid repetition, methods and procedures common to 2 or more chapters are described here in the General Methods section, while the experimental procedures unique to individual experimental chapters are described in the relevant experimental chapter.

3.1 Participants
Participants were recruited using word of mouth, online (Experimental Chapters 3 and 4 only) and newspaper advertisements, flyers in community centres and GP clinics, mail outs to participants of previous studies, posters and flyers dropped in letter boxes and flyers in sporting establishments.

3.1.1 Experimental Chapters 1 & 2
Lean (n=24; BMI range 20-25 kg/m²) and overweight/obese (n=22; BMI range 27-35 kg/m²) men aged 50-70 years were recruited. During recruitment, the originally intended BMI range for obese individuals of 30-35 kg/m² was relaxed to 27-35 kg/m² (overweight/obese) to ensure an adequate sample size in this group. Men were initially interviewed (via telephone) to verify eligibility through
self-reported information pertaining to the inclusion and exclusion criteria (see below for inclusion and exclusion criteria). Eligibility was confirmed on the testing day, at face to face screening, where men were excluded if their BMI fell out of the required ranges.

3.1.2 Experimental Chapters 3 & 4
Women (n=44) aged 30-50 years were recruited. Similar to men, eligibility of women was initially assessed from self-reported information gained via a telephone interview. Given the influence of sex steroids on stress pathway activity (Kajantie & Phillips, 2006), all women who were on any form of steroidal contraception (including oral contraceptives, steroidal implants and steroidal IUDs) were excluded from the studies. Post-menopausal and perimenopausal women were also excluded from the studies. Eligibility was confirmed at Day 1 testing based on baseline blood pressure and anthropometric measurements.

3.2 Inclusion and exclusion criteria
Exclusion criteria for men and women included individuals who had been diagnosed with Cushing’s syndrome, any stress related disorder, anxiety disorder, depression, any diseases of the adrenal gland, type 2 diabetes, hypertension, heart disease (including use of a pacemaker), high cholesterol, stroke or cancer. Furthermore, potential participants were excluded if their resting blood pressure exceeded 160 mmHg for systolic blood pressure or 90 mmHg for diastolic blood pressure.
Chapter 3 - Methods

Every participant provided written informed consent prior to participation in this research. All procedures were approved by the Human Research Ethics Committee of Deakin University (Project code: EC00213 Experimental Chapters 1 & 2; 2011-242 Experimental Chapters 3 & 4) and conformed to the guidelines of the National Health and Medical Research Council’s National Statement on Ethical Conduct in Human Research (2007).

3.3 Anthropometrics

The same anthropometric measurement procedures were implemented for men and women. Weight was recorded in kilograms to the nearest 0.1 kg with digital scales (TANITA, Wedderburn, Melbourne, Australia) on a firm surface. Height was measured to the nearest millimetre using a freestanding stadiometer (Measurement Concepts, North Bend, Australia). Participants were not wearing shoes in both measurements. BMI was calculated as weight (kg) divided by height (m) squared. Percentage body fat, lean muscle content (kg), total body water content (kg), fat mass (kg) and impedance (Ω) were measured using Bio Electrical Impedance/TANITA (Wedderburn, Melbourne, Australia). Waist circumference was measured at the midpoint between the last rib and the anterior superior iliac spine using a tape measure. Hip circumference was measured at the widest point (at the level of greater trochanters) of the gluteal area (Dettwyler, 1993).
3.4 Lunch
Lunch, which was also the test meal in Experimental Chapters 2 (men) and 4 (women), consisted of a meal made by the participants from a choice of standardised ingredients including bread, margarine, processed meat (ham or chicken), tomato, cucumber, cheese, nuts, fruit bars and a fruit drink (juice box). Water was available ad libitum. Records were collected of foods and quantities consumed in household measures and total energy, macronutrient and sodium intake was determined using FoodWorks professional edition (Version 7; Xyris software, Brisbane, Queensland, Australia).

3.5 Stress test
The Trier Social Stress Test is a psychological stress paradigm used in laboratory settings which consists of preparation and delivery of a speech and verbally responding to a mental arithmetic challenge in front of an evaluative audience (Birkett, 2011; Kirschbaum et al., 1993). This test is capable of eliciting substantial increases in both SAM system and HPA axis activity levels (Kirschbaum et al., 1993). Endocrine responses that are generated in response to the TSST are influenced by sex (Kirschbaum et al., 1992), hereditary factors (Kirschbaum et al., 1992), as well as lifestyle choices such as nicotine consumption (Kirschbaum et al., 1993). It is believed that the high degree of ego involvement and the anticipation of negative consequences of being interviewed by a small group of confederates is the mechanism behind the ability of TSST to generate substantial stress pathway reactivity (Birkett, 2011). This procedure was used in Experimental Chapters 1 (men) and 3 (women). In both instances, TSST was imposed at 1500h-1530h (Figure 3.1).
Saliva (men) and blood (women) samples and physiological (heart rate and heart rate variability) measurements were obtained throughout the procedure. The TSST protocol used in this research contained two differences compared to the original protocol (Kirschbaum et al., 1993). Biological and physiological measures were obtained between the speaking and the mental arithmetic tasks (not obtained in the original protocol) and there were only two members of the interview panel (3 members in the original protocol).

Briefly, after measurements were obtained at 1500h, the test commenced with an introduction during which participants were introduced to the panel and were given instructions about the speaking task to follow (Figure 3.1). Participants were told that after 10-minutes of preparation, they were to deliver a 5-minute speech in which they were to imagine that they were applying for a position (any position) within the university. This was followed by a 10 minute preparation phase with measurements obtained towards the beginning (1507h) and end (1515h) of the preparation phase. Five minutes of public speaking followed (Figure 3.1B) after which another measurement was taken (1522h). Participants were given instructions regarding the mental arithmetic task at this point and 5 minutes of mental arithmetic followed. Another measurement was taken at the end of the mental arithmetic task (1530h). The purpose of the measurements obtained in-between speaking and mental arithmetic was to capture any of the rapid changes in SAM system activity (Figure 3.1A). All activities during the TSST were filmed and voice recorded (Figure 3.1B).
On occasions when participants stopped their speeches prematurely, Committee Member 1 requested that they continue speaking for the total duration. In the event of failure to continue after this, Committee Member 1 questioned the individual regarding their topic using some standard prompt questions including, “Why do you think you are the best applicant for this position?”, “What other experiences have you had in this area?”, “What is it about your background that provides a special aptitude and motivation for this position?”, “Where else did you apply?”, “What would you do, if your application here would not succeed?”. In the mental arithmetic task, the participants were asked to subtract in steps of 13 starting at 2083. They were told to do this as quickly as they could and as accurately as possible. If the participant made a mistake, they were asked to start again from 2083. Instructions for this task were given by Committee Member 1.
Figure 3.1A Schematic representation of the Trier Social Stress Test (TSST).

S/B = saliva or blood sample

Figure 3.1B Participant (left) delivering their speech to Committee Members 1 (middle) and 2 (right).
3.6 Biological Sampling and assays

3.6.1 Saliva sampling
Saliva samples were collected from men (Experimental Chapters 1 and 2). These samples were collected using Salivette sampling tubes (Sarstedt, Ingle Farm, Australia) consisting of a centrifugation tube and a cotton swab. Participants were asked to hold the cotton swab in their mouth for 2 minutes. They were instructed to keep the cotton swab under their tongue during the first 30-45 seconds and move it around the oral cavity for the remainder of the duration without chewing it or holding it between their teeth. Samples were stored on ice after collection. After the testing period, samples were centrifuged at 2000 rpm for 5 minutes at 4ºC. Resultant saliva was stored at -80 ºC until assayed.

3.6.2 Blood sampling
Blood samples were collected from women (Experimental Chapters 3 and 4). For the analysis of cardio metabolic risk markers (C-reactive protein, fasting glucose, total HDL and LDL cholesterol and triglycerides) and insulin, blood was collected into serum separator tubes (GreinerBio-One GmbH, Kremsmunster, Austria) and FE Sodium Fluoride/K3EDTA tubes (GreinerBio-One GmbH, Kremsmunster, Austria), respectively, through a single venipuncture to a vein of the antecubital fossa of the forearm using a sterile vacuette safety blood collection set (GreinerBio-One GmbH, Kremsmunster, Austria) after an overnight fast. These blood samples was collected at least one week prior to stress and food intake testing. Analysis of cardio-metabolic
risk markers was performed at a commercial pathology laboratory (Dorevitch, Melbourne, Australia).

For the measurement of catecholamines and cortisol, blood was collected on the day of stress and food intake testing into tubes containing reduced glutathione (Sigma-Aldrich, Australia) and Ethylene Glycol Tetra-acetic Acid (EGTA) (Sigma-Aldrich, Australia) and Lithium Heparin tubes (GreinerBio-One GmbH, Kremsmunster, Austria), respectively, through an intra-venous catheter (Smiths Medical, Ohio, USA) which had been inserted into an antecubital vein of the forearm prior to the commencement of any sampling. Blood samples were stored on ice and processed within one hour of collection. All tubes were spun at 3000 rpm for 6 min. Plasma was separated and stored at -80 °C until assay.

3.6.3 Saliva cortisol assays
Saliva concentrations of cortisol were measured in men (Experimental Chapters 1 and 2) using an enzyme immunoassay (Diagnostic Systems Laboratories, TX, USA). Thirty-one assays were conducted with a mean sensitivity of 0.035 μg/dL. The intra-assay coefficient of variation was 6.9% at 0.25 μg/dL and 8.2% at 2.0 μg/dL. The inter-assay coefficient of variation was 9.4% at 0.28 μg/dL and 7.7% at 1.8 μg/dL.
3.6.4 Salivary alpha amylase assays
Saliva concentrations of alpha amylase were measured in men (Experimental Chapters 1 and 2) using a kinetic assay kit (Salimetrics, PA, USA). Thirty-six assays were conducted with a mean sensitivity of 0.4 U/ml. The intra-assay coefficient of variation was 7.4% at 156.3±4.1 U/ml. The inter-assay coefficient of variation was 7.4% at 20.7 U/ml and 7.0% at 257.3 U/ml.

3.6.5 Plasma cortisol assays
Plasma concentrations of cortisol were measured in women (Experimental Chapters 3 and 4) using a radio immunoassay (Demeditec Diagnostics, Kiel, Germany). Forty-four assays were conducted. The intra-assay coefficient of variation was 9.8% at 92 ng/mL and 9.4% at 193 ng/ml. The inter-assay coefficient of variation was 10.7% at 146 ng/ml and 10.15% at 137 ng/ml.

3.6.7 Plasma insulin assays
Fasting insulin was measured in women (Experimental Chapters 3 and 4) using a human insulin specific RIA kit (Millipore, Darmstadt, Germany). Inter-assay coefficient of variation for the kit is between 2.9-6.0% and the intra-assay coefficient of variation is between 2.2-4.4%.

3.6.8: Plasma catecholamine assays
Stress hormones (adrenaline and noradrenaline), dopamine (which is measured routinely in this assay) and dihydroxybenzylamine (DHPG) were measured in women (Experimental Chapters 3 and 4) by extracting and
separating from plasma and were then quantified using electrochemical detection according to previously validated methods (Lambert & Jonsdottir, 1998). Catecholamines were extracted from plasma with alumina adsorption and separated using high performance liquid chromatography (HPLC).

The HPLC system consisted of a Dionex Ultimate 3000 pump, Dionex ASI-100 refrigerated auto sample injector (Dionex, Germering, Germany), a 25 cm Gemini NX column (ODS 4.60mm x 250mm, 5μ particle size, Phenomenex, CA, USA) and a Dionex STH 585 column oven (Dionex, Germering, Germany). It also contained a Model 5100A coulometric detector with a Model 5021 conditioning cell and a Model 5011 analytical cell (Environmental Sciences Associates, MA, USA). Operating potential for the guard cell was set at +0.35V and -0.35 and +0.29 for detectors 1 and 2 respectively. Analysis was performed at 22 °C.

The mobile phase consisting of sodium dihydrogen phosphate (NaH₂PO₄), EDTA, octane sulfonic acid and HPLC grade acetonitrile was adjusted to the pH 3.4 with phosphoric acid, degassed by vacuum filtration through a 0.22 μm Millipore membrane (Millipore Corp., Bedford, MA, USA), and delivered to the system at 1ml/minute.

Plasma samples of 1ml thawed at room temperature were mixed in 1.6 ml polypropylene Eppendorf tubes for 20 minutes on a rotary mixer (Ratek
Instruments, Victoria, Australia) with 2ng of DHBA (Sigma-Aldrich, St. Louis, MO, USA) as an internal standard, 400µl of 1M TrisHCL (pH 8.6 with EDTA) and approximately 10mg alumina (acid washed and stored at 100ºC before use). After brief centrifugation, the supernatant was aspirated and the alumina was washed with 1ml 0.2M NaHCO₃ (to remove a contaminant eluting with DHPG) and 2ml deionised water. Samples were vortexed and centrifuged each time before discarding the effluent. A 150µl solution consisting of 15% 0.2M phosphoric acid in 0.2M acetic acid was then added to the alumina and the mixture was vortexed for 6 minutes to elute the catecholamines. After centrifugation, the supernatant was transferred into microsample vials and the total volume of acid eluates (150µl) was injected in to the HPLC. The intra-assay coefficients of variation, determined from 3 repeated measurements of pooled venous plasma, were ± 3 % for noradrenaline, ± 5% for adrenaline, ± 3 % for DHPG, ± 3 % for DA. The inter-assay coefficients of variation, determined from 27 consecutive assay runs were ± 7 % for DHPG, ± 5 % for noradrenaline, ± 13 % for adrenaline, ± 10 % for DA. The assay was linear in the physiological range with a sensitivity (signal-to-noise ratio of 3) of approximately 0.1 pmol per 1 ml of plasma assayed. The mean (±SEM) recovery of the DHBA internal standard was 51±3 %.

3.7 Cardiovascular measurements

3.7.1 Blood pressure
Blood pressure was measured using a clinical blood pressure monitor (Criticare systems Inc, Wisconsin, USA) in men and women. During face-to-face screening, four resting blood pressure measurements were obtained from
all participants at 2 minute intervals and the average of the second, third and fourth measurements was used to confirm whether resting blood pressure was within the range pertaining to the eligibility criteria. Blood pressure measures were also obtained simultaneously with saliva or blood samples throughout stress and food intake testing as well.

3.7.2 Heart rate and heart rate variability
Heart rate (HR) and heart rate variability (HRV) was measured using continuous ECG traces (ADinstruments, NSW, Australia) in men and women. ECG electrodes were placed on each of the participants’ wrists and another just above the antecubital fossa of the elbow. For the purposes of data presentation and analysis, HR and HRV was calculated as the means of all data for 5 minute blocks at the same time points as those at which blood pressure measurements, saliva samples and blood samples were collected.

Heart rate variability was defined as the variation over time of the period between consecutive heart beats. The square root of the mean of the sum of squares of differences between adjacent R-R intervals (RMSSD), power in low frequency range (LF), power in high frequency range (HF) and the ratio between high and low frequency power ranges (LF/HF) were assessed during 5 minute blocks (as mentioned above).
Power spectral analysis of heart rate variability was computed using Lab Chart Pro software (ADinstruments, NSW, Australia) from R-R intervals. Software based filters were used for all recordings to remove any confounders including movement artefacts and ectopic beats. ECG recording range was 2mV and 100hz and 0.3hz were used as the upper and lower cut-offs of the band pass filter, respectively. Cubic interpolation for R-R series at 4Hz and spectrum estimation was based on fast Fourier transformation. HF component (0.15 – 4 Hz) and LF component (0.04- 0.15 Hz) of heart rate variability is thought to be related to respiratory activity and vasomotor waves respectively. Area under the curves (i.e. centre frequency for both of these components) were calculated. It is believed that the HF component indicates parasympathetic nervous system activity whereas LF component indicates sympathetic nervous system activity (Perini & Veicsteinas, 2003).

3.8 VO₂ max testing
Maximal aerobic capacity (VO₂ max) was measured via an incremental cycle ergometer test to volitional exhaustion (Lorenzo & Babb, 2012) in women (Experimental Chapters 3 and 4) at least one week prior to stress and food intake testing. Pulse rate was measured continually by a heart rate monitor (Polar Electro, Kempele, Finland). Peak oxygen consumption was determined via measurement of expired air connected to a metabolic measurement cart (Vacumed, Ventura, California, USA). The incremental exercise test was performed on an electronically braked cycle ergometer (Lode B.V., Groningen, Netherlands). Participants started the test at 50W. After 2 minutes of riding,
the workload was increased to 100W for 2 minutes. Then increments of 25W were applied each minute until volitional exhaustion.

