Neurohormonal correlates of altered eating behavior in Depressive Disorders

by

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Depression and obesity are widespread, significant global public health concerns. Weight and appetite disturbances are common symptoms of Major Depressive Disorder that can complicate treatment and prognosis and, similarly, obesity increases vulnerability to depression. Proposed shared etiologies for depression and elevated body weight include altered stress perceptions and eating behaviours, as well as neurohormonal mediation. The latter includes altered hypothalamic-pituitary-adrenal (HPA) axis activity, and changes in hunger and satiety signals, adding further evidence to phenotype overlap. There are few investigations on eating behavior, weight changes, and HPA axis alterations in depression. The aim of this series of interlinked investigation was to examine the influence of stress and of food ingestion on hormones involved in appetite regulation along side HPA axis activity. Cortisol, ghrelin, and leptin secretory response to two physiological challenges, i.e., psychosocial stress, and food, was evaluated in eighteen patients with clinical depression, and seventeen matched healthy controls. Neurocognition, eating behavior, and stress perception measures were obtained. In response to the stress challenge, depressed participants exhibited elevation of leptin levels in comparison to controls. Such elevation was influenced by the chronicity of illness. No differences between...
patients with depression or controls were found in cortisol or ghrelin response to either set of experiments. However, among the participants, emotional eaters exhibited significantly lower levels of ghrelin in response to food. In addition, significant associations between leptin and cognition were noted. Results suggest possible alteration in appetite regulation in both depressed and emotional eaters. It is suggested that appetite and weight increase often seen in chronic depression may be in part be mediated by HPA axis leptin interactions. Potential alterations in feedback systems regulating appetite may be suggested as leading to increased vulnerability for future weight gain in depression.
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List of Abbreviations

ACTH – adrenocorticotropic hormone
AGRP – agouti related peptide
ARC – arcuate nucleus
AUC<sub>G</sub> - Area under the curve, ground formula
AUC<sub>I</sub> – Area under the curve, increase formula
BMI – Body Mass Index
CART – cocaine- and amphetamine- regulated peptide
CCK - cholecystokinin
CRH – corticotropin releasing hormone
CPT-II – Conners’ Continuous Performance Task
DEBQ – Dutch Eating Behavior Questionnaire
DSM-V - Diagnostic Statistical Manual, Fifth Edition
GLP-1 – Glucagon-like peptide
HAMD – Hamilton Depression Rating Scale
HPA – hypothalamic-pituitary-adrenal axis
IGT – Iowa Gambling Task
IL-6 – interleukin 6
LHP – lipid hydroperoxidase
MCH – melanin concentrating hormone
MDD – major depressive disorder
NPY – neuropeptide Y
PANAS – Positive and Negative Affect Scale
POMC – pro-opiomelacortin
PPY – peptide YY
PSS – Perceived Stress Scale
SAD – Seasonal Affective Disorder
SSRI – selective serotonin reuptake inhibitor
SNRI – serotonin norepinephrine reuptake inhibitor
TNFα – tumor necrosis factor alpha
TOMM – Test of Memory Malingering
TRH – thyrotropin-releasing hormone
TSST – Trier Social Stress Test
1 Literature Review

Major depressive disorder (MDD) is a common mental illness that causes significant impairment and burden (Whiteford et al., 2013). Appetite disturbance is one of its common symptoms, with a significant number of individuals experiencing loss of appetite and weight loss, while a smaller subgroup exhibit hyperphagia and weight gain. It is of note that a significant association between chronic depressive disorders and an increased vulnerability to obesity has been well documented (Taylor et al., 2008). However, a clear causal relationship has not been established nor the mechanisms mediating this link been fully elucidated (McElroy et al., 2004). Furthermore, it is well documented that obese individuals with depression often display resistance to treatment and may to represent a distinct subcategory of the disorder (Kloiber et al., 2007).

The neurobiology of eating behaviours is complex and proposed to involve several neurohormones and neurotransmitters that modulate hunger and satiety signals. The neurohormones include those influencing satiety signals, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and ghrelin. In addition, neurohormones with adiposity signals such as leptin and insulin, neuropeptides such as neuropeptide Y (NPY), agouti-related peptide (AGRP), pro-opiomelacortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), as well as hypothalamic releasing hormones such as corticotrophin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH), have been implicated. Neurotransmitters that are thought to have a particular role include monoamines such as serotonin, norepinephrine, and dopamine, among others (for review see Valassi et al., 2008, Zheng and Berthoud, 2008).
It has long been acknowledged that several hypothalamic regions influence eating behaviours. Among these are the lateral hypothalamus, thought to be the site of the hunger centre, and ventromedial hypothalamus, thought to be the satiety centre (Mayer and Thoma, 1967). In addition, recent evidence implicates the arcuate nucleus (ARC) to play a key role in modulating food intake. This region contains neurons that release NPY and AGRP, which enhance food intake, as well as POMC and CART, both of which possess anorexigenic effects (Valassi et al., 2008; Zheng and Berthoud, 2008). Projections from this region to second order...
neurons in the paraventricular nucleus stimulate not only the secretion of CRH and TRH, which also have an anorexogenic effect, but also melanin concentrating hormone (MCH) (Ludwig et al., 1998) and orexin (Sakurai et al., 1998), which, in contrast, promote food intake. Ghrelin and leptin are relatively recently discovered peptides, both of which have been clearly demonstrated to play key roles in hunger and satiety. Ghrelin increases hunger and food intake, is thought to control eating behaviour both centrally and peripherally through its interactions with other neurohormones previously mentioned (Kojima and Kangawa, 2005). On the other hand, leptin is thought to inhibit hunger, food intake and control and eating behavior centrally influencing the same hormones (Zhang et al., 1994).

The two-way relationship between stress and food intake is well documented (Oliver et al., 2000; Epel et al., 2001). Several mediating mechanisms, including psychological, behavioural, and cognitive have been proposed, the exact physiological underpinnings of this relationship are yet to be fully elucidated (Adam and Epel, 2007). It is well accepted that hypothalamic-pituitary-adrenal axis (HPA) serves as the primary mediator of stress reactivity, and that alterations in its activity are a well documented finding in both depression and obesity (e.g. Carroll et al., 1976; Bjorntrop and Rosmond, 2000). The frequent presence of appetite disturbances (both increase and decrease) in depressed populations has led to recent investigations evaluating the role of both leptin (Lu, 2007) and ghrelin (e.g. Kluge et al., 2009) in mediating this symptom. Furthermore, there is early evidence to suggest that both these hormones may even play a role in the pathophysiology of mood disorders, and at least in part through their effect on the HPA axis and stress responsivity (Lu, 2007). With the ubiquitous presence of both stress vulnerability and altered appetite in depressed populations, there is need to evaluate the inter-relationship between the stress response, food intake, as well as hunger and satiety.
Both the pathophysiology of depressive illness and neurobiological regulation of food intake and appetite in obesity are independently complex and involve numerous of overlapping systems that encapsulate a number of potential vulnerabilities to weight disturbances in depression. While it is recognized that several diverse factors contribute to such vulnerability it would not be possible to evaluate their contributions in a single series of investigations. Thus, this research program will focus on the influence of stress reactivity and the appetite/satiety hormones leptin and ghrelin. The following section reviews published literature pertaining to Depressive Disorders and the following: weight disturbances; stress and/or emotional eating; HPA axis activity; energy homeostatic neurohormones related to appetite (ghrelin and leptin); as well as other potential contributing factors.

1.1 Depressive Illness

As previously noted, depression is a common mental illness with significant morbidity, which is expected to have the second highest illness burden worldwide by 2020 (World Health Organization, 2008; Whiteford et al., 2013). It is frequently associated with many comorbid psychiatric and medical conditions, and high rates of suicide (Patten et al., 2009; Levitan et al., 1997). This section provides a brief overview of unipolar depressive disorders and reviews the literature on the association between depression, eating behavior and weight changes.

One-year prevalence rates of major depressive disorder are estimated between 4 to 11% (Patten et al., 2009; Parikh et al., 2001; Hasin et al., 2005), with rates in females approximately twice that of men. Two-thirds of depressed patients have at least one concurrent general medical condition, while two-thirds have a lifetime likelihood of comorbid psychiatric disorder
(Rush, 2006). Lifetime prevalence rates are higher and are estimated to peak between ages 15 to 45 years (Kessler et al., 2005; Patten et al., 2005) with majority of lifetime cases beginning before age 24 (Kessler et al., 2005; Richards, 2011). Often early age of onset is associated with a more severe and recurrent forms of depression, substantial functional impairment, and greater illness burden (Zisook et al., 2007). Indeed, recent epidemiological investigations indicate that unipolar depression is the leading cause of disease burden for individuals under the age of 24 worldwide (Gore et al., 2011).

According to the Diagnostic Statistical Manual, Fourth Edition (DSM-IV-TR), the criteria for the diagnosis of a major depressive episode included the presence of the following key symptoms: depressed mood, loss of interest, appetite disturbances, insomnia or hypersomnia, psychomotor agitation or retardation, loss of energy/fatigue, feelings of worthlessness, concentration difficulties, and recurrent thoughts of death and/or suicide (American Psychiatric Association, 2000).

Five out of the nine symptoms, one of which must be depressed mood or loss of interest, must be present most days for a minimum of 2 weeks. Clusters of symptoms are categorized to form subtypes of depression. One common subtype is the atypical depression, which presents with hypersomnia, hyperphagia, weight gain, and rejection sensitivity while another is the melancholic subtype presenting with insomnia, lack of mood reactivity, and appetite and weight loss (American Psychiatric Association, 2000). Main forms of treatments for depressive disorders are antidepressants (e.g. selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs)), and psychological interventions such as Cognitive Behavioural Therapy and Interpersonal Psychotherapy. While many individuals with depression receive treatment, partial or non-response to treatment is common, with significant residual
symptoms (Hasin et al., 2005), contributing to a chronic course of depression. Individuals with treatment-resistant depression with residual symptoms have a greater likelihood of relapse and recurrences, and often have poor quality of life (Dunner et al., 2006). Residual depressive symptoms are also difficult to treat, and time to remission is greater for those who require more trials of treatment (Rush et al., 2006). It is estimated that approximately 30-50% of those who experience an acute episode of depression will experience residual symptoms (Fawcett, 1994), which has been suggested to be a significant risk factor for relapses as well as impaired social functioning (Kennedy and Paykel, 2004).

Chronic depression in DSM-IV-TR included both a major depressive episode that persists for at least 2 years and dysthymia, which referred to a chronic milder depression with fluctuating course (American Psychiatric Association, 2000). The recent revisions in the DSM-V have included both these diagnostic categories under Persistent Depressive Disorder. Thus, this diagnostic category now encompasses chronic depression in both its milder form (formerly known as Dysthymic Disorder) and the more severe form that met criteria for Major Depressive Disorder ((American Psychiatric Association, 2013). The criterion of a duration of unremitted illness of two years or longer remains.

Often, the term “chronic depression” is used synonymously with “treatment-resistant depression” and strategies to treat chronic depression are often distinct from those used for acute depression. Such strategies involve augmentation with mood stabilizing agents, atypical antipsychotics, and more recently, psychostimulants (Ravindran et al., 2008), as well as psychotherapies adapted for a chronic illness (McCullough, 2003). Due to its differing symptom profile and lack of response to usual antidepressant treatments, chronic depression has been proposed to have distinct neurobiological substrates from that of acute depression (Stahl, 2001).
1.1.1 Depressive Illness and Eating Behaviour

The relationship between depression and eating behaviour is clinically significant as altered appetite is a common symptom of depression, and depression is often comorbid with eating disorders (Hudson et al., 2007). While most individuals with depression often exhibit hypophagia (decreased eating), hyperphagia is more commonly seen in chronic forms of depression, with patients with chronicity often presenting with excessive eating, weight gain and obesity (McElroy et al., 2004). While the literature on eating behaviours in depressed populations is quite extensive, it has mostly focused on comorbidity of eating disorders, and on specific food cravings, namely carbohydrates in subgroups such as seasonal affective disorder. In addition, irregular patterns of eating (e.g. skipped meals) have been noted in depressed patients, leading some to suggest that missed meals may contribute to feelings of low energy in depressed patients, leading to replacement of calories from high fat content foods (Fulkerson et al., 2004). Interestingly, restrained eating (defined as attempting to restrict food intake in order to maintain or lose weight) has been attributed at least in part to poor psychological well-being (Appleton & McGowan, 2006) and preliminary studies have identified an association between restrained eating, depression, and weight gain (Polivy and Herman, 1976, Zielinski, 1978). In addition, emotional eating theory, i.e. eating in response to negative emotions, may be particularly applicable to depressive populations. Several investigations have studied negative affect in relation to emotional eating and weight disturbances (e.g. van Strien et al. 1985; van Strien et al., 2013) yet few have directly investigated emotional eating in depressed populations. In one exception, Ouwens and colleagues (2008) did find an association with self-reported depressive symptoms and emotional eating. They proposed that this association may be mediated by the decreased ability to identify emotions. Preliminary results of an investigation of
emotional eating in a clinically depressed sample in our centre found that patients reported higher levels of emotional eating in comparison to BMI-matched healthy controls (McKay et al., 2009). Thus, while appetite disturbances are one of the core symptoms of depression, it is likely that other factors such as poor eating habits and greater tendency to emotional eating, may also contribute to weight changes found in depression.

Altered monoamnergic (in particular 5HT and dopamine) neurotransmission, thought to underlie the pathophysiology of depression, may also play a role in hyperphagia and carbohydrate cravings (Wurtman and Wurtman, 1989). It is suggested that carbohydrates can relieve depressive symptoms and improve mood via increased serotonergic activity, particularly in those with atypical depression (Wurtman &Wurtman, 1989; Moller, 1992). Others have suggested that carbohydrate cravings found in depressed individuals may also be linked to the opioid system (Parker and Crawford, 2007). Furthermore, it has been proposed that foods commonly the object of cravings (e.g. chocolate) tend to activate the mesolimbic dopamine reward system similar to that of drugs of abuse, though not specifically in depressed populations (Parker et al., 2006).

1.1.2 Depressive Illness and Overweight Status

While there is a reasonable body of literature on the association between depression and weight gain, it is still unclear if the illness precedes weight gain or vice versa (for review see Faith et al., 2002; McElroy et al., 2004; Atlantis and Baker, 2008). The severity of the depression, the degree of obesity and gender, are thought to impact on this association, and stress reactivity, altered eating behavior, and level of physical activity may have specific influences (Stunkard, Faith, and Allison, 2003). As well, both weight loss and gain have been noted as occurring as a result of psychotropic medications used in the treatment of depression.
Interestingly, an association between overweight status and poor response to treatment has been reported, leading to further difficulties in understanding this inter-relationship (Papakostas et al., 2005; Kloiber et al., 2007).

Epidemiological studies have noted higher rates of depression in overweight and obese subjects compared to the general population (Wadden and Stunkard, 1987; Roberts et al., 2000; Dixon et al., 2003). However, these published studies also note the confounding effect of gender on this association. For example, a recent investigation based on North American samples, suggests that the risk of depression increases among women with a BMI greater than 30, and that abdominal obesity further adds to this risk, though being underweight may also be a vulnerability factor (Ma and Xiao, 2009). Another early study reported that obesity in women was associated with a 37% increase in MDD, whereas in males, obesity was associated with a 37% decrease in MDD (Carpenter et al. 2000). Increased BMI was found to be associated with both major depression and suicide ideation for females, but interestingly an opposite relationship was noted in males with decreased BMI being associated with depression, suicide ideation and attempts (Carpenter et al., 2000). A more recent investigation estimated that obese females had a 43% increase risk for depression compared with women in the normal weight range (Chen et al., 2009). Based on published finding, a review concluded that females with atypical depression may be at a particular risk of elevated body weight (McElroy et al., 2004). In spite of these findings from individual studies, recent meta-analyses of epidemiological studies have concluded that while there an association exists between obesity and major depression, there is insufficient evidence to support a causal relationship. This is attributed, at least in part, to lack of studies that employed rigorous methodology (Atlantis and Baker, 2008). However, based on the above-mentioned investigations, it is clear that gender is a key factor influencing the relationship between obesity and depression.
The inverse, i.e. that MDD is a risk factor rather than a consequence of weight change, has also been proposed. For example, a direct correlation between MDD severity and BMI variability was reported in a cohort study spanning 20 years, and noted that in the short to medium term depression was associated with weight change, i.e. in 2-5 years of episode rather than over the long-term (Hasler et al 2005). In another study that followed treatment-naive, lean subjects with depression over a four-year period, an upward shift in BMI was noted over time, suggesting a significant risk of weight increase for this population (Taylor et al., 2008).

Furthermore, females who experienced depression in late adolescence were found to be at greater risk for obesity in adulthood, with multiple depressive episodes experienced in adolescence increasing this risk (Richardson et al., 2003).

Several investigations have suggested that weight status may influence treatment response, and those who have higher body weight or BMI are less likely to benefit from antidepressants. For example, depressed individuals classified as overweight, but not obese, were reported to be less responsive to fluoxetine compared to those of normal body weight, in spite of equivalent symptom severity (Papakostas et al., 2005). Similarly, Kloiber and colleagues (2007) reported that MDD patients with high BMI showed less overall improvement compared to those with low BMI following antidepressant treatment. The former group also showed greater HPA axis dysregulation and less improvement in cognitive performance with treatment (Kloiber et al., 2007). The observation that this association between obesity and poor antidepressant response is supported by at least one report that found that obese men are the group least likely to respond to treatment with SSRIs (Khan et al., 2007).

Gender differences in weight gain following antidepressant therapy have also been reported. While weight gain in females during treatment was associated with better response, the
reverse was seen in males where it was associated with lack of symptom resolution (Ravindran et al., 1997).

While there are many reports confirming the significant occurrence of body weight changes in depression, there are inconsistencies in the detailed findings. These are at least in part due to methodological issues, including failure to control for variables with the potential to influence the trajectory of either condition. Further elucidation of the biological underpinnings of eating behavior in depressed and normal populations, and in particular, evaluation of HPA axis activity and the influence of “eating hormones” like leptin and ghrelin, may provide useful information to better understand this complex relationship.

1.2 Stress, Negative Affect, and Eating Behaviour

Altered eating behavior (either increased or decreased) in response to stress or triggered by negative affect is so common that terms such as “stress eating” or “emotional eating” are part of popular vernacular. This section provides a brief review of literature of the relationship between stress, negative affect and eating behavior, and the role of HPA axis activity on this relationship.

Evidence suggests that the majority of individuals alter food or caloric intake when experiencing stress or negative affect, with approximately 40% reducing food intake, while 40% increase food consumption (Gibson, 2006; Torres and Nowson, 2007). As previously noted, emotional eating, i.e., eating in response to emotional arousal states such as anger, fear, or anxiety (van Strien, 2002), has been linked to overeating and subsequent body weight increase (van Strien et al., 2009).
Several laboratory investigations have confirmed that stress and negative affect often induce greater food intake, particularly for palatable food high in either sugar or fat (Oliver et al., 2000; Epel et al., 2001; Zellner et al. 2007, Wallis and Hetherington, 2009). In addition, naturalistic investigations have shown that many individuals, during periods of stress, shift from healthy to less healthy eating patterns by increased intake of more palatable foods such as carbohydrates (O’Connor et al., 2008; Newman et al., 2007).

Although the terms “stress-induced eating” and “emotional eating” are at times used to describe distinct but overlapping behaviours, it is likely that it is the latter that mediates the relationship between stress and increased eating, as a response to negative affect (Oliver et al., 2000; van Strien et al., 2005; Yeoman and Coughlan, 2009). Recent investigations have noted that in response to a psychosocial stressor, participants identified as high emotional eaters consumed a greater amount of food post stressor in comparison to low emotional eaters (van Strien et al., 2012). Indeed, emotions are included as a key factor in neurophysiological models of obesogenic eating behaviours (e.g. Dallman, 2009).

As previously noted, emotional eating is likely a common phenomenon in depressed populations who endorse greater levels of such behaviour in comparison to BMI-matched healthy controls (McKay et al., 2009). As such, it is potentially an important, yet not well studied, contributor to weight disturbances in depressed populations.

1.2.1 HPA axis response to stress in association with food consumption

Several investigations have explored HPA axis reactivity in association with stress induced food consumption in both naturalistic and laboratory settings (Newman et al., 2007; Epel et al., 2001). In a laboratory investigation, Epel et al. (2001) measured stress reactivity and
post stress caloric intake in premenopausal women following stress induction and after a day of rest. Results indicated that participants who were high cortisol reactors (i.e., exhibited increased cortisol secretion in response to acute stressor) consumed more food than low cortisol reactors post stress induction. In the comparison condition, a day of rest, high cortisol reactors consumed less calories than low cortisol reactors, however, it was reported that high cortisol reactors tended to eat more sweet foods in both experimental conditions. Further, self-report ratings of negative affect were positively correlated with food consumption on the day of the stress induction, while no such association was found following the day of rest. The authors suggest that psychophysiological response to stress may contribute to subsequent obesogenic eating behavior. In another study, Raspapow et al., (2010) noted that individuals high in emotional eating exhibited a greater change in cortisol in response to a psychosocial stressor in comparison to low emotional eaters, though baseline levels of cortisol were lower in emotional eaters than non-emotional eaters. Further, in naturalistic investigations, a positive association between the amount of daily hassles and snack intake was noted only for participants who also exhibited a higher cortisol reactivity to said daily stressors, with no such association in amount of snack intake noted for low cortisol reactors (Newman et al., 2007).

Studies have also examined the influence of stress on cortisol secretory patterns in response to meal consumption and have noted alterations in cortisol response that have been linked with psychological and behavioral characteristics such as stress and anxiety. For example, an investigation of postprandial cortisol levels have indicated that obese individuals with greater subjective reports of stress and anxiety exhibited less of a decline in cortisol levels in response to meal consumption in comparison to participants with lower anxiety and stress (Sarker et al., 2013).
Investigations that evaluated the relationship between HPA axis activity and food intake have reported inconsistent findings. While some have noted a direct correlation (Epel et al., 2001), others found HPA hypoactivity to be associated with increased food and caloric intake. The data may be more applicable when stress is experienced for longer periods of time, in other words, when stress becomes chronic (Dallman et al., 2010).

Findings from animal investigations have led to several key findings that link HPA axis activity, chronic stress, glucocorticoid changes, and food intake. In brief, sustained treatment with glucocorticoids, as a way to mimic chronic overactivity in HPA axis in the absence of concurrent stress, both basal and acutely stimulated activity in the HPA axis were inhibited (Akana et al., 1985), suggesting that persistent activation of HPA axis decreases future diurnal and short term HPA activity. Further, animals exposed to chronic stress and given high fat or sugar food demonstrate an attenuated stress response (reduced CRH expression and ACTH secretion) compared to those given regular, less palatable food (Pecoraro et al., 2004; Foster et al., 2009). In addition, it has been suggested that palatable food, glucocorticoids and insulin influence the regulation of CRH mRNA expression in the paraventricular nucleus of the hypothalamus. This in turns impacts on ACTH secretion and glucocorticoid responses to stress, and subsequent abdominal obesity (Warne et al., 2009). Furthermore, it has been proposed that a signal from adipose tissue, that is not yet fully understood, likely mediates the feedback to HPA axis activity following chronic stress (Dallman et al., 2005; Warne et al., 2009).

Based on a series of animal investigations, Dallman et al. (2006) concluded that HPA responses to novel stimuli in the presence of concurrent stress were sensitized only when the tonic mean concentration of glucocorticoids was elevated above the normal daily mean value. This sensitization is suggested to be a consequence of the recruitment of a “chronic stress network” that requires elevated corticoids and is likely initiated by increased limbic CRF
activity. This network is proposed to involve effect on several brain regions, including the amygdala, mesolimbic dopaminergic system, prefrontal cortex and hippocampus, to stimulate feeding, learning, and memory (Dallman et al., 2006; 2010). This model serves to explain the role of several other key brain regions, beyond the hypothalamus, to the initiation (to relieve distress) and maintenance (reward association, learning, and memory) of stress-induced eating and increased body weight.

While most of the evidence for the above model comes from animal investigations, data from a small number of studies in humans provides some support. For example, Tomiyama and colleagues (2011) examined the impact of chronic stress and body weight on HPA axis activity, and eating behaviours and compared female caregivers of children who were chronically ill to caregivers of healthy children. Women with high levels of perceived stress had greater amount of emotional eating, abdominal obesity, and lower cortisol response to a psychosocial stressor and a physiological challenge (the dexamethasone test [DST]); while the women reporting low stress did not exhibit the association between abdominal obesity and HPA axis functioning. The findings were suggested as evidence of long-term adaption to chronic stress exposure in humans, similar to that seen in animal studies. Van Strien et al. (2013) tested the above suggestion in female participants using the Trier Social Stress Test (TSST). Individuals were categorized as either high or low emotional eaters and exposed to the TSST and a control activity devoid of stress, both followed by a period of ad lib eating. High emotional eaters who demonstrated a blunted cortisol reaction to the stress induction consumed the greatest amount of calories following the stressor, in comparison to the control activity. This group also ate more food compared to similarly high emotional eaters but with high cortisol reactivity. These findings were suggested as supporting the findings from animal models (e.g. Dallman et al., 2010) of the
relationship between blunted cortisol response and increased stress-induced food intake in humans.

Thus, an increase in perceived stress, physiological response to stress, and emotional eating all appear to contribute to stress-induced eating, which may in turn lead to increased body weight, possibly more so for those who experience chronic stress. The above findings may be particularly relevant to depressed patients, who frequently experience a chronic or recurrent course of illness.

1.3 Depressive Illness and Stress

The relationship between stress and depression has long been recognized, and indeed, some suggest that depression itself is better classified as a stress disorder (Anisman, 2008). As well, depressed individuals may be particularly vulnerable to emotional eating and to altered perceptions of and physiological responses to stress, further promoting weight changes. This section will summarize the large body of literature documenting this association, with an emphasis on the physiological response to stress in depressed populations.

Perceived stress is an accepted risk factor for depression. Stressful life events have been shown to precipitate the onset of a depressive episode, with 50-80% of depressed individuals experiencing a major life event in 3-6 months prior to the onset of the episode (Paykel et al., 1978). As well, perceived daily occurrence of minor irritating events are said to contribute to the perpetuation of depressed mood. Such irritating events, also referred to as hassles, are said to contribute to negative affect and distorted perceptions in patients with depression (van Eck et al., 1998). Depressed individuals not only report fewer positive daily events, but interestingly, also appraise both positive and negative events as stressful. Furthermore, a significant relationship
between the number of such stressors with depression severity and treatment resistance has been noted (Peeters et al., 2003, Ravindran et al., 2002, Bockting et al., 2006).

1.3.1 Depressive Disorders and HPA axis activity

Altered activity of the HPA axis in individuals with major depressive disorder has been well documented since the 1950’s (Board et al., 1957). Reports of such alterations on several measures of HPA axis activity have been reported, and include the following: (i) Elevated total and free cortisol concentrations in urine (Carroll et al., 1976a), in plasma (Gibbons & McHugh, 1963; Carroll et al., 1976b) and in cerebrospinal fluid (Carroll et al., 1976b); (ii) Earlier and greater cortisol nadir (Halbreich et al., 1985; Jarrett et al., 1983); (iii) Both elevated levels and blunted awakening cortisol (Huber et al., 2006); (iv) Elevated concentration of cerebrospinal fluid CRF (Nemeroff et al., 1984); (v) nonsuppression in response to dexamethasone challenge or dexamethasone test (DST) (Carroll et al., 1981); (vi) increased cortisol response to ACTH test (Amsterdam et al., 1983); and blunted ACTH response to CRH test (Gold et al., 1984; Holsboer et al., 1984). In addition, other related findings reported include adrenal gland enlargement (Nemeroff et al., 1992) and an increased number of CRH and arginine vasopressin neurons in paraventricular nucleus (Raadsheer et al., 1994; Purba et al., 1996). It has also been reported that persistent dexamethasone and/or CRH non-suppression may be a marker for vulnerability for relapse in depression (Zobel et al., 1999).

Increased levels of cortisol, referred to as hypercortisolemia, are a consistent finding in major depression during the acute episode (e.g. Carroll et al., 2007). It has been proposed that such HPA axis overactivity, as reflected by high levels of cortisol, is mediated by an increased secretion of CRH. Acute depression appears to be associated with a failure of the internal negative feedback system, which would normally suppress the secretion of CRH (see Claes,
2004 for review). However, individuals with chronic depression have been reported to show alterations in the stress responsivity that are variable and distinct from that seen in acute depression. For example, chronically depressed patients did not differ from control participants in cortisol response to dex/CRH challenge (Oshima et al., 2000) or the DST (Watson et al., 2002), or in response to hormone challenges (Shah et al., 1998). Others have reported reduced cortisol levels in elderly long-term depressed populations (Oldehinkel et al., 2001). Furthermore, O’Keane and colleagues (2005) found increased ACTH response to CRH challenge in chronically depressed patients, which is in contrast to findings in acute melancholic depression.

One proposed explanation for such contradictory findings is that clinical depression is a heterogeneous illness made up of distinct subtypes but with overlapping symptoms. For example, studies investigating ACTH and cortisol dynamics in depressed in-patients compared to normal volunteers found that hypercortisolemia was strongly associated with melancholic and psychotic depressive subtypes (Carroll et al., 2007). In a review of the literature, Gold and Chrousos (2002) concluded that while melancholic depression was associated with overactivity of the HPA axis and hypersecretion of CRH, subject with atypical depression exhibited decreased CRH and down-regulated HPA axis. It was suggested that this pathophysiological difference may contribute to the difference in symptom presentation of the two subtypes. Similar suppression of the HPA axis has been reported in females with atypical depression (Levitan et al., 2002) and in patients with early onset, chronic depression with atypical features (Stewart et al., 2005). However, not all investigations support such a difference in HPA axis activity among subtypes of depression, and have reported conflicting findings. For example, Young et al., (2001) examined pulses and amplitude of cortisol and ACTH in premenopausal women. Regardless of depressive subtype, only 24% of the depressed samples displayed evidence of hypercortisolemia. Further, only individuals with melancholic subtype had cortisol
levels that were higher than control participants, while cortisol levels of those characterized as having atypical depression were similar to controls. Another large investigation of both currently depressed and remitted individuals found that both groups exhibited similar elevated cortisol secretion (Vreeburg et al., 2009). In addition, the atypical and melancholic subtypes did not differ in their cortisol levels, and both groups had elevated levels in comparison to control participants.