Participants breathed through a Hans-Rudolph two way non-rebreathing valve and the expired air passed through low resistance plastic tubing through to a 4L mixing chamber. Expired air was measured through a flow transducer (KL engineering, Fitchburg, WI, USA), and mixed expired oxygen and carbon dioxide were analysed by a rapidly responding gas analyser (Vacumed, Ventura, California, USA). Ventilatory gas data were averaged every 30s using TurboFit software (Vacumed, Ventura, California, USA). The gas analyser was calibrated before every testing session using a commercially available gas mixture (Harris Specialty Gas, Brisbane, Australia). The ventilometer was calibrated using a standard 7L syringe (Hans Rudolph INC, Missouri, USA).

3.9 Statistical Analysis
Data were analysed using the Statistical Package for the Social Sciences software version 20.0 and 21.0 for Windows (SPSS. Inc, Chicago, USA). Kolmogorov-Smirnov and Shapiro –Wilk tests were conducted to test for normality. Tests for homogeneity of variance were conducted using Levene’s test of equality of error variances. Descriptive characteristics were compared between groups using univariate analysis of variance. Specific statistical analyses are described in each experimental chapter.
4.1: Introduction

The magnitude of the response to psychological stress can be influenced by the physiological status of an individual. Obesity (a state of elevated adiposity) has reached epidemic proportions in the western world. As such, an understanding of the influence of obesity on the responsiveness of the stress systems is essential (Section 2.5.1). Most of the research done in this area has been in women of different age groups. Therefore a paucity of evidence exist in this area for men. Given that there are sex differences in stress pathway activity (Section 2.3), findings in females cannot be extrapolated to men. Previous research in obesity and stress has reported both increased, decreased as well as comparable stress pathway activation in individuals with increased levels of adiposity compared with lean individuals (Section 2.5.1; Table 2.2). The inconclusive results could be due to differences in the stressor utilised, time of day and low frequency of sampling. Furthermore, very little research has considered the SAM system and the HPA axis together (Table 2.2).

The aims of this study were to identify whether lean and overweight/obese men differ in their cortisol, salivary alpha amylase, heart rate and heart rate variability responses to the TSST. It was hypothesised that overweight/obese men aged 50-70 years will have a greater salivary cortisol, salivary alpha amylase and heart rate response to the Trier Social Stress Test compared with age matched lean men.
4.2: Materials and Methods

4.2.1: Experimental procedure
All participants were asked to abstain from smoking, ingesting any caffeine containing beverages (e.g. tea, coffee, cola), liquorice, alcohol or drugs (except for any regular medications) and from strenuous physical activity during the 12 hours prior to participation in the experimental day.

A schematic representation of the experimental day is presented in Figure 4.1. Briefly, participants arrived at the research laboratory at 1100h. Their eligibility was confirmed through the measurement of anthropometric variables (Section 3.3) and blood pressure (Section 3.7.1). Eligible participants were then given a standardised lunch at 1200h (Section 3.4). A Trier Social Stress Test (TSST) was imposed from 1500h – 1530h (Section 3.5). Saliva samples were collected every 15 min from 1145h until 1700h apart from 1215h (lunch) where no sample was collected and between 1500h-1530h where samples were collected more frequently (1500h, 1507h, 1515h, 1522h, 1530h; Section 3.5; Figure 3.1A). Electrocardiogram (ECG) was measured from 1230h – 1700h (Section 3.7.2) with the exception of a 10 minute break to use the bathroom after the 1330h sample collection. Only data from the period 1400h-1700h are considered in this chapter.
4.2.2: Statistical analyses

Preliminary analysis

Pre-treatment cortisol was defined as the average of the five concentrations from 1400h to 1500h (1400h, 1415h, 1430h, 1445h and 1500h). Pre-treatment salivary alpha amylase was defined as the average of the three concentrations from 1430h to 1500h (1430h, 1445h and 1500h). Pre-treatment heart rate was defined as the average of the four readings from 1400h to 1445h (1400h, 1415h, 1430h and 1445h). Peak height for all parameters was defined as the highest value that was obtained for each individual after the commencement of the stress. Reactivity was calculated by subtracting the pre-treatment value from the peak height for all parameters. Area under the curve (with respect to increase) was calculated for each parameter using all values from 1500h – 1700h after the subtraction of the pre-treatment value from each data point.
Areas under the curve were calculated using the trapezoid rule utilising Sigmaplot graphing software (Systat Software Inc., California, USA).

**Recovery time** for all parameters was defined as the time difference from the commencement of the stressor (1500h) to the point at which the relevant parameter returned to within two standard deviations of its pre-treatment value. We used this as our definition for recovery since 95% of a normally distributed set of data lie within 2 standard deviations of the mean and since 5% error (p=0.05) is the generally accepted cut off level for statistical significance. This suggests that once the value has returned to within 2 standard deviations of the pre-treatment level, it has returned to pre-treatment levels. For recovery time only, those who did not exceed two standard deviations of the pre-treatment value between 1500-1700h (n=1 lean and n=2 overweight/obese for cortisol, n=2 lean and n=5 overweight/obese for salivary alpha amylase, n=0 lean and n=0 overweight/obese for heart rate) were excluded from the analysis. For those who did exceed two standard deviations but did not return to within two standard deviations by 1700h (n=8 lean and n=6 overweight/obese for cortisol, n=4 lean and n=1 overweight/obese for salivary alpha amylase, n=0 lean and n=1 overweight/obese for heart rate), 120 minutes was used as the recovery time in the analyses.

**Analysis**

Salivary cortisol, salivary alpha amylase, heart rate and heart rate variability were compared within and between groups using repeated measures analysis.
of variance. The within subjects factor was time and the between subjects factor was treatment. Derived salivary cortisol, salivary alpha amylase and heart rate parameters (pre- treatment, peak height, reactivity, area under the curve and recovery time) were compared between groups using univariate analysis of variance. Pearson’s correlation was used to test for relationships between pre- treatment, peak height, reactivity and area under the curve and measures of adiposity (BMI, percentage body fat, waist circumference, waist to hip ratio). P<0.05 was considered statistically significant.

4.3: Results

4.3.1: Participants
Data were collected from 24 lean and 22 overweight/obese men who were eligible for the study. Two lean and 5 overweight/obese men were subsequently excluded from analyses due to insufficient saliva volume to undertake the assays for cortisol and alpha amylase. Initial analysis revealed that the lean men were significantly older (64.2±1.1 years) than the overweight/obese men (61.0±1.1 years) (P=0.054). The results of the three oldest lean men were excluded from the analyses to remove the significant effect of age. Consequently, the results from 19 lean and 17 overweight/obese men (7 overweight and 10 obese) were included in the analyses for cortisol and salivary alpha amylase. The individuals that were excluded did not differ significantly from the final cohort in any of the baseline characteristics (data not shown). Including these three men in the final analyses did not change any of the outcomes. Due to technical problems with ECG recordings, data were not available for 2 further lean and 2 further overweight/obese men.
leaving 17 lean and 15 overweight/obese men (6 overweight, 9 obese) for heart rate and heart rate variability analysis. The individuals that were excluded from the final analysis due to technical difficulties in ECG recording did not differ significantly from the final cohort in any of the baseline characteristics (data not shown).

4.3.2: Participant characteristics

Overweight/obese men had a significantly ($p<0.001$) greater body weight and BMI compared to lean men (Table 4.1). On average, overweight/obese men had 7.9% more body fat compared with lean men ($p<0.001$). Overweight/obese individuals had a 19% larger waist circumference ($p<0.001$), a 10% larger hip circumference ($p<0.001$) and a 10% larger waist-to-hip ratio ($p<0.001$) compared with lean men. Resting systolic and diastolic blood pressure and mean arterial pressure were also significantly ($p<0.05$) higher in overweight/obese men compared with lean men (Table 4.1). Nevertheless, age, height and resting heart rate did not differ significantly between the groups (Table 4.1).
Table 4.1. Mean (±SEM) baseline descriptive characteristics in lean and overweight/obese men.

<table>
<thead>
<tr>
<th></th>
<th>Lean (n=19)</th>
<th>Overweight/Obese (n=17)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.3±1.1</td>
<td>61.1±1.1</td>
<td>0.166</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.0±1.4</td>
<td>174.8±1.4</td>
<td>0.179</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.7±1.6</td>
<td>93.8±2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5±0.3</td>
<td>30.6±0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% Fat</td>
<td>20.2±1.1</td>
<td>28.1±0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% Lean</td>
<td>79.3±1.1</td>
<td>72.0±0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>86.1±1.5</td>
<td>106.9±1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>97.5±1.2</td>
<td>109.2±1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.88±0.01</td>
<td>0.98±0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>119.1±3.3</td>
<td>129.0±2.8</td>
<td>0.030</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>67.7±2.1</td>
<td>74.5±2.0</td>
<td>0.027</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>85.3±2.4</td>
<td>93.3±2.2</td>
<td>0.022</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>64.2±3.0</td>
<td>64.4±2.6</td>
<td>0.968</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance
BMI = body mass index; % Fat = percentage body fat; % Lean = percentage lean mass; WHR = waist to hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate.
4.3.3: Cortisol
Saliva concentrations of cortisol in lean and overweight/obese men are shown in Figure 4.2 and Table 4.2. Repeated measures analysis of variance revealed that there was a significant effect of time (p<0.001; Figure 4.2). Overall (both groups combined), the peak height of cortisol concentrations (1.37±0.19 μg/dL) was significantly higher than pre-treatment concentrations (0.29±0.02 μg/dL) (p<0.001). Overall, there was a 406% increase in cortisol concentrations from pre-treatment concentrations to the peak of the response (both groups combined).

Saliva concentrations of cortisol in response to the TSST did not differ significantly between lean and overweight/obese men (time*treatment, p = 0.187; Figure 4.2) and accordingly, there were no significant differences between the groups in peak height, cortisol reactivity or area under the curve for the cortisol response (Table 4.2). The mean time to recovery did not differ significantly between the groups (Table 4.2). There was no significant overall difference between the groups (between subjects effect; p = 0.210).

There were no significant associations (data not shown) between measures of cortisol (pre-treatment cortisol, peak height, cortisol reactivity, area under the curve) and measures of adiposity (BMI, percentage body fat, waist circumference and waist to hip ratio).

The cortisol data presented in Figure 4.2 were presented in the author’s honours thesis entitled “Cortisol response to acute psychological stress in lean and overweight/obese men”. Nevertheless, salivary alpha amylase, heart rate...
and heart rate variability data were not presented in the author’s honours thesis and are presented in thesis format here for the first time. Cortisol data are included here to allow the reader to gain a complete view of the physiological stress response in this study.
Figure 4.2. Mean (±SEM) concentrations of salivary cortisol (μg/dL) in lean and overweight/obese men from 1400h to 1700h (time effect p<0.001; time * treatment interaction p=0.187; treatment effect p=0.210). TSST: Trier Social Stress Test.

Table 4.2. Mean (±SEM) pre-treatment cortisol, peak height of cortisol, cortisol reactivity, area under the curve and recovery time for lean and overweight/obese men.

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Overweight/Obese</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment (μg/dL)</td>
<td>0.29±0.02</td>
<td>0.28±0.02</td>
<td>0.788</td>
</tr>
<tr>
<td>Peak height (μg/dL)</td>
<td>1.52±0.22</td>
<td>1.21±0.15</td>
<td>0.254</td>
</tr>
<tr>
<td>Reactivity (μg/dL)</td>
<td>1.23±0.21</td>
<td>0.93±0.15</td>
<td>0.263</td>
</tr>
<tr>
<td>AUC (μg/dL/min)</td>
<td>55.3±10.3</td>
<td>38.7±7.7</td>
<td>0.118</td>
</tr>
<tr>
<td>Recovery time# (min)</td>
<td>103.3±6.0</td>
<td>101.8±5.9</td>
<td>0.867</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance; # Recovery time only includes individuals who had a significant response to the stressor (n=18 lean and n=15 overweight/obese)
AUC = Area under the curve

**4.3.4: Saliva concentrations of alpha amylase**

Saliva concentrations of alpha amylase in lean and overweight/obese men are shown in Figure 4.3 and Table 4.3. There was a significant time effect (p<0.001; Figure 4.3). Overall (both groups combined), the peak height of salivary alpha amylase concentrations (281.5±48.35 U/ml) was significantly higher than pre-treatment concentrations (126.5±16.3 U/ml) (p<0.001). Overall, there was a 197% increase in salivary alpha amylase from pre-treatment concentrations to the peak height of the salivary alpha amylase response (both groups combined).

Salivary alpha amylase concentrations in response to the TSST did not differ significantly between lean and overweight/obese men (time*treatment, p = 0.288; Figure 4.3) and accordingly, there were no significant differences between the groups in peak height, reactivity or area under the curve of the salivary alpha amylase response (Table 4.3). The mean time to recovery did not differ significantly between the groups (Table 4.3). There was no significant treatment effect indicating that there was no significant overall difference between the groups (p = 0.332).

There were no significant associations (data not shown) between measures of salivary alpha amylase (pre-treatment salivary alpha amylase, peak height, salivary alpha amylase reactivity, area under the curve) and measures of adiposity (BMI, percentage body fat, waist circumference and waist to hip ratio).
Figure 4.3. Mean (±SEM) concentrations of salivary alpha amylase (U/ml) in lean and overweight/obese men from 1400h to 1700h (time effect p<0.001; time * treatment interaction p=0.288; treatment effect p=0.322). TSST: Trier Social Stress Test.

Table 4.3. Mean (±SEM) pre-treatment salivary alpha amylase, peak height, salivary alpha amylase reactivity, area under the curve and recovery time for lean and overweight/obese men.

<table>
<thead>
<tr>
<th></th>
<th>Lean (n=19)</th>
<th>Overweight/Obese (n=17)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment (U/ml)</td>
<td>112.1±16.1</td>
<td>140.8±16.5</td>
<td>0.224</td>
</tr>
<tr>
<td>Peak height (U/ml)</td>
<td>267.3±55.5</td>
<td>295.6±41.2</td>
<td>0.690</td>
</tr>
<tr>
<td>Reactivity (U/ml)</td>
<td>155.1±51.2</td>
<td>154.9±31.6</td>
<td>0.997</td>
</tr>
<tr>
<td>AUC (U/ml/min)</td>
<td>5221±2735</td>
<td>3131±1525</td>
<td>0.523</td>
</tr>
<tr>
<td>Recovery time# (min)</td>
<td>74.1±7.7</td>
<td>60.6±9.2</td>
<td>0.267</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance; AUC = Area under the curve, # Recovery time only includes individuals who had a significant response to the stressor (n=17 lean and n=12 overweight/obese)
4.3.5: Heart rate (HR)
Heart rate data in lean and overweight/obese men are shown in Figure 4.4 and Table 4.4. There was a significant effect of time (p<0.001; Figure 4.4). Overall (both groups combined), peak height of HR (78.6±4.1 bpm) was significantly higher than pre-treatment values (64.5±3.9 bpm) (p<0.001). Overall, there was a 22% increase in heart rate from pre-treatment values to the peak height of the heart rate response (both groups combined).

Heart rate response to the TSST did not differ significantly between lean and overweight/obese men (time*treatment, p = 0.550; Figure 4.4). As with cortisol and salivary alpha amylase, this lack of significant difference in the heart rate response between the groups was also illustrated by there being no significant difference between the groups in peak height, reactivity and area under the curve of the heart rate response (Table 4.4). The mean time to recovery did not differ significantly between the groups (Table 4.4). There was also no significant treatment effect indicating that there was no significant overall difference between the groups (p = 0.838).

There were no significant associations (data not shown) between measures of heart rate (pre-treatment heart rate, peak heart rate, heart rate reactivity, area under the curve) and measures of adiposity (BMI, percentage body fat, waist circumference and waist to hip ratio).
Figure 4.4. Mean (±SEM) heart rate (bpm) in lean and overweight/obese men from 1400h to 1700h (time effect p<0.001; time * treatment interaction p=0.550; treatment effect p=0.838). TSST: Trier Social Stress Test.

Table 4.4. Mean (±SEM) pre-treatment HR, peak height of HR, HR reactivity, area under the curve and recovery time for lean and overweight/obese men.