A recent investigation by Lamer and colleagues (2013) further evaluated HPA activity as well as several inflammatory markers in subtypes of depression. They reported that patients categorized as having melancholic depression displayed hyperactivity of the HPA axis as evidenced in greater area under the curve for cortisol awakening response. In contrast, participants with atypical depression had lower diurnal cortisol slope, and in addition displayed metabolic disturbances and inflammation that were distinct from the melancholic subtype. Specifically, atypically depressed patients exhibited elevated levels of IL-6, C-reactive protein, and TNF-alpha in comparison to both melancholic depressed patients and controls.

In a recent review of the literature, O’Keane and colleagues (2013) have proposed a further elaboration to the current understanding of the pathophysiology of HPA axis dysfunction in depression that may explain the above inconsistent findings in subtypes of depression. It is suggested that when depression follows a chronic course, a switch occurs in the regulation of the HPA system from CRH to arginine vasopressin (AVP) control. This is said to result in an altered homeostasis in the HPA system, resulting in physiological changes that may explain at least in part the changing profile of depression over time (O’Keane et al., 2013).

In spite of extensive investigations, several facets of the complex relationship between stress and depression need further elucidation. There is increasing evidence that many of the
contradictory findings reported in biological studies are due to the heterogeneous nature of the illness. Such heterogeneity is contributed to by the inclusion of subtypes with distinct pathophysiology and time course/chronicity, as well as influenced by the common presence of comorbidities.

1.4 Ghrelin

Ghrelin is a hormone with a 28-amino-acid peptide and whose main function is thought to be to stimulate growth hormone (GH) and to increase appetite and food intake. However, in addition it has been proposed to have several other physiological effects. This section will review the literature on ghrelin in relation to appetitive behaviours, disordered eating and depressive illness.

Ghrelin is mainly produced in gastrointestinal structures (GI), and in particular the stomach, but ghrelin-producing cells have also been found in several GI regions including the duodendum (Kojima et al., 1999). Extra GI ghrelin producing cells are found in organs including the kidney, placenta, and the arcuate nucleus of the hypothalamus, albeit in small quantities. Neurons containing ghrelin send efferent fibers to neurons containing NPY and AGRP, which are thought to control appetite (Kojima and Kangawa, 2006). There is a growing body of literature on the role of ghrelin in multiple physiological functions, including the control of ACTH and prolactin secretion, as well as glucose and lipid metabolism, gastric motility and acid secretion. Furthermore, it has been proposed to have an effect on cardiac function, as well as on sleep and reproductive functions (Valassi et al., 2008).
1.4.1 Role of Ghrelin in appetitive behaviour

Several studies have evaluated the relationship between ghrelin activity and appetite (e.g. Tolle et al., 2003; Tschop et al., 2000). Ghrelin increases by two-fold before a meal and rapidly decreases postprandial (Tschop et al., 2000). Studies evaluating plasma ghrelin and changes in body weight found higher levels in patients with reduced appetite and anorexia, while low levels were reported in obese populations (Tolle et al., 2003; English et al., 2003). Interestingly,
studies report that ghrelin administration may restore normal hunger patterns in both these populations (Huda et al., 2009; Hotta et al., 2009) and furthermore, that food-related environmental cues, such as visual presentation of food, elevate ghrelin levels in healthy participants (Schussler et al., 2012).

The physiological mechanisms by which ghrelin influences appetite and feeding behavior is thought to be mediated centrally through the ARC by the enhancement of NPY/AGRP pathways and the inhibition of POMC neurons, as well as peripherally via blood and vagus nerve (Kojima and Kangawa, 2006). Animal and human investigations have suggested that the effects of ghrelin may also be mediated by its effect on the HPA axis of stimulating the production of CRH and ACTH (Wren et al., 2002; Schmid et al., 2005). Additionally, ghrelin targets cells in the mesolimbic reward circuitry, specifically the ventral tegmental area, that modulate activity of dopamnergic neurons, and it is thought to further increase appetitive behaviours by this mechanism (Abizaid et al., 2006; Abizaid, 2009).

1.4.2 Experimental Studies of Ghrelin Administration

Due to the putative role of ghrelin on energy homeostasis, several investigations have measured endocrine response to ghrelin administration. Ghrelin has been shown to strongly stimulate growth hormone release (Peino et al., 2000; Nagaya et al., 2001; Micic et al., 2002; Broglio et al., 2003; Akamizu et al., 2004), as well as that of several other neurohormones, including prolactin, CRH, ACTH and cortisol (Broglio et al., 2003; Schmid et al., 2005; Vestergaard et al., 2007). Reported effects of ghrelin administration on carbohydrate metabolism in healthy populations include increased glucose levels (Vestergaard et al., 2007) and reduction in insulin levels (Broglio et al., 2003).
The finding from preclinical studies that ghrelin consistently increases hunger, food intake, and adiposity (e.g. Tschop, et al., 2000), led to evaluation of its effects on appetite and food intake in healthy human subjects. For example, Wren and colleagues (2001), in an infusion study, found that both appetite and food intake increased following ghrelin infusion in comparison to placebo (saline infusion) in healthy subjects. In addition to an increase in subjective hunger ratings, a significant increase (28%) in energy intake for a single meal was noted after the infusion of ghrelin compared to that of saline. In subsequent investigations of subjects with extremes of weight, low dose ghrelin infusion was found to increase caloric intake in obese participants only, while higher doses increased food intake in both lean and obese subjects (Druce et al., 2005). Interestingly, only the higher dose of infusion increased hunger ratings, while low dose of ghrelin did not impact subjective ratings of appetite or hunger in either group.

There are also reports of single injection studies of ghrelin in healthy subjects. For example, a single injection of ghrelin was found to increase appetite and stimulate the production of hormones related to HPA axis that included GH, ACTH and cortisol, compared to placebo (Schmid et al., 2005).

### 1.4.3 Ghrelin and Altered Eating Behaviour

Following the delineation of its physiological role in appetite and hunger, ghrelin has been investigated in populations with disordered eating. A recent systematic review and meta-analysis of data of GI hormones of eating disordered populations reports that the largest effect size of differences between the eating disorders (anorexia and bulimia) and healthy populations
was that of ghrelin levels, with patients having higher baseline levels of ghrelin (Prince et al., 2009). There are several other reports on the relationship between ghrelin levels and those with disordered eating. For example, Geliebter and colleagues (2005) measured plasma concentrations of key gut peptides in obese patients with binge eating disorder and found them to have lower plasma concentrations of ghrelin before a meal, and less decline in these levels post meal, compared to norms. No differences in other satiety peptides were found, suggesting that ghrelin may play a key role in the manifestation of binge eating symptoms (Geliebter, Hashim, Gluck, 2009). Furthermore, there are several other reports of ghrelin administration restoring normal hunger patterns in both anorexic and obese populations (Huda et al., 2009; Hotta et al., 2009).

Laboratory investigations have studied the impact of stress on ghrelin in populations vulnerable to weight gain and obesity, including subjects with binge eating disorder, as well as those individuals categorized as emotional eaters. Consistent with previous findings, basal ghrelin levels were significantly higher in normal weight participants in comparison to obese subjects, while levels of those with binge eating disorder were at an intermediate level with no significant difference from the other two groups (Rauch et al., 2007). Across all participant groups, ghrelin levels were elevated only in subjects who had a cortisol response, and a direct correlation was noted between anxiety and stress scores and ghrelin levels. In a similar experiment Raspapow et al., (2010) evaluated the relationship between cortisol response to a social stressor and plasma ghrelin levels in a population of emotional eaters. They report that emotional eaters had a higher cortisol response to a social stressor compared to non-emotional eaters. In addition, while post-meal ghrelin levels remained stable in emotional eaters, non-emotional eaters exhibited a significant decline in ghrelin levels after food ingestion. Furthermore, in a recent investigation, associations between postprandial cortisol and ghrelin
levels were examined in obese women (Sarker et al., 2013). Women whose ghrelin did not
decrease following meal consumption also did not experience a corresponding drop in cortisol,
and despite feeling more satiated reported greater levels of subjective feelings of anxiety and
stress. In contrast, women with normal ghrelin signaling not only experienced a decline in
cortisol following a meal but also experienced a significant improvement in acute subjective
feelings of satiety, anxiety, and stress. The findings of these studies strongly support a
physiological relationship between cortisol stress response and ghrelin activity, providing further
evidence for the notion that the HPA axis and ghrelin interact in the control of hunger and satiety
in humans.

1.4.4 Depressive Illness and Ghrelin

Studies evaluating ghrelin levels and secretion patterns in psychiatric populations are
relatively few, though most of them included patients with depression. In one investigation,
Schanze and colleagues (2008) measured ghrelin levels and eating behaviour in populations with
either schizophrenia or depression compared to controls. No differences in ghrelin levels were
reported among the groups, despite significant differences in weight and BMI. However, in the
whole sample, ghrelin levels, when corrected for BMI differences, predicted higher disinhibition
subscales scores on the Three-Factor Eating Questionnaire, an eating behaviour measure.
Himmerich and colleagues (2005) also reported no differences in ghrelin levels between different
psychiatric groups, including MDD, but did note that ghrelin levels were lower in patients who
reported no changes in food intake or appetite compared to those who reported an alteration
(either an increase or decrease) in either physiological function.
Several other studies investigating the difference in ghrelin secretion and levels between depressed patients and controls have come up with contradictory findings. Kluge et al. (2009) found nocturnal secretion patterns of ghrelin to be similar between patients and healthy controls, though an analysis of the total sample revealed that females overall had higher levels of ghrelin than males. However, this sample of depressed patients included only those in the normal weight range and in fact, the majority of patients endorsed decrease in appetite. While Kurt et al. (2007) reported greater pre-treatment ghrelin levels in depressed subjects compared to controls, others have reported them to be lower. Barim et al. (2009) reported lower levels of ghrelin in patients than controls both before and after treatment with citalopram, and this was in spite of an observed BMI increase in patients following treatment. Interestingly, while lower levels of ghrelin tend to be found in overweight populations, Barim and colleagues (2009) found levels to be decreased in depressed patients with normal BMI in comparison to the control sample. The latter had significantly higher BMIs, and the authors suggest that reduction in ghrelin may have a pathophysiological role in depressive illness possibly related to its antioxidant properties. In this context, it is of note that significant differences in ghrelin gene Leu72Met variants between patients with major depression and healthy controls have been reported, suggesting that Leu72Met polymorphism may be associated with depressive disorder (Nakashima et al., 2008).

### 1.4.5 Impact of interventions on ghrelin secretion and levels

Investigations evaluating the impact of pharmacological and other treatments on ghrelin levels have reported contradictory findings. For example, Himmerich and colleagues (2005) found that individuals taking medications known to induce weight gain, such as mirtazapine and atypical antipsychotics, did not exhibit significant differences in ghrelin levels compared to those receiving other psychotropics. Even more interestingly, they did not find ghrelin levels to be
significantly different between those who gained weight during treatment vs. those who did not (Himmerich et al., 2005). In contrast, Pinar and colleagues (2008) have reported that depressed patients receiving a tetracyclic antidepressant (maprotiline) exhibited significant weight gain accompanied by a parallel increase in total ghrelin levels after only 30 days of treatment. Notably, the overall study sample was lean with an average BMI of 19.47, which increased significantly to 20.94 in the 30 days. Weight gain is a common and well-documented side effect with the atypical antipsychotic, olanzapine (Allison et al., 1999; Zipursky et al., 2005). In a longer-term investigation, Murashita and colleagues (2008) noted a significant increase in serum ghrelin and leptin levels, as well as body fat percentage over 6 months of treatment with this agent. In yet more contrary findings, serum ghrelin levels were reported to decrease significantly in patients with both unipolar and bipolar depression after treatment with ECT; however, BMI, as well as leptin levels, remained stable before and after treatment (Kurt et al., 2008). Treatment with citalopram has also been reported to result in decreased in ghrelin levels (Barim et al., 2009). Furthermore, Schmid and colleagues (2005) found that after 4 weeks of treatment with mirtazapine, (an antidepressant known in induce weight gain), ghrelin and cortisol levels were found to decrease in depressed patients, while leptin and melatonin levels were increased.

While it is accepted that the above investigations provide evidence of altered levels and secretion of ghrelin in populations with depression, the direction of the findings are inconsistent. Such differences are often attributed to the failure to control for potential confounding variables including body weight, gender, and particular subtypes of depression with distinct prolonged neurovegetative symptom profiles (e.g. appetite and weight changes). To our knowledge, there are no published studies of ghrelin levels and or secretion that have categorized depressed patients based on the direction of such prolonged changes.
1.5 Leptin

Leptin is a hormone peptide and member of the adipokine family that was first described in 1994 (Zhang et al., 1994). Its discovery was considered to be one of the most significant in obesity research in the past two decades (Trayhurn, 2013). This section will summarize the literature on the physiology of leptin, as well as its association with obesity, depression and altered HPA axis activity.

1.5.1 Physiological and anatomical connections

Leptin is secreted into the bloodstream from white adipose cells and its main target organ is the brain and most notably, the hypothalamus. The main function of leptin is to regulate food intake and metabolism (Denver et al., 2011). Leptin serves to communicate both short and long-term energy homeostatic information bidirectionally between the brain and the rest of the body, primarily through plasma concentrations (Ahima 2005; Chehab et al., 1997) and is secreted in direct proportion to fat stores (Friedman, 1998). Initially leptin was thought to be a satiety factor due to its capacity to suppress appetite, and low levels evidenced in obese populations. However, more recently it has been conceptualized as a “signaling factor” that alerts the CNS as to the amount of adipose storage in the body, rather than as a satiety signal per se (Jequier et al., 2002). Such notion was based on the observation that leptin expression increases directly with body mass. The observation that starvation leads to a rapid decrease in serum leptin level prior to the depletion of adipose tissue mass, and has led to the suggestion that decrease in leptin may serve to activate the body’s starvation response processes. While its primary role is in energy homeostasis, leptin has also been shown to influence several other physiological functions,
including thermogenesis, reproduction, and immunity, among others (Steiner and Romanofvsky, 2007).

It is of particular note that leptin receptors are expressed in numerous regions of the brain including the amygdala, hippocampus, cerebellum, medulla, neocortex, and basal ganglia (Harvey, 2007; Paz-Filho et al., 2010; Zupancic and Mahajan, 2011). Leptin receptor expression is highest in neurons in the nuclei of the basomedial hypothalamus including the ARC, dorsomedial hypothalamic nuclei and ventromedial hypothalamic nuclei (Elmquist et al., 1998). Leptin acts on POMC and CART neurons in the ARC to increase the expression of both, which produces an anorectic signal via alpha-MSH. It also acts on NPY/AgRG neurons to inhibit expression of their orexigenic signals. Second order neurons located in paraventricular nucleus that synthesize TRH or CRH are regulated indirectly by leptin targets in the ARC, which mediates leptin’s inhibitory actions on food intake (Freidman, 1998).

1.5.2 Leptin and Obesity

The ob gene encodes the protein leptin which is produced and secreted by the white adipose tissue and as previously noted, its circulating levels are closely related to body fat mass (Tartaglia et al., 1995). Obese individuals consistently exhibit elevated serum leptin levels (Schwartz et al., 1996). Experimental administration of leptin has been shown to normalize several metabolic alterations anomalies seen in lipodystrophic patients, including hyperglycemia and dyslipidemia, and thus may reverse the obese phenotype of leptin-deficiency (Friedman, 2010). Recently, the term “leptin resistance” has been used to describe the inability of obese individuals and high fat-fed animals to respond to either endogenous or exogeneous leptin. It is thought to co-occur in many individuals with obesity and insulin resistance (Friedman, 2010; Myers et al., 2010). Although the exact mechanisms by which leptin resistance develops is yet
to be elucidated, alterations in the central system has been proposed to result from intracellular alterations in the ARC, hypothalamus and related regions of the brain (Muzenberg and Myer, 2005). On the other hand, a peripheral subtype of leptin resistance has been attributed to several molecular and intracellular mechanisms (Konner and Bruning, 2012).

1.5.3 Leptin and Reward Mechanisms in the Brain

Leptin receptors are partially well expressed in brain regions associated with reward and motivation. Such receptors have been identified in the structural components of the mesolimbic dopamine system including the VTA and substantia nigra (Figlewicz et al., 2003), suggesting a link between the reward circuitry and leptin signaling (Fulton et al., 2006). Although animal experiments have reported leptin administration to attenuate dopaminergic neuronal activity (Hommel et al., 2006), such effect may differ between acute and chronic leptin exposure in the VTA (DiLeone et al., 2009).

Recent human neuroimaging studies have added further information on the target effect of leptin. Grosshans, et al. (2012) recently reported a significant positive correlation between plasma leptin and fMRI blood oxygen level derived (BOLD) response in ventral striatal regions in response to visual food cues (Grosshans et al., 2012). In addition, individuals with genetic leptin deficiency have been noted to display abnormally high activity in the nucleus accumbens in response to visual food stimuli, with such response normalizing with leptin administration (Farooqi et al., 2007). Further, PET investigations have shown positive associations between circulating leptin levels and dopamine release in response to a pain stressor (Burghart et al., 2012). The authors concluded that leptin regulates nucleus accumbens responsivity to salient stimuli in humans and that this hormone is important for motivational drives related to hunger that may be additionally influenced by stress (Burghart et al., 2012)
1.5.4 Leptin and HPA Axis interaction

The last two decades have seen several reports on the interaction between the HPA axis and leptin. However, most of these reports were derived from animal studies (Ahima et al., 2000; Roubos et al., 2012), with relatively fewer investigations with human subjects (Malendowicz et al., 2008).

In brief, animal studies have shown that leptin interacts with various components along the HPA axis. Leptin and its receptors are expressed in the central region of the HPA axis and modulate CRH and ACTH (Malendowicz et al., 2008). Evidence also indicates that leptin, under acute conditions, stimulates hypothalamic CRH biosynthesis (Costa et al., 1997), though some studies suggest an inhibitory effect in different rodent species (Jang et al., 2000). Another effect of leptin is to influence the basal levels of ACTH and corticosterone (Nowak et al., 2002). In ob/ob mice, leptin deficiency has been associated with elevated corticosterone, which normalized with leptin administration (Ahima et al., 2005). Based on this body of evidence, a negative feedback loop has been proposed between leptin and HPA axis where increases in ACTH blood level raise leptin, which in turn inhibits corticosterone secretion (Spinedi and Gaillard, 1998). Leptin has also been noted to dampen the HPA axis response to a variety of stressors, but the reports have had some contradictory findings (Ahima et al., 2005; Wilson et al., 2005; Heiman et al., 1997). For example, injection of leptin in response to stress has been shown to moderately magnify ACTH response but not corticosterone (Hochol et al., 2000). While the animal models have demonstrated a clear association between leptin and HPA axis activity, the exact molecular mechanisms that mediate or influence this connection remain unclear.

Several other studies have evaluated patterns of leptin secretion relative to HPA axis hormones in healthy human subjects, but have come up with some conflicting findings. For
example, Licino and colleagues (1997) found inverse patterns in circulating leptin vs. ACTH and cortisol in healthy participants in a 24hr period (Licino et al., 1997), while Miell et al. (1996) reported that cortisol or dexamethasone administration resulted in an acute sustained increase in leptin concentration. On the other hand, there are reports of cortisol not influencing circulating levels of leptin (Nye et al., 2000). In addition, it has been observed that glucocorticoid administration resulted in increased energy intake despite elevated leptin levels (Tatarrani et al., 1997). These findings, along with the notion that cortisol may be involved in the accumulation of body fat, has led to the suggestion that cortisol may contribute to the onset of leptin resistance through physiological mechanisms yet to be determined (Bjorntorp, 2001).

The relationship between circulating leptin levels and stress reactivity in humans has also been investigated. For example, Brydon (2011) found that women with a larger waist circumference displayed greater stress-induced increases in plasma leptin, as well as interleukin 1RA. The authors also noted that women with elevated basal leptin also had greater stress-induced increases in IL-6. In addition, a significant association between basal plasma leptin levels and cardiovascular response to stress was noted, with higher leptin levels linked to greater increases in heart rate and a proportionate decrease in heart rate variability during the stress experience (Brydon, 2008; 2011). In another recent report, a modified version of the Trier social stress test was used to study leptin response and food intake in response to a psychosocial stressor (Tomiyama et al., 2012). Higher levels of leptin in response to the stressor were associated with lower consumption of food with high fat and sugar content post stressor. However, there was no correlation noted between plasma leptin and cortisol levels (Tomiyama et al., 2012).
1.5.5 Leptin and Depressive Disorders

As energy and appetite disturbances are core symptoms of depressive disorders, it is not surprising that investigations have focused on physiology of both appetite regulation and energy homeostasis in this population. A proposed corollary is that leptin, a key hormone in the physiology of appetite regulation, may mediate symptoms of depression and contribute to the pathophysiology of depressive illness (Lu, 2007).

Although there are significant number of reports investigating leptin changes in depressed populations compared to normal controls, they have had variable findings, with some noting an increase in patients (Antonijevic et al, 1998), and others a decrease (Kraus et al., 2001) or no difference from controls (Deuschle et al., 1996). Since leptin levels are highly correlated with body mass index, it has been suggested that such inconsistencies may be the result of bidirectional weight disturbances that commonly occur in depression. However, more recent studies noted that even depressed patients who were in the normal BMI range had lower fasting levels of leptin compared to healthy controls (Kraus et al., 2001; Yang et al., 2007; Lawson et al., 2012). In addition, others have noted increased serum leptin levels in depressed patients with atypical features, but with normal BMI, in comparison to typically depressed and control participants (Gecici et al 2005).

There is also significant evidence that gender influences leptin changes in both healthy and depressed populations. For example, females have been reported to have higher leptin levels compared to males in both control and depressed groups (Yang et al., 2007). Studies examining diurnal leptin levels have noted higher levels at all time points over 24 hours in women with depression compared to normal controls (Cizza et al., 2010). In contradiction, at least one study
Several investigations report that increasing severity of depression may be associated with higher leptin levels in plasma, but that weight may have a confounding effect on this relationship (Esel et al., 2005; Lawson et al., 2007). Morris and colleagues (2012) have proposed that high adiposity may mediate the relationship between depression severity and leptin levels. They noted that among depressed patients of normal weight, those with moderate to severe symptoms had lower leptin levels compared to those with mild symptoms. However, among the overweight or obese subjects, those with moderate to severe symptoms had higher levels of leptin compared to those with mild depression (Morris et al., 2012). Another investigation has reported increased leptin levels in female patients with a history of depressive episodes, and interestingly, an association between higher leptin levels and an increased risk of depressive relapse, suggesting a role as a possible “trait” marker (Pasco et al., 2008).

There are several reports on the three-way relationship between depression, HPA activity and leptin’s physiological effects. Early studies reported that increased ACTH and cortisol levels in response to AVP administration significantly suppressed leptin levels in depressed women, but not in depressed males (Rubin et al., 2002). More recently, another study reported inverse relationships between leptin on one side and ACTH and cortisol on the other, in female depressed patients but not in depressed men (Cizza et al., 2010). Similarly, in response to AVP administration, this suppression in leptin levels was positively correlated with somatization symptoms as measured by the HAMD (Rubin et al., 2002).

Several studies have investigated the impact of antidepressant treatment on leptin levels but have come up with inconsistent findings. Some have noted increased leptin levels in both
male and female patients with depression after treatment with various antidepressants (Esel et al., 2005). In other studies, while antidepressants such as mirtazapine produced a modest increase in leptin in depressed patients (Kraus et al., 2002; Schilling et al., 2013), treatment with paroxetine had no such effect (Hinze-Selch et al., 2000; Schilling et al., 2013). However, Schilling and colleagues (2013) also found that there were disproportionate increased leptin levels in subjects who remitted after treatment with amitriptyline and mirtazapine that could not be accounted for by other factors. These authors suggested that leptin resistance is potentially responsible, and possibly linked to antihistaminergic activity of these antidepressants. Further, Himmerich and colleagues (2007) reported that while leptin levels alone did not change from pre to post antidepressant treatment, those patients who exhibited a normalization of HPA functioning also displayed increase in leptin levels. In addition, a decrease in leptin levels was noted in those whose HPA functioning did not normalize (Himmerich et al 2007).

Given the evidence for the association between leptin, metabolic changes, and HPA axis activity, it is of particular importance that this relationship be evaluated in subjects with clinical depression. It is now generally accepted that the more rigorous approach to evaluating hormone changes is the use of challenge techniques to eliminate the effect of other confounding factors. In this case, the use of food and stress challenges has been proposed as the most appropriate strategies. However, to date, there are no previously published reports of leptin response to food or stress challenges in clinically depressed individuals, and as such, filling this gap in the literature was one of the main objectives of this investigation.
1.6 Depressive Disorders and Obesity: Shared Pathophysiology?

The common co-occurrence of obesity and depression, particularly in the more persistent forms of depression, has been well documented. Neurobiological studies have further noted several pathophysiological changes shared by the two conditions, including altered activity of the HPA axis (Bornstein et al., 2006) and changes in inflammatory processes (Anisman, 2008; Perez de Heredia et al., 2012). In addition to neurobiological processes, overlapping behavioral, cognitive, and personality factors between depressed and obese populations have been noted (Elfhag and Morrey, 2008). It has been proposed that several of these factors may contribute to weight disturbances in both populations.

The frequent co-morbidity of obesity and depression and the experimental evidence supporting shared pathophysiology provide a strong rationale for evaluating these conditions together, rather than as separate entities.

1.6.1 HPA axis

One potential target for a common pathophysiology is the HPA axis (Bornstein et al., 2006). Not only have HPA axis alterations been documented in depression, subtle differences among subcategories of depression have been noted as discussed in Section 1.3.1. It is of note that increased activation of HPA axis has also been associated with obesity and abdominal fat (Bjorntorp and Rosmond, 2000; Bjorntorp 2001). In addition, blunted response to HPA axis challenge in overweight individuals has been associated with anxiety and depressed traits (Rosmond and Bjorntorp, 1998). Furthermore, altered postprandial cortisol secretive patterns have been noted in obese individuals in comparison to lean subjects (Svec and Shawar, 1997; Parra et al., 2006; Garcia-Prieto et al., 2007; Sarker et al., 2013). For example, greater
incremental cortisol in response to a meal in obese females (Svec and Shawar, 1997), higher diurnal variation in cortisol secretion associated with higher postprandial cortisol secretion (Garcia-Prieto et al., 2007), but lower cortisol secretions after meal consumption in obese men (Parra et al., 2006) have been noted. Recently, such alterations in postprandial cortisol secretion has been linked with ghrelin levels and coping abilities. Specifically, postprandial ghrelin levels have been associated with higher cortisol response and greater subjective stress and anxiety in obese females (Sarker et al., 2013).

While HPA dysfunction is a noted commonality between obese and depressed populations, the parameters of this relationship have not yet been established.

1.6.2 Cytokine and Inflammatory Hypothesis of Depression: the role of leptin

Depression is associated with increased expression of inflammatory molecules (Maes et al., 1995) and immune system activation (Anisman, 2008; 2011). Based on the evidence supporting the role of cytokines for the pathophysiology of depression, the ‘cytokine hypothesis’ has been proposed. This hypothesis considers that both external stressors (e.g. psychosocial) as well as internal stressors (e.g. inflammatory conditions) may trigger depression via inflammatory processes (Maes et al., 2009).

Initial evidence for this hypothesis came from studies that found increased circulating levels of cytokines depressed populations (reviewed in Anisman and Hayley, 2012), with further support provided by evidence from studies of cancer patients requiring administration of interferon-α, which resulted in the occurrence of depressive symptoms in approximately 50% of patients (Raison et al., 2006). In a comprehensive review of stress and the influence of the
inflammatory immune system on stress-related illnesses, such as depression, Anisman and colleagues (2008) suggested that the combined effect of stress and inflammatory processes may promote pathological outcomes via actions on neuropeptides and neurotransmitters that contribute to the recurrence of depressive illness and comorbid conditions, such as neurodegenerative and cardiovascular disease (Anisman et al., 2008). Though a full review of this association is beyond the scope of this current research program, the interaction between certain neurohormones like leptin and inflammatory markers may represent one of the underlying commonalities between depressed and obese populations.

The substantial body of literature on the inflammatory markers in obesity and obesity-related disorders (e.g. Type 2 diabetes) has been well reviewed (e.g. Black, 2006). As with depressive illness, alterations in adipokines such as adiponectin, leptin, and resistin, as well as TNF$\alpha$, IL-6 and IL-1, have been reported in obesity (Bornstein et al., 2006). Further, insulin resistance in obesity is linked to activation of specific inflammatory and insulin signaling pathways (Thaler et al., 2010). In a parallel finding, it has been suggested that adiposity may mediate the elevated levels of IL-6 and C-reactive protein (CRP) in patients with clinical depression (Miller et al., 2003). Though multiple pro- and anti-inflammatory markers have been implicated in depressive disorders, TNF$\alpha$ and IL-6 have been proposed as the most relevant. However, medications and coexisting medical conditions, common in depression, may confound results of investigations of immune functioning. A recent meta-analysis controlled for these factors by focusing on studies that investigated physically healthy and unmedicated depressed patients, and found that concentrations of TNF$\alpha$ and IL-6 had the greatest effect size (Dowlati et al., 2010).
Given the wide range effects of leptin on the immune system, Miller and colleagues (2009) have proposed a model of depression that encompasses inflammatory response, leptin and weight increase. This model proposes that depressive symptoms (e.g. appetite changes) promote weight increase, which in turn activates an inflammatory response through two pathways. The first pathway involves the release of IL-6 at elevated concentrations synthesized from the increased adipose tissue, which in turn induces expression of CRP from the liver. The second pathway involves expanded adipose tissue releasing more leptin concentrations into circulation. Leptin then upregulates the expression of IL-6 from white blood cells and or vascular endothelial cells, which in turn stimulates hepatic release of CRP. However, this model notes that the differences in inflammatory response arise from leptin and adiposity and do not necessarily promote the expression of depressive symptoms, i.e. there is no loop back to the depressive trigger of the inflammatory response (Miller et al., 2009).

1.6.3 Oxidative Stress

Oxidative stress can be defined as the disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses, leading to damage in lipids, DNA, and proteins (Betteridge, 2000). Closely linked with inflammatory processes, oxidative stress has been the focus of study in obese and depressed populations. Several oxidative stress markers, including reduced plasma concentrations of antioxidants such as vitamin E, tryptophan, tyrosine, albumin, zinc, and CoQ10, have been associated with clinical depression. Reduced antioxidant defense is said to weaken protection against damage to fatty acids, proteins, DNA and mitochondria. Increased oxidative stress has been well documented in obese subjects (Furukawa et al., 2004), and evidence for the association between increased oxidative stress and metabolic
syndromes such as insulin resistance (Urakawa et al., 2003; Katsuki et al., 2004) and related illnesses (e.g. diabetes type 2) are now substantial (Evans et al., 2002).

Increased levels of oxidative stress markers have also been noted in patients with depression (Maes et al., 2011). Similarly, accumulation of excessive ROS has been reported in depression. One such study found increased peripheral oxidative damage to lipids in patients with mood disorders regardless of the phase of the illness (Andreazza et al., 2010). Another study found concentrations of 8-iso-PGF 2α, a marker of lipid oxidation, was twice as high in depressed patients compared to matched controls, even after controlling for demographic and lifestyle factors (Yager et al., 2010). Several gender differences have been reported, with biomarkers of lipid peroxidation reported to be significantly higher in males (but not females) with late onset depression in comparison to controls (Milaneschi et al., 2013). Interestingly, self-report scores of depressive symptoms have been shown to correlate positively with serum lipid peroxidase levels, but only in depressed female subjects (Tsubio et al., 2004).

It is of note that peripheral activity of antioxidants enzymes is increased in depressed patients compared to healthy controls, an effect not seen during remission (Gawryluk et al., 2011). In addition, preliminary data indicate that changes in oxidative stress markers may normalize following pharmacological treatment (Tramontina et al., 2009; Berk et al., 2011). For example, MDD patients with melancholic features had higher antioxidative enzyme activities and lipid peroxidation in comparison to controls, which normalized after three months of treatment with an SSRI (Bilici et al., 2001).

Relevant to both depressed and obese populations, previous reports have suggested a positive association between cortisol levels and oxidative stress damage, in both clinical and non-clinical samples of individuals who experience chronic stress (Wolkowitz et al., 2010;
Aschbacher et al., 2013). For example, participants who experience chronic stress have shown greater oxidative stress damage, which was positively associated with cortisol reactivity to an acute stressor (Aschbacher et al., 2013).

The above data provided the rationale to evaluate peripheral (serum) markers of abnormally elevated oxidative stress in patients with MDD and their relationship to stress reactivity, as part of this research program.

1.6.4 Neurocognitive Commonalities

A reasonable body of literature has evaluated neurocognitive function (using both neuroimaging and neuropsychological tests) in subjects with elevated BMI and/or obesity, further adding to information on the neural correlates of ingestive behavior (e.g. Rolls et al. 1999). The evidence from neuroimaging studies has reported both structural and functional changes in this population. Several investigations have reported that higher BMI and waist/hip ratio have been associated with temporal lobe and hippocampal atrophy as well as reduced whole brain volume (Ward et al., 2005; Gustafson et al., 2004; Jagust et al., 2005; Taki et al., 2008). More recently, Walther et al. (2009) found grey matter reductions in left orbitalfrontal, right inferior frontal, right precentral gyrus, and right cerebellar regions, and increased volumes in white matter in the frontal, temporal and parietal lobes, in relation to higher BMI (Walther et al., 2009). Even in younger populations, obese males exhibited reduced grey matter in the frontal lobe noted (Taki et al., 2008). Functionally, individuals who are overweight or obese have been shown to have reduced metabolic activity in the prefrontal lobe, as well as to exhibit atrophy of the superior, middle, inferior frontal, and orbitofrontal gyri (Volkow et al., 2009). Neuroimaging investigations have implicated many of these same regions in the pathophysiology of depressive illness (as reviewed in Drevets et al., 2000)
Neuropsychological evaluations have noted associations between increased body weight and both self-report and behavioural measures of impulsivity, with poor performance noted on tests of response inhibition, mental flexibility and decision-making skills (Verdejo-Garcia et al., 2010). A recent meta-analysis found that decision making, measured through such tasks as the Iowa Gambling Task, are among the most impaired functions in eating disordered populations (Zakzanis et al., 2010). Further, a systematic review reported that poor performance on tests of executive functioning, particularly the Stop Signal and Stroop tasks, to be a reliable and robust finding in populations with elevated BMI (Vainuk et al., 2013). It is of note that many of these neurocognitive findings, particularly impaired executive functioning, have been similarly consistently noted in patients with depression (Snyder, 2012).

Of relevance to this particular investigation, is the observation that both leptin and ghrelin may have a neuroprotective function that may relate to cognitive functioning. Specifically, leptin has been shown to play roles in neurogenesis, axonal growth, synaptogenesis, and dendritic morphology (Bouret 2010). It is thought to exert its neuroprotective actions by inhibiting apoptotic cell death, and by improving cell survival by protecting against glutamatergic cytotoxicity and oxidative stress (Paz-Filho et al., 2010). In animal models, leptin administration has been shown to have positive effects on cognitive functions such as spatial learning and memory (Li et al., 2002). Similarly, an accumulating body of evidence from human studies suggests leptin, in individuals with normal body weight, might provide some degree of protection against cognitive decline associated with neurodegenerative disorders, such as Alzheimer’s Disease (Holden, et al., 2009).

Ghrelin receptors are also well expressed in the hippocampus, though not as widespread as leptin receptors, and are similarly thought to provide neuroprotection and enhance cognition
(McNay, 2007). Animal investigations have found that ghrelin administration to be associated with enhanced spatial, aversive and novel learning and memory, and that animals who are ghrelin deficient show impairment on such tasks (Diano et al., 2006; Carlini et al., 2002).

The exact roles of leptin and ghrelin in modulating cognitive function are not fully understood, and the leptin/ghrelin-cognition relationship in depressed populations is even less clear. However, given the significant comorbidity of depression and obesity and overlapping neurocognitive deficits, it was proposed to evaluate the relationship between these hormones and cognitive functioning in these populations in the current research program.
2 Key Findings from Published Literature and Rationale for Proposed Investigations

2.1 Key Findings from Published Literature

This section provides a summary of key findings in the literature pertaining to Depressive Disorders and the following: weight disturbances; stress and/or emotional eating; HPA axis activity; energy homeostatic neurohormones related to appetite (ghrelin and leptin); as well as other potential contributing factors.

DEPRESSION AND WEIGHT DISTURBANCES

Section 1.1

Depression is a common illness with high illness burden (Gore et al., 2011; Whiteford et al., 2013). Appetite and weight disturbances are common core symptom, and hyperphagia is prominent in certain subtypes of depression (e.g. atypical). It is often recurrent with many individuals experiencing a chronic course of illness. Eating disturbances have been noted in depressed populations and include excessive carbohydrate cravings, irregular meal patterns, and possible tendency to eat in response to negative affect (i.e. emotional eating) (Wurtman & Wurtman, 1989; Fulkerson et al., 2004; Ouwens et al., 2008). As such, a strong association between depression and obesity exists, however, the direction of this association is not yet clear (McElroy et al., 2004; Atlantis & Baker, 2008). This association is of clinical concern given that obese patients with depression have shown to be less likely to improve with treatment (Ravindran et al., 1997; Papakostas et al., 2005; Kloiber et al., 2007).

STRESS AND EATING BEHAVIOURS

Section 1.2

The majority of individuals alter eating behavior by either reducing or increasing consumption in response to stress or negative affect (Gibson, 2006; Torres & Nowson, 2007). Both laboratory and naturalistic investigations have shown that stress elicits greater intake of food high in sugar and/or fat (Epel et al., 2001; Newman et al., 2007). Individuals high in emotional eating tend to consume greater amounts of food in response to stress (van Strien et al., 2009). HPA axis activity and food intake in
response to stress has been examined with inconsistent findings. Greater
cortisol reactivity has been associated with greater food intake in response
to stress (Epel et al., 2001) while others have noted that those with blunted
cortisol response display greater food intake post stressor (Tomiyama et al.,
2011; van Strien et al., 2012). The duration of stress may be an important
factor in explaining the discrepancies in literature. Dallman and colleagues
have proposed a model that describes mechanisms that underlie chronic
stress, glucocorticoid action and food intake. The proposed “chronic stress
network” involves CRH activation in various regions in the brain including
the amygdala, mesolimbic dopaminergic system, prefrontal cortex and
hippocampus to stimulate feeding, learning, and memory (Dallman et al.,
2006; 2010). In human investigations, high levels of perceived stress were
associated with greater amount of emotional eating, abdominal obesity, and
lower cortisol response to a psychosocial stressor and a physiological
challenge (Tomiyama et al., 2011). Furthermore, high emotional eaters
who demonstrated a blunted cortisol reaction to the stress induction
consumed the greatest amount of calories (van Strien et al., 2013).

**GHRELIN**

Section 1.4

Ghrelin is a robust orexigenic signal, with levels rising before a meal and
rapidly decline post-prandial (Tschop et al., 2000). Low levels of ghrelin
have been consistently found in obese individuals and high levels found in
those with low BMI (Tolle et al., 2003; English et al., 2003). Obese
patients with binge eating disorder show lower plasma concentrations of
ghrelin before a meal and less of a decline post meal (Geliebter et al.,
2009).

Ghrelin is associated with HPA axis activity and has been found to
stimulate CRH, ACTH, and cortisol (Broglio et al., 2003; Vestergaard et
al., 2007). For example, single injection of ghrelin shown to stimulate GH,
ACTH, and cortisol in comparison to placebo (Schmid et al., 2005).
Further, ghrelin levels positively associated with cortisol response to
stressor (Rouach et al., 2007). In addition, emotional eating status has been
associated with increased cortisol response coupled with absence of decline
in post-prandial ghrelin levels (Raspapow et al., 2010) as well as subjective feelings of stress and anxiety in obese individuals (Sarker et al., 2013).

There are relatively few studies investigating ghrelin in depressed samples, however, those conducted yield inconsistent findings (Schanze et al., 2008; Kluge et al, 2009). Lower ghrelin levels found in depressed patients absent of appetite disturbances compared to those who endorse appetite disturbances in a depressive episode (Himmerich et al., 2005).

No difference in ghrelin levels were noted between those who gain weight during treatment vs. those who did not in patients receiving treatments known to increase body weight (Himmerich et al., 2005). Others have noted rapid increase in ghrelin and in body weight in depressed patients receiving tetracyclic antidepressant treatment (Pinar et al., 2008). Similarly, treatment with SGA resulted in increase in serum levels of both ghrelin and leptin as well as body fat percentage (Murashita et al., 2008). Interestingly, others have noted decreased ghrelin levels after treatment with SSRI (Barim et al., 2009). Further, some have noted decrease in ghrelin and cortisol, while leptin levels increased (Schmid et al., 2005).

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**LEPTIN**

Section 1.5

One of the main functions of leptin is to regulate food intake and metabolism (Ahima, 2005; Denver et al., 2011). Leptin communicates energy homeostatic primarily through plasma concentrations and is secreted in proportion to fat stores (Friedman, 1998). It is not necessarily a short-term satiety signal but CNS signal of adipose stores (Jequier et al., 2002). It is also involved in many other physiological functions including thermogenesis, reproduction and immunity (Steiner & Romanofsky, 2007). Ob gene encodes the protein leptin, and obese individuals consistently exhibit elevated levels of leptin (Tartaglia et al., 1995; Schwartz et al., 1996).

Leptin receptors are expressed throughout regions of the brain including amygdala, hippocampus, basal ganglia, medulla (Elmquist et al., 1998; Friedman, 1998; Harvey et al., 2007). In the hypothalamus,
leptin receptors are expressed in basomedial hypothalamus including ARC, dorsomedial and ventromedial hypothalamic nuclei (Elmquist et al., 1998). It acts on POMC, [pro-opiomelanocortin] and CART [cocaine and amphetamine regulated transcript] in the ARC to increase anorectic signal via alpha MSH [melanin stimulating hormone] and also on NPY/Agouti related peptide neurons to inhibit expression of orexigenic signals. CRH is indirectly regulated by leptin targets in ARC to mediate leptin’s inhibitory actions on food (Friedman, 1998).

Administration of leptin to individuals with leptin deficiencies results in normalization of metabolic functioning and body weight (Friedman, 2010). Leptin resistance is a term used to define the inability to response to either endogenous or exogeneous leptin and is thought to co-occur in many individuals with obesity and insulin resistance, yet exact mechanisms of leptin resistance are not yet known (Muzenberg & Myer, 2005; Myer et al., 2010)

A link between leptin and mesolimbic dopaminergic system has been suggested (Fulton et al., 2006). Evidence from animal studies suggests that leptin attenuates dopaminergic activity, which has been supported by evidence found in human studies (Hommel et al., 2006; Grosshans et al., 2012). Imaging investigations have shown that patients with genetic leptin deficiency show abnormally high activity in the nucleus accumbens elicited by visual food stimuli which normalizes with leptin administration (Farooqi et al., 2007) and circulating leptin levels were positively associated with dopamine release in response to physical stressor (Burghart et al., 2012).

Evidence from numerous animal studies of HPA axis activity and leptin suggest that leptin stimulates hypothalamic CRH biosynthesis, and reduces basal ACTH and corticosterone (Costa et al., 1997; Nowak et al., 2002). In response to stress, leptin has been shown to dampen HPA axis response in animal studies (Ahima et al., 2005). Similarly in human investigations leptin has been shown to have an inverse association with HPA axis as evidenced in inverse patterns of
circulating levels of leptin vs. ACTH and cortisol over a 24hr period have been found in human studies (Licino et al., 1997) and increased leptin concentration in response to cortisol or dexamethasone administration (Miell et al., 1996).

In response to stress, some have found positive associations between waist circumference and leptin response to stress that was further associated with greater stress-induced increases in IL-6 (Brydon, 2011). While others have not found an association between leptin and cortisol response to stress (Tomiyama et al., 2012).

Investigations comparing leptin levels in depressed patients vs., healthy controls have yielded inconsistent findings (Dueschle et al., 1996; Antonijevic et al., 1998; Kraus et al., 2001). Subtype of depression, namely atypical or severity of depression may influence levels (Gecici et al., 2005). Severity of depression, along with body weight may contribute to inconsistencies in findings as some evidence suggests that severely depressed patients of normal weight had the lowest levels while overweight or obese severely depressed patients had the highest leptin levels in comparison to mildly depressed of the same weight categories (Morris et al., 2012).

Further, in studies investigating HPA axis activity and leptin interaction in depressed populations have noted this inverse association between leptin and both ACTH as well as cortisol, particularly in female patients (Cizza et al., 2010).

Investigations of the impact on antidepressants on leptin levels have also yielded inconsistent findings. Some have suggested that improvement in response to treatment is linked with HPA axis normalization that correlates with leptin levels (Himmerich et al., 2007).

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<th>Depressive Disorders and Obesity:</th>
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<td>“Cytokine Hypothesis” of depression may be applicable to this investigation given the associations between leptin, and cytokines supported by strong evidence of increased expression of inflammatory and</td>
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Inflammatory Markers, Oxidative Stress Markers, Neurocognitive Functioning

Section 1.6

immune system activation in depressed populations. Meta-analysis results indicate that IL-6 and TNF alpha concentrations have the greatest effect size in healthy unmedicated depressed populations in comparison to healthy controls. Alterations in adipokines such as adiponectin, resistin, TNF alpha, IL-6, and IL-1 have been reported in obese populations (Bornstein et al., 2006). Some have proposed a model of depression that attempts to explain appetite and weight disturbances as a result of increased leptin levels associated with elevated inflammatory response (IL-6 and CRP) (Miller et al., 2009).

Increased oxidative stress is linked with both obese and depressed populations. A substantial body of literature that documents oxidative stress damage in patients with depression has lead some to propose that oxidative damage promotes neurodegeneration and could be considered the final common pathway underlying the pathophysiology of depression (Maes et al., 2011).

Distinctions in cognitive functioning have been noted in both depressed and obese populations. Individuals with elevated BMI have been reported to perform poorly on neuropsychological measures of impulsivity, such as response inhibition, mental flexibility and decision-making skills (Verdejo-Garcia et al., 2010). Many of these neurocognitive findings in populations with elevated BMI, particularly impaired executive functioning, have also been consistently noted in patients with depression (Snyder, 2013). Both leptin and ghrelin have been shown to have neuroprotective actions that positively effect cognitive functioning in animal models (Harvey, 2007; McNay, 2007; Morrison, 2009). Of the few investigations in human populations, higher leptin levels have been associated with less cognitive decline in elderly patients (Holden et al., 2009) and lower incidence of Alzheimer Disease (Lieb et al., 2009). Similarly, higher levels of ghrelin are associated with reduced cognitive performance in elderly populations (Spitznagel et al., 2010), while ghrelin administration has been shown to increase memory for food related items in younger samples (Malik et al., 2008).
2.2 Rationale for Proposed Investigations

The review of scientific literature in Chapter 1 describes several components of a complex neurohormonal system that controls energy homeostasis, which is dysregulated in individuals with depression, contributing to its key symptoms of appetite and weight disturbances. Figure 2-1 is a schematic representation of the inter-relationship of several neurophysiological, psychological and behavioural factors proposed as contributing to such symptoms.

Depression is a common recurrent condition with a significant burden of illness at an individual, familial and societal level. The prevalence of depression is also on the rise globally, making it a significant public health concern. Many symptoms of depression, such as appetite disturbances, promote other chronic conditions, including obesity, which adds further to the burden of disease. While the high co-morbidity rate between depression and obesity is well established, the mechanistic basis of this association is not fully understood. Improving the understanding of this comorbidity is a major objective of the current investigation.

Both the pathophysiology of depressive illness and neurobiological regulation of food intake and appetite in obesity are independently complex and involve numerous of overlapping systems that encapsulate a number of potential vulnerabilities to weight disturbances in depression. While it is recognized that several diverse factors contribute to such vulnerability it would not be possible to evaluate their contributions in a single series of investigations. Thus a decision was made to focus on the influence of stress reactivity and appetite/satiety hormones. Stress reactivity, both physiologically and psychologically, is altered in depressed populations, and has a known influence on food consumption. In addition, two recently discovered hormones, leptin and ghrelin, are both involved in energy homeostasis, have known influences
appetite regulation, and are associated with stress reactivity, specifically cortisol secretion. Furthermore, both leptin and ghrelin have been studied in association with depressive illness, however, not in the context of stress reactivity or food consumption.

Figure 2-1 Potential neurophysiological, psychological, and behavioural contributions to appetite and weight disturbances found in depression. These may include: alterations in HPA axis activity, greater stress perception, tendency to eat in response to emotions, increased pro-inflammatory molecule signaling, oxidative stress markers, and disturbed cognitive functioning.
The primary goal of this research program was to investigate a few keys pathways that might link depression and obesity in the more chronic forms of depression that typify tertiary care settings. While there are a very large number of pathways that might contribute in this regard, I chose three major foci for investigation as described below:

1. The HPA-axis, specifically cortisol response, was chosen as the first focus based on the role of stress in depressive disorders itself, and its impact on food consumption. In addition, it was of equal priority to investigate cortisol reactivity in association with stress induced eating behaviours, such as emotional eating, likely to be present in depressed patients.

2. Appetite and satiety hormones, ghrelin and leptin. This focus was chosen for reasons mentioned above, but also because offers the opportunity to investigate appetite and satiety signals that have known associations with stress reactivity, as well as, proposed associations with stress induced eating behaviours such as emotional eating.

3. The third focus of investigation was cognitive contributions to appetite regulation in association with stress reactivity. Leptin, ghrelin and elevated body weight have been linked with cognitive performance, and as such, it was chosen to explore this relationship in patients with depressive disorders.

**Methodology:** In choosing between several possible methodologies to implement for the current study, given the complex nature of the study population, consideration was given to a balance between theoretical relevance and practicality. To study stress reactivity, it was decided to use the TSST because it has been shown in many investigations to elicit a strong, and measurable cortisol response. As well, individuals with depression are likely to be sensitive to interpersonal rejection, and the TSST can evoke a similar reaction in a controlled laboratory
setting. In addition, this model of stress induction provides an opportunity to evaluate ghrelin and leptin response to stress independent of food consumption. This was seen as a significant advantage, as most published studies combined a stress induction procedure followed by immediate food consumption, causing difficulty in determining the independent effects of stress vs. food consumption on these hormones. This is particularly relevant to ghrelin, as its secretion changes quickly in response to food consumption but also has documented associations to stress reactivity.

To study response to food, a food challenge that consisted of a small meal, was implemented. This challenge method was chosen as alterations in cortisol response to food consumption has been linked with psychological and behavioral characteristics such as stress, anxiety and emotional eating, and as such would be relevant to a depressed populations. Similarly, differing patterns of postprandial ghrelin secretion has been linked with the presence of emotional eating behaviours. While leptin has been shown to remain stable for a few hours in response to food consumption, it was chosen in order to explore any deviations in postprandial leptin secretion that may be indicative of a disturbance in an intermediate satiety signal in patients with depression.

And finally, the cognitive measures chosen are those that are particularly relevant to individuals with elevated body weight, in that such individuals exhibit impairment on measures of attention and decision-making. The particular measures have also been used to study associations between hormone levels (cortisol, leptin, ghrelin) in other populations, and thus was of interest to explore in individuals with depression.
The overall research program was developed based on the above literature review and was comprised of three overlapping sets of investigations with inter-related themes. They are outlined below along with the specific rationale and aim for each investigation.

1) Cortisol response to psychosocial stress and food consumption stimuli in depressed and control participants

Rationale for the investigation exploring the relationship between stress and altered eating behaviour

Stress induced eating is also common and has been linked with eating behaviours that promote body weight increase. Emotional eating, intertwined with stress induced eating, may provide the link between increase in perceived stress and a tendency to eat highly palatable and caloric rich food, therefore leading to body weight increase. Further, HPA activity has been linked with increased likelihood of consuming high caloric food in response to stress. Emotional eating status has been associated with increased cortisol response coupled with absence of decline in post-prandial ghrelin levels, which is a known physiological cue to cease food consumption. In addition, it has been proposed that the length of time that stress is endured may influence the type of HPA axis reactivity and the degree of comfort food consumption. Proposed models, based on animal investigations, have described a “chronic stress network” that involves CRH activation in various regions in the brain including the amygdala, mesolimbic dopaminergic system, prefrontal cortex and hippocampus to stimulate feeding, learning, and memory that explains comfort food consumption and abdominal obesity. In this model, an attenuated stress response is thought to be elicited through “comfort eating” of palatable food, which in turn dampens the stress response while promoting abdominal obesity via an unknown signal from
adipose stores. The latter may be of particular relevance to depressed populations who tend to respond increased perceived stressors as well as an increased tendency towards emotional eating. As depression often follows a long-term course, they tend to endure extended periods of stress.

**Altered Stress Perceptions and Physiological Stress Response in Depression**

Individuals with depression not only experience increased levels of perceived stress, but also exhibit significant alterations in HPA axis activity with both increased and decreased activation being documented. Among the reasons suggested for such inconsistencies include the influence of chronicity and the subtypes of depression. Those who experience atypical depression may be the most vulnerable to weight gain, as there is some evidence to suggest they experience an attenuated stress response, and is said to exhibit alterations in inflammatory markers promoting increase in body weight. *How alterations in stress perception and stress reactivity are linked with other hormones involved in appetite regulation has not yet been studied in individuals with clinical depression.*

**Aim of Investigation I**

To evaluate cortisol response to stress induction and food consumption in depressed subjects and normal controls, and to determine if such differs in a) the presence of emotional eating behaviour, b) duration of illness and c) by subtypes of depression. In addition, as oxidative stress damage is common to both depression and obesity, it was interest to explore peripheral markers of oxidative stress (lipid hydroperoxidase) in response to stress within this investigation.
**Primary Hypothesis (1.1):** It is hypothesized that participants with depression will experience altered HPA axis reactivity following stress and food consumption compared to control participants.

**Secondary Hypotheses**

(i): Participants with a chronic course or atypical subtype of depression will experience blunted HPA axis reactivity in response to experimental challenges in comparison to control participants.

(ii) HPA axis reactivity will vary by emotional eating status. Thus participants categorized as emotional eaters will experience blunted HPA axis reactivity in response to experimental challenges compared to non-emotional eaters.

(iii) Participants with depression and elevated body weight will exhibit the greatest oxidative stress damage.

2) **Impact of social stress and food challenge on appetite and satiety hormones**

Rationale for the Investigation:

*Orexigenic effects of Ghrelin: HPA axis activity and Emotional Eating*

Ghrelin is a robust orexigenic hormone that has been associated with increased appetite, food intake and body weight. It has been shown to modulate HPA activity and has been implicated in eating behaviours associated with negative affect (e.g. emotional eating), and thus, is a logical candidate for investigations of appetite and weight changes. So far, the few published studies of ghrelin in depressed populations have yielded inconsistent results, *and to date, there are no*
studies evaluating ghrelin response following stress induction of food challenge in depressed subjects. It is proposed that such an investigation may help elucidate the neurobiological basis of appetite and weight disturbances in depression.

Leptin: adipose signal linked with HPA axis activity

Leptin, the satiety hormone, is said to function as an adipose signal and anorexic agent. It impacts HPA axis activity by indirectly regulating CRH and attenuating stress reactivity. As such, it is a likely candidate for investigating stress induced eating, particularly in relation to depressive disorders (Anisman et al., 2008). Investigations of leptin concentrations in depressed subjects and changes following treatment have so far yielded inconsistent findings. Changes in ghrelin or leptin concentrations following stress induction and food challenge have not previously been evaluated in subjects with depression.

Aim of Investigation II:

To determine if the impact of stress induction or food challenge on serial ghrelin and leptin production differ in subjects with depression compared to healthy controls and if the hormonal response are influenced by the presence of emotional eating, and duration of illness.

Primary Hypothesis 2.1: It is hypothesized that subjects with depression will exhibit greater leptin and ghrelin secretary responses following stress induction and food consumption compared to healthy control participants.
Secondary Hypotheses:

(i) Secretion of leptin and ghrelin in response to food challenge will vary by chronicity and subtype of depressive episode with greater response among participants with chronic depression.

(ii): Altered secretion of ghrelin in response to food consumption will vary by emotional eating status. Participants categorized as emotional eaters will exhibit less of decline in post-prandial ghrelin levels in comparison to non-emotional eaters.

3) Investigation III: Neurocognitive alterations in depression and obesity: Association with appetite and satiety hormones

Rationale for Investigation:

Commonalities in neurocognition in depression and obese populations

Impaired cognitive function has been noted in both depressed and obese populations. The appetite and satiety hormones, ghrelin and leptin, have been shown to have neuroprotective functions and beneficial effects on cognitive functioning in animal studies. However, this relationship has not been fully demonstrated in human subjects (and even less in the depressed population), nor has the mechanism been fully elucidated. To date, there are no studies that evaluate the relationship between these hormones and cognitive functioning in individuals with depression. Given the significant comorbidity of depression and obesity and presence of shared neurocognitive deficits, it appears rational to evaluate this link between the two conditions.
Pilot Study Aim: To determine if any associations exists between the degree of neuropsychological deficits and neurohormonal responses in subjects with depression and to evaluate the impact of BMI as a contributing factor to such association.

**Hypothesis 3.1:** Greater deficits in neurocognitive functioning will be seen in depressed participants in comparison to controls. The greatest deficits in cognitive functioning will be seen in individuals with lower levels of leptin and ghrelin and higher levels of cortisol and in individuals with elevated BMI.

**Summary of Rationale and Clinical Relevance**

While eating disturbances in mood disorders have been well documented, the interaction between eating and stress neurohormones has not. The majority of investigations of hormones related to eating in depressed populations have focused on patients with loss of appetite or of normal body weight; however a significant percentage of depressed patient experience hyperphagia, weight gain, and obesity that can complicate recovery. It is important to investigate depressed patients who experience increased appetite, weight gain and/or obesity, as it may contribute to the our understanding of a) the pathophysiology of major depression; b) the interaction of stress and eating hormones in depressed patients, c) the role of emotional eating and weight gain in depression, and e) identify potential interventions for those who experience comorbid depression and obesity.
3 Design, Study Population and Methods

The following section outlines study design and methodology common to all investigations in this research program. Each study will provide a description of procedures specific that particular investigation in their respective sections. The study population was the same across all three studies and is described in the section below.

3.1 Common Method Section

Outline of Design:

Serial plasma cortisol, ghrelin, and leptin were measured in subjects with major depressive disorder and age and gender matched control sample during to two experimental conditions: 1) metabolic induction by food intake and 2) psychosocial stress induction. Serial blood samples were collected during each experiment for the analysis of cortisol, ghrelin, leptin, as well as measures of oxidative stress. Several psychological, physiological, and cognitive measures were also obtained from participants to determine associations with hormonal response.

3.1.1 Participants:

The individual participants had a primary diagnosis of unipolar major depressive disorder criteria for a unipolar depressive disorder and the individuals in the control group were volunteers with
no history of psychiatric disorders. All healthy controls were paired by gender, BMI, and age to patients with depression. Approval for this study was obtained from CAMH Research Ethics Board and all study procedures were conducted in compliance with the Declaration of Helsinki.

3.1.2 Inclusion/Exclusion Criteria for Participants with Depressive Disorder

Participants were included in the depressive disorder group if they met the following criteria: Male or female between 19-65 years of age; fulfilled diagnostic criteria (DSM-IV-TR) for current Major Depressive Disorder or Dysthymic Disorder based on the Structured Clinical Interview for DSM-IV, Axis I Disorders (SCID-I) (First et al., 1996); were physically healthy; and able to provide informed consent. They were excluded if they had a diagnosis of Bipolar I & II, or any other Axis I disorder as primary diagnosis. In addition, subjects with significant substance abuse/dependence; acute risk of suicidality or past history of psychotic symptoms were also excluded. Other exclusionary diagnoses included eating disorders, presence of significant medical illnesses (e.g. heart disease, rheumatoid arthritis, diabetes, Hepatitis C, etc.) as well as medications likely to impact on the validity of the experiment. These included medications such as atypical antipsychotics, psychostimulants, mood stabilizers, benzodiazepine and mirtazapine, as well as ones likely to affect metabolic factors or cortisol measurement (e.g. antibiotics and steroid medications). Currently pregnant or breastfeeding; major recent life stressors (deaths, divorce, job loss, etc.); food sensitivities or allergies were also exclusion criteria.

The healthy control participants were in the 19-65 years of age range; physically healthy; and able to provide informed consent. Participants were excluded from the healthy volunteer group
if they reported any current or past history of symptoms associated with any Axis I or II disorder (DSM-IV-TR) based on SCID-I, including history of substance abuse/dependence or history of major medical illnesses (e.g. heart disease, rheumatoid arthritis, diabetes, Hep C, etc.). Medications such as antibiotics and steroids; being currently pregnant or breastfeeding; experience of major recent life stressors (deaths, divorce, job loss, etc.); and the presence of food sensitivities or allergies were other exclusion criteria.