<table>
<thead>
<tr>
<th></th>
<th>Lean (n=17)</th>
<th>Overweight/Obese (n=15)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment (bpm)</td>
<td>64.4±2.2</td>
<td>64.6±3.5</td>
<td>0.964</td>
</tr>
<tr>
<td>Peak height (bpm)</td>
<td>80.2±4.1</td>
<td>76.9±4.0</td>
<td>0.570</td>
</tr>
<tr>
<td>Reactivity (bpm)</td>
<td>15.8±2.5</td>
<td>12.3±1.7</td>
<td>0.270</td>
</tr>
<tr>
<td>AUC (beats/min²)</td>
<td>148.1±113.6</td>
<td>31.3±71.6</td>
<td>0.406</td>
</tr>
<tr>
<td>Recovery time# (min)</td>
<td>40.1±3.7</td>
<td>45.5±5.9</td>
<td>0.440</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance, # Recovery time only includes individuals who had a significant response to the stressor (n=17 lean and n=15 overweight/obese)
4.3.6: Heart rate variability

RMSSD
Mean (±SEM) RMSSD in lean and overweight/obese men from 1400-1700h are shown in Figure 4.5a. Repeated measures analysis of variance revealed a significant time effect ($p < 0.001$), no time * treatment interaction ($p = 0.566$) and no significant treatment effect ($p = 0.125$).

HF power
Mean (±SEM) HF in lean and overweight/obese men from 1400-1700h are shown in Figure 4.5b. Repeated measures analysis of variance revealed a significant time effect ($p < 0.001$), no time * treatment interaction ($p = 0.215$) and no significant treatment effect ($p = 0.411$).

LF power
Mean (±SEM) LF in lean and overweight/obese men from 1400-1700h are shown in Figure 4.5c. Repeated measures analysis of variance revealed a significant time effect ($p < 0.001$), no time * treatment interaction ($p = 0.211$). Nevertheless, there was a significant treatment effect ($p = 0.039$) indicating that overall, overweight/obese men had a higher LF power than those who were lean.

LF/HF ratio
Mean (±SEM) LF/HF ratio in lean and overweight/obese men from 1400-1700h are shown in Figure 4.5d. Repeated measures analysis of variance revealed
a significant time effect (p < 0.001), no time * treatment interaction (p = 0.718) and no significant treatment effect (p = 0.142).

Figure 4.5. Mean (±SEM) a) RMSSD, b) HF power, c) LF power and d) LF/HF ratio in lean and overweight/obese men from 1400h to 1700h (time effect p<0.001 for all; time * treatment interaction p=0.566, 0.215, 0.211, 0.718; respectively, treatment effect p=0.125, 0.411, 0.039, 0.142; respectively). TSST: Trier Social Stress Test.
4.4: Discussion

The hypothesis that overweight/obese men will have a greater salivary cortisol, salivary alpha amylase and heart rate response to the Trier Social Stress Test compared with age matched lean men was not supported. While there was a substantial increase in salivary cortisol, salivary alpha amylase and heart rate during the stress, this increase did not differ between the two groups. These findings show that, for men with a moderate level of obesity (BMI=30.6±0.6 kg/m²) who were otherwise healthy (men with severe illnesses were excluded from the study), the response of salivary cortisol, salivary alpha amylase and heart rate to a potent acute psychological stress was not compromised compared to that in lean men (BMI=23.5±0.3 kg/m²). Nevertheless, it is possible that differences between the groups may have been seen if there had been a higher degree of difference between the groups in the level of adiposity. Although counter-intuitive, it may also be possible that there is no influence of increased adiposity on HPA axis and SAM system responses in men. Furthermore, there may be other stress-sensitive factors that are involved in the development of chronic disease such as cytokines and/or opioids. While these factors may have been influenced differently by the stressor in the two different groups, these factors were not measured in the current study. As such, it remains to be determined if these other factors may respond differently to stress.

It is also possible that subtle differences in HPA axis activity between the groups would have been evident with a less stressful task. The magnitude of the cortisol response that was found in the current experiment was substantial...
compared with the response in cortisol concentrations reported in past experiments. When both groups were combined, there was a 406% increase in cortisol in the current experiment. In humans, Therrien and co-workers reported a 82% increase in cortisol after subjecting men and women with different body weight and body fat profiles to the TSST (Therrien et al., 2010). Benson and co-workers (2009) reported a 83% increase of cortisol in premenopausal obese and lean women after exposure to the TSST (Benson et al., 2009). The magnitude of the cortisol response observed in the current experiment also surpassed the increments of cortisol observed in experiments where exogenous agents have been used to stimulate the stress pathways. Ljung and colleagues reported increments of around 200% in cortisol concentrations following treatment with CRH (Ljung et al., 2000) and Pasquali and colleagues reported increases in cortisol concentrations of 325% after CRH and AVP stimulation of age matched premenopausal eumenorrheic women with different obesity phenotypes (Pasquali et al., 1996). Nevertheless, it is possible that more subtle differences in HPA axis activity would become evident with a less stressful task. Salivary alpha amylase also had a substantial reactivity (197%) in this experiment compared with earlier human studies that used salivary alpha amylase as a marker of the SAM system activity. For instance, Nater and colleagues (2005) found a 50% increment in salivary alpha amylase levels when 24 healthy adults were exposed to the TSST (Nater et al., 2005). Increments in heart rate were also greater in the current experiment compared with some previous work. The heart rate response to stress in the current experiment (22%) is within the range of previously reported significant heart rate responses to TSST (Ljung
et al., 2000; Rimmele et al., 2009; Rimmele et al., 2007). These comparisons with other studies indicate that the response elicited by our Trier Social Stress Test protocol was substantial and robust and may also indicate that the long rest period prior to stress in the current study had allowed these stress parameters to reach low resting levels.

The results also suggest that moderate levels of adiposity as observed in the current experiment may not compromise the response to acute psychological stress. We have shown in sheep that a large (23%) difference in the levels of adiposity (31.7 ± 3.4% body fat vs 8.9 ± 0.6%) is capable of eliciting a difference in adrenocorticotrophic hormone, cortisol and adrenaline reactivity to psychological stress (Tilbrook et al., 2008). By comparison, the differences in percentage body fat in the groups in the current study were more modest at 8% (Lean = 20.2 ± 1.1% body fat vs overweight/obese = 28.1±0.9%). Benson and colleagues also reported a significantly enhanced cortisol response to psychological stress in severely obese (38.2 ± 1.5 kg/m²) pre-menopausal women compared with their leaner (23.1 ± 0.6 kg/m²) counterparts (Benson et al., 2009). BMI in the current study was 23.5 ± 0.3 kg/m² for lean and 30.6 ± 0.6 kg/m² for overweight/obese men. Similar to our findings, Ljung and colleagues (Ljung et al., 2000) did not find a difference in HPA axis responses to psychological stress in individuals who had a BMI between 25-33kgm⁻². Furthermore, Therrien and co-workers also did not find a difference in HPA axis responses between individuals (both men and women) who had a BMI of <27 kgm⁻² and between 30-35 kgm⁻² (Therrien et al., 2010). Thus, it appears that there may need to be a bigger difference in BMI or percentage body fat to
observe a clear distinction in stress responsiveness between lean and obese individuals. Now that a significant proportion of the current population is either overweight or obese, the study groups of this current experiment may be particularly clinically relevant. Nevertheless, this experiment provides no evidence that salivary cortisol, salivary alpha amylase and heart rate responsiveness to psychological stress are influenced by this level of adiposity. Further research might need to compare morbidly obese individuals with lean individuals to further characterise differences in stress responsiveness.

It is also possible that factors other than those associated with the SAM system and the HPA axis are important in determining health risks of responses to psychological stress. For instance, inflammatory cytokine responses (Chrousos, 2009) and opioid pathways may play a role in mediating the stress response (Tilbrook, 2007). Therefore, it is possible that various factors that were not investigated in this experiment are involved in mediating the effects of stress in lean and overweight/obese men. Further research is required to consider a potential role for these other mediators.

A common criticism of stress experiments is the lack of potency of the stressor (Jackson & Dishman, 2006; Kirschbaum et al., 1993). The stressor implemented in this experiment was able to elicit substantial responses from both the HPA axis and the SAM system. Another strength of this study was the frequent sampling during the period of stress which enabled detailed
profiling of how the stress parameters responded during the stressor. The lengthy rest time prior to the administration of the stressor was another benefit given that this reduces the likelihood of the stress systems being already activated at the commencement of the sampling period. Furthermore, age is known to influence stress responsiveness (Kudielka et al., 2004b). The participants in the current experiment were all 50-70 years. Therefore, younger age groups could also be considered.
Chapter 5.0: Experimental Chapter 2 - Adiposity in men and responses to food intake.

5.1: Introduction
Food intake is a physiological challenge that can activate the HPA axis as well as the SAM system (Section 2.2.3). Nevertheless, available evidence in humans in this area is inconclusive. There is also a paucity of evidence on SAM system reactivity to consumption of food (Section 2.2.3). Furthermore, no previous studies have investigated the effects of adiposity on HPA axis and SAM system activity in lean and overweight/obese men in response to food intake (Section 2.2.3). Since food intake is a challenge experienced by the human body several times per day, a greater understanding of the influence of adiposity on the physiological response to food intake is of fundamental importance. If overweight/obese men have a greater activation of the stress pathways every time they consume food it is plausible that they may be more susceptible to the development of stress-related diseases.

The aim of this investigation was to identify differences between lean and overweight/obese men in HPA axis responses (measured via salivary cortisol) and SAM system responses (measured via salivary alpha amylase) to the consumption of a standardised lunch. It was hypothesised that overweight/obese men will have greater salivary cortisol and salivary alpha amylase responses to the standardised lunch compared with lean men.
5.2: Materials and Methods

5.2.1: Experimental procedure
The experimental procedure for this study is explained in detail in Experimental Chapter 1 (Section 4.2.1). Data from 1400h-1700h were considered in Experimental Chapter 1 to investigate the response to psychological stress whereas data from 1145h-1400h are considered in this chapter (Experimental Chapter 2) to consider the response to food intake.

As described in Section 4.2.1, the first saliva sample was collected at 1145h, the second one at 1200h (immediately before lunch), third at 1230h and subsequent samples were collected every 15 minutes until 1400h. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) were measured using a clinical blood pressure monitor (Criticare systems Inc, Wisconsin, USA) at 1145h, 1245h and every 15 minutes from there onwards until 1400h. Participants were allowed a break to use the bathroom immediately after the 1330h saliva and blood pressure/heart rate sampling.

5.2.2: Test meal
The test meal consisted of lunch made by the participants from a choice of standardised ingredients (a full description can be found in Section 3.4).
5.2.3: Statistical analysis

Preliminary analysis

Pre-treatment salivary cortisol and pre-treatment salivary alpha amylase were defined as the concentrations of the hormones in the samples collected at 1200h. Pre-treatment SBP, DBP, MAP and HR were defined as the value recorded at 1145h. Peak height for cortisol was defined as the highest value obtained for each individual between 1230h-1400h, inclusive. Since the SAM system appears to have had a secondary activation at the time of the break to use the bathroom, peak height for salivary alpha amylase was defined as the highest value obtained from 1230h-1330h. Peak height for all cardiovascular parameters was defined as the highest value obtained between 1245h-1330h. Reactivity was calculated by subtracting the pre-treatment value from the peak height for all parameters. Area under the curve with respect to increase was calculated for cortisol using values between 1200h – 1400h, for salivary alpha amylase using values between 1200h – 1330h and for SBP, DBP, MAP and HR using values between 1145h – 1330h after the subtraction of the pre-treatment value from each data point. Area under the curve for all parameters were calculated using the trapezoid rule utilising Sigmaplot graphing software (Systat Software Inc., California, USA).

Analysis

Salivary cortisol, salivary alpha amylase, blood pressures and heart rate were compared within and between subjects using repeated measures analysis of variance. The within subjects factor was time and the between subjects factor
was treatment. When the time effect for salivary cortisol was considered separately in each group, only data from 1200h-1400h were included. Derived salivary cortisol, salivary alpha amylase and cardiovascular parameters (pre-treatment, peak height, reactivity and area under the curve) were compared between groups using univariate analysis of variance. P<0.05 was considered statistically significant.

5.3: Results
Descriptive characteristics of this study cohort were analysed and reported in Experimental Chapter 1 (Section 4.3.2 and Table 4.1).

5.3.1: Participants
Nineteen lean and 17 overweight/obese were included in the final study cohort (Section 4.3.1).

5.3.2: Participant characteristics
Overweight/obese men had significantly higher body weight, BMI, percentage body fat and significantly lower percentage lean mass compared with lean men (p <0.001 for all). Girth measurements were also higher in overweight/obese men with a 19% larger waist circumference (p<0.001), a 10% larger hip circumference (p<0.001) and a 10% larger waist-to-hip ratio (p<0.001) compared with lean men (Table 4.1, Section 4.3.2).
5.3.3: **Total energy and macronutrient intake**

Lean and overweight/obese men consumed similar amounts of total energy, protein, carbohydrate and fat (Table 5.1). There were no significant differences between the groups in these parameters. Overall (both groups combined), the meal consumed by the participants consisted of 22% protein, 53% carbohydrates and 25% fat.

<table>
<thead>
<tr>
<th>Table 5.1. Mean (±SEM) total energy and macronutrients consumed by lean and overweight/obese men.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean (n=19)</td>
</tr>
<tr>
<td>-----------------------------------</td>
</tr>
<tr>
<td><strong>Total energy (kJ)</strong></td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
</tr>
</tbody>
</table>

*Univariate analysis of variance

5.3.4: **Salivary Cortisol**

Saliva concentrations of cortisol in lean and overweight/obese men are shown in Figure 5.1 and Table 5.2. In response to lunch, saliva concentrations of cortisol differed significantly between lean and overweight/obese men (time*treatment, p = 0.008; Figure 5.1). Further, separate analysis of the groups revealed a significant effect of time in overweight/obese men (p = 0.005) but not in lean men (p = 0.384). The peak height of cortisol
concentrations in the overweight/obese group (0.654 ± 0.09 μg/dL) was significantly higher than pre-treatment concentrations (0.345 ± 0.03 μg/dL) (p = 0.003). This corresponded to an 86% increase in cortisol concentrations from pre-treatment concentrations to the peak height of the response in overweight/obese men. There were no significant differences between the groups in peak height for the cortisol response (Table 5.2). Area under the curve for cortisol was significantly higher in the overweight/obese men compared with lean men (p = 0.039) and there was a trend towards cortisol reactivity being higher in overweight/obese men compared with lean men (p = 0.083; Table 5.2). Repeated measures analysis of variance revealed that there was a significant overall effect of time (p < 0.001; Figure 5.1). There was no significant between subjects effect indicating that there were no significant overall differences between the groups (p = 0.643).
Chapter 5 – Food intake and adiposity in men

Figure 5.1. Mean (±SEM) concentrations of salivary cortisol (μg/dL) in lean and overweight/obese men from 1145h to 1400h (time effect p<0.001; time * treatment interaction p=0.008; treatment effect p=0.643). The box labelled “lunch” represents the timing of the lunch period and the hashed box represents the timing of the break to use the bathroom.

Table 5.2. Mean (±SEM) pre-treatment salivary cortisol, peak height of salivary cortisol, salivary cortisol reactivity and area under the curve for lean and overweight/obese men.

<table>
<thead>
<tr>
<th></th>
<th>Lean (n=19)</th>
<th>Overweight/Obese (n=17)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment (μg/dL)</td>
<td>0.411±0.04</td>
<td>0.345±0.03</td>
<td>0.204</td>
</tr>
<tr>
<td>Peak height (μg/dL)</td>
<td>0.532±0.06</td>
<td>0.654±0.09</td>
<td>0.251</td>
</tr>
<tr>
<td>Reactivity (μg/dL)</td>
<td>0.121±0.06</td>
<td>0.309±0.08</td>
<td>0.083</td>
</tr>
<tr>
<td>AUC (μg*min/dL)</td>
<td>-3.250±19.49</td>
<td>10.182±18.03</td>
<td>0.039</td>
</tr>
</tbody>
</table>

* Univariate analysis of variance; AUC = Area under the curve
5.3.5: Salivary alpha amylase

Saliva concentrations of alpha amylase in lean and overweight/obese men are shown in Figure 5.2 and Table 5.3. Repeated measures analysis of variance revealed that there was a significant effect of time (p<0.001; Figure 5.2). Overall (both groups combined), the peak height of salivary alpha amylase concentrations (235±24.6μg/dL) was significantly higher than pre-treatment concentrations (131±12.8 μg/dL) (p<0.001). Overall, there was an 80% increase in salivary alpha amylase concentrations from pre-treatment concentrations to the peak of the response (both groups combined).

Saliva concentrations of alpha amylase in response to the lunch did not differ significantly between lean and overweight/obese men (time*treatment, p=0.195; Figure 5.2) and accordingly, there were no significant differences between the groups in peak height, reactivity or area under the curve for the salivary alpha amylase response (Table 5.3). There was no significant between subjects effect (p = 0.898) indicating that there were no significant overall differences between the groups.
Figure 5.2. Mean (±SEM) concentrations of salivary alpha amylase (U/ml) in lean and overweight/obese men from 1145h to 1400h (time effect p<0.001; time * treatment interaction p=0.195; treatment effect p=0.898). The box labelled “lunch” represents the timing of the lunch period and the hashed box represents the timing of the break to use the bathroom.

Table 5.3. Mean (±SEM) pre-treatment salivary alpha amylase, peak height of salivary alpha amylase, salivary alpha amylase reactivity and area under the curve for lean and overweight/obese men.

<table>
<thead>
<tr>
<th></th>
<th>Lean (n=19)</th>
<th>Overweight/Obese (n=17)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment (U/ml)</td>
<td>135±19.6</td>
<td>126±16.5</td>
<td>0.716</td>
</tr>
<tr>
<td>Peak height (U/ml)</td>
<td>228±33.7</td>
<td>242±37.1</td>
<td>0.771</td>
</tr>
<tr>
<td>Reactivity (U/ml)</td>
<td>93±25.0</td>
<td>117±26.4</td>
<td>0.510</td>
</tr>
<tr>
<td>AUC (U*min/ml)</td>
<td>2288±1427</td>
<td>2863±4254</td>
<td>0.751</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance
AUC = Area under the curve
5.3.6: Cardiovascular parameters

Cardiovascular parameters in lean and overweight/obese men are shown in Figure 5.3 and Table 5.4.

**Systolic blood pressure**

There was a significant effect of time for systolic blood pressure (p=0.001; Figure 5.3a). Overall (both groups combined), the peak height of systolic blood pressure (129±3 mmHg) was significantly higher than pre-treatment systolic blood pressure (124 ±3 mmHg) (p=0.03).

Systolic blood pressure in response to the lunch did not differ significantly between lean and overweight/obese men (time*treatment, p= 0.726; Figure 5.3a). Systolic blood pressure reactivity and area under the curve did not differ between the two groups (Table 5.4). However, overweight/obese men had significantly higher pre-treatment and peak height values for systolic blood pressure compared with the lean men (p < 0.05 for both; Table 5.4). There was also a trend towards systolic blood pressure being significantly higher overall in the overweight/obese men compared with the lean men (between subjects effect, p = 0.058).

**Diastolic blood pressure**

Repeated measures analysis of variance revealed that there was a significant effect of time for diastolic blood pressure (p<0.001; Figure 5.3b). Overall (both groups combined), the peak height of diastolic blood pressure (74±2mmHg) was significantly higher than pre-treatment diastolic blood pressure (71 ±2 mmHg) (p=0.03).
Diastolic blood pressure in response to the lunch did not differ significantly between lean and overweight/obese men (time*treatment, p= 0.898; Figure 5.3b). This finding was consistent with a lack of significant difference in reactivity and area under the curve of diastolic blood pressure between lean and overweight/obese men (Table 5.4). Nevertheless, overweight/obese men had a significantly higher pre-treatment diastolic blood pressure and peak height compared with the lean men (Table 5.4). There was a significant between subjects effect indicating that the overweight/obese men had higher diastolic blood pressure overall than the lean men (p = 0.022).

**Mean arterial pressure**

There was a significant effect of time for mean arterial pressure (p=0.007; Figure 5.3c). Overall (both groups combined), there was a trend towards the peak height of mean arterial pressure (92 ±2 mmHg) being significantly higher than pre-treatment (89 ±2 mmHg) (p=0.06).

Mean arterial pressure in response to the lunch did not differ significantly between lean and overweight/obese men (time*treatment, p= 0.713; Figure 5.3c). Mean arterial pressure reactivity and area under the curve were similar in overweight/obese men compared with lean men (Table 5.4). Overweight/obese men had a significantly higher pre-treatment and peak height for mean arterial pressure compared with lean men (Table 5.4). There was also a significant between subjects effect indicating that the overweight/obese men had higher mean arterial pressure overall compared with lean men (p = 0.026).
Heart rate

There was no significant effect of time for heart rate ($p=0.087$; Figure 5.3d). Overall (both groups combined), the peak height of heart rate (68 ±3 bpm) was not significantly different to pre-treatment heart rate (64 ±3 bpm) ($p=0.16$).

Heart rate following lunch did not differ significantly between lean and overweight/obese men (time*treatment, $p=0.620$; Figure 5.3d) and accordingly, there were no significant differences between the groups in peak height, heart rate reactivity or area under the curve for heart rate (Table 5.4). There were also no significant differences between the groups in pre-treatment heart rate (Table 5.4) and there was no significant overall between subjects effect indicating that there were no significant overall differences between the groups for heart rate ($p = 0.709$).
Figure 5.3. Mean (±SEM) a) systolic blood pressure (mmHg), b) diastolic blood pressure (mmHg), c) mean arterial pressure (mmHg) and d) heart rate (bpm) in lean and overweight/obese men from 1145h to 1400h (time effect p=0.001, 0.000, 0.007, 0.087, respectively; time * treatment interaction p=0.726, 0.898, 0.713, 0.620, respectively; treatment effect p=0.058, 0.022, 0.026, 0.709, respectively). The boxes labelled “lunch” represent the timing of the lunch period and the hashed boxes represent the timing of the break to use the bathroom.
Table 5.4. Mean (±SEM) pre-treatment, peak height, reactivity and area under the curve for SBP, DBP, MAP and HR in lean and overweight/obese men.

<table>
<thead>
<tr>
<th></th>
<th>Lean (n=19)</th>
<th>Overweight/Obese (n=17)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmHg)</td>
<td>119±3</td>
<td>129±3</td>
<td>0.030</td>
</tr>
<tr>
<td>Peak height (mmHg)</td>
<td>125±3</td>
<td>134±4</td>
<td>0.046</td>
</tr>
<tr>
<td>Reactivity (mmHg)</td>
<td>6±2</td>
<td>5±3</td>
<td>0.820</td>
</tr>
<tr>
<td>AUC (mmHg*min)</td>
<td>-173±143</td>
<td>-19±178</td>
<td>0.489</td>
</tr>
<tr>
<td>DBP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmHg)</td>
<td>68±2</td>
<td>75±2</td>
<td>0.027</td>
</tr>
<tr>
<td>Peak height (mmHg)</td>
<td>71±1</td>
<td>76±3</td>
<td>0.017</td>
</tr>
<tr>
<td>Reactivity (mmHg)</td>
<td>3±2</td>
<td>3±2</td>
<td>0.956</td>
</tr>
<tr>
<td>AUC (mmHg*min)</td>
<td>-15±108</td>
<td>33±131</td>
<td>0.780</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmHg)</td>
<td>85±2</td>
<td>93±2</td>
<td>0.022</td>
</tr>
<tr>
<td>Peak height (mmHg)</td>
<td>89±1</td>
<td>95±2</td>
<td>0.026</td>
</tr>
<tr>
<td>Reactivity (mmHg)</td>
<td>3±2</td>
<td>2±2</td>
<td>0.588</td>
</tr>
<tr>
<td>AUC (mmHg*min)</td>
<td>55±118</td>
<td>254±142</td>
<td>0.289</td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (bpm)</td>
<td>64±3</td>
<td>64±3</td>
<td>0.968</td>
</tr>
<tr>
<td>Peak height (bpm)</td>
<td>67±2</td>
<td>69±3</td>
<td>0.628</td>
</tr>
<tr>
<td>Reactivity (bpm)</td>
<td>3±2</td>
<td>5±1</td>
<td>0.483</td>
</tr>
<tr>
<td>AUC (bpm*min)</td>
<td>56±134</td>
<td>-109±99</td>
<td>0.337</td>
</tr>
</tbody>
</table>

*Univariate Analysis of Variance; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; MAP = Mean arterial pressure; HR = Heart rate; AUC = Area under the curve
5.4: Discussion
Our hypothesis that overweight/obese men will have greater cortisol and alpha amylase responses to the challenge of food consumption compared with lean men was partially supported. Overweight/obese men showed a greater activation of the HPA axis to food intake compared with lean men as indicated by the higher response of salivary cortisol. Contrary to our hypothesis, both groups showed a similar salivary alpha amylase response to consumption of food. Systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate responses were also similar between the groups in response to lunch indicating that there is no difference in SAM system activation in response to food intake between lean and overweight/obese men.

The finding that only overweight/obese men showed a significant HPA axis response to food intake is of particular importance. This is in accord with the findings of Vicennati and colleagues in women with abdominally distributed body fat (waist to hip ratio > 0.85) in response to the ingestion of a high carbohydrate meal (Vicennati et al., 2002). Nevertheless, it should be noted that the meal used by Vicennati et al. contained 89% carbohydrates whereas ours only contained 53% carbohydrates. This suggests that even moderate levels of carbohydrates can elicit a significant HPA axis response in overweight/obese men. The notion that even moderate levels of carbohydrates can elicit significant HPA axis activity is of particular significance, especially in the context of the modern day western diets including large quantities of refined carbohydrates (Bengmark, 2013). This could even be more relevant to individuals who are already overweight/obese
and are on a high carbohydrate diet because regular elevations of cortisol may further complicate metabolic anomalies that may already exist. Findings by Martens et al. further reiterate the importance of the carbohydrate content in eliciting cortisol responses to food intake (Martens et al., 2010). They reported that protein or fat intake can decrease cortisol responses, whereas ingestion of carbohydrates increased cortisol responses. Therefore, it appears that the carbohydrate content of a meal can play a significant role in activating the HPA axis.

Lean men did not show a significant elevation in cortisol after consumption of the standardised lunch in the current experiment. This is contrary to the findings of Gibson and colleagues in 1999 where they investigated cortisol responses to a high protein (39%) lunch in healthy men (Gibson et al., 1999). It should be noted that the contribution of protein towards the total energy in the current experiment was only 22%. Therefore, it appears that a protein content as low as 22% can elicit significant HPA axis response in overweight/obese men. This also suggests that a significant HPA axis response is dependent on a higher protein content only in lean men. The energy intake in the current study is also similar to that of the average Australian energy intake for men (22% protein, 45% carbohydrate and 32% fat) as reported in the 1995 National Nutrition Survey (McLennan, 1995). Therefore, it is highly likely that overweight/obese men will consume a diet that can elicit a significant cortisol response every time they eat.
Abdominal obesity can be associated with disrupted endocrine as well as neural feedback from the brain-gut axis to the HPA axis (Hussain & Bloom, 2013). Food can stimulate the secretion of certain peptides/hormones such as insulin, leptin, ghrelin, cholecystokinin, and pancreatic peptide Y which can in turn have an impact on the HPA axis activity (Ishizuka et al., 1983; Schwartz et al., 2000). It has been reported that these feedback pathways are malfunctioning in obese individuals (Velloso & Schwartz, 2011). Overweight/obese men in the current study had significantly higher waist to hip ratios compared with the lean men suggesting that they had a higher proportion of abdominally distributed fat. Therefore, it is possible that abdominally based body fat distribution in the overweight/obese men may have contributed towards the higher HPA axis response to the lunch. There are several mechanisms that may play a role in overweight/obese individuals having a higher cortisol response to food intake. For example, differences in stimulation of the HPA axis compared with lean individuals, perhaps through increased noradrenergic drive, decreased negative feedback and/or increased corticotrophin releasing hormone and arginine vasopressin synthesis and secretion are plausible mechanisms for the pattern of results observed (Tilbrook & Clarke, 2006). Cortisol plays a major role in metabolism (Tempel & Leibowitz, 1994) and it is well known that variations in cortisol can have a significant impact on nutrient absorption (Divertie, Jensen, & Miles, 1991; Simmons, Miles, Gerich, & Haymond, 1984). Cortisol contributes towards lipolysis and proteolysis thus it increases the plasma free fatty acid availability. This could potentially accentuate the deposition of abdominal fat or further complicate existing manifestations of obesity. Furthermore, hyperactivity of
the HPA axis has been shown to be associated with cardiovascular disease (Seldenrijk, Hamer, Lahiri, Penninx, & Steptoe, 2012), type 2 diabetes (Steptoe & Brydon, 2005) and anxiety and depression (Yoon & Joormann, 2012).

None of the previous studies in this area has investigated the effects of food consumption on the activity of the SAM system in lean and overweight/obese men. Our results indicate that, with the exception of heart rate, all of the SAM system parameters that were measured in the current experiment (salivary alpha amylase, systolic blood pressure, diastolic blood pressure and mean arterial pressure) increased in response to lunch (time effect for all parameters p <0.05). These findings partially concur with the reports of Harthoorn et al who found increases in sympathetic nervous system activity after ingestion of a standardised meal (15-20% protein, 35-40% fat and 40-45% carbohydrate) in a group of healthy men and women (Harthoorn & DransWeld, 2008). Increases in sympathetic nervous system activity are to be expected as eating places a demand on the cardiovascular system. It has been reported that the postprandial increase in heart rate together with the subsequent increase in cardiac output facilitates the rise in demand for blood flow to the visceral areas (Jager, Bollinger, Valli, & Ammann, 1986). However, there was no such effect on heart rate in the current experiment. The reduction in systolic blood pressure, diastolic blood pressure and mean arterial pressure in the postprandial period (i.e., 1245h-1330h in the current experiment) may be a result of the reduction in resistance to blood flow in the mesenteric vessels. Another plausible explanation could be that the satiety hormones are having an inhibitory effect on the sympathetic nervous system during this period.
(Burcelin, 2005; Fan et al., 2004). These findings suggest that there is no
differential SAM system response to food intake in men based on the level of
adiposity.

It has previously been reported that sympathetic nervous system response to
food intake can be bi-phasic in nature (Harthoorn & DransWeld, 2008). 
Harthoorn et al reported a secondary increase in sympathetic nervous system
activity about 45-50 minutes post ingestion of a meal. This could be attributed
to the physiological changes that happen due to the gastrointestinal
mechanisms of digestion of food. While this previously reported increase is
somewhat similar to the secondary rise in salivary alpha amylase and blood
pressure parameters in the current experiment, the increase in the current
experiment occurred with a greater delay (75-90 minutes) after the ingestion
of the meal. The secondary increase in our experiment coincided with the
bathroom break that was offered to participants after the 1330h sample
collection. Consequently, it is not possible to determine in the current
experiment if this secondary increase in SAM system activity is a result of
gastrointestinal mechanisms of food ingestion or merely an artefact of the
participants physically moving during the bathroom break.

The current experiment is the first of its kind to investigate both SAM system
and HPA axis responses simultaneously in response to food intake. While the
measurement of saliva cortisol (which is in a high concentration per volume
range; μg/dL range) has been shown to be unaffected by blood contamination
due to micro-injury of the oral cavity (Kivlighan et al., 2004), blood contamination has been shown to influence the measurement of steroid hormones present in the very low concentration per volume range (pg/ml range) including salivary testosterone (Kivlighan et al., 2004), salivary oestradiol (Kivlighan, Granger, & Schwartz, 2005) and salivary progesterone (Kivlighan et al., 2005). Therefore, to support the outcomes of the current study, future research could confirm these findings in a similarly designed study that measures plasma cortisol instead of salivary cortisol. Future experiments could also consider females and participants of different age groups.

In conclusion, this experiment showed that increased adiposity in men was associated with hyperactivity of the HPA axis after the ingestion of a meal consisting of 22% protein, 53% carbohydrates and 25% fat. This suggests that ingesting a standardised meal can result in differential HPA axis but not SAM system activation in overweight/obese men of 50-70 years compared with age matched lean men.
Chapter 6.0: Experimental Chapter 3 - Physical activity in women and responses to psychological stress

6.1: Introduction
Regular physical activity is thought to be associated with biological adaptations that moderate the physiological response to stress (Section 2.5.2). Nevertheless, the role of physical activity in the regulation of HPA axis and SAM system responsiveness is not fully understood. Women are underrepresented in the investigations in relation to physical activity and stress responsiveness (Section 2.5.2; Table 2.3). However, the limited research done in women has provided interesting but inconclusive evidence (Section 2.5.2). The inconclusiveness of the results could mainly be due to the inconsistencies in methodologies that were implemented. These include, differences in the stressor utilised, low frequency of sampling, differences in hormone status of women, suboptimal measures of the SAM system and underestimation of the level of physical activity undertaken. Furthermore, very little research has considered the SAM system and the HPA axis together (Section 2.5.2; Table 2.3). Therefore, the relationship between levels of physical activity and responsiveness to psychological stress in women requires further investigation.