3.1.3 Allowances for Medications

The depressed group included those who were currently taking psychotropics (in particular, first line antidepressants) as well as those who were drug naïve. However, it was stipulated that individuals receiving SSRI or SNRI treatment must have been on a stable dose for a minimum of 2 weeks in order to be included the study. Participants taking benzodiazepines were also included in the study; however, participants were asked to refrain from taking such medication from the evening prior to the day of the experiments. Those patients taking atypical antipsychotics, psychostimulants, mood stabilizers, and mirtazapine in the two weeks prior to enrolment were excluded from the study. Further, any participants who took medications containing steroids were also included in the study if they were taking them on an infrequent PRN basis, and if they were medically able to refrain from taking such medications for a period of 3 days prior to any experimental visits. This was assessed on a case-by-case basis. Those who were required to take steroid medications on a daily basis were excluded from the study.
3.1.4 Description of Study Measures

**Hamilton Depression Rating Scale 29 item (HAMD-29)** (Hamilton, 1967)

This is a clinician-rated scale designed to measure depressive symptom severity and has been shown to be highly valid and reliable. Scores were obtained for the first 17-items, representing the core symptoms of depression, as well as 29 –item scores which includes items specific to subtypes of depression (e.g. atypical). Neurovegetative symptoms were measured using the 8-item Seasonal Affective Disorder (SAD) subscale (Williams et al., 1988). All raters were required to successfully complete training specific to this measure.

**Dutch Eating Behaviour Questionnaire-R (DEBQ)** (Van Strien et al., 1986)

Dutch Eating Behaviour Questionnaire-Revised is a 33-item self-report scale that measured emotional, external, and restraint eating. This scale generated three main subscales: Emotional Eating, Restrained Eating, and External Eating respectively. Two additional subscales; a) eating in response to emotions and b) eating to diffuse emotions were calculated from the Emotional Eating subscale. Participants were asked to assess the accuracy of each item in relation to their own eating behaviour on a 5-point likert scale (1- “Never” to 5 “Very Often”). Scores for these three types of eating were generated.

**Perceived Stress Scale (PSS)** (Cohen, Kamarack, and Mermelstein, 1983)

Perceived Stress Scale is a 14-item scale that measured the degree to which life situations are perceived as stressful. Participants were asked to indicate the frequency at which they experience various thoughts/feelings related to stress on a 5-point likert scale (0 - “never” to 4 “very often”).
Positive and Negative Affect Scale (PANAS) (Watson and Clark, 1988): 20-item self-report measure containing two scales; one of positive emotions, and one of negative emotions. Participants indicated to what extent they felt the various emotions on a 5-point likert scale (1-“Very slightly or not at all” to 5-“Extremely”). Separate scores for positive and negative affect were obtained.

Neuropsychological Measures

Iowa Gambling Task (IGT) (Bechara et al., 1994)

The IGT is a computerized task designed to test motivation and risk-taking in relation to decision-making abilities using rewards and penalties. Participants are presented with a card game in which they are given four decks of cards and a sum of fake money (e.g., $2000). They are instructed to select cards one at a time and that the goal is to lose the least amount of money and win the most. Each card results in a reward, or a penalty with some decks producing a big gain with bigger overall loss, while others decks have a smaller win but bigger overall gain. Over one hundred trials, participants learn to avoid the decks with large losses. On average, the task takes approximately 15 to 20 minutes and the examiner remains in the room with the participant for the full duration of the task.

Conners’ Continuous Performance Task (CPT-II) (Conners et al., 2000)

This computerized task assesses sustained attention and response inhibition. Participants are presented with letters one at time, at varying speeds and asked to respond as quickly as possible by pressing the space bar each time they are presented with a stimuli. However, there is one exception in that they are instructed to refrain from responding when presented with a target
letter (‘X’). The task is approximately 14 minutes in length, and is completed in solitude in an environment devoid of any distractions.

**Test of Memory Malingering (TOMM) (Tombaugh, 1996)**

The TOMM was designed to assess exaggeration or faking of memory impairment and is often used as a symptom validity test in clinical neuropsychological assessments.

The test consists of two learning trials and one retention trial. During each learning trial, participants are presented with 50 line drawings of common objects for 3 seconds each. After the participant has been presented with all 50 images, they are then presented with a forced recognition task. Each recognition panel includes an image previously shown along with a new image and participants are required to select the target picture previously shown. Verbal feedback is given on response correctness for each item. In Trial 2, the same 50 images are presented, but in a different order and participants are again asked to complete the forced recognition task with verbal feedback regarding correctness. For the purposes of this study, only Trial 1 and 2 were completed and the retention trial was omitted. This was to assess level of effort and motivation, rather than memory malingering itself.

**Trail Making Test (A and B) (Reitan, 1955)**

This test is a measure of attention, speed, and mental flexibility. In Part A, participants are required to connect 25 encircled numbers by drawing a line between randomly arranged numbers in the correct order and are instructed to do so as quickly as possible. Part B is similar, and requires participants to alternate between 25 encircled numbers and letters in order. The examiner is present to interrupt and correct when any errors are made.
**Stroop (Golden Version; Golden, 1978)**

The Stroop task is a measure of selective attention and cognitive flexibility. It is a timed task comprised of 3 trials, each 45 seconds in length. The first trial is a word-reading task and requires participants to read out loud the names of colours (red, green, blue) printed on a stimulus card in black ink arranged in five rows with 20 words per row. The second trial involves a colour-naming task during which participants are presented with a stimulus card that contains “X’s” printed in red, blue, and green in five rows with 20 per row. The third trial is the colour-word page, which contains colour words from the first page (red, green, blue) printed in colours from the second trial (red, green, blue), however the word itself and colour of ink are incongruent. Participants are instructed to read out loud the word itself, ignoring the colour that it is printed in. The level of interference caused by the incongruent stimuli presented in the 3\textsuperscript{rd} trial is calculated using scores from the first two trials as predictors.

**Stress Induction Paradigm**

**Trier Social Stress Test** (Kirschbaum et al, 1993): Based on the procedures developed by Kirschbaum et al. (1993), social stress was induced by placing participants in a novel situation in which they believed they were being socially evaluated. The task required participants to make a 5-minute speech and to complete a 5-minute arithmetic challenge (counting backwards in 13’s from 1021) in front of a panel of three confederates. Participants were informed that the panel members were behavioral analysts who would be assessing for signs of anxiety. To heighten the feeling of evaluation, participants were also led to believe that both video and audio recordings were being obtained through a microphone and video camera for assessment of anxiety symptoms.
Trained volunteers fulfilled the role of the “experimenter”. The purpose of this role was to provide instructions regarding the experimental procedures, obtain saliva samples, and introduce panel members to the participant all while adhering to the study schedule. The experimenter kept a neutral stance and kept conversation to a minimum deferring any questions until the end of the experiment.

3.1.5 Study Procedures

3.1.5.1 Participant Recruitment

Participants were recruited through various measures. Study advertisement flyers were posted around the Centre for Addiction and Mental Health, hospitals in close proximity (e.g. University Health Network) and on notice boards in the surrounding neighborhood. Additionally, a one-time advertisement was posted in Toronto Transit System for a period of one month. Further, participants who were seen in the Mood Disorders Clinic and who indicated they were interested in participating in research were referred to the study by staff psychiatrists. All recruitment methods and materials were approved by CAMH’s research ethics board and public affairs office.

Participants were initially contacted via telephone and were provided with verbal information about the study. A phone screen was also administered to identify participants who fulfilled main exclusion criteria or who reported difficulties with blood/injection procedures in order to reduce inconvenience to participants.
3.1.5.2 Screening/Baseline Procedures (Visit 1)

All participants underwent an informed consent process during which they were provided with verbal and written information about the study. Participants were given the opportunity to ask questions, and as much time to contemplate and/or discuss with family or friends as they desired.

Once participants agreed to participate in the study and signed consent, screening procedures were initiated. A diagnostic interview (Structured Clinical Interview, DSM-IV-TR) was conducted to confirm diagnosis of Major Depression Disorder or Dysthymic Disorder and in the case of controls, the absence of any psychiatric disorders. All patients who met criteria for Major Depressive Disorder or Dysthymic Disorder were seen by a psychiatrist in consultation to confirm diagnosis. Consultations occurred either on the same day, or prior to initial visit (in the case of those referred from the Mood Disorders Clinic).

Demographic information, medical history, psychiatric history and current treatment information was obtained. Height was measured in centimeters using a stadiometer and weight was measured in pounds with a mechanical scale. Waist and hip measurements were taken in centimeters using a stretch resistant tape. Waist circumference measurement was obtained from the smallest circumference of the natural waist, usually just above the belly button, and the hip circumference was measured at its widest part of the buttocks or hips. Body Mass Index (BMI) was calculated using the following formula: (Weight in Kilograms / (Height in Meters x Height in Meters)). Vitals (blood pressure, heart rate) were also obtained. Information regarding menstrual cycles was obtained from female participants. In addition, participants were given self-report questionnaires to complete.
3.1.5.3 Experimental Procedures

The stress induction and food challenge occurred on two separate visits with a minimum of three days apart. The order in which the experiments occurred varied based on scheduling factors. For the majority of participants, the food challenge was administered first as order effects were thought to more likely occur if the stress induction took place first. All experimental visits were conducted in the same room. This room contained only furniture necessary for the experiment, and was absent of decoration to maintain a neutral environment. To limit stimuli, the blinds were kept closed during all experiments.

3.1.5.3.1 Stress Induction: Trier Social Stress Test (Kirschbaum et al., 1993)

On a separate day, participants were asked to come in for what they believe to be a mild social stress task in the afternoon. They were not provided with any information prior to the experimental day and believed they would experience a mild social stressor. To control for diurnal cortisol secretion, all experiments were conducted at approximately 1400h. Participants were asked to refrain from ingesting a meal or rigorous exercise in 2 hours prior to the appointment. Information regarding food consumption for that day was collected prior to any experimental procedures. Once participants were seated comfortably, an indwelling catheter was inserted by a physician into participant’s lower arm or hand, and was attached to an Ambulatory Withdrawal pump for blood sampling.

Participants were introduced to an individual who fulfilled the role of the “experimenter” and was provided with an introduction to the procedures for the visit. They were then left in the
room to relax while the experimenter prepared while the pump operator remained in the room behind the screen.

Based upon the time of needle insertion, a schedule for sample extraction and heart rate variability recording for the experiment was immediately calculated. The first blood sample was taken 2 minutes after needle insertion. In addition, the first measure of heart rate variability recording was obtained. The participants are also asked to complete the Positive and Negative Affect Scale (PANAS). Twenty minutes after the participant arrived, panel members entered the room and the participant was given instructions about their task. Participants were asked to pretend that they were applying for a job and to prepare a speech explaining why they are the best candidate for that job. In addition, participants were informed that they would have to perform a second task but that those instructions will be given immediately following the speech. The participants were then given the opportunity to ask the experimenter questions for clarification and then panel members exit the room while the participant prepared for the task.

After a period of 10 minutes, the panel members were brought back into the experimental room. A microphone was placed in front of the participant while one panel member turned on the video camera, and a second panel member initiated the audio recording. Once the panel members were seated behind a table, participants were instructed to begin their speech. If participants finished before the time limit of five minutes, panel members remained silent for 20 seconds and then asked a list of scripted questions for the remainder of time.

After the speech task, participants were then given the instructions for arithmetic challenge. If participants made an error, a panel member informed them of the error and
instructed them to begin again. This continued until the time limit (5 minutes) was completed. Participants were then instructed to await further instructions and the panel members exited the room. At this time, the participants completed another PANAS.

The experimenter instructed the participant to rest while serial blood samples were obtained over a period of 90 minutes. Samples were taken 2 min after arrival, immediately prior to the stress induction, and then 10 min, 30min, 45min, and 60min post stressor. The total volume of blood drawn in this challenge was approximately 40 ml.

3.1.5.3.2 Food Challenge Procedures

Participants were asked to return on a separate day to complete the food challenge and asked to arrive between 1130 and 1145h. All participants were asked to fast for 3 hours prior to the start of the appointment. Upon arrival, participants were asked to report all food and drink intake for that morning. They were also asked to order their sandwich from a selected menu that controlled for caloric intake. Participants were allowed to choose sandwich and topping options that fell in a caloric range of 350 to 400 cal. Sandwiches came from a local restaurant (Mr. Sub) and were ordered and prepared with uniform instructions the day of the experiment. Participants were asked to order their sandwich with toppings of their preference from the limited menu to reduce confounding effects of food subtypes.

Participants were seated and a physician inserted the needle into their lower arm or hand for blood sampling. The blood sampling was then initiated as the participants were encouraged to relax by reading, or if preferred, by watching videos on their cell phone. In the latter case, participants were asked to view videos that were of neutral content (e.g. absent of negative or
stressful content) and were asked to refrain from communicating with anyone outside the experiment.

At 1200h participants were provided with lunch that consisted of a 6-inch sandwich (as per their order) and a bottle of water. They were left alone to consume the sandwich in private, though the door was left ajar for safety reasons. They were instructed to let study staff know when they had finished eating sandwich and amount of consumption time was recorded. Amount (weight) of food consumed was also recorded. Immediately following the completion of the meal, a sampling schedule was calculated so that samples were drawn 20, 40, 60min. after food consumption. The total volume of blood drawn in this challenge was approximately 35 ml. Again, participants were encouraged to relax by reading until the last sample was taken.

After needle removal, trained study staff administered a small battery of cognitive tasks in a separate room. This battery included Trail Making A and B; Stroop, TOMM, IGT, and CPT-II; and were administered in this order. The battery took 30-40 minutes to administer. After completion of the last test, participants were provided with compensation and allowed to leave.

3.1.5.3.3 Blood Sampling Procedures and Sample Processing

For all experimental visits, blood samples were obtained via a Dakmed Ambulatory Withdrawal Pump (Dentifax/Dakmed Inc., Buffalo NY). A screen placed behind the participant concealed both the blood pump and pump operator from the participants’ view. The operator remained in the room behind the screen and kept conversation to a minimum during all experiment visits.

At the beginning of each experimental visit and once the participant was seated comfortably, an indwelling a catheter was inserted into the non-dominant forearm by a physician. In cases where
a suitable vein was not found, the needle was inserted into either the dominant forearm, or hand. The needle was connected to the pump via a 5ft or 8ft heparinized tube, and blood samples were collected into 6mL tubes containing EDTA. Blood was drawn at a very slow continuous rate throughout the experiment and speed was increased in order to extract a sample at the scheduled time points in the experimental visit. Each sample took approximately 4 minutes to collect. Participants remained seated throughout both of the experiments.

All blood samples were processed in a laboratory within 45-60 minutes ($M = 51.86$ minutes, $SD = 5.32$) of being drawn. Two milliliters of whole blood of each 6mL sample was transferred equally into 3 polypropylene conical tubes, one of which was labeled for the analysis of ghrelin. One hundred micrograms of AEBSF (EMD Millipore, Inc.) solution was added to 2mL of whole blood of labeled for ghrelin analysis. All three conical tubes were then centrifuged at 3000g for 15 minutes. Plasma was then aliquoted into 6 microtubes and two of the microtubes were labeled for ghrelin analysis. Two hundred and fifty micrograms of 1N HCI was added to each of the microtubes labeled for ghrelin analysis. This procedure was repeated for each sample time point, and was processed at the same duration as the first sample for that particular experimental visit. For example, if the duration between being collection and centrifugation was 45 minutes for sample 1, then each subsequent sample was centrifuged in the same duration (e.g. 45 minutes). Whole blood was kept at room temperature until it was ready to be processed and all plasma was kept on ice. Once processing was complete, plasma was transferred to an -80 degrees freezer where it remained until assay analysis was completed.
3.2 Subject Description, Demographics, and Group Characterization

Fifty-five individuals consented to participate in the study. Of those, two participants were lost to follow-up after the consent process, and three participants were determined to be inappropriate for the study due to comorbid psychiatric issues. Ten participants completed the initial screen visit only due to a variety of reasons, including scheduling difficulties, previous exposure to stress induction procedures, and difficulties with needle insertion. The latter included difficulties with location of suitable vein or positioning of the needle, or a reaction to needle insertion that included dizziness, nausea, and in two cases, fainting. As such, partial data were collected on 50 participants (n= 24 depressed; n=26 control). Thirty-five participants continued on to participate in one or both experimental visits. This sample was comprised of 18 patients with depression (12 females, 6 males), and 17 (10 females, 7 males) comparable participants. See Figure 3-1.

The demographic information of the sample (n=50) are presented in Table 3.1. There were no significant differences between groups in terms of demographic information, with one exception. A significantly greater proportion of depressed participants were unemployed compared to non-depressed participants, $\chi^2 (1, N=49) = 4.85, p=.03$. 
Figure 3-1 Study Participant Recruitment Process

Table 3.1 Demographic Information for Study Participants

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<th></th>
<th>Depressed</th>
<th>Control</th>
<th>t</th>
<th>p</th>
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<td>SD</td>
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<td>SD</td>
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<td>BMI</td>
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<td>7.51</td>
<td>25.27</td>
<td>4.58</td>
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</table>
Table 3.2  Demographic Information for Study Participants

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<th>Depressed</th>
<th>Control</th>
<th>$\chi^2$</th>
<th>$p$</th>
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</thead>
<tbody>
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<td>.33</td>
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<td>3.02</td>
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<td>19.2</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Family History</strong></td>
<td>55.6</td>
<td>44.4</td>
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</tr>
<tr>
<td><strong>Handedness (Right)</strong></td>
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<td>94.7</td>
<td>.36</td>
<td>.55</td>
</tr>
<tr>
<td><strong>Antidepressant Medication</strong></td>
<td>54.2</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Comorbid Anxiety Diagnosis</strong></td>
<td>37.5</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Recurrent</strong></td>
<td>91.3</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Chronic Episode</strong></td>
<td>47.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

While the mean BMI for the depressed group was numerically higher, it did not statistically significantly differ between the groups. Further, all participants were categorized into Health Canada’s weight classification based on BMI and is presented in Table 3.3. Chi
square analysis indicated that there were no significant differences in the proportion of participants in each BMI category between groups, $\chi^2 (5, N=49) = 3.99, p=.55$.

Table 3.3  BMI Category by Study Group

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>Depressed</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>4.2</td>
<td>0</td>
</tr>
<tr>
<td>Normal Weight</td>
<td>37.5</td>
<td>48.0</td>
</tr>
<tr>
<td>Overweight</td>
<td>29.2</td>
<td>36.0</td>
</tr>
<tr>
<td>Obese I</td>
<td>12.5</td>
<td>12.0</td>
</tr>
<tr>
<td>Obese II</td>
<td>8.3</td>
<td>4.0</td>
</tr>
<tr>
<td>Obese III</td>
<td>8.3</td>
<td>0</td>
</tr>
</tbody>
</table>

Over half of the sample (57%) indicated that they had a comorbid medical condition, though this did not significantly differ between patients and controls ($\chi^2 (1, N=49) = .25, p=.62$). These conditions were considered to be minor in nature and included disorders such as asthma, dermatological conditions (e.g. eczema), and seasonal allergies. The study protocol dictated that participants who endorsed more severe medical conditions would be excluded from the study; however, none of the participants who consented to the study met this exclusion criterion.
### i. Characterization of Clinical Depression

Descriptive details of the depressed group are presented in Table 3.4. Based on the diagnostic interview (SCID) and DSM-IV-TR criteria, the majority of the depressed sample was categorized as having typical depression (65.2%), while 26.1% of the sample met criteria for the atypical subtype, and 8.7% met for melancholic subtype.

Table 3.4 Clinical Features of Depression by Subtype

<table>
<thead>
<tr>
<th>Type of Depression</th>
<th>Overall (n=15)</th>
<th>Typical (n=2)</th>
<th>Melancholic (n=6)</th>
<th>Atypical (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAMD 17 Total</td>
<td>17.64 (3.77)</td>
<td>18.00 (3.56)</td>
<td>20.5 (6.36)</td>
<td>17.17 (3.37)</td>
</tr>
<tr>
<td>HAMD SAD</td>
<td>7.64 (4.06)</td>
<td>5.80 (2.40)</td>
<td>5.5 (.71)</td>
<td>12.0 (4.98)*</td>
</tr>
<tr>
<td>HAMD 29</td>
<td>25.84 (5.41)</td>
<td>24.4 (4.7)</td>
<td>27.5 (7.78)</td>
<td>29.17 (6.43)</td>
</tr>
<tr>
<td>Age of Onset</td>
<td>15.92 (6.02)</td>
<td>16.07 (7.11)</td>
<td>17.5 (3.53)</td>
<td>15.67 (4.32)</td>
</tr>
<tr>
<td>Duration of Illness (years)</td>
<td>8.46 (7.54)</td>
<td>7.9 (8.86)</td>
<td>13.00 (7.47)</td>
<td>7.5 (2.51)</td>
</tr>
<tr>
<td>Number of Episodes</td>
<td>5.43 (6.23)</td>
<td>3.93 (2.57)</td>
<td>2.5 (.70)</td>
<td>11.00 (11.78)*</td>
</tr>
<tr>
<td>Number of Previous Medication Trials</td>
<td>1.04 (1.29)</td>
<td>.78 (1.19)</td>
<td>0 (.00)</td>
<td>2.2 (1.30)*</td>
</tr>
</tbody>
</table>

* p<.05 Typical vs. Atypical subtype of depression
Since appetite disturbances were relevant to this investigation, frequencies of those who endorsed appetite disturbances as part of their depressive symptomatology were calculated based on responses to appetite and weight items of the clinical diagnostic interview (SCID). Twenty-five percent of the sample did not endorse any change in appetite or weight over the past month, while 35.7% of the sample endorsed decreased appetite and/or weight loss, and 28.6% of the sample endorsed increased appetite and weight gain. Interestingly, based on items in the clinical interview of depression severity (i.e. HAMD-29), 23.07% of depressed participants endorsed weight gain, 30.8% endorsed increased appetite and increased eating, while 73.1% reported increased carbohydrate cravings over the past week.

Few participants met for melancholic subtype of depression (n=2), and therefore were excluded from all analyses involving subtype of depression. Univariate analysis of variance of severity of depression, as measured by the HAMD 17 item, did not differ between participants with typical, and atypical subtypes of depression ($F(1, 21) = .24, p = .63$). However, patients with atypical subtype had significantly higher scores on the Seasonal Affective Disorder (SAD) subscale in the HAMD than those with typical subtype ($F(1,20)=15.31, p=.001, \eta^2$). In addition, patients with atypical subtype had a greater number of previous depressive episodes ($F(1,19)= 5.19, p=.04, \eta^2=.22$) and greater number of previous antidepressant medication trials ($F(1,18)= 4.98, p=.04, \eta^2=.23$), than patients with typical depression. The means are presented in Table 3.4

**ii. Characterization of Eating Behaviour**

Independent samples t-tests were used to assess group differences in eating behavior, as measured by the DEBQ, between depressed participants and healthy controls. Means, standard
deviations, and t statistics with p-values are presented in Table 3.5. Participants with depression had significantly higher scores on all emotional eating subscales. No significant differences in external or restrained eating scores were observed. In addition, simple Pearson correlations (two-tailed) were performed on BMI and DEBQ subscale scores, and did not reveal any significant associations for both the depressed and control groups.

Table 3.5 Participant Group Differences in Eating Behaviour (DEBQ) Scores

<table>
<thead>
<tr>
<th></th>
<th>Depressed (n=19)</th>
<th>Control (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td><strong>DEBQ</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emotional Eating</td>
<td>2.60</td>
<td>.92</td>
</tr>
<tr>
<td>Emotional Eating - Diffuse</td>
<td>2.97</td>
<td>.93</td>
</tr>
<tr>
<td>Emotional Eating - Response</td>
<td>2.44</td>
<td>.99</td>
</tr>
<tr>
<td>External Eating</td>
<td>3.04</td>
<td>.74</td>
</tr>
<tr>
<td>Restrainted Eating</td>
<td>2.63</td>
<td>.85</td>
</tr>
</tbody>
</table>
DEBQ scores were categorized into degrees of eating behavior (low to very high) based on normative data (van Strien et al., 2002). The proportion of depressed and control participants per eating behavior categories are presented in Table 3.6. Chi square analysis revealed significant differences in the proportion of Emotional Eating categories and participant group. A greater proportion of depressed participants fell in the very high and high categories of emotional eating than control participants. No differences in the proportion of external eating or restrained eating between depressed and control participants were found.

Table 3.6 DEBQ Categories by Participant Group

<table>
<thead>
<tr>
<th>DEBQ Category</th>
<th>Very High (%)</th>
<th>High (%)</th>
<th>Above Mean (%)</th>
<th>Mean (%)</th>
<th>Below Mean (%)</th>
<th>Low (%)</th>
<th>(\chi^2)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DEPR</td>
<td>CTRL</td>
<td>DEPR</td>
<td>CTRL</td>
<td>DEPR</td>
<td>CTRL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emotional Eating</td>
<td>26.3</td>
<td>0</td>
<td>36.8</td>
<td>19.0</td>
<td>5.3</td>
<td>19.0</td>
<td>5.3</td>
<td>28.6</td>
</tr>
<tr>
<td>External Eating</td>
<td>21.1</td>
<td>4.8</td>
<td>26.3</td>
<td>42.9</td>
<td>1.5</td>
<td>4.8</td>
<td>26.3</td>
<td>28.6</td>
</tr>
<tr>
<td>Restrained Eating</td>
<td>10.5</td>
<td>23.8</td>
<td>21.1</td>
<td>0</td>
<td>21.1</td>
<td>19.0</td>
<td>21.1</td>
<td>42.9</td>
</tr>
</tbody>
</table>

***Characterization of Stress Perception***

Independent samples t-tests were employed to assess group differences in perceived stress as measured by self-report questionnaires. Results are presented in Table 3.7. Depressed
participants reported statistically greater levels of perceived stress than control participants as indicated by Perceived Stress Scale scores, with a strong effect size. As well, depressed participants reported a statistically greater number of daily hassles in comparison to control participants. While control participants reported a greater number of daily uplifts than depressed participants, it did not reach statistical significance.

Table 3.7 Stress Perception Measures by Participant Group

<table>
<thead>
<tr>
<th></th>
<th>Depressed (n=19)</th>
<th>Control (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Perceived Stress</td>
<td>33.94</td>
<td>6.70</td>
</tr>
<tr>
<td>Hassles Scale</td>
<td>44.67</td>
<td>19.85</td>
</tr>
<tr>
<td>Uplifts Scale</td>
<td>32.78</td>
<td>17.24</td>
</tr>
</tbody>
</table>

Associations between Neurohormonal Measures and Experimental Challenge

Partial correlations (two-tailed), controlling for BMI, were performed to assess associations between neurohormonal responses in and between experimental challenges. In addition, partial correlations (two-tailed), controlling for BMI, were performed separately on each participant group. A Bonferroni correction was applied to each analysis to control for multiple correlations.
When the entire sample was included in the analysis significant associations in hormones between experimental challenges were noted. The results are presented in Table 3.8. Positive associations for each hormone were found between experimental challenges. In other words, levels of cortisol in the stress induction positively correlated with levels of cortisol in response to the food challenge, and the same was true for both leptin and ghrelin measures.

3.8 Partial Correlations – AUC ground Neurohormones by Experiment Visit

<table>
<thead>
<tr>
<th></th>
<th>AUCg Cortisol Stress Visit</th>
<th>AUCg Ghrelin Stress Visit</th>
<th>AUCg Leptin Stress Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All (n=28)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCg Cortisol Food Visit</td>
<td>.75**</td>
<td>-.21</td>
<td>-.38</td>
</tr>
<tr>
<td>AUCg Ghrelin Food Visit</td>
<td>-.04</td>
<td>.60**</td>
<td>.16</td>
</tr>
<tr>
<td>AUCg Leptin Food Visit</td>
<td>-.40</td>
<td>.11</td>
<td>.85**</td>
</tr>
</tbody>
</table>

*uncorrected (p<.05); ** remained significant after correction (p<.02)

However, when participant groups were analyzed separately, not all associations remained significant. For example, in the depressed group, only the between experiment associations for ghrelin and leptin remained significant, while no significant association between cortisol levels in response to stress and in response to food consumption were found. However, when the control was analyzed separately, the opposite pattern was found. No significant associations between experimental challenges were noted for leptin and ghrelin, yet, a very strong significant positive association was found for cortisol. The results are presented in Tables 3.9 and 3.10.
Table 3.9  Partial Correlations – AUC ground Neurohormones by Experiment Visit (Depressed Only)

<table>
<thead>
<tr>
<th>Depressed (n=16)</th>
<th>AUCg Cortisol Stress Visit</th>
<th>AUCg Ghrelin Stress Visit</th>
<th>AUCg Leptin Stress Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$r$</td>
<td>$r$</td>
</tr>
<tr>
<td>AUCg Cortisol Food Visit</td>
<td>.23</td>
<td>.24</td>
<td>-.50</td>
</tr>
<tr>
<td>AUCg Ghrelin Food Visit</td>
<td>-.44</td>
<td>.73*</td>
<td>.23</td>
</tr>
<tr>
<td>AUCg Leptin Food Visit</td>
<td>-.51</td>
<td>.16</td>
<td>.92**</td>
</tr>
</tbody>
</table>

*uncorrected (p<.05); ** remained significant after correction (p<.02)

Table 3.10  Partial Correlations – AUC ground Neurohormones by Experiment Visit (Control Group Only)

<table>
<thead>
<tr>
<th>Control (n=12)</th>
<th>AUCg Cortisol Stress Visit</th>
<th>AUCg Ghrelin Stress Visit</th>
<th>AUCg Leptin Stress Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$r$</td>
<td>$r$</td>
</tr>
<tr>
<td>AUCg Cortisol Food Visit</td>
<td>.99**</td>
<td>-.57</td>
<td>-.75* (p=.03)</td>
</tr>
<tr>
<td>(p&lt;.01)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCg Ghrelin Food Visit</td>
<td>.19</td>
<td>.47</td>
<td>.22</td>
</tr>
<tr>
<td>AUCg Leptin Food Visit</td>
<td>-.45</td>
<td>.19</td>
<td>.68</td>
</tr>
</tbody>
</table>

*uncorrected (p<.05); ** remained significant after correction (p<.02)
Focusing on hormonal response to stress induction, associations between hormones were noted in response to a psychosocial stressor. When all participants were included in the analysis, significant negative associations were noted between leptin and cortisol, as well as, ghrelin and cortisol (Table 3.11). However, only the latter remained statistically significant after correction for multiple correlations was applied. In addition, when the depressed group was analyzed separately, only a negative association between cortisol and leptin was noted, but again, this is did not survive the correction for multiple correlations (Table 3.12). And finally, when the control group was analyzed separately significant inverse associations were found between leptin and cortisol, as well as leptin and ghrelin, though neither remained statistically significant after the correction was applied (Table 3.13).