It has also been reported that high levels of physical activity are associated with healthy profiles of cardio-metabolic risk markers (Tsatsoulis & Fountoulakis, 2006). To the best of our knowledge, none of the previous experiments in this area of research have investigated the association between cardio-metabolic risk markers and acute physiological responses to
psychological stress in women who differed in their physical activity status (Table 2.3). If left untreated, unhealthy levels of cardio metabolic risk markers combined with excessive stress pathway activity may pose substantial health risk especially in the context of progression to chronic disease. Therefore, it is of fundamental importance to investigate this relationship in women who participate in varying levels of activity.

The aims of this study were 2-fold. The first aim was to measure both HPA axis (plasma cortisol) and SAM system (plasma adrenaline and noradrenaline, blood pressure heart rate and heart rate variability) responses to the TSST in women in the follicular phase of the menstrual cycle who engaged in high or low levels of physical activity. The second aim was to measure chronic disease status via the levels of cardio-metabolic risk markers in the same cohort of women and investigate any relationships between the risk markers and stress responsiveness. Given that levels of physical activity and cardiorespiratory fitness (measured by maximal oxygen consumption) are significantly correlated, we used maximal oxygen consumption (VO₂ max) as an objective measure of physical activity status. It was hypothesised that:

1. Women who engage in higher levels of physical activity will have lower cortisol (HPA axis), catecholamine, blood pressure and heart rate (SAM system) responses to the TSST compared with their relatively inactive counterparts.
2. Lower physiological stress reactivity will be associated with a more desirable profile of cardio metabolic risk markers.

6.2: Materials and Methods
Forty-four women were recruited for this experiment (Section 3.1.2). Given the influence of sex steroids on stress pathway activity (Kajantie & Phillips, 2006), post-menopausal women, peri-menopausal women and all women who were on any form of steroidal contraception (including oral contraceptives, steroidal implants and steroidal IUDs) were excluded from the study.

6.2.1 Experimental procedure
Each woman (n=44; aged 30-50 years) reported to the laboratory on two separate days. The first visit was to obtain additional health information (details below), to obtain a fasting blood sample for the measurement of cardio-metabolic risk markers and to measure maximum oxygen consumption (VO₂ max). The stress test (Section 3.5) was conducted on the second visit which occurred at least one week after the first visit.

Day 1 testing
Participants were given instructions to fast overnight (for at least 10 hours) prior to attending the laboratory. All Day 1 testing was completed between 0600h – 1200h. Weight, height, BMI and resting blood pressure were recorded to determine eligibility (Section 3.3 and 3.7.1). A blood sample was collected after anthropometric measures (Section 3.6.2).
Participants were then offered a snack (a selection of foods from muesli bars, nuts, dried fruit and juice boxes were made available) and asked to fill in a Physical Activity Readiness Questionnaire (PAR-Q), an International Physical Activity Questionnaire (IPAQ) (Bauman et al., 2009), a State-Trait Anxiety Inventory (STAI) (Spielberger, 2010) and a Beck Depression Inventory (BDI-ii) (Beck, Steer, & Brown, 2005). The PAR-Q was used to assess if it was safe for each participant to undertake a VO2 max test. Based on the results of the PAR-Q, it was considered safe for all women to undertake a VO2 max test. Information from the IPAQ was used to calculate the total time spent by women in moderate and vigorous intensity physical activity in a normal week. The STAI and the BDI-ii were used to measure baseline levels of anxiety and depression, respectively. Water was available *ad libitum* to all participants throughout the testing session. A thorough explanation of the requirements of the fitness test was provided to all participants after the snack. This was followed by a graded VO2 max test (Section 3.8). After ranking women by VO2 max score, a median split was used to allocate women evenly into a group with high VO2 max (high fit group; n=22) and a group with high VO2 max (low fit group; n=22).

**Day 2 testing**

The testing day was booked such that participants were in their mid-follicular phase of the menstrual cycle at the time of the stress testing session. Mid follicular phase was defined as Days 5-9 of the menstrual cycle, inclusive, where Day 1 was the first day of menses onset (Lustyk, Olson, Gerrish, Holder, & Widman, 2010).
All participants were asked to abstain from smoking, ingesting any caffeine containing beverages (e.g. tea, coffee, cola), liquorice, alcohol or drugs (except for any regular medications) and from strenuous physical activity during the 12 hours prior to participation in Day 2 testing.

The experimental procedure for Day 2 is shown in Figure 6.1. Participants arrived at our clinical research facility at 1100h. Between 1100h-1145h, anthropometric measurements (Section 3.3) were obtained and participants were asked to fill in a background questionnaire which entailed questions about their alcohol consumption and physical activity in the week preceding to the stress testing day. Also during this period, an intra-venous catheter (Smiths Medical, Ohio, USA) was inserted into an antecubital vein of the forearm for subsequent sampling of blood. The sampling procedures began at 1145h. Participants made their own lunch from standardised ingredients (Section 3.4) during 1200h-1230h. A familiarisation period took place between 1230h-1330h. A TSST (Section 3.5) was imposed between 1500h-1530h and a recovery period extended from 1530h-1700h.

Blood samples (10ml) were collected every 15 minutes from 1145h -1700h except for during and immediately after stress when blood was collected more frequently (1500h, 1507h, 1515h, 1522h, 1530h, 1537h, 1545h, 1552h, and 1600h; Figure 6.1). Blood samples were stored on ice and processed within one hour of collection. Only data from 1400h-1700h is presented in this experimental chapter.
Figure 6.1: Schematic representation of the stress testing day. ‘B’ and black arrows= blood samples, TSST= Trier Social Stress Test, ECG = Electrocardiogram

6.2.2: Statistical analysis

Preliminary analysis

Pre-treatment for cortisol, adrenaline (Adr) noradrenaline (NA) and dopamine (DA) was defined as the average of the four concentrations from 1415h to 1500h (1415h, 1430h, 1445h and 1500h). Pre-treatment for systolic (SBP) diastolic (DBP) and mean arterial pressure (MAP) was defined as the average of the five readings from 1400h-1500h (1400h, 1415h, 1430h, 1445h and 1500h). Pre-treatment for heart rate was defined as the average from the four readings from 1400h to 1445h (1400h, 1415h, 1430h, and 1445h). Peak height for cortisol, catecholamines and blood pressures was defined as the highest value obtained for each individual after commencement of stress
Peak height for heart rate was defined as the highest reading recorded between 1500h and 1700h inclusive. **Reactivity** was calculated by subtracting the pre-treatment value from the peak height for all parameters. **Area under the curve** (with respect to increase) was calculated for each parameter using all values from 1500h – 1700h after the subtraction of the pre-treatment value from each data point. Areas under the curve were calculated using the trapezoid rule utilising Sigmaplot graphing software (Systat Software Inc., California, USA).

**Recovery time** for all parameters was defined as the time difference from the commencement of the stressor (1500h) to the point at which the relevant parameter returned to within two standard deviations of its pre-treatment value. We used this as our definition for recovery since 95% of a normally distributed set of data lie within 2 standard deviations of the mean and since 5% error \((p=0.05)\) is the generally accepted cut off level for statistical significance. This suggests that once the value has returned to within 2 standard deviations of the pre-treatment level, it has returned to pre-treatment levels. Those who did not exceed two standard deviations of the pre-treatment value between 1500-1700h \((n=5 \text{ low fit and } n=3 \text{ high fit for cortisol}, n=5 \text{ low fit and } n=1 \text{ high fit for Adr}, n=1 \text{ low fit and } n=1 \text{ high fit for NA}, n=9 \text{ low fit and } n=12 \text{ high fit for DA}, n=3 \text{ low fit and } n=0 \text{ high fit for SBP}, n=5 \text{ low fit and } n=2 \text{ high fit for DBP}, n=1 \text{ low fit and } n=1 \text{ high fit for MAP}, n=2 \text{ low fit and } n=1 \text{ high fit for heart rate}) were excluded from analyses of recovery time. For those who did exceed two standard deviations but did not return to within two standard deviations by 1700h \((n=3 \text{ low fit and } n=3 \text{ high fit for cortisol}, n=3 \text{ low fit and } n=3 \text{ high fit for Adr}, n=0 \text{ low fit and } n=0 \text{ high fit for NA}, n=1 \text{ low fit and } n=1 \text{ high fit for DA,
n=1 low fit and n=1 high fit for SBP, n=0 low fit and n=1 high fit for DBP, n=1 low fit and n=1 high fit for MAP, n=1 low fit and n=0 high fit for heart rate), 120 minutes was used as the recovery time in the analyses.

Analysis
Plasma cortisol, plasma catecholamines, blood pressure, heart rate and heart rate variability were compared within and between groups using repeated measures analysis of variance. The within subjects factor was time and the between subjects factor was treatment. Derived plasma cortisol, plasma catecholamine, blood pressure and heart rate parameters (pre- treatment, peak height, reactivity and area under the curve) were compared between groups using univariate analysis of variance. Pearson’s correlation was used to test for relationships between stress reactivity, cardiorespiratory fitness and cardio metabolic risk markers. P<0.05 was considered statistically significant.

6.3: Results

6.3.1 Participants
A total of 44 women were tested in this study. Women were ranked by VO$_2$ max and a median split was used to allocate women to either the low fit (n=22) or the high fit group (n=22). One woman from the high fit group was excluded from cortisol, NA, Adr and DA analysis due to cannula failure during sampling. Nine other women (3 from the low fit group and 6 from the high fit group) women were excluded from the analysis of Adr due to complications in measuring levels of adrenaline or not having detectable amounts of adrenaline
6.3.2: Participant characteristics

Descriptive characteristics of the participants are detailed in Table 6.1. VO₂ max and number of hours of physical activity were significantly higher in women in the high fit group compared with the women in the low fit group (Table 6.1). VO₂ max and the number of hours of physical activity per week were significantly positively correlated (r= 0.598; p<0.001). BMI was similar in both groups, although the low fit women had significantly more (p=0.013) body fat than the high fit women (Table 6.1). Waist circumference and WHR were also significantly higher in women in the low fit group (p=0.021 and 0.004; respectively). Levels of triglycerides (p = 0.024), CHOL/HDL ratio (p = 0.001), levels of glucose (p = 0.024) and insulin resistance (p = 0.049) were also significantly higher in low fit women compared with high fit women (Table 6.2). C-reactive protein, total cholesterol levels, insulin levels and depression and anxiety scores were comparable between the groups (Table 6.2).
### Table 6.1: Mean (±SEM) Descriptive characteristics of Low and High fit women

<table>
<thead>
<tr>
<th></th>
<th>Low fit (n= 22)</th>
<th>High fit (n=22)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.4±1.4</td>
<td>38.1±1.3</td>
<td>0.233</td>
</tr>
<tr>
<td>Amount of physical activity (hours)</td>
<td>2.7±0.5</td>
<td>7.1±1.3</td>
<td>0.004</td>
</tr>
<tr>
<td>VO₂ max (ml/kg*min)</td>
<td>27.4±1.0</td>
<td>41.9±1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.4±1.4</td>
<td>167.5±1.4</td>
<td>0.127</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.6±2.3</td>
<td>62.2±1.6</td>
<td>0.833</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.1±0.7</td>
<td>22.2±0.4</td>
<td>0.241</td>
</tr>
<tr>
<td>% Fat</td>
<td>30.3±1.4</td>
<td>25.5±1.2</td>
<td>0.013</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>82.5±2.2</td>
<td>76.4±1.3</td>
<td>0.021</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>97.5±1.6</td>
<td>96.1±1.2</td>
<td>0.481</td>
</tr>
<tr>
<td>WHR</td>
<td>0.84±0.0</td>
<td>0.79±0.0</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance
Table 6.2: Mean (± SEM) cardio-metabolic risk markers and mental health scores in low fit and high fit women

<table>
<thead>
<tr>
<th></th>
<th>Low fit (n=22)</th>
<th>High fit (n=22)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>1.4±0.7</td>
<td>0.6±0.2</td>
<td>0.279</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.0±0.1</td>
<td>4.8±0.2</td>
<td>0.256</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.9±0.1</td>
<td>0.7±0.1</td>
<td>0.024</td>
</tr>
<tr>
<td>CHOL/HDL ratio</td>
<td>3.3±0.2</td>
<td>2.6±0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.3±0.1</td>
<td>4.7±0.2</td>
<td>0.024</td>
</tr>
<tr>
<td>Insulin</td>
<td>12.7±0.9</td>
<td>11.3±0.5</td>
<td>0.165</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.0±0.2</td>
<td>2.5±0.1</td>
<td>0.049</td>
</tr>
<tr>
<td>BDI-ii score</td>
<td>4.4±1.1</td>
<td>2.8±0.9</td>
<td>0.271</td>
</tr>
<tr>
<td>STAI score (trait)</td>
<td>31.9±1.5</td>
<td>31.7±1.9</td>
<td>0.469</td>
</tr>
<tr>
<td>STAI score (state)</td>
<td>32.4±1.2</td>
<td>30.7±2.1</td>
<td>0.475</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance
6.3.3: Cortisol

Plasma concentrations of cortisol in low and high fit women are shown in Figure 6.2 and Table 6.3. Repeated measures analysis of variance revealed that there was a significant effect of time (p<0.001; Figure 6.2). Overall (both groups combined), the peak height of cortisol concentrations (221±86 ng/ml) was significantly higher than pre-treatment concentrations (107±46 ng/ml) (p<0.001). Overall, there was a 107% increase in cortisol concentrations from pre-treatment concentrations to the peak of the response (both groups combined).

Plasma concentrations of cortisol in response to the TSST did not differ significantly between low fit women and high fit women (time*treatment, p = 0.987; Figure 6.2) and accordingly, there were no significant differences between the groups in peak height, reactivity or area under the curve for the cortisol response (Table 6.3). The mean time to recovery did not differ significantly between the groups (Table 6.3). There was no significant between subjects effect indicating that there were no significant overall differences between the groups (p = 0.524).
Figure 6.2: Mean (± SEM) plasma cortisol concentrations in low and high fit women from 1400h- 1700h.
TSST: Trier Social Stress Test

Table 6.3. Mean (±SEM) pre-treatment cortisol, peak height of cortisol, cortisol reactivity, area under the curve and recovery time for low and high fit women.

<table>
<thead>
<tr>
<th></th>
<th>Low fit (n=22)</th>
<th>High fit (n=21)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment (ng/ml)</td>
<td>105±9.3</td>
<td>108±10.7</td>
<td>0.825</td>
</tr>
<tr>
<td>Peak height (ng/ml)</td>
<td>217±17.5</td>
<td>225±20.0</td>
<td>0.755</td>
</tr>
<tr>
<td>Reactivity (ng/ml)</td>
<td>112±15.9</td>
<td>117±16.8</td>
<td>0.825</td>
</tr>
<tr>
<td>AUC (ng*min/ml)</td>
<td>4010±1083</td>
<td>5325±1463</td>
<td>0.471</td>
</tr>
<tr>
<td>Recovery time (min)#</td>
<td>93±6.4</td>
<td>87±6.2</td>
<td>0.523</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance, #n = 16 (low fit), 18 (high fit) for recovery time; AUC = Area under the curve
6.3.4: Catecholamines
Plasma concentrations of adrenaline (Adr), Noradrenaline (NA) and Dopamine (DA) in low fit women and high fit women are shown in Figure 6.3 and Table 6.4. Repeated measures analysis of variance revealed that there was a significant effect of time for all of the catecholamines (p<0.002 for Adr; p<0.001 for NA; p<0.001 for DA; Figure 3). Overall (both groups combined), the peak height of Adr concentrations (133±27 pg/ml), NA concentrations (609±37 pg/ml) and DA concentrations (44±4 pg/ml), were significantly higher than their pre-treatment concentrations (54±14, 317±21 and 30±3 pg/ml; respectively) (p<0.001 for all). Overall, there was a 146% increase in Adr, a 92% in NA and a 44% in DA concentrations from pre-treatment concentrations to the peak of the response (both groups combined).