Table 3.11 Partial Correlations between Neurohormones during Stress Induction

<table>
<thead>
<tr>
<th></th>
<th>All (n=28)</th>
<th>AUCg Cortisol Stress Visit</th>
<th>AUCg Ghrelin Stress Visit</th>
<th>AUCg Leptin Stress Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>r</td>
<td>r</td>
<td></td>
</tr>
<tr>
<td>AUCg Cortisol</td>
<td>1.00</td>
<td>-.50** (p=.018)</td>
<td>-.42* (p=.05)</td>
<td></td>
</tr>
<tr>
<td>Stress Visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCg Ghrelin</td>
<td>-.50** (p=.018)</td>
<td>1.00</td>
<td>.19</td>
<td></td>
</tr>
<tr>
<td>Stress Visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCg Leptin</td>
<td>-.42* (p=.05)</td>
<td>.19</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Stress Visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*uncorrected (p<.05); ** remained significant after correction (p<.02)
Table 3.12 Partial Correlations between Neurohormones during Stress Induction (Depressed Group Only)

<table>
<thead>
<tr>
<th></th>
<th>AUCg Cortisol Stress Visit</th>
<th>AUCg Ghrelin Stress Visit</th>
<th>AUCg Leptin Stress Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressed (n=16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r )</td>
<td>1.00</td>
<td>-.43</td>
<td>-.58*</td>
</tr>
<tr>
<td>( AUCg Cortisol Stress Visit )</td>
<td>( r )</td>
<td>1.00</td>
<td>.21</td>
</tr>
<tr>
<td>( AUCg Ghrelin Stress Visit )</td>
<td></td>
<td>-.43</td>
<td>.21</td>
</tr>
<tr>
<td>( AUCg Leptin Stress Visit )</td>
<td>( r )</td>
<td></td>
<td>1.00</td>
</tr>
</tbody>
</table>

*uncorrected (p<.05); ** remained significant after correction (p<.02)

Table 3.13 Partial Correlations between Neurohormones during Stress Induction (Control Group Only)

<table>
<thead>
<tr>
<th></th>
<th>AUCg Cortisol Stress Visit</th>
<th>AUCg Ghrelin Stress Visit</th>
<th>AUCg Leptin Stress Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r )</td>
<td>1.00</td>
<td>-.59</td>
<td>-.73*</td>
</tr>
<tr>
<td>( AUCg Cortisol Stress Visit )</td>
<td>( r )</td>
<td>1.00</td>
<td>-.74*</td>
</tr>
<tr>
<td>( AUCg Ghrelin Stress Visit )</td>
<td></td>
<td>-.59</td>
<td>.73* (p=.04)</td>
</tr>
<tr>
<td>( AUCg Leptin Stress Visit )</td>
<td>( r )</td>
<td></td>
<td>1.00</td>
</tr>
</tbody>
</table>

*uncorrected (p<.05); ** remained significant after correction (p<.02)

Associations between hormones were also examined in response to food consumption.

When all participants were included in the analysis, significant negative associations were noted between leptin and cortisol (Table 3.14), however, only the latter remained statistically significant after correction for multiple correlations was applied. In addition, when the
depressed group was analyzed separately, only a negative association between cortisol and leptin was noted, but again, this is did not survive the correction for multiple correlations (Table 3.15). And finally, when the control group was analyzed separately significant inverse associations were found between leptin and cortisol, as well as leptin and ghrelin, though neither remained statistically significant after the correction was applied (Table 3.16).

Table 3.14 Partial correlations between neurohormones in response to food challenge

<table>
<thead>
<tr>
<th></th>
<th>$AUC_g$ Cortisol</th>
<th>$AUC_g$ Ghrelin</th>
<th>$AUC_g$ Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Food Visit</td>
<td>Food Visit</td>
<td>Food Visit</td>
</tr>
<tr>
<td>All (n=31)</td>
<td>$r$</td>
<td>$r$</td>
<td>$r$</td>
</tr>
<tr>
<td>$AUC_g$ Cortisol Food Visit</td>
<td>1.00</td>
<td>.12</td>
<td>-.39*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(p=.038)</td>
</tr>
<tr>
<td>$AUC_g$ Ghrelin Food Visit</td>
<td>.12</td>
<td>1.00</td>
<td>.07</td>
</tr>
<tr>
<td>$AUC_g$ Leptin Food Visit</td>
<td>-.39*</td>
<td>.07</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>(p=.038)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*uncorrected (p<.05); ** remained significant after correction (p<.02)

Table 3.15 Partial correlations between neurohormones in response to food challenge

<table>
<thead>
<tr>
<th></th>
<th>$AUC_g$ Cortisol</th>
<th>$AUC_g$ Ghrelin</th>
<th>$AUC_g$ Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Food Visit</td>
<td>Food Visit</td>
<td>Food Visit</td>
</tr>
<tr>
<td>Depressed (n=16)</td>
<td>$r$</td>
<td>$r$</td>
<td>$r$</td>
</tr>
<tr>
<td>$AUC_g$ Cortisol Food Visit</td>
<td>1.00</td>
<td>.19</td>
<td>-.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(p=.08)</td>
</tr>
<tr>
<td>$AUC_g$ Ghrelin Food Visit</td>
<td>.19</td>
<td>1.00</td>
<td>.21</td>
</tr>
<tr>
<td>$AUC_g$ Leptin Food Visit</td>
<td>-.46</td>
<td>.21</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>(p=.08)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.16 Partial correlations between neurohormones in response to food challenge

<table>
<thead>
<tr>
<th>Control (n=15)</th>
<th>AUCg Cortisol Food Visit</th>
<th>AUCg Ghrelin Food Visit</th>
<th>AUCg Leptin Food Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>r</td>
<td>r</td>
</tr>
<tr>
<td>AUCg Cortisol Food Visit</td>
<td>1.00</td>
<td>-.01</td>
<td>-.39</td>
</tr>
<tr>
<td>AUCg Ghrelin Food Visit</td>
<td>-.01</td>
<td>1.00</td>
<td>-.05</td>
</tr>
<tr>
<td>AUCg Leptin Food Visit</td>
<td>-.39</td>
<td>-.05</td>
<td>1.00</td>
</tr>
</tbody>
</table>
4 Study 1: Cortisol Reactivity to Psychosocial Stress and Food Challenge

4.1 Synopsis of Published Literature

Stress and depression are well documented to have a strong relationship. Major life events commonly precede the onset of a depressive illness (Paykel et al., 1978), and have been suggested to act often as a trigger for onset of illness (Kendler et al., 1999). It has been proposed that stressful events or external stressor kindle a cascade of internal stressors, i.e., inflammatory processes that are part of the pathophysiology of both onset and maintenance of depression (Anisman, 2008; Miller et al., 2009). In addition, depressed individuals appear to be vulnerable to daily irritants or “daily hassles” as evidenced greater levels of perceived stress scores reported. Alterations in HPA axis activity have long been noted in patients with depression, with research documenting abnormalities in many components of the HPA axis in these patients (Stetler and Miller, 2011). Specifically, they include changes in awakening cortisol response, diurnal patterns and response to stress induction and physiological challenges (e.g. DST). However, while there is general consensus that HPA axis functioning is disrupted in patients with depressive disorders (Carroll et al., 1976; Carroll et al., 1981; Nemeroff et al., 1984; Claus, 2004), not all findings agree on the direction of such alterations. For example, while most studies report that HPA axis is over active in depression, there is evidence to suggest that some depressed patients exhibit basal levels of hypoactivity, as well as blunted responses to stress induction (Levitan et al., 2002; O’Keane et al., 2013). Such inconsistencies have been attributed to both depression subtype based on symptom pattern (i.e. melancholic vs. atypical), as well as length of illness (Gold and Chrousos, 2002; Stewart et al, 2005). In studies of laboratory stress inductions, both hyper- and hypoactivity have been noted depressed participants in comparison
with healthy control participants (Burke et al., 2005). Subjects with atypical depression or chronic depression are often linked with hypoactivity and blunted reaction to acute stress (O’Keane et al., 2005; Stewart et al., 2005), while those with melancholic type of depression are thought to have an overactive HPA axis (Gold and Chrousos, 2002; O’Keane et al., 2013).

As noted in the general introduction, neurobiological links between stress and the underlying pathophysiology of depression include inflammatory markers, among which are several cytokines. The ‘cytokine hypothesis’ proposes that both external stressors (e.g. psychosocial) as well as internal stressors (e.g. inflammatory conditions) may trigger depression via a cascade of inflammatory processes (Maes et al., 2009). Further, the interrelationship between appetite and satiety neurohormones like leptin and inflammatory markers may represent one of the pathological links between depressed and obese populations.

Closely linked with inflammatory processes, oxidative stress has been the focus of study of investigation in both obese and depressed populations. Several oxidative stress markers are associated with clinical depression. Reduction in antioxidant defenses and accumulation of excessive reactive oxygen species (by several peripheral (serum) measures) of oxidative stress has been reported in depression (Maes et al., 2011). Increased oxidative stress has also been noted in obese patients (Furukawa et al., 2004), and the association between increased oxidative stress and metabolic syndromes such as insulin resistance (Urakawa et al., 2003; Katsuki et al., 2004) as well as related illnesses (e.g. diabetes type 2) have been well documented (Evans et al., 2002). In addition, previous reports have suggested a positive association between cortisol levels and oxidative stress damage, in both clinical and non-clinical samples of individuals who experience chronic stress (Wolkowitz et al., 2010; Aschbacher et al., 2013). With this rationale, serial peripheral (serum) markers of oxidative stress were included as an exploratory objective in
this program to evaluate their relationship to stress reactivity in depressed and control participants.

Finally, the association between stress and eating behavior is well documented, with most individuals exhibiting altered eating behavior in response to stress or negative affect (Gibson, 2006). Investigations have shown that stress tends to elicit greater intake of food high in sugar and/or fat (Oliver et al., 2000) and that individuals with high emotional eating tendencies consume greater amounts of food in response to stress (van Strien et al., 2012). While there are reports evaluating the relationship between the stress hormone, cortisol, and change in food intake following response to stress, findings have not been consistent. For example, greater cortisol reactivity was associated with greater food intake in response to stress in one report (Epel et al., 2001), but in other studies, blunted cortisol response correlated with greater food intake post stressor (Tomiyama et al., 2011; van Strien et al., 2013). Variation in the duration of the stressor may provide some explanation for such discrepancies. In this context, Dallman and colleagues have proposed a model to explain the interrelationship between chronic stress, glucocorticoid changes and food intake via a proposed “chronic stress network” (Dallman et al., 2006; 2010). This model may be of particular relevance to depressed populations, who not only experience disturbances in stress perceptions and HPA axis activity, but also often have a tendency to emotional eating, and are likely to endure extended periods of stress due to the chronic or recurrent course of illness.

**Aim of Investigation I**

To evaluate cortisol response to both stress induction and food consumption in depressed subjects and normal controls and to determine if such differs by a) the presence of emotional eating behaviour, b) duration of illness and c) subtypes of depression.
Primary Hypothesis (1.1): It is hypothesized that participants with depression will experience altered HPA axis reactivity following stress and food consumption compared to control participants.

Secondary Hypotheses

(i): Participants with a chronic course or atypical subtype of depression will experience blunted HPA axis reactivity in response to experimental challenges in comparison to control participants.

(ii) HPA axis reactivity will vary by emotional eating status. Thus participants categorized as emotional eaters will experience blunted HPA axis reactivity in response to experimental challenges compared to non-emotional eaters.

(iii) Participants with depression and elevated body weight will exhibit the greatest oxidative stress damage.

4.2 Methods

The methods and procedures have been previously explained in detail in section 3.1. In brief, serial plasma cortisol was measured in subjects with major depressive disorder and matched control sample during to two experimental conditions: 1) psychosocial stress induction and 2) food challenge. Specific only to this study are analysis of cortisol and lipid hydroperoxidase samples and those methods are as follows:
a) Cortisol Analysis

Cortisol analysis was conducted at a well-established neuroscience laboratory. Cortisol determinations were performed using commercial radioimmunoassay (I$^{125}$) kits in accordance to manufacturers instruction (Linco, St. Charles, MO and MP Biomedicals, Solon, OH). All of the procedures were conducted in duplicate, and less than 8% inter- and intra-assay variability was observed. Wherever possible, assays were performed in a single run to minimize intra-assay variability. The sensitivity of the assays was in the vicinity of 8pg/mL.

b) LHP Analysis

Using plasma samples, levels of LHP were measured by the following method. In brief, levels of LPH (Cayman; Item No. 705003) were extracted from samples (150uL) by the addition of 150uL of the extraction buffer (2:1 methanol: acetic acid) and 300uL of cold chloroform (4oC) per 150uL of sample. Assay tubes were then centrifuged (1500g, 5') to isolate the bottom chloroform extract layer, which were then mixed with 20uL chromogen mixture and incubated for 45 min in the dark at room temperature. Samples were then loaded into 96-well plates and absorbance was read at 500 nm.

4.3 Results

There are a several variables known to influence the cortisol response, however, given the small sample size only variables most likely to impact results were examined. As such, age, gender, and medication status were analyzed to determine whether differences in cortisol response to stress induction and food consumption were evident.
First, age was included as a covariate in a 2 (participant group) x 6 (time over experiment) repeated measures ANCOVA analysis of cortisol response to both stress induction and food consumption. No significant interactions between age and cortisol response were found, as well, no significant between group effects of age were noted. In a subsequent analysis, gender was included as a covariate in a 2 (participant group) x 6 (time over experiment) repeated measures ANOVA analysis of cortisol response to both stress induction and food consumption. No significant interactions between gender and cortisol response were found, as well, no significant between group effects of gender were noted. To assess the influence of antidepressant medication on cortisol response, the depressed group was analyzed separately. One-way ANOVA was employed to assess differences in cortisol response to psychosocial stress and food consumption between depressed individuals receiving medication vs. those who were not. No significant differences in $AUC_G$, $AUC_I$, or peak percent change in response to both stress induction and food consumption were noted between depressed participants receiving or not receiving antidepressant medication. The results are presented in Appendix 1.

Based on these results, it was decided to proceed without including age, gender, or medication status in the subsequent analyses.

a) Cortisol Response to Psychosocial Stress induction

Cortisol response to a psychosocial stress induction was collected from 28 participants (Depressed, n=16; Control, n=12). Kolmogorov-Smirnov tests of normality were conducted to assess distribution of cortisol data and results indicated that it was not distributed normally. As such, log transformations were performed on all raw cortisol data. In addition, area under the curve (AUC) was calculated using both $AUC_G$ and $AUC_I$ formulas by Pruessner et al., (2003).
As well, for cortisol data, peak percentage change in cortisol was calculated (maximum cortisol measure – baseline / baseline x100).

**Affective response to Psychosocial Stress Induction**

The Positive and Negative Affect Scale (PANAS) was administered before and after the stress induction to measure affect changes occurring as a result of the stress induction. A 2 (participant group) x 2 (pre-post stressor) mixed measures ANOVA was conducted to examine change in positive and negative affect pre-post stress induction. The results are listed in Table 4.1. With respect to positive affect, there was no main effect of time \( F(1, 31)=.01, p=.96, \eta^2 = .00 \), or group \( F(1, 31)=1.93, p=.17, \eta^2 = .06 \), and no significant interaction \( F(1, 31)=.07, p=.80, \eta^2 = .01 \). Further, no main effect of time was found \( F(1, 31)=1.31, p=.26, \eta^2 = .04 \) for negative affect subscale scores. However, a main effect of group was noted, in that depressed participants reported greater levels of negative affect both before and after the stress induction \( F(1, 31)=11.24, p<.01, \eta^2 = .27 \), but no significant interaction was found \( F(1, 31)=.03, p=.87, \eta^2 = .01 \).
Table 4.1 PANAS Scores Pre and Post Psychosocial Stress Induction by Participant Group

<table>
<thead>
<tr>
<th>Measure</th>
<th>Depressed (n=16)</th>
<th>Control (n=12)</th>
<th>M</th>
<th>SD</th>
<th>M</th>
<th>SD</th>
<th>t</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANAS Positive Subscale – Pre Stressor</td>
<td>21.31</td>
<td>7.47</td>
<td>25.74</td>
<td>7.31</td>
<td>-1.77</td>
<td>.09</td>
<td>.60</td>
<td></td>
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<tr>
<td>PANAS Positive Subscale – Post Stressor</td>
<td>21.60</td>
<td>9.65</td>
<td>25.67</td>
<td>8.66</td>
<td>2.98</td>
<td>p&lt;.01</td>
<td>.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANAS Negative Subscale – Pre Stressor</td>
<td>17.13</td>
<td>7.49</td>
<td>11.78</td>
<td>1.66</td>
<td>-1.28</td>
<td>.21</td>
<td>.45</td>
<td></td>
<td></td>
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<tr>
<td>PANAS Negative Subscale – Post Stressor</td>
<td>18.33</td>
<td>7.53</td>
<td>12.67</td>
<td>3.12</td>
<td>2.91</td>
<td>p&lt;.01</td>
<td>.98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

i. *Cortisol Response to Stress Induction*

As alterations in cortisol response to stress have been noted in depressed populations, it was important to determine whether any such differences existed in this study sample. A 2 (participant group) x 6 (time over experiment) mixed measures ANOVA was conducted to assess group differences in mean cortisol levels during the stress induction. This analysis revealed significant main effect of time, $F(5, 130)=3.00, p=.01, \eta^2 = .10$, however, no significant interaction was found ($F(5, 130)=0.64, p=.67, \eta^2 = .02$). The results are presented in Figure 4-1.
Figure 4-1 Mean cortisol levels (SD) at each time point in the stress induction protocol. While levels significantly differed over the course of the experiment, there were no differences between depressed and control groups.

Independent-samples t-tests were used to assess differences in AUC cortisol and peak percent change in cortisol between participant groups in the stress induction experiment. No significant differences were found between depressed participants (M=177.29, SD=26.49) and control participants (M=184.22, SD=48.64) for AUC\textsubscript{G} cortisol (t(26)=-.48, p=.63). Similarly, there were no significant differences found between depressed participants (M=-23.90, SD=20.51) and control participants (M=-26.88, SD=17.35) for AUC\textsubscript{I} cortisol (t(26)=.41, p=.69). Further, no significant differences in peak percent change in cortisol in response to the stress
induction were found between depressed participants \((M=43.97, SD=33.92)\) and control participants \((M=26.30, SD=24.69)\) \((t(25)=1.48, p=.15)\). The results are presented in Table 4.2.

Table 4.2 Depressed vs. Control Cortisol Measures in Response to Psychosocial Stress Induction

<table>
<thead>
<tr>
<th>Cortisol Measures</th>
<th>Depressed (n=19)</th>
<th>Control (n=21)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>AUC ground</td>
<td>177.29</td>
<td>26.49</td>
<td>184.22</td>
<td>48.64</td>
</tr>
<tr>
<td>AUC increase</td>
<td>-23.90</td>
<td>20.51</td>
<td>-26.90</td>
<td>17.35</td>
</tr>
<tr>
<td>Peak % change</td>
<td>43.97</td>
<td>33.92</td>
<td>26.30</td>
<td>24.69</td>
</tr>
</tbody>
</table>

**ii. Depression Subtype and Cortisol Reactivity to Stress Induction**

The literature to suggest that cortisol response may differ based on depression subtype (e.g. Gold and Chrousos, 2002), and therefore, a separate analysis was conducted on the depressed group alone to determine if there were any differences based on subtype of depression.

Univariate analysis of variance was employed to assess whether any differences in \(\text{AUC}_G\), \(\text{AUC}_I\), and peak percent change in cortisol response to stress induction between the two main subtypes of depression, i.e., typical \((n=11)\) and atypical \((n=4)\) (only one participant met diagnostic criteria for melancholic subtype, and therefore, was not included in comparisons). No
differences were found in AUC\textsubscript{G} cortisol between typical ($M=172.11$, $SD=23.74$) and atypical ($M=183.14$, $SD=33.28$) subtypes ($F(2, 15)=1.14$, $p=.35$). The results were similar with AUC\textsubscript{I} cortisol between typical ($M=-25.09$, $SD=24.32$) and atypical ($M=-25.00$, $SD=4.64$) subtypes ($F(2, 15)=.36$, $p=.70$) with no significant differences noted. Similarly, no differences in peak percent change in cortisol response to stress induction between typical ($M=46.95$, $SD=39.45$) and atypical ($M=30.42$, $SD=11.41$) subtypes ($F(2, 15)=.53$, $p=.60$) were seen. However, given the extent to which the groups are unequal and the overall small sample size, comparisons between subtypes of depression should be interpreted with caution.

\textit{iii. Cortisol Response and Duration of Depressive Illness}

In addition to depressive subtype, duration of illness has been reported as influencing HPA axis activity. As such, exploratory analyses were conducted to see if chronicity of illness was associated with cortisol reactivity following stress induction.

Univariate analysis of variance was also employed to assess whether AUC\textsubscript{G}, and AUC\textsubscript{I} cortisol response to psychosocial stress was influenced by the chronicity of current depressive episode. Duration of two years was used as a cut off (as per DSM-IV criteria). No significant differences were found in AUC\textsubscript{G} cortisol between patients with a current episode $\geq 2$ years (n=10) ($M=175.23$, $SD=27.53$) compared to those with a current episode $< 2$ years (n=6) ($M=180.74$, $SD=26.80$), ($F(1, 14)=.15$, $p=.70$). Similar lack of difference between patients with depressive episode $\geq 2$ years ($M=-19.59$, $SD=18.76$) and those with an episode $< 2$ years ($M=-31.07$, $SD=23.01$) was noted in AUC\textsubscript{I} cortisol following the psychosocial stress induction ($F(1, 14)=1.19$, $p=.29$). As well, no significant difference in peak percent change were noted between participants with a depressive episode $\geq 2$ years ($M=51.79$, $SD=37.65$) compared to those with a current episode of shorter duration ($M=30.94$, $SD=23.95$), ($F(1, 14)=1.46$, $p=.25$).
In addition simple Pearson correlations (two-tailed) were employed to assess associations between duration of depressive illness (total years) and measures of cortisol reactivity. No significant associations were found between AUC$_G$, AUC$_I$, and duration of depressive illness. However, a significant negative association was found between duration of illness and peak percent change ($r(16)=-.53$, $p=.03$, two-tailed). Given the focus of this investigation, BMI was also included in this analyses and a marginally significant negative correlation was found between BMI and peak percent change ($r(16)=-.45$, $p=.08$, two-tailed). Subsequently, a linear regression of peak percent change in cortisol, with BMI and duration of illness included in the model, was conducted and was found to significantly predict peak cortisol change with duration of illness accounting for a significant proportion of the variance ($R^2=.44$, $F(2,13)=5.03$, $p=.02$). Duration of illness significantly predicted peak cortisol change $\beta=-2.17$, $t(13)=-2.30$, $p=.04$, while with BMI, it showed a trend for significance, ($\beta=-1.81$, $t(13)=-1.87$, $p=.08$).

**iv. Cortisol Response to Stress Induction by Emotional Eating Status**

It was also of interest to determine whether eating behavior style had any influence on cortisol response to a psychosocial stressor (van Strien et al., 2013). Therefore, using scores from DEBQ Emotional Eating subscale, participants were defined as an emotional eater if the emotional eating subscale score on the DEBQ fell above normative means (van Strien et al. 2002). Given that no differences in cortisol response were found between depressed and control participants, the combined sample was divided on the basis of emotional eating status and comparisons were then made between emotional (n=15) and non-emotional eaters (n=10) in AUC cortisol and peak cortisol change in response to the stress induction procedures.

A 2 (participant group) x 6 (time over experiment) mixed measures analysis of variance (ANOVA) was conducted to assess differences in mean cortisol levels between emotional and
non-emotional eaters during the stress induction. This analysis did reveal a significant main effect of time \((F(5, 115)=3.91, p<.01, \eta^2 = .15)\). However, there was no main effect of group \((F(1, 22)=1.30, p=.27)\), and no significant interaction was found \((F(5, 110)=.55, p=.74)\). The results are presented below in Figure 4-2.

Figure 4-2 Mean cortisol levels (SD) at each time point in the stress induction protocol by emotional eating category. While levels significantly differed over the course of the experiment, there were no differences between emotional and non-emotional eaters.
Independent samples t-tests were employed to assess differences in cortisol measures between emotional and non-emotional eaters. The results are presented in Table 4.3. No significant differences were found between emotional eaters ($M=176.44$, $SD=29.01$) and non-emotional eaters ($M=193.84$, $SD=47.61$) in $AUC_G$ cortisol ($t(23)=-1.14$, $p=.27$). Again, no significant differences found between emotional eaters ($M=-24.19$, $SD=17.95$) and non-emotional eaters ($M=-20.35$, $SD=15.99$) in $AUC_I$ cortisol ($t(23)=-.55$, $p=.59$). Similarly, no significant change in peak cortisol change was noted between emotional eaters ($M=40.98$, $SD=36.64$) and non-emotional eaters ($M=54.77$, $SD=55.98$) in $AUC_I$ cortisol ($t(23)=-.75$, $p=.46$).

Table 4.3 Emotional vs. Non-Emotional Eaters Cortisol Measures in response to Psychosocial Stress Induction

<table>
<thead>
<tr>
<th></th>
<th>Emotional Eaters (n=15)</th>
<th>Non-Emotional Eaters (n=10)</th>
<th>$t$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol Measures</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AUC ground</td>
<td>176.44</td>
<td>193.84</td>
<td>-1.14</td>
<td>.27</td>
</tr>
<tr>
<td>AUC increase</td>
<td>-24.19</td>
<td>-20.35</td>
<td>-.55</td>
<td>.59</td>
</tr>
<tr>
<td>Peak % change</td>
<td>40.98</td>
<td>54.77</td>
<td>.75</td>
<td>.46</td>
</tr>
</tbody>
</table>

In addition, the possible influence of stressors outside the experimental environment on cortisol response to the stress induction procedures was evaluated. Pearson correlations (two-tailed) were performed to determine whether there were any significant associations between
stress reactivity and stress-related self-report measures (e.g. perceived stress scores). Only perceived stress scores were significantly associated with peak percent change in cortisol \((r(25)=.41, p=.04)\), suggesting that higher perceived stress was associated with greater peak change in cortisol in response to a stressor for the entire study sample. Further, given the nature of this investigation, it was of interest to assess a possible contribution of BMI to this significant association. As such, a linear regression of peak percent change in cortisol, with BMI and perception of stress scores included in the model, was conducted. This model was found to significantly predict peak cortisol change, accounting for a significant proportion of the variance \((R^2=.59, F(2,24)=5.91, p<.01)\), with both BMI \((\beta=-1.99, t(24)=-2.45, p=.02)\) and perceived stress scores \((\beta=1.59, t(24)=2.80, p=.01)\) significantly predicting peak cortisol change during the stress induction.

\[Oxidative \ Stress \ Marker \ in \ response \ to \ Stress \ Induction\]

With recent literature providing a rationale (Maes et al., 2011), changes in peripheral oxidative stress markers, specifically lipid hydroperoxidation (LHP), in response to stress induction, were evaluated. A total of 31 subjects (Depressed n=16; Control n=15) were evaluated and 3 time points were included in the analysis: 1) baseline, 2) 10 minutes post stressor, and 3) end of recovery period (60 minutes post stressor). Kolmogorov-Smirnov tests of normality were conducted to determine the distribution all LHP data and results indicated that it was distributed normally.

A 2 (participant group) \times 3 \ (time \ over \ experiment) mixed measures analysis of covariance (ANCOVA) was conducted to assess group differences in mean LHP levels during
the stress induction. Given the well-documented association between age and oxidative stress markers (for recent reviews see Vitale et al., 2013; and Jacobs et al., 2013), age was included as a covariate in the model. A marginally significant main effect of time was noted ($F(2, 27)=2.63$, $p=.09$, $\eta^2 = .16$), but no effect of group was found ($F(2, 27)=.05$, $p=.81$). No significant interaction of group by time was noted ($F(2, 56)=.07$, $p=.92$), however, a significant age by time interaction was found ($F(2, 27)=3.12$, $p=.05$, $\eta^2 = .10$). Results are presented in Figure 4-3.

Figure 4-3 Mean LHP levels (SD) at each time point in the stress induction protocol by participant group. While levels significantly differed over the course of the experiment, there were no differences between depressed and control participants.
Based on the association found between age and LHP levels, additional exploratory analyses were utilized to investigate whether differences in LHP could be identified based on age. The sample was split into two age groups (<= 30 years of age (n=14) vs. >30 years of age (n=17)) based on age distribution of study participants. Independent samples t-tests were used to detect differences between the two age groups at each time point. No significant difference were found at baseline between participants <= 30 years (\(M=11.24, SD=.1.16\)) compared to participants >30 years (\(M=11.33, SD=1.52\)), at baseline \(t(29)=-.18, p=.86\). Similarly, at 60 minutes post stressor, no significant differences were found between participants <= 30 years (\(M=11.83, SD=1.35\)) and participants >30 years (\(M=11.37, SD=1.71, t(29)=.82, p=.42\)). However, 10 minutes following the stressor, a marginally significant difference between participants <= 30 years (\(M=12.00, SD=1.76\)) and participants >30 years (\(M=10.74, SD=1.95\)), was found \(t(29)=1.87, p=.07\), suggesting that those >30 years exhibited lower levels of LHP, and as such, less evidence of oxidative stress.

To further explore this finding, Pearson correlations (two-tailed) were performed on age and LHP levels at each time point. No significant associations were found between age and baseline \(r(31)=-.17, p=.37\), or age and 60 minutes post stressor \(r(31)=-.22, p=.24\) time points. However, a significant inverse association was found between age and LHP levels 10 minutes post-stressor \(r(31)=-.46, p=.01\), of which depressed participants accounted for 36% of the total variation \(R^2=.36\). The results are presented in Figure 4-4. Based on these results, it was of interest to determine if age of onset of illness or duration of illness were associated with LHP measures for depressed participants. However, no significant associations were found between
LHP post stressor and age of onset ($r(16)=-.10, p=.72$, two-tailed) or duration of illness ($r(16)=-.34, p=.19$, two-tailed).

Figure 4-4 LHP by Age Immediately following Stress Induction by Participant Group

It was also of interest to determine if there were any associations between oxidative stress and cortisol response to stress in the study sample. Partial correlations were used to determine if there were any significant associations between AUC$_G$ LHP and cortisol. Results indicate that a significant positive association between AUC$_G$ LHP and AUC$_G$ cortisol was found for all
participants. When the sample was divided into depressives and controls, this association remained significant for depressed participants but only approached significance for control participants. The results are presented in Table 4.4.

Table 4.4 Partial Correlations for LHP and Cortisol in Response to Psychosocial Stress

<table>
<thead>
<tr>
<th></th>
<th>All participants (n=28)</th>
<th>Depressed (n=16) AUC LHP</th>
<th>Control (n=12) AUC LHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC Cortisol</td>
<td>.40*</td>
<td>.55*</td>
<td>.60+</td>
</tr>
</tbody>
</table>

* p<.05; + p=.08

b) Food Challenge

Pre and post-prandial cortisol, ghrelin, and leptin measures were obtained from 31 participants (Depressed, n=16; Control, n=15). Kolmogorov-Smirnov tests of normality were conducted to determine the distribution of cortisol data collected in the food challenge, and results indicated a lack of normal distribution. As such, log transformations were performed on all raw cortisol data. In addition, area under the curve (AUC) was calculated using the AUC_G and AUC_I formulas by Pruessner et al., (2003).

Cortisol in Response to Food Challenge

The results of mean cortisol response to the food challenge by participant group are presented in Figure 4-5. A 2 (participant group) x 4 (time over experiment) repeated measures ANOVA was conducted to assess group differences in mean cortisol levels during the food challenge visit. This analysis revealed a main effect of time that approached significance (F(3,
87)=2.52, \( p=.06, \eta^2 = .10)\), but no group effects and no significant time by group interaction \((F(3, 87)=.34, p=.80)\).

Figure 4-5 Mean cortisol levels (SD) at each time point during the food challenge protocol. A marginally significant decrease in post-prandial cortisol levels was observed. No group differences or interaction was found.

Independent-samples t-tests were used to assess differences in AUC cortisol in the food challenge experiment. No significant differences found between the depressed \((M=176.82, SD=18.07)\) and control participants \((M=178.02, SD=24.99)\) for \(AUC_G\) cortisol \(t(29)=-.15,\)
Similarly, there were no significant differences for AUC$_I$ ($t(29)=-.84, p=.41$), between depressed participants ($M=-8.04, SD=16.71$) and control participants ($M=-3.43, SD=13.73$).

Cortisol Response to Food Challenge: Depression Subtype and Duration of Depressive Illness

As previously noted, it may be important to determine if differences in cortisol response can be attributed to subtype of depression. Univariate analysis of variance was employed to assess whether AUC$_G$, and AUC$_I$ cortisol response to meal ingestion differed between the main subtypes of depression (Typical n=12; Atypical n=3). Unfortunately only one participant who completed the food challenge was categorized as melancholic subtype and thus, was excluded from the analysis. However, among the other two i.e., typical ($M=178.51, SD=15.70$) and atypical ($M=171.18, SD=31.99$) subtypes of depression, no differences were found in AUC$_G$ cortisol ($F(2, 15)=.19, p=.82$). Similarly, no significant differences were noted in AUC$_I$ cortisol between the two (typical ($M=-4.43, SD=17.17$) and atypical ($M=-21.48, SD=10.86$); ($F(2, 15)=1.32, p=.30$). However, given the extent to which the groups are unequal and the overall small sample size, comparisons between subtypes of depression should be interpreted with caution.

Univariate analysis of variance was also employed to assess whether AUC$_G$, and AUC$_I$ cortisol response to meal ingestion was influenced by the chronicity of current depressive episode. Duration of two years was used as a cut off (as per DSM-IV criteria). No significant differences were found in AUC$_G$ cortisol between patients with an episode $\geq$ 2 years (n=10) ($M=181.85, SD=16.21$) compared to those with an episode $< 2$ years (n=6).
(M=168.45, SD=19.30), (F(1, 14)=2.23, p=.16). Similar lack of difference between patients with depressive episode >= 2 years (M=-9.96, SD=14.21) and those with an episode < 2 years (M=-4.82, SD=21.31) was noted in AUC₁ cortisol following the food challenge (F(1, 14)=.34, p=.57).

**Cortisol Response to Food Challenge by Emotional Eating Status**

Results from previous investigations suggest that cortisol response may be influenced by emotional eating behaviours (Raspopow et al., 2010). As previously described, the sample was divided based on into two groups based on the presence of emotional eating pattern. A 2 (participant group) x 4 (time over experiment) mixed measures ANCOVA, with BMI as a covariate, was conducted to assess group differences in mean cortisol levels between emotional and non-emotional eaters during the food challenge experimental visit. No main effect of time found (F(3, 69)=1.02, p=.39), suggesting that cortisol levels did not change significantly over the course of the food challenge experiment for either emotional or non-emotional eaters. However, there was a significant main effect of group (F(1, 23)=5.27, p=.03, $\eta^{2} = .18$), but no significant time by group interaction (F(3, 69)=1.27, p=.29. Participants categorized as emotional eaters exhibited lower levels of cortisol in comparison to non-emotional eaters throughout the food challenge. The results are presented in Figure 4-6.
Figure 4-6 Mean cortisol levels (SD) at each time point in the food challenge protocol with study sample divided by emotional eating status (emotional vs. non-emotional eater). Cortisol levels did not change over the course of the experiment. However, emotional eaters had significantly lower levels of cortisol in comparison to non-emotional eaters throughout the experiment.

Independent samples t-tests were employed to assess differences in cortisol measures in response to food ingestion between emotional (n=13) and non-emotional eaters (n=13). Emotional eaters (M=171.69, SD=18.13) had significantly lower significant AUCG cortisol in response to meal ingestion compared to non-emotional eaters (M=187.75, SD=20.45) (t(24)=-2.12, p=.04). However, no significant differences were found between emotional eaters (M=-
7.27, SD=11.85) and non-emotional eaters (M=-7.20, SD=13.60) in AUC cortisol (t(23)=-.01, p=.99). These results are presented in Table 4.5.

Table 4.5 Emotional vs. Non-Emotional Eaters Cortisol Measures in Response to Food Challenge

<table>
<thead>
<tr>
<th>Cortisol Measures</th>
<th>Emotional Eaters (n=13)</th>
<th>Non-Emotional Eaters (n=13)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC ground</td>
<td>171.69 18.13</td>
<td>187.75 20.45</td>
<td>-2.12</td>
<td>.04</td>
</tr>
<tr>
<td>AUC increase</td>
<td>-7.27 11.85</td>
<td>-7.20 13.60</td>
<td>.01</td>
<td>.99</td>
</tr>
</tbody>
</table>

4.4 Discussion

Stress Perception

In the current investigation, depressed participants reported greater levels of perceived stress compared to controls, with a strong effect size. Depressed participants also reported a greater number of perceived daily hassles than healthy controls. Both these findings are consistent with previous reports (Peeters et al., 2003; Ravindran et al., 2002). In addition, depressed participants also reported greater degree of emotional eating in comparison to healthy controls, which is also consistent with previous reports (McKay et al., 2009) Further, elevated
stress perception was significantly associated with higher tendency to emotional eating in the combined samples of controls and depressed participants, which could be attributed to “comfort eating”. But when the sample was divided by participant groups and analyzed separately, this association was no longer significant. As the total sample was small, larger numbers of subjects may have confirmed the association. It is also worth considering that participants who reported high levels of emotional eating did so more in response to other specific affectively charged circumstances, rather than in response to higher levels of non-specific perceived stress. This may be particularly relevant to depressed individuals, who experience persistent global negative affect. Investigations with a larger sample size, and that involve different types of stressors, may help to clarify the relationship between stress perception and emotional eating in depression.

*Cortisol response to experiments*

1.1 Participant Group Differences in Cortisol Response to Stress Induction and Food Consumption

No significant differences were found in cortisol response to stress induction or food consumption between depressed and control participants. In addition, no significant differences in all cortisol measures, AUC and peak percent change, were found between depressed participants and healthy controls, in response to psychosocial stress or food consumption.

One probable explanation for these finding may be the small sample size. It is possible that there were too few participants in each group to detect a difference, especially with a measure such as cortisol, the levels of which are very sensitive to change (Kudielka et al., 2009). Further, some reports indicate that only a small proportion of depressed patients, regardless of subtype, exhibit overactivity of HPA axis, and that some patients are indistinguishable from
control participants based on measures of cortisol secretion (Young et al., 2001). The current patient sample may have contained depressed participants who are more similar to healthy controls in terms of HPA axis activity. With respect to cortisol response to the stress induction, it is possible that it may have been influenced by the bias towards atypical subtype and lack of melancholic subjects in the current sample. Previous investigations have shown that melancholic depressives tend to display overactivity or an exaggerated cortisol response in comparison to atypically depressed individuals (Gold and Chrousos, 2002). Given that there was only one depressed participant who met criteria for melancholic subtype of depression (who was subsequently excluded from subtype analyses), patients who are known to exhibit HPA axis overactivity were under represented in this current study sample. Indeed, when the depressed sample was analyzed separately, there were no differences in cortisol measures (AUC and peak response) between depressive subtypes. Specifically, there were no significant differences between in cortisol response to either stress or food consumption between the typical and atypical subtype groups. However, these results should be interpreted with caution given the small sample size and unequal subsample sizes. In addition, given the exploratory nature of this research program and its main focus on neurohormonal response to experimental challenges, depressed participants were recruited based on core diagnosis. Thus, it would be premature to draw any firm conclusions about subtype differences in neurohormonal response to stress and food consumption, based on this current investigation.

Another explanation for the lack of difference in the cortisol response between control and depressed participants may be the influence of longer duration of the illness of the depressed sample as a whole. It has been noted that individuals with a longer or recurrent course of depression often exhibit a blunted cortisol response to stress, while those experiencing an acute depressive episode may exhibit an exaggerated one. The current study sample consisted of
depressed participants who either experienced a recurrent or chronic course of depression, with few currently experiencing an acute depressive episode. With such an overrepresentation of participants with chronic or recurrent course of illness, it is perhaps unsurprising that no difference in cortisol reactivity to a psychosocial stressor was found between controls and depressed participants.

Findings related to chronicity of illness as a domain are also noteworthy. The chronicity of the illness was defined using two parameters: 1) total duration of depressive illness (in years), and 2) duration of current depressive episode (>= 2 years vs. < 2 years). When the sample was divided based on the duration of current episode, with a cut off of two years, no differences in cortisol measures (AUC, peak response) were evident. However, when duration of illness (measured in years) was analyzed, it showed a negative correlation with the magnitude of cortisol response following the social stressor. In other words, the longer the duration of the depressive illness, the weaker was the cortisol response following the social stressor. This finding is consistent with several previous reports, which have reported attenuated or blunted cortisol in chronic depression or in humans and animals subjected to repeated stress (Burke et al., 2005; Dallman et al., 2010).

The difference in findings between two measures of chronicity may simply be a reflection of the rigidity of diagnostic criteria (chronic depressive episode >= 2 years in length) and may include those with so-called double depression, i.e., superimposition of an acute episode in a persistent depressive illness. Thus, the duration of illness would capture both groups but not subjects with recurrent (but not continuous) illness. Regardless of diagnostic definitions, the two depressive categories may be better conceptualized as a joint category, as most patients with
recurrent depression do not experience full inter-episodic remission but often experience residual symptoms between episodes.

1.2 Emotional Eating and Cortisol Response

When the sample was divided by emotional eating status, no significant differences in cortisol response to the stress induction were found between emotional and non-emotional eaters. However there are several previous reports noting lower cortisol levels in emotional eaters (Tomiyama et al., 2011; van Strien et al., 2013) and the discrepancy with our findings is difficult to explain. However, we did note that in response to food consumption, emotional eaters exhibited lower total cortisol (AUC_G) in comparison to non-emotional eaters. To our knowledge, this is the first report evaluating the association between emotional eating and several cortisol levels, including postprandial ones, and the first to examine this relationship in depressed and matched control populations. As such, there are few earlier reports that may shed light on the above inconsistencies. In addition, the subgroup numbers were somewhat unequal, and together with the small sample size, restricted the extent of analyses performed. Additional investigations with a larger sample are warranted.

1.3 Associations with Cortisol Response, Perceived Stress, and BMI

Across all participants, a significant robust association was found between cortisol response to the stressor (as measured by peak percent change) and the degree of self-reported perceived stress. Specifically, participants who reported higher levels of perceived stress had a higher peak percent change in cortisol following the social stressor. Furthermore, BMI and perceived stress scores were found to predict cortisol response to stress, with an inverse relationship noted between BMI and cortisol response, i.e. greater BMI was associated with
reduced cortisol response to stress. This finding is of particular interest, and may be consistent with proposed models of stress and obesity (Dallman et al., 2010).

Several longitudinal investigations support the notion that greater levels of perceived stress are associated with higher BMI (Harding et al., 2013; Mouchacca et al., 2013; Wardle et al., 2011). It has been proposed that this association may be tempered by gender and age, with a stronger relationship between perceived stress and BMI found in younger, female populations (Brandheim et al., 2013). Indeed, the contribution of physiological response to stress has also been investigated in association with increased BMI (Bornstein et al., 2000), although a recent systematic review suggests a somewhat weaker association between perceived stress, cortisol, BMI and metabolic syndrome (Abraham et al., 2013). While there is overlap, it should be noted that these studies investigated diurnal and basal levels of cortisol, rather than reactivity to an acute stressor. Our current finding is consistent with at least one report that investigated abdominal obesity, levels of perceived stress, and cortisol reactivity to an acute stressor in a non-clinical sample, which found that females experiencing chronic stress, who also reported higher levels of perceived stress, were found to have greater BMI and waist circumference, and exhibited a blunted cortisol reaction to an acute stressor (TSST) (Tomiyama et al., 2011).

However, the perceived stress was reported in the time frame of the past week, and therefore, it is unclear whether the perceived stress scores were related to acute or chronic stressors.

In the current study, when analysis included depressed participants only, it was noted that BMI and duration of illness were found to predict peak percent change in cortisol in response to stress. Specifically, greater BMI and longer duration of illness were predictive of a lower cortisol response. This supports the notion that the ingestion of comfort food in depression is to be considered a physiological form of a “chronic stressor”. Others have proposed that
depression, itself, is a stressor, and as such triggers a cascade of internal processes leading to the onset and maintenance of the illness (Anisman, 2008). If so, those who experience a longer duration of illness may experience prolonged adverse physiological consequences similar to that those associated with exposure to chronic stress. Current results could be explained in the context of such theories, which also suggest that an attenuated stress response may be elicited through “comfort eating” of palatable food and abdominal obesity resulting from exposure to chronic stress. In this current study, longer duration of illness (chronic stress) and greater BMI predict attenuated stress response to an acute stressor. However, fluctuations in appetite disturbances over the course of depressive illness, and resulting impact on stress response, has not been well examined thus far, and such information may provide further elucidation of this relationship.

1.4 Oxidative Stress Markers

In an exploratory component of this investigation, the impact of stress on the peripheral oxidative stress marker, lipid hydroperoxidase (LHP), was examined. No significant differences in LHP following stress induction were found between depressed and control participants. However, when age was included as a covariate in the model, a marginally significant difference was noted in LHP between younger and older participants (regardless of group status) immediately following the stress induction, with participants >= thirty years exhibiting marginally less peripheral oxidative stress, as measured by LHP, than those < 30 years. However, LHP measures at baseline and recovery appear to be unaffected by age, suggesting that the immediate impact of stress is more pronounced in younger participants. In addition, when participant group was taken into consideration, the depressed group accounted for 34% of the
total variation, while controls accounted for only 11\%, which suggests that depressed individuals provided a greater contribution to this relationship.

As it is often commonly held that the combination of greater age and presence of depression increases vulnerability to oxidative stress, this anomalous finding with age in the current study was unexpected (Wolkowitz et al., 2010). One possible explanation may be the role of ‘homeostatic adaptation’. Stress, a disruption to equilibrium, triggers physiological and behavioural responses that are aimed at restoring homeostasis (Seyle, 1936). Through processes like allostasis, “maintaining stability through change”, balance is maintained through alterations in activity of any number of physiological in response change in environment or challenge (McEwen, 2000). In other words, changes in glucocorticoid activity, among other mediators, promote adaptation to a stressor if only required for a short duration of time, otherwise it can become a burden and result in allostatic overload (McEwen, 2005). Given that people with depression report greater levels of perceived stress and daily hassles, they may not respond to an acute stressor in the same manner as individuals with fewer perceived environmental stressors. In other words, response to an acute stressor may not be as pronounced in individuals who are accustomed to persistent stressors, i.e., older participants with depression, in comparison to individuals who less accustom to stress. Over time, adaptation to acute stressors may occur at the cellular level. However, future studies are required to support such speculation.

In alignment with theories regarding allostatic overload (McEwen, 2005), previous reports have suggested a positive association between cortisol levels and oxidative stress damage, in both clinical and non-clinical samples of individuals who experience chronic stress (Wolkowitz et al., 2010; Aschbacher et al., 2013). For example, participants who experience chronic stress have shown greater oxidative stress damage, which was positively associated with
cortisol reactivity to an acute stressor (Aschbacher et al., 2013). Thus, again, our findings appear counter intuitive.

One alternate explanation may be the effect of psychotropic medication received by the subjects. Antidepressant medication has been shown to have antioxidant properties and neuroprotective effects that may weaken the impact of acute stress on cellular processes (Dodd et al., 2013). Overall, when patients receiving medication were compared to those not currently receiving antidepressant medications, no differences in LHP measures (AUC) were found (See Appendix 1). However, it is possible that in the current study, depressed participants >= 30 years, if taking medications, may have been doing so for longer periods of time, and therefore, any additive benefits of antidepressant medications against oxidative stress damage are more pronounced. However, these suggestions remain speculative, and future studies are needed to assess the effect of illness chronicity and the use of antidepressant medication on stress response.

In further exploration of associations between oxidative stress markers and neurohormone levels in response to stress, partial correlational analyses (controlling for BMI) were performed in the current investigation. A positive correlation between plasma cortisol and LHP was noted for all participants, in keeping with the published literature, suggesting that as the cortisol levels increased, so did oxidative stress, as measured by LHP. However, when the sample was divided by participant group, the effect was significant for only the control group, but not for the depressed group. This was surprising as the expectation was that this relationship would be stronger in the depressed population through the additive effect of illness on both cortisol and LHP parameters. This latter result may in part accounted for by the effect of antidepressant medication received by the depressed group. Antidepressant treatment is said to impact on oxidative stress damage in two potential ways: 1) antidepressant treatment may alter
HPA activity for a proportion of depressed patients (McKay and Zakzanis, 2010), which then in turn reduces oxidative stress damage; and/or, 2) antidepressant treatment protects against oxidative stress damage independently of HPA axis activity (Dodd et al., 2013). The role of antidepressants can only be resolved by the evaluation of oxidative stress damage in parallel between medicated and unmedicated patients.

**Clinical implications and Future Directions**

While no significant differences in cortisol reactivity were found in patients with depression compared to normal controls in response to experimental challenges, there are several key findings from this study that are likely of translational value. One is a relationship between chronicity of depression and increased vulnerability to obesity, which may be mediated by HPA axis activity. Furthermore, we found that higher BMI and longer duration of illness predicted blunted cortisol reaction to stress induction, which are consistent with the proposed model of chronic stress that suggests that chronic stress increases the likelihood of comfort eating. Current findings further support the notion that depressed participants have a greater likelihood of emotional eating and higher levels of perceived stress, both of which raise vulnerability to weight gain and obesity. A strong association between obesity and depression is well documented, and as such, identification of vulnerabilities that could contribute to this association is of considerable clinical value. Further, this investigation found that for all participants, increased BMI and higher levels of perceived stress predicted a blunted cortisol reaction to stress induction, which is not only consistent with proposed models of chronic stress and comfort eating, but also highly relevant to the study of obesity itself. While it has been suggested that increased age and depression individually may increase vulnerability to oxidative stress damage,
we did not observe this effect, though antidepressant medication use by a significant number of depressed participants may have contributed to the negative result.

Taken together, the results of the current investigation suggest that depressive illness, and particularly chronic forms of depression, maybe a key pathological contribution to emotional eating and, in turn, to weight gain and obesity. Obesity is a serious public health issue and these findings highlight the need for aggressive intervention, particularly for milder chronic forms of depression.

**Limitations and Future directions:**

One limitation of this study was small sample size, and future replication studies with a larger sample size are required. In addition, given the novelty of this investigation, depressed participants were not selected based on subtype of depression or duration of illness. Future investigations should stratify the sample based on duration of illness (e.g. acute, recurrent, persistent), as well as select for subtype of depression (e.g. atypical and melancholic) in order to determine if any of these subpopulations are particularly vulnerable to weight gain. In addition, it may be beneficial to assess how eating behavior changes over time as depressive illness persists.
5 Study 2: Appetite and Satiety Neurohormones: effect of Psychosocial Stress and Food Challenge

5.1 Synopsis of Published Literature

The neurohormones, leptin and ghrelin, serve multiple physiological functions. Among these, their appetite and satiety functions are particularly linked to obesity. Both hormones are also thought to play roles in the pathophysiology of both depression and the stress response.

Leptin is considered to be one of the most important discoveries to the study of obesity due to its function as an adipose signal and anorexic influence (Zhang et al., 1994; Trayhurn, 2013). Individuals functioning under positive energy stores, i.e., obese individuals, exhibit high concentrations of leptin (Schwartz et al., 2005). Leptin has also been shown to dampen HPA axis activity and as such, is a likely candidate in the study of stress induced eating (Ahima et al., 2000; Licino et al., 1996). In addition, leptin is thought to be an important consideration in the pathophysiology of depression due to its role in energy homeostasis and relation to HPA axis activity (Lu, 2007). However, investigations of leptin levels in depressed populations have yielded inconsistent findings, with elevated levels, low levels and no difference noted in comparison to healthy participants (Antonijevic et al., 1998; Kraus et al., 2001; Deuschle et al., 1996).

The discovery of ghrelin and its role in appetite regulation has been a similarly important contribution to the study of obesity (Kojima and Kangawa, 2006). Low levels are noted in patients with obesity (English et al., 2003), while high levels found in patients of low body weight and in individuals with eating disorders such as anorexia (Tolle et al., 2003). Ghrelin also interacts with the HPA axis and as such, has also been a focus in the study of stress induced
eating (Wren et al., 2002; Schmid et al., 2005). Levels of ghrelin generally decline rapidly after
food ingestion, but differences have been noted in individuals with disrupted eating behaviors
(Tschop et al., 2000). For example, obese patients with binge eating disorder (Geliebter et al.,
2009), as well as individuals identified as emotional eaters both exhibit an absence of decline in
post-prandial ghrelin levels (Raspapow et al., 2010). As to its role in depression, few
preliminary studies have investigated ghrelin concentrations in subjects with depression, and
they have had inconsistent findings with both higher (Kurt et al., 2007) and lower levels (Barim
et al., 2009) reported in this population compared to healthy controls.

There are very few investigations of ghrelin and leptin response to stress reactivity and to
date, there are no reports of studies directly investigating the role of ghrelin and leptin in appetite
disturbances in subjects with depression. It is suggested that data from such an investigation
would contribute significantly to understanding of the biological substrates of the key symptoms
of appetite and weight change that occurs in depression and other mood disorders. To our
knowledge, this is the first investigation that evaluates both leptin and ghrelin responses to stress
induction and food ingestion in subjects with clinical depression and comparable controls.

Aim of Investigation II:

To determine if the impact of stress induction or food challenge on serial ghrelin and
leptin production differ in subjects with depression compared to healthy controls and if
the hormonal response are influenced by the presence of emotional eating, and duration
of illness.
Primary Hypothesis 2.1: It is hypothesized that subjects with depression will exhibit greater leptin and ghrelin secretion responses following stress induction and food consumption compared to healthy control participants.

Secondary Hypotheses:

(i) Secretion of leptin and ghrelin in response to food challenge will vary by chronicity and subtype of depressive episode with greater response among participants with chronic depression.

(ii): Altered secretion of ghrelin in response to food consumption will vary by emotional eating status. Participants categorized as emotional eaters will exhibit less of decline in post-prandial ghrelin levels in comparison to non-emotional eaters.

5.2 Methods Specific To This Set Of Experiments

The overall methods and procedures have been previously described in detail in section 3.1. In brief, serial plasma leptin and ghrelin was measured in subjects with major depressive disorder and matched control sample during to two experimental conditions: 1) psychosocial stress induction and 2) food challenge. Procedures specific to this study - the blood sampling and processing procedures, and the assay analysis procedures related to ghrelin and leptin – are described in the following paragraphs.
Blood Sampling Procedures

Time Series to determine Sample viability

Given the fact that ghrelin switches from its acylated to des-acylated form quickly (Kojima et al., 1999), in order to measure active ghrelin, duration of the viability of the sample in acylated form was determined. Further, a time series was conducted prior to the initiation of the experiment to ensure accurate results for assay analyses.

Blood samples were obtained from one healthy volunteer and were processed at 30, 60, 120, and 180 minutes post withdraw. Eight millilitres of blood was collected into an EDTA container. At each processing time point, 2 mL of whole blood was transferred into a conical polypropylene tube and 100 mcg of AEBSF (EMD Millipore, Inc.) solution was added. For the first time point (30 minute), AEBSF solution was added to whole blood immediately after the sample was drawn and left for 30 minutes before centrifugation (3000g x 15 minutes) to determine impact on sample analysis. For all other time processing time points, the AEBSF solution was added to whole blood within one minute preceding centrifugation. All samples were centrifuged at 3000g for 15 minutes. Plasma was then transferred into a polypropylene microtube and 250ug of 1N HCI solution was added. All samples were stored at -80 degree Celsius until the time of assay analysis.

Active ghrelin was analyzed in triplicate using commercially available ELISA kit (EMD Millipore, Merck). The mean intra-assay coefficient variance was .037%. Detectable levels of active ghrelin were greatest 40 minutes post withdraw (M=95.28 pg/mL, SD = .004) and slightly reduced at 60 minutes post withdraw (M=82.19 pg/mL, SD = .015). Levels were lowest for the sample left to sit with AEBSF solution for 30 minutes before centrifugation (M= 3.66 pg/mL,
SD=. 004) and substantially decreased in samples processed 120 minutes (M=21.43 pg/mL; SD = .004) and 180 minutes (M = 31.71 pg/mL, SD = .006) post withdraw. See Figure 5-1

Figure 5-1 Decrement in Active Ghrelin levels over Wait Times to Processing

Based on these results, the following was determined: 1) AEBSF solution should be added to whole blood in minutes immediately preceding centrifugation; 2) sample processing procedures were acceptable, and 3) optimal duration between sample draw and processing was in between 0 to 60 minutes.

Assay Procedures

i. Ghrelin and Leptin

Active ghrelin and leptin levels were assessed together using a commercially available assay kit (Milliplex ® Map) and MAGPIX ® equipment (Luminex Corporation). Assays were completed in duplicate and under blinded conditions. Multi-analyte profiling human metabolic
hormone Milliplex ® Map kits (EMD Millipore, Merck KGaA, Darmstadt, Germany) were used. Briefly, Luminex uses technology to internally color-code microspheres with two fluorescent concentrated dyes and are coated specifically with ghrelin and leptin antibodies. Expression levels are determined after incubation with biotinylated detection antibody and Streptavidin PE conjugate, the reporter molecule. Using MAGPIX ®, the microspheres pass rapidly through lasers exciting internal microsphere dyes as well as fluorescent dye on reporter molecule. Quantification of the bioassays is based on fluorescent reporter signals.

The assay kits were completed in compliance with the manufacturer’s instructions over a 2- day period to allow for the required 16-hour incubation time. All plates were read using MAGPIX equipment. The sensitivity of the assays were 4 pg/mL for ghrelin and 184 pg/mL for leptin.

1) Stress Induction Experiment

Subjects

Ghrelin and leptin response to a psychosocial stress induction was collected from twenty-eight participants (Depressed, n=16; Control, n=12).

Analysis Outline

Kolmogorov-Smirnov tests of normality were conducted to determine normality of all ghrelin and leptin data, and results indicated that data was not distributed normally. As a result, log transformations were performed on all raw ghrelin and leptin data, and area under the curve (AUC) was calculated using both ground (AUCG) and increase (AUCl) formulas by Pruessner et
al., (2003). In addition, because leptin and ghrelin concentrations are greatly influenced by body weight, it has been recommended that BMI be controlled when conducting analyses (Marshall et al., 2000), and as such, BMI was included as a covariate in all comparisons based on leptin and ghrelin concentrations. Therefore, using scores from DEBQ Emotional Eating subscale, participants were defined as emotional eaters based on whether or not the emotional eating subscale score on the DEBQ fell above normative means (van Strien et al. 2002). Individuals with emotional eating scores that fell in the very low to average ranges were categorized as non-emotional eaters.

5.3 Results

There are a several variables known to influence the ghrelin and leptin levels, however, given the small sample size only variables most likely to impact results were examined. As such, age, gender, and medication status were analyzed to determine whether differences in leptin or ghrelin response to stress induction and food consumption were evident.

First, age was included as a covariate in 2 (participant group) x 6 (time over experiment) repeated measures ANCOVA analysis of ghrelin and leptin response to both stress induction and food consumption. No significant interactions between age and leptin response to stress or food consumption were found, as well, no significant between group effects of age were noted. However, a significant time by age interaction in ghrelin levels in response to food consumption was noted, though no significant findings were noted between groups or in response to stress induction. This finding did not alter overall results and therefore was not considered in all subsequent analyses.
In a subsequent analysis, gender was included as a covariate in 2 (participant group) x 6 (time over experiment) repeated measures ANOVA analysis of ghrelin and leptin response to both stress induction and food consumption. No significant interactions between gender and ghrelin response were found, as well, no significant between group effects of gender were noted. In addition no significant time by gender interaction was noted, however, there was a significant between group difference in leptin levels based on gender. However, this finding did not alter overall results and therefore was not included in any of the main analyses due to small sample size.

One-way ANOVA was employed to assess differences in leptin and ghrelin response to psychosocial stress and food consumption between depressed individuals receiving medication vs. those who were not. No significant differences in $AUC_G$, $AUC_I$, leptin or ghrelin in response to both stress induction and food consumption were noted between depressed participants receiving or not receiving antidepressant medication. The results are presented in Appendix 1. Based on these results, it was decided to proceed without including age, gender, or medication status in the subsequent analyses.

\textit{a) Ghrelin Response}

\textit{i. Ghrelin Response to Stress Induction by Participant Group}

A 2 (participant group) x 6 (time over experiment) mixed measures analysis of covariance (ANCOVA) was conducted to assess group differences in mean ghrelin levels during the stress induction, using BMI as a covariate. This analysis did not reveal a significant main effect of time ($F(5, 125)=1.11, p=.36$), or a main effect of participant group ($F(1,25)=0.30$,}
As well, no significant time by participant group interaction ($F(5, 125)=.31, p=.85$) or time by BMI interaction were found ($F(5, 125)=1.02, p=.41$). The results are presented in Figure 5-2.

Figure 5-2 Mean ghrelin levels ($SD$) at each time point in the stress induction protocol. No significant differences in ghrelin levels over time or between participant groups was noted.

Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC ghrelin between participant groups in the stress induction experiment. There were no significant differences found between depressed participants ($M=197.76, SD=58.00$) and control
participants ($M=220.37, SD=57.94$) for AUC\textsubscript{G} ghrelin ($F(2, 25)=2.33, p=.12$). Similarly, there were no significant differences found between depressed participants ($M=-41.22, SD=44.88$) and control participants ($M=-38.75, SD=42.42$) for AUC\textsubscript{I} ghrelin ($F(2, 25)=1.44, p=.26$).

\textit{ii. Ghrelin Response to Stress Induction by Duration of Illness}

Some have proposed that chronicity of depressive illness may contribute to particular depressive symptoms such as increased appetite (e.g. O’Keane et al., 2013). As such, it was of interest to explore differences in ghrelin levels in response to stress based on length of current depressive episode. The subsample of depressed participants was divided based on DSM-IV-TR diagnostic criteria for chronic episode specifier (\(\geq 2\) years). Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC ghrelin between the participants experiencing a chronic depressive episode (\(n=10\)) and participants experiencing a depressive episode of shorter duration (\(n=6\)). There was no significant difference in AUC\textsubscript{G} ghrelin between depressed participants experiencing a shorter episode of depression ($M=188.27, SD=56.57$) than depressed participants experiencing a chronic depressive episode ($M=203.45, SD=61.11$), $F(2, 15)=2.00, p=.17$). Similarly, no significant difference in AUC\textsubscript{I} ghrelin were found between depressed participants experiencing a shorter episode of depression ($M=-49.63, SD=41.90$) and those experiencing a chronic depressive episode ($M=-36.17, SD=48.02$) ($F(2, 15)=2.15, p=.16$).

\textit{iii. Ghrelin Response to Stress Induction by Depression Subtype}

In order to determine if there were any differences based on type of depression, a separate analysis of ghrelin response to the stress induction was conducted using the depressed group only.
Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC ghrelin between the subtypes of depression (Typical n=11; Atypical n=4). Only one participant met for the melancholic subtype and was excluded from these comparisons. No significant differences were found between participants with typical ($M=665.37$, $SD=99.30$) and atypical subtype ($M=749.54$, $SD=69.53$) for $AUC_G$ ghrelin ($F(2, 16)=1.72$, $p=.21$). As well, no significant differences were found between participants with typical ($M=-116.08$, $SD=37.21$) and atypical subtype ($M=-137.15$, $SD=6.58$) for $AUC_I$ ghrelin ($F(3, 16)=2.00$, $p=.17$). However, given the extent to which the groups are unequal and the overall small sample size, comparisons between subtypes of depression should be interpreted with caution.

iv. Ghrelin Response to Stress Induction by Emotional Eating Status

In order to determine if there were any differences based on emotional eating status, a separate analysis of ghrelin response to the stress induction between emotional (n=15) and non-emotional eaters (n=10) was conducted. Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC ghrelin in response to stress based emotional eating status. No significant differences were found between emotional eaters ($M=198.39$, $SD=55.81$) and non-emotional eaters ($M=226.01$, $SD=63.85$) for $AUC_G$ ghrelin ($F(1, 22)=.60$, $p=.45$). As well, no significant differences were found between emotional eaters ($M=-34.45$, $SD=35.22$) and non-emotional eaters ($M=-52.10$, $SD=42.01$) for $AUC_I$ ghrelin ($F(1, 22)=.57$, $p=.46$).
b) Leptin Response

i. Leptin Response to Stress Induction by Participant Group

A 2 (participant group) x 6 (time over experiment) mixed measures analysis of covariance (ANCOVA) was conducted to assess group differences in mean leptin levels during the stress induction, using BMI as a covariate. This analysis did not reveal any significant main effect of time ($F(5, 125)=.85, p=.52$), suggesting that leptin levels did not change significantly over the course of the stress induction. However, a significant main effect of group was noted ($F(2, 25)=4.58, p=.04, \eta^2 = .16$). Specifically depressed participants exhibited higher levels of leptin in comparison to control participants throughout the stress induction, even while holding BMI constant. No significant time by participant group interaction ($F(5, 125)=.47, p=.50$) or time by BMI interaction were found ($F(5, 125)=1.04, p=.32$). The results are presented in Figure 5-3.
Figure 5-3 Mean leptin levels (SD) at each time point during the stress induction protocol. Leptin levels did not significantly differ over the course of the stress induction. However, depressed participants had significantly elevated leptin levels, controlling for BMI, in comparison to control participants throughout the stress induction.

Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC leptin between participants in the stress induction experiment. Results indicated that depressed participants \((M=684.42, SD=95.45)\) had greater AUC\(_G\) leptin than control participants \((M=598.65, SD=73.13)\) \((F(2, 25)=4.81, p=.04, \eta^2 = .16)\), in response to a psychosocial stressor. However, there was no significant difference between depressed participants \((M=-121.00, SD=32.03)\) and control participants \((M=-122.02, SD=35.10)\) for AUC\(_1\) leptin \((F(2, 25)=.36, p=.55)\) during the stress induction.
ii. Leptin Response to Stress Induction by Depression Subtype

To determine if there were any differences based on type of depression, a separate analysis of leptin response to the stress induction was conducted using the depressed group only. Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC leptin between the subtypes of depression (Typical n=11; Atypical n=4). Only one participant met for the melancholic subtype, and was excluded from these comparisons. No significant differences were found between participants with typical ($M=665.37$, $SD=99.30$) and atypical subtype ($M=749.54$, $SD=69.53$) for AUC$_G$ leptin ($F(2, 16)=1.72$, $p=.21$). As well, no significant differences were found between participants with typical ($M=-116.08$, $SD=37.21$) and atypical subtype ($M=-137.15$, $SD=6.58$) for AUC$_I$ leptin ($F(3, 16)=2.00$, $p=.17$). However, given the extent to which the groups are unequal and the overall small sample size, comparisons between subtypes of depression should be interpreted with caution.

iii. Leptin Response to Stress Induction by Duration of Illness

Differences in leptin levels in response to stress, based on length of current depressive episode, were explored. Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC leptin between the participants experiencing a chronic depressive episode, i.e. $\geq$ 2 years (n=10) and participants experiencing a depressive episode of shorter duration (n=6). See Figure 5-4. Those experiencing a shorter episode of depression ($M=783.09$, $SD=33.25$) exhibited a significantly greater AUC$_G$ leptin than participants experiencing a chronic depressive episode ($M=625.22$, $SD=64.69$), $F(2, 16)=14.24$, $p<.01$, $\eta^2 = .69$). Similarly, participants experiencing a shorter episode of depression ($M=-148.73$, $SD=22.95$) exhibited a
significantly greater AUCₜ leptin than participants experiencing a chronic depressive episode (\( M=-104.36.22, SD=24.44 \)) \( (F(2, 16)=7.03, p<.01, \eta^2 = .52) \).

Figure 5-4  Total Leptin by Duration of Depressive Episode

iv. Leptin Response to Stress Induction by Emotional Eating Status

In order to determine if there were any differences based on emotional eating status, a separate analysis of leptin response to the stress induction between emotional (n=15) and non-emotional eaters (n=10) was conducted. Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC leptin in response to stress based emotional eating status. No significant differences were found between participants categorized as emotional eaters \( (M=647.60, SD=95.48) \) and participants categorized as non-emotional eaters \( (M=624.22, SD=95.80) \) for AUC₇ leptin \( (F(1, 22)=.01, p=.98) \). However, a marginally significant difference was found between emotional eaters \( (M=-111.16, SD=27.49) \) and non-emotional eaters \( (M=-124.45, SD=29.69) \) for AUCₗ leptin \( (F(1, 22)=3.28, p=.08) \).
In addition, exploratory partial correlations were conducted in order to determine whether negative affect contributed to the association between emotional eating and leptin levels. Partial correlations between pre-stress negative affect (as measured by PANAS Negative Affect Scale) and AUC leptin in response to stress, controlling for BMI, were conducted. This analysis revealed a significant inverse association between AUC\(_t\) leptin and pre-stressor negative affect, for emotional eaters only (\(r=-.66, p=.04\), two-tailed). This same association was not significant for non-emotional eaters (\(r=-.09, p=.83\), two-tailed). See Figures 5-5 and 5-6. This finding suggests that for individuals categorized as emotional eaters, higher levels of negative affect were associated with a greater magnitude of change in leptin levels in response to stress. Because the AUC\(_t\) of greater magnitude were numerically negative, it indicated a decrease in leptin levels from baseline in response to stress.

Figure 5-5 Leptin AUC\(_t\) in Response to Stress by Negative Affect Score – Emotional Eaters
II) Food Challenge Experiment

Subjects

Pre and post-prandial cortisol, ghrelin, and leptin measures were obtained from thirty-one participants (Depressed, n=16; Control, n=15).

Procedure

Since ghrelin levels are influenced by food ingestion (Druce et al., 2005), it was necessary to account for food intake prior to the food challenge experiment. Participants were asked to fast for a minimum of three hours prior to the food challenge experiment and reported
all food intake for that day upon arrival. Dietary information, specifically macronutrient information, was derived from reported food intake prior to the experiment, as well as from the meal provided during the food challenge. The results are presented in Table 5.1. Depressed participants reported significantly less caloric intake prior to arrival for the experiment than controls. This may be attributed to the fact that a number of depressed participants reported that they did not consume any food on the day of the experiment prior to arrival. However, there were no significant differences in protein, fat, or carbohydrate intake prior to the experiment. Similarly, there were no significant differences in caloric, protein or carbohydrate intake for the meal consumed during the experiment. Interestingly, even though options for the experimental meal were controlled, depressed participants chose options that had a higher amount of fat than control participants, though this difference did not reach statistical significance.

Kolmogorov-Smirnov tests of normality were conducted to determine normality of all ghrelin, and leptin data collected in the food challenge, and results indicated that all hormone data was not distributed normally. As such, log transformations were performed on all raw ghrelin and leptin data. Area under the curve (AUC) was also calculated using the $AUC_G$ and $AUC_I$ formulas by Pruessner et al., (2003). In addition, because leptin and ghrelin concentrations are greatly influenced by body weight, it has been recommended that BMI be controlled when conducting analyses (Marshall et al., 2000), and as such, BMI was included as a covariate in all comparison based on leptin and ghrelin concentrations. Further, previous studies have provided some evidence to suggest that emotional eating may influence ghrelin levels in response to food (e.g. Raspapow et al., 2010), and as such it was of interest to explore in this investigation. Given that there were no differences in ghrelin responses based on participant groups, the sample was then divided by emotional eating status, using methods previously
described, to determine if any differences in ghrelin response to food ingestion were present in this current investigation.

Table 5.1 Meal and Macronutrients by Participant Group

<table>
<thead>
<tr>
<th>Meal and Macronutrients</th>
<th>Depressed (n=16)</th>
<th>Control (n=15)</th>
<th>t</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast – Calorie (kcal)</td>
<td>253.91</td>
<td>403.12</td>
<td>-2.04</td>
<td>.05</td>
<td>.75</td>
</tr>
<tr>
<td>Breakfast – Protein (g)</td>
<td>11.24</td>
<td>18.15</td>
<td>-1.62</td>
<td>.12</td>
<td>.60</td>
</tr>
<tr>
<td>Breakfast – Carbohydrate (g)</td>
<td>33.38</td>
<td>53.70</td>
<td>-1.90</td>
<td>.07</td>
<td>.70</td>
</tr>
<tr>
<td>Breakfast – Fat (g)</td>
<td>9.25</td>
<td>13.74</td>
<td>-1.27</td>
<td>.21</td>
<td>.46</td>
</tr>
<tr>
<td>Study Meal – Calorie (kcal)</td>
<td>387.21</td>
<td>364.97</td>
<td>1.63</td>
<td>.11</td>
<td>.61</td>
</tr>
<tr>
<td>Study Meal – Protein (g)</td>
<td>23.41</td>
<td>21.28</td>
<td>1.68</td>
<td>.10</td>
<td>.52</td>
</tr>
<tr>
<td>Study Meal – Carbohydrate (g)</td>
<td>49.84</td>
<td>51.94</td>
<td>-1.51</td>
<td>.14</td>
<td>.55</td>
</tr>
<tr>
<td>Study Meal – Fat (g)</td>
<td>11.91</td>
<td>8.61</td>
<td>1.95</td>
<td>.06</td>
<td>.72</td>
</tr>
</tbody>
</table>

Results

a) Ghrelin Response

i. Ghrelin Response to Food Challenge by Participant Group

The results of mean ghrelin response to the food challenge by participant group are presented in Figure 5-7. A 2 (participant group) x 4 (time over experiment) mixed measures ANCOVA, with BMI as a covariate was conducted, to assess group differences in mean ghrelin
levels during the food challenge experimental visit. This analysis revealed a significant main effect of time \((F(3, 81)=5.36, p<.01, \eta^2 = .17)\), suggesting that ghrelin levels decreased over the course of the food challenge experiment. However, there was no main effect of participant group \((F(3, 81)=.23, p=.64)\), with no significant time by group interaction \((F(3, 87)=.46, p=.70)\).

Figure 5-7 Mean ghrelin levels (SD) at each time point during the food challenge protocol. Ghrelin levels significantly decreased pre to post prandial for both depressed and control participants. No group differences or interactions were observed.
Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC ghrelin between participant groups in the food challenge experiment. There were no significant differences found between depressed participants \( (M=191.59, SD=66.57) \) and control participants \( (M=212.85, SD=49.63) \) for AUC\(_G\) ghrelin \( (F(2, 27)=2.46, p=.10) \). A significant difference was found between depressed participants \( (M=-16.46, SD=28.26) \) and control participants \( (M=-19.34, SD=27.77) \) for AUC\(_I\) ghrelin \( (F(2, 27)=7.25, p<.01, \eta^2 = .35) \). However, the effect of participant group in this analysis was not significant and accounted for very little of the variance \( (F(1, 27)=.22, p=.64, \eta^2 = .01) \), suggesting that significant effect is attributable to the covariate, BMI \( (F(1, 27)=14.38, p<.01, \eta^2 = .35) \).

**ii. Ghrelin Response to Food Challenge by Depression Subtype**

In order to determine if there were any differences based on type of depression, a separate analysis of ghrelin response to the food challenge was conducted using the depressed group only (Typical \( n=12; \) Atypical \( n=3 \)). Only one participant met for the melancholic subtype, and was excluded from these comparisons. Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC ghrelin between the subtypes of depression. No significant differences were found between participants with typical \( (M=204.68, SD=68.41) \) and atypical subtype \( (M=137.74, SD=31.30) \) for AUC\(_G\) ghrelin \( (F(2, 15)=1.26, p=.32) \). However, a significant difference was found between participants with typical subtype \( (M=-27.05, SD=22.59) \) and atypical subtype \( (M=20.06, SD=18.80) \) for AUC\(_I\) ghrelin \( (F(3, 16)=5.39, p=.01, \eta^2 = .57) \). However, given the extent to which the groups are unequal and the overall small sample size, comparisons between subtypes of depression should be interpreted with caution.
iii. Ghrelin Response to Food Challenge by Duration of Illness

Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC ghrelin in between the participants experiencing a chronic depressive episode (n=10) and participants experiencing a depressive episode of shorter duration (n=6) in response to the food challenge. There was no significant difference in AUC\(_G\) ghrelin between depressed participants experiencing a shorter episode of depression (\(M=185.57, SD=75.17\)) than depressed participants experiencing a chronic depressive episode (\(M=195.20, SD=64.87\), \(F(2, 15)=.83, p=.46\)) in response to the food challenge. Similarly, no significant difference in AUC\(_I\) ghrelin were found between depressed participants experiencing a shorter episode of depression (\(M=-21.53, SD=28.17\)) and those experiencing a chronic depressive episode (\(M=-8.00, SD=28.81\)) (\(F(2, 15)=.06, p=.81\)).

iv. Ghrelin and Emotional Eating in response to Food Challenge

All participants who completed the food challenge experiment were divided into “Emotional Eaters” (n=15) and “Non-emotional” eaters (n=10) regardless of participant group, using methods previously described. The results of mean ghrelin response to the food challenge by Emotional Eating status are presented in Figure 5-8. A 2 (emotional vs. non-emotional eating) x 4 (time over experiment) mixed measures ANCOVA, with BMI as a covariate was conducted, to assess group differences in mean ghrelin levels during the food challenge experimental visit. This analysis revealed a significant main effect of time (\(F(3, 66)=4.94, p<.01, \eta^2 = .18\)), suggesting that ghrelin levels decreased over the course of the food challenge experiment for both emotional and non-emotional eaters. As well, there was also a main effect of group (\(F(3, 22)=4.46, p=.05, \eta^2 = .17\)), suggesting that non-emotional eaters exhibited higher levels of ghrelin than emotional eaters throughout the food challenge experiments. However,
there was no significant time by group interaction ($F(3, 66)=.20, p=.66$), yet, a significant time by BMI interaction ($F(3, 66)=8.07, p<.01, \eta^2 = .27$) was noted.

Figure 5-8 Mean ghrelin levels ($SD$) at each time point in the food challenge protocol with study sample divided by emotional eating status (emotional vs. non-emotional eater). Ghrelin levels significantly decreased pre to post prandial. Emotional eaters had significantly lower levels of ghrelin in comparison to non-emotional eaters throughout the experiment, even after controlling for BMI.

Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC ghrelin between emotional and non-emotional eaters in AUC ghrelin in the food challenge experiment. Emotional eaters ($M=186.96, SD=39.94$) had significantly lower
AUC<sub>G</sub> ghrelin in response to a meal in comparison non-emotional eaters (\(M=233.85, SD=49.63\)), (\(F(2, 22)=3.74, p=.04, \eta^2 = .25\)), while holding BMI constant. Similarly, emotional eaters (\(M=-17.06\), \(SD=32.15\)) exhibited significantly lower AUC<sub>I</sub> ghrelin in response to a meal in comparison to non-emotional eaters (\(M=-20.88, SD=25.27\)), while holding BMI constant (\(F(2, 22)=5.57, p=.01, \eta^2 = .34\)).

\(b\) Leptin Response

\(i\). Leptin Response to Food Challenge by Participant Group

Leptin samples in response to food ingestion were collected from thirty participants (Depressed, \(n=16\); Control, \(n=14\)). A 2 (participant group) x 4 (time over experiment) mixed measures analysis of covariance (ANCOVA) was conducted to assess group differences in mean leptin levels during the food challenge, using BMI as a covariate. This analysis did not revealed any significant main effect of time (\(F(3, 81)=.22, p=.88\)), suggesting that leptin levels did not change significantly before and after ingesting a meal. A significant main effect of group approached significance (\(F(2, 27)=3.52, p=.07, \eta^2 = .12\)), with depressed participants showing higher levels of leptin in comparison to control participants, even while holding BMI constant. In addition, the time by participant group interaction also approached significance (\(F(3, 81)=2.36, p=.07, \eta^2 = .10\)), however, the time by BMI interaction was not significant (\(F(3, 81)=.27, p=.85\)). The results are presented in Figure 5-9.
Mean leptin levels (SD) at each time point in the food challenge protocol. Leptin levels did not change pre to post prandial for all participants. Depressed participants exhibited significantly elevated levels of leptin, controlling for BMI, in comparison to control participants.

Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC leptin between participants in response to meal ingestion. A marginally significant difference between depressed participants ($M=662.29$, $SD=81.74$) and control participants ($M=600.99$, $SD=64.07$) in AUC$_C$ leptin was noted ($F(2, 27)=3.48$, $p=.07$, $\eta^2=.11$). Similarly, a marginally significant difference in AUC$_I$ leptin was noted between depressed ($M=-6.75$, $SD=18.41$) and control participants ($M=4.65$, $SD=12.68$) ($F(2, 25)=3.11$, $p=.09$) during the food challenge.
ii. Leptin Response to Food Challenge by Depression Subtype and Duration of Illness

In order to determine if there were any differences based on type of depression, a separate analysis of leptin response to the food challenge was conducted using the depressed group only. Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC leptin between the subtypes of depression. Since only one participant met for the melancholic subtype, they were excluded from these comparisons. No significant differences were found between participants with typical ($M=645.83$, $SD=86.61$) and atypical subtype ($M=726.74$, $SD=31.30$) for AUC$_G$ leptin ($F(2, 16)=2.28, p=.13$). As well, no significant differences were found between participants with typical ($M=-7.07$, $SD=15.84$) and atypical subtype ($M=-5.85$, $SD=34.05$) for AUC$_I$ leptin ($F(3, 16)=.12, p=.95$). However, given the extent to which the groups are unequal and the overall small sample size, comparisons between subtypes of depression should be interpreted with caution.

iii. Leptin Response to Food Challenge by Duration of Illness

Univariate analysis of covariance, with BMI as a covariate, was used to assess differences in AUC leptin between the participants experiencing a chronic depressive episode (n=10) and participants experiencing a depressive episode of shorter duration (n=6). Those experiencing a shorter episode of depression ($M=721.82$, $SD=77.73$) exhibited greater AUC$_G$ leptin than participants experiencing a chronic depressive episode ($M=626.57$, $SD=63.23$), that approached significance $F(2, 16)=3.30$, $p=.09$, $\eta^2=.20$). Furthermore, no difference in AUC$_I$ leptin between participants experiencing a shorter episode of depression ($M=-10.41$, $SD=26.32$) and participants experiencing a chronic depressive episode ($M=-4.55$, $SD=12.88$) ($F(2, 16)=7.03$, $p<.01$, $\eta^2=.52$) was noted in response to the food challenge.
iv. Leptin to BMI Ratio by Participant Group

Due to the dependence of circulating leptin levels on amount of adipose tissue, it was of interest to explore whether any differences in the proportions of body weight and leptin concentrations existed between depressed and control participants. Based on procedures described in Schilling et al. (2013), a BMI/leptin ratio was calculated and compared between depressed and control participants. The first sample collected in the food challenge visit was used in this calculation, as it was obtained after a period of fasting and devoid of any stressors. Since the data violated limits of normality, square root transformation was performed on the data. Univariate analysis of variance revealed significantly higher BMI/leptin ratio in depressed participants compared to healthy controls ($F(1, 28)=7.97$, $p<.01$, $\eta^2 = .22$). Results are presented in Figure 5-10.

Figure 5-10  BMI-Leptin Ratio by Participant Group
v. Leptin and Emotional Eating in response to Food Challenge

In an exploratory analysis, it was of interest to determine whether or not leptin response to food ingestion differed based on emotional eating status. As such, the sample was divided into emotional vs. non-emotional eaters, as previously described. The results of mean leptin response to the food challenge by Emotional Eating status are presented in Figure 5-11. A 2 (emotional vs. non-emotional eating) x 4 (time over experiment) mixed measures ANCOVA, with BMI as a covariate, was conducted to assess group differences in mean leptin levels during the food challenge experimental visit. No significant main effect of time ($F(3, 66)=.82, p=.48$) and no main effect of emotional eating status ($F(1, 22)=.22, p=.64$) was found. Further, no significant time by group interaction ($F(3, 66)=.71, p=.55$) nor significant time by BMI interaction ($F(3, 66)=1.16, p=.33$) was noted.
Figure 5-11 Mean leptin levels (SD) at each time point in the food challenge protocol with study sample divided by emotional eating status (emotional vs. non-emotional eater). Leptin levels did not change pre to post meal consumption. No difference in leptin levels between emotional eaters and non-emotional eaters were noted.

5.4 Discussion

*Leptin response to experimental conditions*

No significant variation in leptin levels were noted in response to the psychosocial stress induction or food consumption for both depressed and control participants. This is consistent with previous findings from investigations of healthy participants that assessed leptin response to stress induction (Appelhans et al., 2010) as well as leptin response to food consumption.
(Korbonits et al., 1997). The latter finding is not unexpected given that leptin levels have been found to take 4-6 hours to respond to food ingestion (Havel et al., 1999). No significant interaction between time and group was noted either, suggesting that both depressed and control participants responded to stress and food consumption in a similar manner, as evidenced by leptin concentrations. However, interestingly, depressed participants exhibited elevated levels of leptin throughout the stress induction in comparison to control participants, even when allowing for BMI differences. A trend for similar change was noted in leptin levels of depressed patients in response to food consumption, though it did not reach statistical significance. Several previous reports have also noted elevated leptin levels in depressed populations (Gecici et al., 2005; Pasco et al., 2008; Cizza et al., 2010), though this is the first report of elevated leptin levels in response to acute stress in a depressed population. This is also the first investigation to measure leptin response to both social stress as well as food challenge in depressed patients. Findings from previous investigations employing stress induction paired with subsequent food intake in healthy populations suggest that acute stress is a trigger that influences circulating levels of leptin, and that even slight variations in leptin may be associated with stress-induced eating (Appelhans et al., 2010; Tomiyama et al., 2012).

Elevated levels in total leptin secretion (AUC) were also found in depressed participants compared to healthy controls. However, findings from previous studies have not been consistent, with some reporting higher concentrations of leptin in depressed patients (Gecici et al., 2005; Pasco et al., 2008), while others reported lower basal levels of leptin (Lawson et al., 2012) or no difference noted by yet other researchers in comparison to healthy controls (Deuschle et al., 1997; Schilling et al., 2013). These inconsistencies have been attributed at least in part to the heterogeneous nature of depression. One example is the elevated levels seen with the atypical subtype (Gecici et al., 2005), and in severe depression (Esel et al., 2005; Morris et al., 2012).
The majority of depressed participants in our investigation were categorized as having typical depression, and with mild to moderate severity, limiting comparisons. Interestingly, we found that patients experiencing an episode of depression that was less than 2 years in duration had significantly higher levels of leptin (AUC) in response to an acute stressor. In other words, overall, depressed participants exhibited higher leptin levels than healthy controls, but among them, patients with a current depressive episode of less than 2 years exhibited the highest leptin levels. Thus, there is some early signal that the duration of illness may impact leptin levels, which needs further evaluation. Given leptin’s interaction with the HPA axis, this may be an important area to pursue in the study of chronic depression. Previous studies of depressed populations that have investigated HPA axis activity and leptin interaction have noted an inverse relationship between leptin and both ACTH and cortisol secretion in female patients (Cizza et al., 2010). Interestingly, it has been suggested that this is state related, as improvement after treatment with antidepressants has led to both HPA axis normalization and changes in leptin levels (Himmerich et al., 2007).

An alternate explanation for differences in leptin levels based on duration of depressive illness is that elevated leptin levels are a consequence of an overactive HPA axis. Previous reports (Farabaugh et al., 2004) and results from the current study indicate that depressed participants report greater levels of perceived stress, likely contributing to HPA axis overactivity and greater leptin levels. Since with chronicity, HPA axis activity shifts from hyperactive to hypoactive, the leptin levels may shift downward, as well (Carroll et al., 2007; Stewart, 2005; O’Keane et al., 2013). On the other hand, leptin has been shown to inhibit glucocorticoids (Ahima et al., 2005), and therefore, the observed higher leptin levels may be suggested to be part of a homeostatic attempt to normalize an overactive HPA axis. It is possible that over time, as depression becomes chronic, HPA activity may decrease in part due to leptin’s inhibitory effects.
Indeed, blunted HPA activity is noted in both individuals who experience chronic stress and those who experience a chronic course of depression. However, given the complexity of these homeostatic processes and physiological response to stress, it is likely that leptin is but one of many factors contributing to the pathophysiology of both the stress response and depression.

A potential consequence of elevated leptin levels, referred to here as “leptin resistance”, has been proposed (Munzberg and Myers, 2005). If leptin levels are consistently elevated due to contributing factors other than the amount of adipose tissue, (e.g. depression), it is suggested that its physiological effect decreases, leading to “leptin resistance”. This is said to lead to a reduction of its anorexic and satiety influence, therefore promoting prolonged eating behavior and increased body weight. Presence of elevated leptin levels in obese individuals is a consistently reported finding (Schwartz et al., 2005), and leptin administration does not always seem to lead to reduction of appetite or feeding behavior in obese subjects (Mantzoros and Flier, 2000). This anomaly has been partly explained by this phenomenon of “leptin resistance” present in individuals with high BMIs (Munzberg and Myers, 2005; Jung and Kim, 2013). It can be argued that a similar phenomenon may be occurring in depressed patients. In this current investigation, a greater leptin to BMI ratio was observed in patients with depression in comparison to BMI matched healthy control participants. This suggests that even participants in the normal BMI range may have higher leptin levels that are disproportionate to their adipose stores, suggesting some degree of impairment in signaling processes due to the influence of other, yet to be understood, factors.

The boundaries of and the exact mechanisms leading to leptin resistance are not yet clear (Morrison, 2008), though impaired transport to the brain, and impaired signaling in hypothalamic neurons, have been suggested as possible pathways (Jung and Kim, 2013). In particular, the
pro-inflammatory cytokine, TNF alpha, which inhibits leptin transport to the brain (Oh-I et al., 2005), as well as signaling inhibitor suppressor of cytokine signaling-3 (SOCS3) and protein tyrosine phosphate 1B (PTP1B), have been implicated in hypothalamic inflammation, which is in turn suggested to lead to central leptin resistance (Munzberg and Myers, 2005; Jung and Kim, 2013). Thus, inflammatory markers, including cytokines such as IL-6 and TNF alpha, may mediate leptin resistance (Munzberg and Myers, 2005), though exact mechanism needs elucidation.

Interestingly, it has been suggested that leptin resistance may be specific in its influence of appetite and food intake, as leptin’s other targets, such as cardiovascular function, remain intact (Rahmouni et al., 2005). It has also been suggested that an increase in “leptin sensitivity” may be protective against weight gain, while its loss predisposes to obesity (Morrison, 2008). Evidence from animal investigations suggests that leptin sensitivity is biologically regulated during times when weight gain is required (e.g. pregnancy and hibernation) (Krol et al., 2007; Augustine et al., 2007). Specifically, leptin normally inhibits weight gain, but is biologically programmed to induce leptin resistance during times when positive energy stores are necessary (Krol et al., 2007; Augustine et al., 2007). As such, it has been suggested that factors, yet to be identified, disrupt this biological mechanism and may contribute to loss of sensitivity and weight gain in humans (Morrison, 2008).

Based on the findings of this current investigation, i.e. the elevated leptin levels and greater leptin to BMI ratio in depressed participants, it can be argued that a dysregulation of the leptin feedback system may lead to an increase in leptin sensitivity, resulting in obesogenic vulnerability in this population. Only one other published report (Schilling et al., 2013) noted increased leptin to BMI ratio and suggested the possibility of leptin resistance in depressed
patients. However, the authors also suggested that the occurrence of such resistance may be caused by the use of pharmacological agents like mirtazapine, which are known to induce weight gain (e.g. mirtazapine), mediated via histaminergic pathways (Schilling et al., 2013). As such, additional investigations are warranted.