Plasma concentrations of Adr and NA in response to the TSST did not differ significantly between low fit women and high fit women (time*treatment, p = 0.118 and p = 0.169; respectively; Figure 3) and accordingly, there were no significant differences between the groups in peak height, reactivity or area under the curve for the Adr and NA response between low and high fit women (Table 6.4). While repeated measures analysis of variance showed no significant difference between the groups in the DA response to the TSST (time*treatment, p = 0.392; Figure 3), DA reactivity was shown to be significantly higher in the low fit women compared with the high fit women (Table 6.5). Nevertheless, peak height of DA and area under the curve did not differ significantly between the groups. The mean time to recovery did not differ significantly between the groups for any of the parameters (Table 6.4).
There was no significant between subjects effect for Adr and DA indicating that there were no significant overall differences between the groups ($p = 0.242$ and 0.848; respectively). However, NA for low fit women showed an overall trend of being higher than the values for high fit women (treatment effect $p = 0.090$).
Figure 6.3: Mean (±SEM) a) Adrenaline (Adr), b) Noradrenaline (NA) and c) Dopamine (DA) in low fit and high fit women from 1400h-1700h.

TSST: Trier Social Stress Test
### Table 6.4: Mean (±SEM) pre-treatment, peak height, reactivity, area under the curve and recovery time for Adr, NA and DA in low and high fit women

<table>
<thead>
<tr>
<th></th>
<th>Low fit</th>
<th>High fit</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adr</strong> (n=19)</td>
<td>(n=15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (pg/ml)</td>
<td>72±23</td>
<td>31±9</td>
<td>0.131</td>
</tr>
<tr>
<td>Peak height (pg /ml)</td>
<td>144±43</td>
<td>120±29</td>
<td>0.664</td>
</tr>
<tr>
<td>Reactivity (pg /ml)</td>
<td>72±22</td>
<td>89±23</td>
<td>0.598</td>
</tr>
<tr>
<td>AUC (pg *min/ml)</td>
<td>1945±631</td>
<td>2610±1093</td>
<td>0.584</td>
</tr>
<tr>
<td>Recovery time (min)#</td>
<td>66±10</td>
<td>54±10</td>
<td>0.373</td>
</tr>
<tr>
<td><strong>NA</strong> (n=22)</td>
<td>(n=21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (pg/ml)</td>
<td>349±31</td>
<td>283±27</td>
<td>0.121</td>
</tr>
<tr>
<td>Peak height (pg /ml)</td>
<td>644±57</td>
<td>572±47</td>
<td>0.333</td>
</tr>
<tr>
<td>Reactivity (pg /ml)</td>
<td>295±38</td>
<td>288±27</td>
<td>0.889</td>
</tr>
<tr>
<td>AUC (pg *min/ml)</td>
<td>7551±1937</td>
<td>4459±1254</td>
<td>0.192</td>
</tr>
<tr>
<td>Recovery time (min)#</td>
<td>52±3</td>
<td>47±4</td>
<td>0.412</td>
</tr>
<tr>
<td><strong>DA</strong> (n=22)</td>
<td>(n=21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (pg/ml)</td>
<td>29±3</td>
<td>32±4</td>
<td>0.530</td>
</tr>
<tr>
<td>Peak height (pg /ml)</td>
<td>47±5</td>
<td>40±6</td>
<td>0.312</td>
</tr>
<tr>
<td>Reactivity (pg /ml)</td>
<td>19±4</td>
<td>8±2</td>
<td>0.009</td>
</tr>
<tr>
<td>AUC (pg *min/ml)</td>
<td>50±147</td>
<td>-215±157</td>
<td>0.225</td>
</tr>
<tr>
<td>Recovery time (min)#</td>
<td>55±9</td>
<td>66±11</td>
<td>0.461</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance, #n = 14 (low fit), 14 (high fit), 21 (low fit), 20 (high fit), 13 (low fit), 9 (high fit) for Adr (adrenaline), NA (noradrenaline) and DA (dopamine) recovery times, respectively.

AUC = Area under the curve
6.3.5 Blood pressure
Systolic, diastolic and mean arterial pressures in low fit and high fit women are shown in Figure 6.4 and Table 6.5. Repeated measures analysis of variance revealed that there was a significant effect of time for all blood pressure parameters (p<0.001 for all; Figure 6.4). Overall (both groups combined), the peak height of SBP (133±2 mmHg), DBP (80±2 mmHg) and MAP (102±2 mmHg) were significantly higher than their respective pre-treatment values (105±2, 58±1 and 75±1 mmHg) (p<0.001). Overall, there was a 27% increase in SBP, a 37% increase in DBP and a 35% increase in MAP from pre-treatment pressures to the peak of the response (both groups combined).

None of the blood pressure parameters in response to the TSST differed between low fit women and high fit women (time*treatment, p = 0.513 (SBP), p = 0.384 (DBP) and p = 0.263 (MAP); Figure 4) and accordingly, there were no significant differences between the groups in peak height, reactivity or area under the curve for the blood pressure responses (Table 6.5). The mean time to recovery of all blood pressure parameters were also similar between the groups (Table 6.5). There was no significant between subjects effect in SBP, DBP or MAP indicating that there were no significant overall differences between the groups for any of these variables (p = 0.233, 0.251 and 0.164 for SBP, DBP and MAP; respectively).
Figure 6.4: Mean (±SEM) systolic, diastolic and mean arterial pressures in low and high fit women from 1400h-1700h
TSST: Trier Social Stress Test, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure
<table>
<thead>
<tr>
<th></th>
<th>Low fit (n=22)</th>
<th>High fit (n=21)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmHg)</td>
<td>106±3</td>
<td>103±2</td>
<td>0.312</td>
</tr>
<tr>
<td>Peak height (mmHg)</td>
<td>132±4</td>
<td>133±3</td>
<td>0.812</td>
</tr>
<tr>
<td>Reactivity (mmHg)</td>
<td>26±2</td>
<td>30±3</td>
<td>0.202</td>
</tr>
<tr>
<td>AUC (mmHg *min)</td>
<td>730±125</td>
<td>544±155</td>
<td>0.355</td>
</tr>
<tr>
<td>Recovery time (min)#</td>
<td>45±6</td>
<td>42±5</td>
<td>0.614</td>
</tr>
<tr>
<td><strong>DBP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmHg)</td>
<td>59±2</td>
<td>58±1</td>
<td>0.478</td>
</tr>
<tr>
<td>Peak height (mmHg)</td>
<td>80±2</td>
<td>80±3</td>
<td>0.858</td>
</tr>
<tr>
<td>Reactivity (mmHg)</td>
<td>21±1</td>
<td>23±3</td>
<td>0.525</td>
</tr>
<tr>
<td>AUC (mmHg *min)</td>
<td>779±102</td>
<td>601±108</td>
<td>0.237</td>
</tr>
<tr>
<td>Recovery time (min)#</td>
<td>48±4</td>
<td>45±4</td>
<td>0.689</td>
</tr>
<tr>
<td><strong>MAP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmHg)</td>
<td>77±2</td>
<td>74±2</td>
<td>0.208</td>
</tr>
<tr>
<td>Peak height (mmHg)</td>
<td>100±2</td>
<td>102±4</td>
<td>0.609</td>
</tr>
<tr>
<td>Reactivity (mmHg)</td>
<td>24±2</td>
<td>29±4</td>
<td>0.187</td>
</tr>
<tr>
<td>AUC (mmHg *min)</td>
<td>839±108</td>
<td>773±149</td>
<td>0.721</td>
</tr>
<tr>
<td>Recovery time (min)#</td>
<td>43±4</td>
<td>45±5</td>
<td>0.773</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance, #n = 19 (low fit), 22 (high fit), 17 (low fit), 20 (high fit), 21 (low fit), 21 (high fit) for SBP, DBP and MAP recovery times respectively; AUC = Area under the curve
6.3.6: Heart rate (HR)

Heart rate data in low and high fit women are shown in Figure 6.5 and Table 6.6. There was a significant effect of time (p<0.001; Figure 6.5). Overall (both groups combined), peak height of HR (82±2 bpm) was significantly higher than pre-treatment values (69±1 bpm) (p<0.001). Overall, there was a 20% increase in heart rate from pre-treatment values to the peak height of the heart rate response (both groups combined).

Heart rate response to the TSST did not differ significantly between low and high fit women (time*treatment, p = 0.832; Figure 6.5). This lack of difference was further illustrated by there being no significant differences in peak height, reactivity, AUC and mean time to recovery between the two groups (Table 6.6). Overall, low fit group had higher heart rates (treatment effect p = 0.030) compared with the women in the high fit group (Figure 6.5). This overall difference between the groups was reflected in there being a significant difference (p = 0.011) in the pre-treatment heart rates between the groups (Table 6.6).
Figure 6.5: Mean (± SEM) heart rate in low and high fit women from 1400h-1700h. TSST: Trier Social Stress Test

Table 6.6. Mean (±SEM) pre-treatment HR, peak height of HR, HR reactivity, area under the curve and recovery time for low and high fit women.

<table>
<thead>
<tr>
<th></th>
<th>Low fit</th>
<th>High fit</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=22)</td>
<td>(n=21)</td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (bpm)</td>
<td>72±2</td>
<td>65±2</td>
<td>0.011</td>
</tr>
<tr>
<td>Peak height (bpm)</td>
<td>86±3</td>
<td>79±3</td>
<td>0.125</td>
</tr>
<tr>
<td>Reactivity (bpm)</td>
<td>13±2</td>
<td>14±2</td>
<td>0.716</td>
</tr>
<tr>
<td>AUC (beats/min)</td>
<td>106±88</td>
<td>229±86</td>
<td>0.323</td>
</tr>
<tr>
<td>Recovery time (min)#</td>
<td>47±6</td>
<td>41±4</td>
<td>0.375</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance, #n = 19 (low fit), 21 (high fit) for recovery time; AUC = Area under the curve
6.3.7 Heart rate variability

6.3.7.1: RMSSD
Mean (±SEM) RMSSD in low and high fit women from 1400-1700h are shown in Figure 6.6a. Repeated measures analysis of variance revealed a significant time effect (p < 0.001) and no time * treatment interaction (p = 0.606). However, there was trend of high fit women having significantly high RMSSD (treatment effect p = 0.081).

6.3.7.2: HF power
Mean (±SEM) HF in low and high fit women from 1400-1700h are shown in Figure 6.6b. Repeated measures analysis of variance revealed no significant time effect (p = 0.282), no significant time * treatment interaction (p = 0.394) and no significant treatment effect (p = 0.889).

6.3.7.3: LF power
Mean (±SEM) LF in low and high fit women from 1400-1700h are shown in Figure 6.6c. Repeated measures analysis of variance revealed no significant time effect (p = 0.144), no time * treatment interaction (p = 0.521) and no significant treatment effect (p = 0.417).

6.3.7.4: LF/HF ratio
Mean (±SEM) LF/HF ratio in low and high fit women from 1400-1700h are shown in Figure 6.6d. Repeated measures analysis of variance revealed a significant time effect (p < 0.001) and no time * treatment interaction (p = 0.125). However, there was trend of low fit women having higher LF/HF ratio (treatment effect p = 0.078).
6.3.8: Correlations/Associations

**VO₂ max and stress reactivity**

There were no significant associations between VO₂ max scores and reactivity of cortisol, Adr, and NA (data not shown). Nevertheless, there was a significant ($p = 0.007$) negative correlation (Pearson’s $r = -0.407$) between VO₂ max and DA reactivity. There were no significant associations between VO₂ max scores...
and reactivity of any of the blood pressure parameters or heart rate in response to the TSST (data not shown).

**VO₂ max and cardio metabolic risk markers**

VO₂ max scores were significantly (p<0.05 for all) correlated with triglyceride levels, LDL cholesterol, HDL cholesterol, CHOL/HDL ratio and HOMA-IR (Pearson’s r = -0.368, -0.467, 0.335, -0.533 and -0.380; respectively).

**Stress reactivity and cardio metabolic risk markers**

Adr reactivity was significantly correlated with HDL cholesterol (Pearson’s r = 0.359, p= 0.037) and DA reactivity was significantly correlated CRP (Pearson’s r = 0.327, p= 0.033). There were no other significant associations between catecholamine reactivity and cardio metabolic risk markers. No significant relationships between cortisol reactivity and any of the cardio metabolic risk markers were found. Systolic and diastolic blood pressure reactivity were not significantly associated with any of the cardio metabolic risk markers. However, MAP reactivity was significantly correlated with fasting glucose levels (Pearson’s r = -0.473, p= 0.001). Heart rate reactivity was significantly correlated with levels of insulin (Pearson’s r = -0.367, p= 0.015) and HOMA-IR (Pearson’s r = -0.350, p= 0.023).
6.4: Discussion

Results revealed that women who engaged in higher levels of moderate and vigorous intensity physical activity have comparable HPA axis and SAM system responses to a potent psychological stress compared with aged matched women who engaged in low levels of activity. This suggests that physiological responses to psychological stress are not influenced by engaging in moderate and vigorous intensity physical activity ranging from 3 to 8 hours a week. Nevertheless, a significant negative correlation was observed between fitness levels and DA reactivity which aligned with low fit women having a significantly high DA reactivity to stress. Furthermore, reactivity of Adr, DA, MAP and HR were significantly correlated with HDL cholesterol, CRP, fasting glucose and insulin kinetics (facing insulin and HOMA-IR); respectively. This provides some support for an association between cardio metabolic risk markers and an individual’s susceptibility to over activation of the SAM system.

Findings from previous studies partially concur with the results of the current experiment. Our results are in accordance with Monya and colleagues findings in men with varying levels of aerobic fitness and more recently with the findings of Poole and colleagues where they tested young women against a combined mirror tracing task and a socially evaluative speech task (Moyna et al., 1999; Poole et al., 2011). Moyna et al 1999 reported that neuroendocrine responses to mental stress were independent from the aerobic fitness levels. Similarly
Poole et al 2011 reported psychophysiological responses to stress to be independent of physical activity levels. In line with the findings of the aforementioned investigations and the findings of this research, it is also possible to speculate that HPA axis and SAM system activity in response to psychological stress is independent of the physical activity status of an individual. However, further testing is required in this area to determine if this is true as there is evidence suggesting that increased physical activity can be beneficial in mediating physiological responses to psychological stress.

Results from the current study contrast both Klaperski and colleagues and Traustadottir and colleagues work in young females and postmenopausal women, respectively (Klaperski et al., 2013; Traustadottir, Bosch, & Matt, 2005). Nevertheless, it should be noted that in Klaperski and colleagues work, the women who showed the highest cortisol levels were participating in as low as 22 minutes of physical activity per week. Compared to this, low fit women in the current study took part in an average of 2.5 hours physical activity a week. Therefore, it may be possible that physical activity status will only have a negative effect on stress responsiveness in individuals who take part in extremely low levels of activity only. Findings of Traustadottir and colleagues may imply that the changes in the hormone milieu in women after the cessation of menses might be the primary mechanism behind the attenuated neuroendocrine stress responsiveness to stress (Traustadottir et al., 2005). Nevertheless, direct comparisons of the aforementioned research and the current study should be approached with caution due to the differences in methodology.
The lack of significant reductions of HPA axis activity in the high fit group in response to stress in the current cohort of women contradicts the findings of Rimmele and colleagues in untrained men, male elite and male amateur athletes (Rimmele et al., 2009; Rimmele et al., 2007) and more recently with the findings of Webb and colleagues in men with below average and higher than average fitness levels in response to a combination of physical and mental stress as well (Webb et al., 2013). In all instances it was reported that higher activity/fitness significantly attenuates HPA axis responses to psychological stress. Therefore, it could be speculated that attenuation in HPA axis activity in response to psychological stress mediated by the physical activity status is sex specific and may only occur in males.

We performed an array of direct (catecholamines) and indirect (heart rate, blood pressure and heart rate variability) SAM system measurements to unravel any underlying capabilities of regular physical activity to attenuate physiological responses to psychological stress. Summers and colleagues reported no differences in urinary catecholamine responses to psychological stress in fit and unfit women (Summers, Lustyk, Heitkemper, & Jarrett, 1999). This is in line with the comparable changes in Adr and NA that were observed in the current experiment in low fit and high fit women. However, a direct comparison of the studies requires a cautious approach due to the difference in the stressors utilised (colour-word stroop vs TSST) and the differences in the medium used to measure catecholamines (urine vs plasma). Furthermore,
these investigators found that fitter women had lower baseline/resting heart rates compared with unfit women which is confirmed by our findings as well. The finding that low fit women had a significantly higher DA reactivity compared with high fit women is of interest and warrants further investigation. Both the source of DA circulating in the periphery and the relevance of DA in stress responsiveness are worthy of further investigation.

The majority of past research in the area, albeit mostly in men, reveals that increased fitness/levels of regular physical activity can attenuate heart rate responses to psychological stress (Klaperski et al., 2013; Rimmiele et al., 2009; Rimmmele et al., 2007; Webb et al., 2013). It could be argued that this is a further illustration of a sex difference in the role of regular exercise exerting its capabilities to attenuate physiological responses to mental stress. Current results indicate that despite higher baseline HR in low fit women, the HR responses to TSST were not affected by the physical activity status. Current results also indicate that there is no difference in the HRV or blood pressure responsiveness to TSST in women with low and high levels of cardiorespiratory fitness. To the best of our knowledge, HRV and BP in the context of protective effects of regular physical activity against mental stress has received minimal attention in past research. Comparable changes in blood pressure and HRV in response to stress further compliments findings of the direct catecholamine measures of the SAM system in this instance. It is also evident from the current results that all parameters (both HPA and SAM) that were measured showed substantial increases in responses to the TSST. This highlights the potency of TSST to adequately activate both SAM system
and HPA axis. Nevertheless, it cannot be discounted that the lack of differences observed may have been a result of the TSST fully activating and acutely exhausting stress pathways. Therefore, it might be possible to elucidate subtle differences in stress pathway activity in response to stress if a less potent stressor was used.

Lack of associations between cortisol reactivity and VO₂ max scores confirms the inability of regular exercise to influence HPA responses to psychological stress in 30-50 year old women. However, the relationship between SAM system responses to psychological stress seem to be complicated given that there was a significant negative association between VO₂ max scores and DA reactivity but not Adr or NA reactivity. Although it is a part of a routine catecholamine measurement, DA is not considered as a classic stress hormone. Exposure to psychological stress can activate mesocorticolimbic DA system in humans (Nagano-Saito et al., 2013). Therefore, this relationship may merit further investigation. It should however be noted that the VO₂ max scores were still associated with a more desirable cardio metabolic risk marker profile as indicated by significant negative correlations between VO₂ max and triglycerides, LDL cholesterol, CHOL/HDL ratio and HOMA-IR and a significant positive correlation between VO₂ max and HDL cholesterol. This suggests that regular exercise can be beneficial in forms other than moderating the HPA axis and SAM system responses to stress. Although it is not clear as to which precedes the other, previous research shows that increases in sympathetic activity can be associated with metabolic anomalies (Flaa et al., 2008). The relationship between reactivity of HR and insulin and HOMA-IR and reactivity
of MAP and glucose in this research may indicate early signs of such anomalies. This perhaps is also suggestive of the need of further research into potential profound metabolic effects on excess sympathetic activation.

In conclusion, while some associations were found between stress reactivity and levels of cardio metabolic risk factors, findings of this research do not support there being a differential effect of the level of physical activity on SAM system and HPA axis activity in response to psychosocial stress in healthy women between 30-50 years of age.
7.1: Introduction

Food intake is a physiological challenge experienced by the human body several times a day. We (Chapter 5.0) and others have shown that food intake is a challenge that can activate both the SAM system and the HPA axis (Section 2.2.3). Thus, there lies the possibility that excessive SAM system and HPA axis responses to food intake may place individuals at increased risk of developing chronic conditions. Therefore, investigation of acute physiological responses of both SAM system and HPA axis to food intake is of utmost importance.

Influence of physical activity levels on physiological responses (both HPA axis and SAM system) to food intake has not been investigated before (Section 2.2.3). Exercise brings about many health benefits including lowering progression to chronic disease by influencing heart rate, blood pressure and vascular endothelial functioning (Hamer, 2012; Throne et al., 2000; Tsatsoulis & Fountoulakis, 2006). Moderating the HPA axis and SAM system responses to food intake may well be another avenue by which exercise exerts its protective capabilities against the development of chronic disease.

The aims of this study were to measure HPA axis and SAM system responses to food intake in women who differed in physical activity status (i.e. levels of
Chapter 7 – Food intake and physical activity in women

participation in moderate and vigorous intensity physical activity). It was hypothesised that women who participated in higher levels of activity will have lower HPA axis and SAM system responses to food intake. Given that physical activity levels significantly correlate with VO2 max scores, we used VO2 max as an objective measure to categorise women into high fit and low fit groups.

7.2: Materials and methods
Forty-four women participated in this experiment (Section 3.1.2). As explained in Section 6.2, post-menopausal women, peri-menopausal women and all women who were on any form of steroidal contraception (including oral contraceptives, steroidal implants and steroidal IUDs) were excluded from the study.

7.2.1: Experimental procedure
The experimental procedure for this study is explained in detail in Section 6.2.1. Data from 1400h-1700h are used in Experimental Chapter 3 to consider the response to psychological stress whereas data from 1145h-1400h are used in this chapter (Experimental Chapter 4) to consider the response to food intake. Testing was done on two separate days with VO2 max and cardio-metabolic risk marker testing on Day 1 and testing of HPA axis and SAM system responses to lunch (Section 3.4) on Day 2.
7.2.2: Statistical analysis

Preliminary analysis

Pre-treatment salivary cortisol was defined as the concentrations of the cortisol in the sample collected at 1200h. Pre-treatment SBP, DBP, MAP and HR were defined as the average of values recorded at 1145h and 1200h. Peak height for cortisol was defined as the highest value obtained for each individual between 1215h-1400h, inclusive. Peak height for all cardiovascular parameters was defined as the highest value obtained between 1215h-1330h. Data from 1345h-1400h were not used in this calculation because of the apparent effects on cardiovascular parameters of physical movements during the bathroom break. Reactivity was calculated by subtracting the pre-treatment value from the peak height for all parameters. Area under the curve with respect to increase was calculated for cortisol using all values between 1200h – 1400h and for SBP, DBP, MAP and HR using values between 1200h-1330h after the subtraction of the pre-treatment value from each data point. Areas under the curve for all parameters were calculated using the trapezoid rule utilising Sigmaplot 12.5 graphing software (Systat Software Inc., California, USA).

Analysis

Descriptive characteristics of this study cohort were analysed and reported in Experimental Chapter 3 (Section 6.3.2 and Tables 6.1 and 6.2). Plasma cortisol, blood pressure and heart rate were compared within and between subjects using repeated measures analysis of variance. The within subjects
factor was time and the between subjects factor was treatment. Derived plasma cortisol and cardiovascular parameters (pre-treatment, peak height, reactivity and area under the curve) were compared between groups using univariate analysis of variance. \( P<0.05 \) was considered statistically significant.

### 7.3: Results

#### 7.3.1: Participants

A total of 44 women were tested in this study. Women were ranked according to their VO\(_2\) max score and a median split was used to allocate women to two even groups (low fit group; \( n = 22 \) and high fit group; \( n = 22 \)). One woman had to be excluded from the cortisol analyses due to a blocked cannula which prevented the collection of several blood samples.

#### 7.3.2: Participant characteristics

As indicated previously (Section 6.3.2), women in the high fit group had significantly higher VO\(_2\) max levels and participated in a significantly higher number of hours of moderate and vigorous intensity physical activity compared with the women in the low fit group (\( p<0.05 \) for both; Section 6.3.2). Percentage body fat and waist circumference were significantly lower in the high fit group compared with the low fit group (\( p<0.05 \) for both). Furthermore, the low fit group had significantly more abdominal body fat compared with the high fit group as indicated by the waist to hip ratio (\( p=0.004 \); Section 6.3.2). High fit women had significantly lower serum triglyceride levels, serum CHOL/HDL ratio, serum glucose concentrations and HOMA-IR compared with
low fit women (p<0.05 for all; Section 6.3.2). There were no significant differences between the groups in serum C-reactive protein levels, serum cholesterol levels, plasma insulin levels, depression and state or trait anxiety scores (Section 6.3.2, Table 6.2).

7.3.4: Test meal
Low fit and high fit women consumed similar amounts of total energy, protein, carbohydrate, fat and sodium (Table 7.1). There were no significant differences between the groups in these parameters. Overall, both groups combined, the meal consumed by the participants consisted of 20% protein, 61% carbohydrates and 19% fat.

Table 7.1: Mean (± SEM) energy, macro nutrient and sodium intake in low and high fit women

<table>
<thead>
<tr>
<th></th>
<th>Low fit (n=22)</th>
<th>High fit (n=22)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (kJ)</td>
<td>2047±162</td>
<td>2094±116</td>
<td>0.811</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>19.7±1.8</td>
<td>19.3±1.6</td>
<td>0.847</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>58.9±4.6</td>
<td>59.6±3.3</td>
<td>0.901</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>17.5±2.3</td>
<td>19.2±2.0</td>
<td>0.592</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>1047±115</td>
<td>860±72</td>
<td>0.176</td>
</tr>
</tbody>
</table>

* Univariate Analysis of Variance
7.3.5: Plasma cortisol

Plasma concentrations of cortisol in low fit and high fit women are shown in Figure 7.1 and Table 7.2. Repeated measures analysis of variance revealed that there was a significant effect of time ($p=0.005$; Figure 7.1). Overall, both groups combined, the peak height ($174.4 \pm 9.8$ ng/ml) of cortisol concentrations was significantly higher ($p<0.001$) than the pre-treatment ($137.4 \pm 10.4$ ng/ml) cortisol concentrations. This represented a 27% increase from the pre-treatment cortisol concentrations.

In response to lunch, plasma concentrations of cortisol did not differ significantly between low fit and high fit women (time*treatment, $p = 0.882$; Figure 7.1). This lack of difference in cortisol response between low and high fit women was further illustrated by there being no significant differences in peak height, cortisol reactivity and area under the curve ($p>0.05$ for all; Table 7.2). There was also no significant between subjects effect indicating that overall, high fit women had similar cortisol levels compared with low fit women ($p = 0.839$).
Chapter 7 – Food intake and physical activity in women

**Figure 7.1**: Mean (±SEM) plasma cortisol concentrations in low and high fit women from 1145h-1400h. The boxes labelled “lunch” represents the timing of the lunch period and the hashed box represents the timing of the break to use the bathroom.

**Table 7.2.** Mean (±SEM) pre-treatment cortisol, peak height of cortisol, cortisol reactivity and area under the curve for low fit and high fit women

<table>
<thead>
<tr>
<th></th>
<th>Low fit</th>
<th>High fit</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=22)</td>
<td>(n=21)</td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (ng/ml)</td>
<td>138.7±16.7</td>
<td>136.1±12.7</td>
<td>0.900</td>
</tr>
<tr>
<td>Peak height (ng/ml)</td>
<td>172.9±13.8</td>
<td>175.9±14.2</td>
<td>0.881</td>
</tr>
<tr>
<td>Reactivity (ng/ml)</td>
<td>34.2±10.3</td>
<td>39.8±8.3</td>
<td>0.673</td>
</tr>
<tr>
<td>AUC (ng*min/ml)</td>
<td>-433.1±1045</td>
<td>-263.3±822</td>
<td>0.900</td>
</tr>
</tbody>
</table>

* Univariate analysis of variance

AUC = Area under the curve
7.3.6: Cardiovascular parameters
Cardiovascular parameters in low fit and high fit women are presented in Figure 7.2 and Table 7.3.

Systolic blood pressure
There was a significant effect of time (p<0.001) for systolic blood pressure (Figure 7.2a). Overall (both groups combined), the peak height of systolic blood pressure (120±3 mmHg) was significantly higher than the pre-treatment systolic blood pressure (108 ±2 mmHg) (p<0.001). This represents a 12% increase.

Systolic blood pressure in response to the lunch did not differ significantly between low fit and high fit women (time*treatment, p= 0.472; Figure 7.2a). This lack of difference of the response between groups was further illustrated by there being no difference in reactivity and area under the response curve for systolic blood pressure between the two groups (p > 0.05 for both; Table 7.3). Nevertheless, peak height showed a trend of being significantly higher (p=0.057) in the low fit group. Overall, low fit women showed a trend of having significantly higher systolic blood pressure compared with the high fit women (treatment effect, p = 0.064).

Diastolic blood pressure
There was a significant effect of time (p<0.001) and no treatment effect (p=0.180) for diastolic blood pressure (Figure 7.2b). Overall (both groups
combined), the peak height of diastolic blood pressure (72±2 mmHg) was significantly higher than the pre-treatment diastolic blood pressure (63 ±1 mmHg) (p<0.001). This represents a 14% increase.

Diastolic blood pressure in response to the lunch did not differ significantly between low fit and high fit women (time*treatment, p= 0.864; Figure 7.2b). Furthermore, diastolic blood pressure peak height, reactivity and area under the curve did not differ between the two groups (Table 7.3).

**Mean Arterial pressure**
There was a significant effect of time (p<0.001) for mean arterial pressure (Figure 7.2c). Overall (both groups combined), the peak height of mean arterial pressure (88±2 mmHg) was significantly higher than the pre-treatment mean arterial pressure (80 ±2 mmHg) (p<0.001). This represents an 11% increase.

Mean arterial pressure in response to the lunch did not differ significantly between low fit and high fit women (time*treatment, p= 0.805; Figure 7.2c). Peak heights, reactivity and area under the response curve for mean arterial pressure were similar between the groups (p > 0.05 for both; Table 7.3). There was a trend for overall mean arterial pressure being significantly higher (p=0.081) in the low fit group compared with the high fit group (Figure 7.2c).
Heart rate

There was a significant effect of time (p<0.001) for heart rate (Figure 7.2d). Overall (both groups combined), the peak height of heart rate (75±2 mmHg) was significantly different from the pre-treatment heart rate (66±2 mmHg) (p<0.001). This represents a 14% increase.

Heart rate in response to the lunch did not differ significantly between low fit and high fit women (time*treatment, p= 0.225; Figure 2d). High fit women had a significantly lower (p=0.005) peak height of the heart rate response compared with low fit women and heart rate reactivity showed a trend (p=0.084) of being significantly higher in the low fit women compared with the high fit women. Pre-treatment values and Area under the curve did not differ between the two groups (Table 7.3). Overall, low fit women had significantly higher levels of heart rate compared with the high fit women as indicated by the significant treatment effect (p=0.008).
Figure 7.2: Mean (±SEM) systolic (a), diastolic (b), mean arterial pressure (c) and heart rate (d) in low fit and high fit women from 1145h-1400h. The boxes labelled “lunch” represent the timing of the lunch period and the hashed boxes represent the timing of the break to use the bathroom.
### Table 7.3: Mean (±SEM) pre-treatment, peak height, reactivity and area under the curve for heart rate and blood pressures in low fit and high fit women

<table>
<thead>
<tr>
<th></th>
<th>Low fit (n=22)</th>
<th>High fit (n=22)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmHg)</td>
<td>112±4</td>
<td>104±2</td>
<td>0.115</td>
</tr>
<tr>
<td>Peak height (mmHg)</td>
<td>125±4</td>
<td>116±3</td>
<td>0.057</td>
</tr>
<tr>
<td>Reactivity (mmHg)</td>
<td>14±3</td>
<td>12±3</td>
<td>0.642</td>
</tr>
<tr>
<td>AUC (mmHg*min)</td>
<td>188±163</td>
<td>104±118</td>
<td>0.688</td>
</tr>
<tr>
<td><strong>DBP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmHg)</td>
<td>65±2</td>
<td>62±2</td>
<td>0.374</td>
</tr>
<tr>
<td>Peak height (mmHg)</td>
<td>72±2</td>
<td>72±3</td>
<td>0.833</td>
</tr>
<tr>
<td>Reactivity (mmHg)</td>
<td>8±2</td>
<td>10±3</td>
<td>0.607</td>
</tr>
<tr>
<td>AUC (mmHg*min)</td>
<td>-166±90</td>
<td>-216±112</td>
<td>0.730</td>
</tr>
<tr>
<td><strong>MAP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (mmHg)</td>
<td>82±3</td>
<td>77±2</td>
<td>0.119</td>
</tr>
<tr>
<td>Peak height (mmHg)</td>
<td>90±3</td>
<td>87±3</td>
<td>0.507</td>
</tr>
<tr>
<td>Reactivity (mmHg)</td>
<td>7±2</td>
<td>10±3</td>
<td>0.503</td>
</tr>
<tr>
<td>AUC (mmHg*min)</td>
<td>-148±115</td>
<td>-165±124</td>
<td>0.920</td>
</tr>
<tr>
<td><strong>HR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment (bpm)</td>
<td>69±2</td>
<td>64±2</td>
<td>0.113</td>
</tr>
<tr>
<td>Peak height (bpm)</td>
<td>80±2</td>
<td>71±2</td>
<td>0.005</td>
</tr>
<tr>
<td>Reactivity (bpm)</td>
<td>11±2</td>
<td>7±1</td>
<td>0.084</td>
</tr>
<tr>
<td>AUC (bpm*min)</td>
<td>239±129</td>
<td>54±69</td>
<td>0.212</td>
</tr>
</tbody>
</table>

*Univariate analysis of variance

SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate
7.4: Discussion
According to the results of this experiment, our hypothesis that women who engaged in high levels of physical activity will have lower SAM system and HPA axis responses to the ingestion of a standardised lunch compared with women who engaged in low levels of activity was not supported. While all of the parameters tested (plasma cortisol, heart rate and blood pressure) increased during lunch, none of these responses differed between the groups confirming the comparable SAM system and HPA axis activity to food intake in women with different physical activity status. Alternatively, it is also possible that SAM system and HPA axis responsiveness to food intake is independent of physical activity status in 30-50 year old women.

Results indicate that women in this research did have a substantial elevation of cortisol to lunch as indicated by the time effect and the significant increase in reactivity (both groups combined), despite there being no difference between low fit and high fit groups in this response. In an earlier experiment (Experimental Chapter 2) we observed a significant HPA axis response (salivary cortisol) to food intake in overweight/obese men compared with lean men. However, the percentage increase of cortisol in lean men (86%) were substantially higher than the percentage increase of cortisol in women (27%; both groups combined) in response to lunch. In the men’s study, salivary cortisol was used to measure HPA axis activity. Whereas in the current experiment, plasma cortisol was the measure of choice. Salivary cortisol indicates the free fraction of cortisol (a small proportion of total cortisol). Therefore, it is possible the differences in percentage increases that were
observed may have been due to the differences in HPA axis activity measures that were implemented. The age range of the current experiment (30-50 years) was also different to the age range (50-70 years) of the men. Since age has a significant influence on HPA axis activity (Pardon, 2007), we cannot discount that women in the current experiment may show a different cortisol reactivity pattern with age.

In the current experiment, all SAM system parameters in both groups elevated in response to lunch. These increases are in accordance with the reports of Harthoorn et al who found increases in sympathetic nervous system (heart rate and salivary alpha amylase) activity after ingestion of a standardised meal (15-20% protein, 35-40% fat and 40-45% carbohydrate) in a group of healthy men and women (Harthoorn & DransWeld, 2008). These elevations are indicative of the physiological demands that food intake places on the sympathetic nervous system activity (Jager et al., 1986). Furthermore, it is noteworthy that the low fit women showed a trend towards a higher heart rate reactivity to food intake compared with the high fit group. This suggests that low fit women may find food intake physiologically more challenging than the high fit women. There is also a reduction in systolic blood pressure, diastolic blood pressure and mean arterial pressure in the postprandial period in both groups (i.e., 1230h-1330h in the current experiment) which may indicate a reduction in resistance to blood flow in the mesenteric vessels and perhaps even an indication that the satiety hormones are having an inhibitory effect on the sympathetic nervous system during this period (Burcelin, 2005; Fan et al., 2004). Nevertheless, the absence of any significant differences in SAM
parameters between high fit and low fit women in the current experiment suggests that the post prandial sympathetic activity is independent of the physical activity status of an individual.

Abdominal obesity can have significant impact on the activity of the stress pathways (Epel et al., 2000; Katz et al., 2000). Vicennati and colleagues reported that high carbohydrate meals (89% carbohydrate, 11% protein, 0% fat) can results in a significant HPA axis response in women who predominantly had a visceral body fat distribution compared with women with peripheral body fat distribution and normal weight healthy controls (Vicennati et al., 2002). Despite there being no difference between the groups in BMI in the current study, waist to hip ratios and percent body fat levels indicate that the low fit women had significantly more abdominally based body fat compared with their fitter counterparts. It should be noted that the WHR of women who had visceral body fat in Vicennati and colleagues research, was much higher (0.92±0.01) than the WHR of low fit women (0.84±0.01) in the current experiment. However, given that there are no differences between the groups in SAM and HPA axis activity, it suggests that having greater quantities of abdominally based body fat does not accentuate SAM system and HPA axis responses to food intake in low fit women. Furthermore, low fit individuals in the current experiment had significantly high HOMA-IR values compared with the high fit women. Elevated HOMA-IR is suggestive of potential anomalies in insulin action (Wallace et al., 2004). Insulin has both a vasodilatory role and can also enhance sympathetic nervous system activity (Baliga, Burden, Sidhu, Rampling, & Kooner, 1997; Baron, 1994). Thus, it is unexpected to see
comparable sympathetic nervous system activity in the two groups in response to lunch.

This was the first experiment of its kind which investigated the effects of physical activity status (objectively measured via maximal oxygen consumption) on response to food intake. Women in the current study had low to moderate fitness levels (average VO$_2$ max in high fit and low fit 41.9±1.6 and 27.4±1.0 ml/kg*min; respectively) (Medicine, 2013). Therefore, it would be interesting to investigate if women with greater maximal oxygen consumption levels show a different response pattern to food intake. It would also be interesting to see if physical activity status has an effect on SAM system and HPA axis responses to food intake of women in different phases of the menstrual cycle other than mid follicular phase. Men could also be considered in testing the same hypothesis. Ideally, future experiments would also include a longer lead in time prior to the administration of lunch.
Chapter 8: General Discussion
This thesis tested the general hypothesis that adiposity in men and physical activity in women can influence SAM system and HPA axis responsiveness to psychological stress and food intake. Contrary to the expected pattern of stress reactivity, this research provided little to no support for the notion that adiposity in men (50-70 years) and physical activity status in women (30-50 years) can influence the physiological responses to psychological stress. With the exception of DA reactivity in women, neither men with differing levels of adiposity nor women with varying levels of physical activity status showed a differential HPA axis or SAM system reactivity to a potent psychological stress. Nevertheless, food intake, conformed partially with the predicted outcomes. Food intake was capable of eliciting significantly higher HPA axis responses in overweight/obese men compared with lean men but did not elicit a significantly different response in low fit and high fit women (although there was a trend towards heart rate reactivity being higher in low fit women than in high fit women). This suggests that minimising stress pathway reactivity to food intake is unlikely to be one of the ways that exercise exerts its protective capabilities in women. The results also provided little to no support for the hypothesis in women that low stress reactivity will be associated with a more desirable profile of cardio metabolic risk markers. The lack of associations between reactivity levels of the major stress parameters and desirable levels of cardio-metabolic risk markers is somewhat suggestive of a dissociation between stress reactivity and progressive disease status measured via cardio-metabolic risk markers. However, it should be noted that this research did not measure all of the available metabolic risk markers. Nevertheless, there were
some significant associations observed between SAM system measures and cardio-metabolic risk markers. Adrenaline and DA reactivity were significantly positively correlated to HDL cholesterol and CRP, respectively and negative associations between MAP reactivity and fasting insulin and between HR reactivity and insulin kinetics (fasting insulin and HOMA-IR) were also observed. Further testing is required in this regard prior to determining the exact relationship between stress reactivity and markers of chronic disease.

While outcomes of this research have provided little support for the general hypothesis of this thesis, it would be premature to reject the hypothesis outright. Only the HPA axis and SAM system were investigated in this research. Nevertheless, it should be acknowledged that there are other stress sensitive systems that can mediate the physiological stress response that were not part of the investigative process of this research. These systems may have significant implications on human health. For instance, inflammatory cytokine responses (Chrousos, 2009) and opioid pathways may play a role in mediating the stress response (Tilbrook, 2007). Hence, there is much to be investigated prior to arriving at definitive conclusions. The physiological stress response is a collective result of different physiological systems acting cohesively in response to challenges to homeostasis. Stress can have far reaching consequences in numerous physiological systems throughout the body (Chrousos, 2009). For instance, activation of the stress pathways can have significant influences on arousal and sleep (Chrousos, 2007), metabolism (Picard et al., 2014a), growth, reproduction, thyroid function (Chrousos, 1998a), gastrointestinal function (Taché & Bonaz, 2007) and the immune
system (Elenkov & Chrousos, 1999). It is of utmost importance that future stress experiments are designed in a way in which the measurement of multiple stress pathways is viable.

Findings of this research are highly applicable to modern populations given that all investigations in this research were conducted in clinically relevant study populations. Prevalence of obesity is high in many societies around the world (James, 2004) and therefore, the men subjected to investigation in the initial experiments of this research is particularly clinically relevant. There are more men in the general population in the overweight/obese category than there are in the morbidly obese category (Australian Bureau of Statistics, 2013). As such, the men used in this study were representative of the greatest portion of men in the general population who have weight above the normal range. Recruitment of men with extreme levels of obesity would have limited the findings to a relatively smaller proportion of the population. The same applies for the women tested in the latter experiments of this research. Women were recruited from the general population without excluding based on the levels of physical activity thus giving us the best possible reflection of physical activity levels in modern populations. Recruitment of women from the high and low end of the physical activity spectrum would have made the findings less relevant to the general population. The women in this study were, old enough to show early signs of development of chronic disease, yet young enough for a health intervention strategy to be implemented that could have significant benefits.
It is possible that we may have seen differences in SAM system and HPA axis responses had we chosen to use participants in this research whose adiposity or physical activity levels between the experimental groups was more extreme. The overweight/obese men in this research only had a moderately high level of adiposity, although, it was significantly higher compared with the lean men. Outcomes may have been different if men with extreme levels (e.g. morbid) of adiposity were investigated. Previously, our research group has shown in animal models that greater differences in adiposity levels can elicit significant differences in stress pathway activity in response to psychological stress (Tilbrook et al., 2008). Similarly, the high fit women only participated in moderately high levels of activity even though, it was significantly higher than the levels in low fit women. Therefore, it cannot be discounted that greater levels of physical activity may have resulted in significant differences in stress pathway activity to psychological stress. Previous investigations have shown that men who participated in more than ten hours of high intensity physical activity a week can yield attenuated stress system reactivity to psychological stress (Rimmele et al., 2009; Rimmele et al., 2007). By comparison, the high fit women in this research only participated in approximately seven hours of vigorous and moderate intensity activity a week. Nevertheless, as stated earlier, the participants used in this research were most representative of adiposity and physical activity levels in the general populations meaning that the results of this research are applicable to a larger proportion of the general population.
Investigating the effects of psychological stress under the influence of varying physiological conditions is timely especially due to the negative consequences associated with frequent exposure to psychological stress in modern life (Alwan, 2011; Brown et al., 2009; Mendis et al., 2011). Physiological reactivity generated within the body in response to psychological stress is often unnecessary because psychological stressors usually don’t demand a physical response. It is evident throughout this research that the TSST protocol implemented was potent in eliciting both HPA and SAM system responses, sometimes to a level far exceeding the reactivity levels reported previously (Benson et al., 2009; Ljung et al., 2000; Therrien et al., 2010). Thus, there may have been a ceiling effect which prevented the observation of any differences in SAM system and HPA axis responses to TSST in both men and women. While this research cannot be criticised for using a stressor that wasn’t stressful enough, it is possible that such a potent stressor could have masked subtle differences in stress pathway activity. Therefore, it cannot be discounted that a different outcome would have been generated if a less potent stressor had been used. It would be a challenge in this area of research to come up with a stressor that is potent enough to elicit a substantial response from both HPA axis and the SAM system but at the same time, does not acutely exhaust the response potential of either of these pathways.

The ability of adiposity and physical activity levels to influence responses to stress may differ from one stressor to another. There may be stressor-specific outcomes. In sheep, our lab found that differences between the sexes in the cortisol response to stress were stressor-specific (Turner, Rivalland, Clarke, &
Tilbrook, 2010). Findings in men from this research provides partial support for this notion. Current results indicated that in men with different levels of adiposity, only food intake but not psychological stress could elicit significant differences in HPA axis activity. Therefore, ideally, stress pathway reactivity in response to multiple stressors should be investigated prior to arriving at final conclusions. The stressor-specific nature of physiological responses highlights the enormity of the intricacies in investigating mechanisms of human stress responsiveness.

While it is clear that outcomes from one sex cannot be extrapolated to the other sex, it also important to consider that within each sex, changes in sex steroid milieu may influence outcomes. Age related changes in sex steroid milieu in either sex may influence outcomes (Kudielka et al., 2004b), while changes in sex steroid status with the stages of the menstrual cycle in women may influence outcomes as well (Lustyk et al., 2010). Men and women of different age groups could be considered in future research to obtain a better understanding of the influence of age related changes in sex steroids. Women in the follicular phase were investigated in this research, but further research would be required to consider if there are similar or different outcomes at other times during the menstrual cycle or during steroidal contraceptive use. To negate any potential influences of differing levels of sex steroids, this research only recruited men in the initial experiments and has adequately controlled for the menstrual cycle phase and steroidal contraceptive use in women in the latter experiments.
Given that the modifiable factors tested in this research proved to be of little or no significant influence (with the exception of food intake in overweight/obese males) on the HPA axis and SAM system reactivity, the question still beckons as to what could be the underlying factor/s that exacerbate the stress response in some humans and perhaps lead to the development of stress related chronic disease. Thus, future investigations should be directed at different metabolic (Picard et al., 2014b), innate (Storbeck, Swart, Slabbert, & Swart, 2007) and physiological systems such as the immune system (Besedovsky & del Rey, 2007) that are stress sensitive.

Immune system is closely linked with the activity of the HPA axis and the SAM system (Chrousos, 2000b; Elenkov, Papanicolaou, Wilder, & Chrousos, 1996). Therefore, investigating activity of inflammatory markers such as tumor necrosis factor, IL-6, IL-8 and IL-12 in response to different stressors may be of particular importance. Investigating innate mechanisms that are involved in the production of stress hormones may also provide a new perspective about stress responsiveness in humans. For instance, the influence of genes that are involved in the cortisol production pathway such as CYP17 could be investigated. Emerging evidence suggests that variations in the amino acid sequencing of alleles of CYP17 can lead to differences in cortisol production in goats (Storbeck, Swart et al. 2007). This is yet to be investigated in humans and therefore could form a worthwhile direction for future stress research.
It is also possible to look at more metabolically oriented mechanisms such as reducing the allostatic load to the mitochondria, as potential mediators of stress. Increases in allostatic load have been associated with changes in mitochondrial DNA subsequently leading to mitochondrial anomalies that could generate toxins, promote inflammation and hasten cell aging through influencing gene expression (Picard, Juster et al. 2014). These metabolic endpoints/mechanisms could also be useful as potential therapeutic targets.

8.1: Conclusion
Excess stress pathway activation has been shown to be an integral component of the development of cardiovascular disease, type 2 diabetes, depression and anxiety (Section 2.6). Understanding the influence of peripheral factors that can interfere with the activity of the stress pathways may be of fundamental importance in discovering avenues to implement health strategies that could slow or overcome the progression of stress related chronic disease. Increased adiposity is a worldwide epidemic that is associated with a multitude of chronic diseases. It is not well known if obesity can accentuate the activity of stress pathways (to unnecessary proportions) in humans (Section 2.5.1). Physical activity/fitness is another modifiable factor that can have significant influences on stress system activity. Elevated levels of physical activity have been shown to attenuate the reactivity of the stress pathways in response to external stimulation (Section 2.5.2). This research investigated the effects of both adiposity in men and physical activity in women on HPA axis and SAM system activity in response to a potent psychological stress (Section 3.5) using an optimal methodological approach which included an array of reliable direct and
indirect measures of HPA axis and SAM system (Sections 2.1.3 and 2.1.4) and frequent sampling optimised to capture all changes during stress (Figure 3.1A). The results provide little to no support for the notion that excess adiposity in men or lack of physical activity in women influences HPA axis and SAM system reactivity to psychological stress.

Food intake is seldom considered as a challenge that could significantly alter the activity levels of the physiological stress systems. Nevertheless, it is a physiological challenge to the stress pathways that people generally experience on multiple occasions each day (Section 2.2.3). Therefore, investigating the potential influences of food intake on stress pathway activity was of fundamental importance. This research found that overweight/obese men of 50-70 years, had a significant HPA axis response to food intake whereas their leaner counterparts had no such response. In women, results indicated that physical activity status does not play a role in modifying the HPA axis and SAM system responses to food intake.
Chapter 9: References


Chapter 9 – References


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Chapter 9 – References