Taken together, the results of the current investigation provide evidence of abnormal leptin concentrations in depressed patients, which may be indicative of a disrupted leptin feedback system that may be at least in part influenced by stress, which promotes increased eating behaviour. Thus, there is a reasonable theoretical rationale to evaluate in the future the benefit of leptin as a therapeutic agent in depressed subjects, with BMI being evaluated as a covariate. This investigation, however, does not rule out the contribution of other factors, such as circulating cytokines and inflammatory markers, which have been shown to influence metabolic status. Indeed, such agents have been implicated in both depression (Anisman and Hayley, 2012) and obesity (Black, 2006). A link between depression and adiposity, proposed to be mediated by inflammatory markers such as IL-6 and CRP, as well as leptin, has been proposed (Miller et al, 2003). As a corollary, certain inflammatory markers and cytokines, such as IL-6 and TNF alpha, have been proposed as mediators of leptin resistance (Munzberg and Myers, 2005). Measurement of these markers as part of future investigations may provide useful information.

**Ghrelin response to experimental conditions**

This investigation found that in response to food consumption, levels of ghrelin significantly decreased in sixty minutes post-prandial. However, ghrelin levels did not vary significantly in response to a psychosocial stressor. Further, no significant differences in ghrelin response to either challenge were noted between depressed and control participants.
The literature suggests that in general, levels of ghrelin decline rapidly after food ingestion in healthy subjects (Tschop et al., 2000). However, lower plasma concentrations of ghrelin before a meal and less of a decline post meal were noted in obese participants with binge eating disorder (Geliebter et al., 2009). In addition, there are previous reports of emotional eaters not exhibiting the decline in post-prandial ghrelin levels (Raspapow et al., 2010). Given depressed participants’ altered eating behaviors, tendency towards emotional eating and association with obesity, the current investigation anticipated significant differences in post-prandial evidence between depressed subjects and controls. However, the results did not show such differences in ghrelin levels after meal consumption between the two groups, which were matched for BMI.

Further, no differences in ghrelin levels were noted between depressed and healthy controls in response to stress, nor did ghrelin levels change significantly in response to a psychosocial stressor in either group. At least one previous study, albeit not in depressed subjects but in binge eating disorder subjects, reported that ghrelin levels increased with cortisol response to an acute stressor (Rouach et al., 2007). Further, in an investigation of healthy participants and emotional eating, ghrelin levels were found to vary in response to a psychosocial stressor across all participants regardless of emotional eating status (Raspapow et al., 2010). The results from this current investigation suggest that ghrelin response following either stress induction or food consumption is not distinguishable from that of healthy controls, and as such, ghrelin levels may not be influenced by depressive illness under these conditions. Future replicative investigations that include a larger sample are warranted.
Emotional Eating Status and Ghrelin response to Food Consumption

When study subjects were divided by emotional eating status, significant differences in ghrelin levels were noted. Specifically, those who were categorized as emotional eaters exhibited lower levels of ghrelin in comparison to non-emotional eaters in response to the food challenge experiment, while controlling for BMI. In the literature, it has consistently been shown that individuals with higher BMI or obesity exhibit low levels of ghrelin (English et al., 2003). However, in the current investigation, individuals categorized as an emotional eater exhibited lower ghrelin levels yet did not have significantly higher BMI than non-emotional eaters. Interestingly, no difference in ghrelin levels in response to stress between emotional and non-emotional eaters was found.

Findings from this current investigation are in keeping with at least one previous report. Raspapow and colleagues (2010) examined ghrelin response to a psychosocial stressor in emotional eaters and found lower levels of ghrelin compared to non-emotional eaters. They also reported an absence of decline in ghrelin levels in response to food consumption post stress for emotional eaters only, a finding not replicated in our study, as we found that emotional eaters and non-emotional eaters exhibited similar decline in post-prandial ghrelin levels. This contradiction may be explained at least in part by the fact that the food challenge was conducted as a separate procedure to the stress induction. As well, Raspapow et al. (2010) only included healthy participants who were also emotional eaters, while the current study included both healthy and depressed emotional eaters.

Nonetheless, it is interesting that the current investigation is the second to report lower levels of ghrelin in emotional eaters, who by definition consume food in response to emotional rather than physiological cues. Ghrelin concentrations rise prior to meal consumption
throughout the day, and is thought to be a hunger signal that initiates food intake (Cummings et al., 2004). If food intake is consumed regardless of diurnal rise and fall of ghrelin (e.g. eating in response to negative affect, when ghrelin levels are low), perhaps a ‘flattening’ of this diurnal rise and fall occurs, leading to persistent low levels of active ghrelin. Thus, it could be argued that if emotional eaters are indeed eating in response to affect rather than physiological cues, the lack of reliance on hunger signals could result in low ghrelin concentrations. As there are very few published human studies of ghrelin response to food consumption in non-obese emotional eaters and none with depressed patients, it may be premature to speculate on the specific mechanisms. However, evidence from animal investigations indicates that long-term exposure to high fat diet attenuates the association between active ghrelin concentrations and both BMI and food intake (Sugiishi et al., 2013), suggesting alterations in ghrelin response over time, referred to as “ghrelin resistance” (Briggs et al., 2010). Briggs and colleagues (2010) noted a reduction in hypothalamic NPY and AgRP circuit responsiveness to ghrelin induced food intake in obese (diet induced) mice. Subsequent investigations have suggested that weight loss from a calorically restricted diet can restore ghrelin sensitivity and increase hypothalamic NPY and AgRP expression (Briggs et al., 2013). Taken together, it suggests that the neuroendocrine ghrelin axis feedback system is potentially sensitive to diet and adjusts based on energy stores. However, the emotional eaters in the current study were not all obese, and BMI was held constant in comparison analyses of ghrelin levels between emotional and non-emotional eaters. It is possible that the ghrelin feedback system is also sensitive to energy intake in absence of physiological cues, and adjusts not only in response to diet and body weight, but also to maintain energy homeostasis in absence of response to hunger signals. Thus, it may be suggested that if energy homeostasis can be maintained (likely in a positive balance) without rise and fall of ghrelin levels, then such diurnal variations may become redundant to initiate food intake, and
over time results in low or more stable diurnal levels over the day, such as those exhibited by emotional eaters. Further, as previously stated, future investigations are necessary to provide experimental evidence to support such speculation. In addition, given that the few investigations to date measure food intake either post-stress induction, or after ingestion of one meal, measuring diurnal variation in ghrelin concentrations in a naturalistic manner may confirm differences in individuals with emotional eating tendencies.

It must be noted, however, that there was an overrepresentation of depressed participants in the emotional eating group in the current study. Based on this sample, the majority of depressed participants had emotional eating scores that fell above the mean, suggesting that they, as a group, have emotional eating tendencies, yet ghrelin levels of depressed participants in response to both experimental conditions did not differ from control participants. It is possible that the small sample size contributed to this discrepancy. In this context, it might be useful to evaluate the characteristics of depressed participants who did not endorse emotional eating. For example, to determine if depressed non-emotional eaters exhibit different symptom clusters, and if they tend to have anorexic symptoms and weight loss. Nonetheless, it is likely that emotional eating represents a vulnerability to excessive body weight for individuals with depressive disorders. Future investigations should include both emotional and non-emotional depressed participants, and matched controls, so distinctions and unique vulnerabilities to weight gain could be identified.

*Leptin, negative affect and emotional eating in response to stress*

In the exploratory analysis, an association between negative affect, emotional eating and the magnitude of leptin response to stress was noted. However, this association appears to be unique to those with emotional eating tendencies, and not amongst non-emotional eaters.
Specifically, the magnitude of variation in leptin levels was determined using $AUC_T$, which measures increase over time, rather than total area (Pruessner et al., 2003), and while controlling for BMI. Because the $AUC_T$ was negative numerically, it denotes a decrease from baseline, suggesting that for emotional eaters, degree of negative affect prior to the stress induction was correlated with a greater reduction in leptin levels in response to stress. Given that leptin is dependent on adiposity and the fact that this effect is evident while controlling for BMI, suggests that leptin may be influenced by other factors, including emotional ones.

Leptin has anorexic effects, and if levels are lower in response to stress, it may partly explain the physiological basis of emotional eating. Emotional eaters tend to eat more in response to stress, and if satiety or anorexic signals are significantly influenced by stress, in a decremented manner, then it could lead to continued and/or greater food consumption, with increased perception of stress. Leptin levels did not vary over the course of the stress induction in the current study, and the variation became evident only when associated with pre-stressor negative affect. This suggests that experiencing negative affect might be a key trigger for leptin reactivity. However, this is only a preliminary finding on the effect of stress on emotional eating in clinically depressed participants, as such, it is premature to speculate as to its underlying mechanisms. Further, it is difficult to control for all potential confounding variables that may influence this association, and the small sample size limits statistical applications. An investigation with a larger sample size would help elucidate the association between negative affect and leptin levels in those with emotional eating tendencies in relation to weight increases.

**Clinical implications and Future research**

This investigation found that leptin levels did not change significantly over the course of the stress induction, nor did the changes differ between depressed participants and healthy
controls. However, depressed participants had significantly greater levels of leptin in comparison to control participants. Similarly, leptin levels did not change significantly in response to food consumption for either group, and no significant interaction was noted. In addition, greater BMI to leptin ratio was found in depressed participants in comparison to healthy controls. This suggests that even participants in the normal BMI range may have higher leptin levels that are disproportionate to their adipose stores. Taken together, these results suggest a dysregulation of the leptin feedback system, as evidenced by elevated levels, in the depressed participants in this sample. In addition, differences in leptin levels based on chronicity of depressive illness were also noted, indicating that those who experienced a chronic course exhibited the lowest levels of leptin. Given the role of leptin in appetite and satiety functions, this may indicate a vulnerability that favors obesogenic tendencies for those who experience chronic depression. However, it is still unclear, based on these results, whether or not stress contributes to this increased vulnerability. While overall findings did not reveal differences between depressed and controls in several leptin measures over the course of the stressor, elevated leptin levels were found in the depressed group only with the stress induction, and not the food challenge. However, there is indication that leptin levels may vary over the time course of depression and may represent a particular vulnerability for those patients who experience a chronic course. Thus, identifying vulnerability to excessive weight in those who experience a chronic course is likely to be of value in the long-term management of this population. It would influence treatment decision, particularly as they relate to metabolic considerations, which are important in such care, due to their significant contribution to increased morbidity and mortality. Identification of depressed patients with increased vulnerability to leptin resistance prior to onset of obesity, and determining the potential of first-line antidepressants to impact on the leptin pathway and promote weight changes, may of
significant value in long-term management. As such, it may be useful for future investigations to measure leptin levels over the course of illness, and to identify any distinctions between acute and chronic course of illness. In addition, future investigations may explore the usefulness of leptin as an intervention. It may also be relevant to include measures of inflammatory markers (IL-6, TNF-alpha, CRP, etc.) in such investigations.

It is highly likely that emotional eating represents a vulnerability to weight gain, not only for depressed patients but also for individuals who exhibit this tendency. Noted distinctions in ghrelin levels associated with emotional eaters may be indicative of loss of sensitivity in the neuroendocrine ghrelin axis, which may promote increased body weight. In addition, an association between stress-induced leptin response and negative affect was found for emotional eaters only in the current study. This may provide some insight to the physiological underpinnings of emotional eating, though future investigations are needed. Given that emotional eating scores were significantly higher for depressed patients in comparison to healthy controls, vulnerability to emotional eating and weight gain may be particular relevant to depressed populations. As such, development of interventions that address emotional eating tendencies may be helpful.

Limitations

First, the small sample size of this investigation limits the generalizability of results. Future investigations need to be conducted with larger sample sizes in order to account for a number of statistical considerations as well as confounding factors (e.g. gender). In addition, not all participants were medication free, which may present a confound. However, when analyses were conducted based on medication status, there were no differences in any study measures were found. Also, this study did not include a basal measurement of leptin or ghrelin with 12hr
fasting, but only measured these hormones in response to experimental conditions, which may limit generalizability. Control of food intakes on experiment days is also a source of potential confounds. Participants were asked to fast 3 hours prior to the food challenge, but they did so outside the hospital, therefore it is possible that not all participants adhered to this requirement. Similarly, participants were asked not to eat 2 hours prior to the stress induction, but they did not arrive until 10-15 minutes prior to the initiation of the experiment, and as such, again, non-adherence cannot be ruled out. As well, food intake was measured on a separate day without any stress component, and though it was done so purposely, any inferences of stress-induced food intake in the immediate-term are limited.

5.5 Conclusions

This investigation provides evidence of abnormal leptin concentrations in depressed patients, which may be indicative of a disrupted leptin feedback system that may be partly influenced by stress, and which promotes increased eating behaviour. This disruption may be particularly pronounced for those who experience a chronic course of depression. Further, emotional eating may represent a particular vulnerability for weight increase in these patients, via reduced ghrelin levels. As well, negative affect and elevated leptin in response to stress may constitute triggers for emotional eaters. These findings have important clinical implications, both to the pathophysiology underlying the association between depression and weight disturbances, as well as for intervention purposes.
6 Study 3: Appetite and satiety neurohormones and cognition in Depressive Disorders: a pilot investigation

6.1 Synopsis of the Published Literature

Distinctions in cognitive functioning have been noted in both depressed and obese populations. Individuals with excess body weight have been reported to perform poorly on neuropsychological measures of impulsivity, such as response inhibition, mental flexibility and decision-making skills (Verdejo-Garcia et al., 2010). In addition, decision-making tasks are among the most impaired in eating disordered populations, specifically with those with binge eating symptoms (Zakzanis et al., 2010). Further, a recent systematic review reported that impaired performance on tests of executive functioning, particularly the Stop Signal and Stroop tasks, is a robust finding in populations with elevated BMI (Vainuk et al., 2013). It is of note that many neurocognitive findings in overweight populations have also been noted consistently in patients with depression, particularly impaired executive functioning (Snyder, 2013).

Both leptin and ghrelin have been shown to have neuroprotective actions that positively affect cognitive functioning in animal models (Harvey, 2007; McNay, 2007; Morrison, 2009). Animal models suggest that leptin promotes neuronal survival through its effects on synaptic function and neuronal structure, as well as on plasticity by attenuation of cell death (Morrison, 2009; PazFilho et al., 2010). The few published investigations in human populations have yielded inconsistent findings thus far. For example, higher leptin levels were associated with less cognitive decline in elderly patients (Holden et al., 2009) and lower incidence of Alzheimer Disease (Lieb et al., 2009) in some reports, but higher levels were associated with worse cognitive performance in other elderly samples (Gunstad et al., 2008). Further, in studies of HIV
infected men, low leptin levels were associated with poorer performance on memory and learning tasks (Huang et al., 2007), while in investigations of patients with type II diabetes, higher leptin levels were associated with poorer executive functioning (Labad et al., 2012).

Evidence from animal studies suggests that ghrelin may also support cognitive function (McNay, 2007). For example, investigations have noted that circulating ghrelin levels promote dendritic spine synaptic formation in hippocampal neurons, and that increased ghrelin levels are associated with improved learning and memory in rodents (Carlini et al., 2004; Diano et al., 2006). Though human studies are few, findings indicate that higher levels of ghrelin are associated with reduced cognitive performance in elderly populations (Spitznagel et al., 2010), while ghrelin administration has been shown to increase memory for food related items in younger samples (Malik et al., 2008).

To date, there are no published studies investigating the associations between these energy homeostatic neurohormones and cognitive functioning in depressed individuals.

_Pilot Study Aim:_ To determine if any associations exists between the degree of neuropsychological deficits and neurohormonal responses in subjects with depression and to evaluate the impact of BMI as a contributing factor to such association.

**Hypothesis 3.1:** Greater deficits in neurocognitive functioning will be seen in depressed participants in comparison to controls. The greatest deficits in cognitive functioning will be seen in individuals with lower levels of leptin and ghrelin and higher levels of cortisol and in individuals with elevated BMI.
6.2 Methods

The methods and procedures have been previously described in detail in section 3.1, the common methods section. In brief, serial plasma leptin and ghrelin was measured in subjects with major depressive disorder and matched control sample during to two experimental conditions: 1) psychosocial stress induction and 2) food challenge. Trained study staff administered a small battery of cognitive tasks in a separate room than where the experiment was conducted. This battery included Trail Making A and B; Stroop, TOMM, IGT, and CPT-II; and were administered in this order. The battery was administered over a period of 30-40 minutes.

Subjects

Thirty-eight participants completed the neuropsychological measures (Depressed, n=19; Control, n=19), though only thirty-one of the participants went on to complete one or both experiments for reasons previously described in Section 3.1. The majority of the sample was right handed (92.1%). A description of the study sample, including education and clinical information, is presented in Table 3.1 of the common methods section.

Procedure

For the majority of participants (60.5%), neurocognitive measures were administered following the food challenge visit. However, due to scheduling complications, a number of participants completed the neuropsychological measure during the screen visit (23.7%), while a few completed the battery during a separate visit (15.8%). Univariate analysis of variance was employed to determine if any differences could be attributed to time of administration in the study. No significant differences based on administration visit were found for any of the neuropsychological scores. The results are presented in Appendix 1.
6.3 Results

i. Depressed vs. Control Participants

Independent samples t-tests were employed to assess differences in neuropsychological scores between depressed and control participants. No significant group differences were found for any of the neuropsychological test scores. The results are presented in Table 6.1.

Further, differences in neurohormones between depressed and control participants have been previously presented in Section 4 and 5. Briefly, no significant differences in cortisol response or ghrelin response were noted between the two groups. However, elevated leptin levels were noted in depressed participants in comparison to healthy controls, even after controlling for BMI.

ii. Group Differences based on BMI

Given that no group differences in cognitive measures between depressed and controls were noted, the sample was then divided based on BMI categories to determine if there were any differences in neuropsychological performance based on body mass. Due to the small sample size, BMI categories were collapsed into two groups: 1) normal, (n=18), and 2) overweight/obese (n=19). Independent samples t-tests were employed to assess differences in neuropsychological scores between these two groups. The results are presented in Table 6.2.
Table 6.1 Neuropsychological performance by participant group

<table>
<thead>
<tr>
<th>Neurocognitive Measures</th>
<th>Depressed</th>
<th>Control</th>
<th>t</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trails A (seconds)</td>
<td>24.28</td>
<td>24.76</td>
<td>-.18</td>
<td>.85</td>
<td>.06</td>
</tr>
<tr>
<td>Trails B (seconds)</td>
<td>54.75</td>
<td>61.26</td>
<td>-.74</td>
<td>.46</td>
<td>.24</td>
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<tr>
<td>Stroop-Interference Score</td>
<td>5.43</td>
<td>2.82</td>
<td>1.02</td>
<td>.32</td>
<td>.33</td>
</tr>
<tr>
<td>TOMM Trial 1 Score</td>
<td>48.63</td>
<td>46.84</td>
<td>1.64</td>
<td>.11</td>
<td>.53</td>
</tr>
<tr>
<td>TOMM Trial 2 Score</td>
<td>50.00</td>
<td>49.37</td>
<td>1.64</td>
<td>.11</td>
<td>.53</td>
</tr>
<tr>
<td>IGT – Net Total Score</td>
<td>19.75</td>
<td>24.67</td>
<td>-.57</td>
<td>.57</td>
<td>.20</td>
</tr>
<tr>
<td>IGT – Total Money Won</td>
<td>-$267.19</td>
<td>$50.28</td>
<td>-.69</td>
<td>.49</td>
<td>.16</td>
</tr>
<tr>
<td>CPT – Clinical CI</td>
<td>40.22</td>
<td>42.12</td>
<td>-.25</td>
<td>.80</td>
<td>.09</td>
</tr>
<tr>
<td>CPT – Number of Commissions</td>
<td>11.64</td>
<td>11.94</td>
<td>-.10</td>
<td>.92</td>
<td>.04</td>
</tr>
<tr>
<td>CPT – Number of Omissions</td>
<td>2.14</td>
<td>3.41</td>
<td>-.71</td>
<td>.48</td>
<td>.26</td>
</tr>
<tr>
<td>CPT – Hit Reaction Time</td>
<td>379.82</td>
<td>386.80</td>
<td>-.28</td>
<td>.78</td>
<td>.10</td>
</tr>
<tr>
<td>CPT – Variability Score</td>
<td>8.94</td>
<td>6.62</td>
<td>1.05</td>
<td>.30</td>
<td>.36</td>
</tr>
<tr>
<td>CPT – Detectability Score</td>
<td>.84</td>
<td>.86</td>
<td>-.09</td>
<td>.92</td>
<td>.04</td>
</tr>
<tr>
<td>CPT – Response Style Score</td>
<td>.56</td>
<td>.86</td>
<td>-.65</td>
<td>.52</td>
<td>.62</td>
</tr>
<tr>
<td>CPT – Perseveration Score</td>
<td>.36</td>
<td>.71</td>
<td>-.62</td>
<td>.54</td>
<td>.23</td>
</tr>
<tr>
<td>CPT – Hit Reaction Time Block Change Score</td>
<td>.01</td>
<td>.02</td>
<td>.003</td>
<td>.02</td>
<td>.82</td>
</tr>
</tbody>
</table>
Table 6.2 Neuropsychological Performance by BMI Category

| Neurocognitive Measures | BMI Category | | | | | |
|-------------------------|--------------|-----------------|----------------|-----------------|-----------------|-----------------|-----------------|
|                         | Normal (n=18) | Overweight/Obese (n=19) | | | | | |
|                         | M | SD | M | SD | t | p | d |
| Trails A (seconds)      | 24.01 | 8.84 | 24.23 | 6.95 | -.08 | .93 | .02 |
| Trails B (seconds)      | 49.50 | 13.19 | 66.38 | 34.24 | -2.00 | .06 | 1.35 |
| Stroop- Interference Score | 5.73 | 7.67 | 2.47 | 8.14 | 1.24 | .22 | .41 |
| IGT – Net Total Score   | 31.38 | 22.56 | 12.82 | 24.46 | 2.26 | .03 | .79 |
| IGT – Total Money Won ($) | 259.69 | 1404.01 | 509.41 | 1145.75 | 1.72 | .10 | .20 |
| CPT – Number of Commissions | 11.19 | 6.64 | 12.92 | 9.09 | -.59 | .56 | .22 |
| CPT – Number of Omissions | 1.69 | 2.57 | 4.14 | 6.65 | -1.37 | .18 | .49 |
| CPT – Hit Reaction Time (ms) | 389.61 | 79.33 | 370.36 | 52.89 | .77 | .45 | .28 |
| CPT – Perseveration Score | .62 | 1.99 | .50 | .94 | .21 | .83 | .20 |

The overweight/obese group performed significantly worse than the normal/underweight group on the IGT as evidenced by total scores ($t(31)=2.26, p=.03$). In addition, marginally significant differences were found in Trails B Scores, with the overweight/obese group
performing worse on this set-shifting task than the normal/underweight group ($t(35)=-2.00$, $p=.06$).

In an exploratory analysis, Pearson correlations (two-tailed) were performed to assess associations between BMI and neuropsychological performance on the total study sample, and then separately on the depressed and control groups. A Bonferroni correction was applied to control for multiple correlations. No significant associations between BMI and neuropsychological performance were found for the total sample or for the depressed group. However, significant negative associations between BMI and IGT total scores, as well as IGT Total money scores, were found for the control group, such that healthy controls with higher BMIs performed worse on this decision making task. In addition, a negative association between BMI and reaction time was noted in the CPT-II for control subjects with higher BMIs, suggesting that they were slower to respond during this attention task. However, this association did not remain significant after correction for multiple tests was applied. The results are presented in Table 6.3.
Table 6.3 Partial Correlations: BMI and Neuropsychological Performance

<table>
<thead>
<tr>
<th></th>
<th>BMI – All (n=38)</th>
<th>BMI-Depressed (n=20)</th>
<th>BMI-Control (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trails A (seconds)</td>
<td>-.04</td>
<td>.07</td>
<td>-.25</td>
</tr>
<tr>
<td>Trails B (seconds)</td>
<td>.21</td>
<td>.32</td>
<td>-.02</td>
</tr>
<tr>
<td>Stroop-Interference Score</td>
<td>-.11</td>
<td>-.32</td>
<td>.17</td>
</tr>
<tr>
<td>IGT – Net Total Score</td>
<td>-.26</td>
<td>-.08</td>
<td>-.63*</td>
</tr>
<tr>
<td>IGT – Total Money Won ($)</td>
<td>-.17</td>
<td>.06</td>
<td>-.65*</td>
</tr>
<tr>
<td>CPT – Number of Commissions</td>
<td>.01</td>
<td>-.21</td>
<td>.21</td>
</tr>
<tr>
<td>CPT – Number of Omissions</td>
<td>.17</td>
<td>-.12</td>
<td>.41</td>
</tr>
<tr>
<td>CPT – Hit Reaction Time (ms)</td>
<td>-.10</td>
<td>.25</td>
<td>-.55*</td>
</tr>
<tr>
<td>CPT – Perseveration Score</td>
<td>-.14</td>
<td>.12</td>
<td>-.29</td>
</tr>
</tbody>
</table>

*uncorrected (p<.05); ** remained significant after correction (p<.01)

iii. Cognitive Measures in Association with Baseline Neurohormone Measures

The first sample obtained during the food challenge visit was used as a “baseline” measure as this experiment visit was purposely devoid of any stress, and also, participants were asked to fast for a minimum of three hours preceding the visit. Baseline samples were not
collected during any other visits due to logistics and potential burden on participants. As such, it was decided that this sample was the most appropriate substitute for a “baseline” measure for the analysis of associations between hormones and neurocognitive measures.

To assess whether there were any associations between baseline neurohormonal and neurocognitive measures, partial correlations (two-tailed), controlling for BMI, were performed using a Bonferonni correction for multiple correlations. Results are presented in Table 6.4. In addition, partial correlations (two-tailed), controlling for BMI, with correction for multiple correlations, were performed separately on each participant group and results are presented in Tables 6.5 and 6.6.

Table 6.4 Partial Correlations of Baseline Neurohormone and Neuropsychological Performance

<table>
<thead>
<tr>
<th>ALL (n=31)</th>
<th>Leptin</th>
<th>Ghrelin</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$r$</td>
<td>$r$</td>
</tr>
<tr>
<td>Trails A (seconds)</td>
<td>.09</td>
<td>-.29</td>
<td>.09</td>
</tr>
<tr>
<td>Trails B (seconds)</td>
<td>-.18</td>
<td>-.18</td>
<td>.30</td>
</tr>
<tr>
<td>Stroop- Interference Score</td>
<td>-.34</td>
<td>.01</td>
<td>.24</td>
</tr>
<tr>
<td>IGT – Net Total Score</td>
<td>.10</td>
<td>.02</td>
<td>.09</td>
</tr>
<tr>
<td>IGT – Total Money Won ($)</td>
<td>.15</td>
<td>-.11</td>
<td>.04</td>
</tr>
<tr>
<td>CPT – Number of Commissions</td>
<td>-.24</td>
<td>-.32</td>
<td>.11</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>Ghrelin</td>
<td>Cortisol</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>Trails A (seconds)</td>
<td>.06</td>
<td>-.33</td>
<td>-.04</td>
</tr>
<tr>
<td>Trails B (seconds)</td>
<td>-.06</td>
<td>-.14</td>
<td>-.05</td>
</tr>
<tr>
<td>Stroop- Interference Score</td>
<td>-.62*</td>
<td>-.41</td>
<td>.68*</td>
</tr>
<tr>
<td>IGT – Net Total Score</td>
<td>-.14</td>
<td>.26</td>
<td>.32</td>
</tr>
<tr>
<td>IGT – Total Money Won ($)</td>
<td>.10</td>
<td>-.40</td>
<td>.13</td>
</tr>
<tr>
<td>CPT – Number of Commissions</td>
<td>-.35</td>
<td>-.56</td>
<td>-.43</td>
</tr>
<tr>
<td>CPT – Number of Omissions</td>
<td>-.07</td>
<td>-.25</td>
<td>-.28</td>
</tr>
<tr>
<td>CPT – Hit Reaction</td>
<td>.13</td>
<td>.45</td>
<td>-.19</td>
</tr>
</tbody>
</table>

Table 6.5 Partial Correlations of Baseline Neurohormone and Neuropsychological Performance – Depressed Group Only
Table 6.6 Partial Correlations of Baseline Neurohormone and Neuropsychological Performance – Control Group Only

<table>
<thead>
<tr>
<th>Control (n=15)</th>
<th>Leptin</th>
<th>Ghrelin</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trails A (seconds)</td>
<td>.06</td>
<td>-.37</td>
<td>.11</td>
</tr>
<tr>
<td>Trails B (seconds)</td>
<td>-.03</td>
<td>-.24</td>
<td>.48</td>
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<tr>
<td>Stroop- Interference Score</td>
<td>-.02</td>
<td>.36</td>
<td>.06</td>
</tr>
<tr>
<td>IGT – Net Total Score</td>
<td>.05</td>
<td>.11</td>
<td>-.15</td>
</tr>
<tr>
<td>IGT – Total Money Won ($)</td>
<td>.19</td>
<td>.06</td>
<td>-.11</td>
</tr>
<tr>
<td>CPT – Number of Commissions</td>
<td>.44</td>
<td>-.05</td>
<td>.44</td>
</tr>
<tr>
<td>CPT – Number of Omissions</td>
<td>.17</td>
<td>.03</td>
<td>.07</td>
</tr>
<tr>
<td>CPT – Hit Reaction Time (ms)</td>
<td>.02</td>
<td>-.18</td>
<td>-.01</td>
</tr>
<tr>
<td>CPT – Perseveration Score</td>
<td>.37</td>
<td>-.34</td>
<td>.27</td>
</tr>
</tbody>
</table>
When the entire sample was included in the analysis, no significant associations between baseline hormone measures and cognitive performance were found. However, when the depressed group was analyzed independently, associations between leptin, cortisol and performance on the Stroop task were noted. Specifically, higher leptin levels were associated with lower scores of interference on the Stroop task. In addition, higher levels of cortisol were correlated with higher interference scores. However, neither of these associations remained significant after the correction for multiple correlations was applied. Finally, no significant associations were noted when the control group was analyzed separately.

iv. Cognitive Measures in Association with Neurohormonal Response to Stress Induction and Food Consumption

Neurohormonal levels were measured in response to both the stress induction and food challenge. Kolmogorov-Smirnov tests of normality were conducted to determine normality of all cortisol, ghrelin and leptin data, and results indicated that data was not distributed normally. As such, log transformations were performed on all raw cortisol, ghrelin and leptin data. In addition, area under the curve (AUC) was calculated using the ground formula (AUC$_G$) as described in Pruessner et al., (2003). Partial correlations (two-tailed), controlling for BMI, were performed to assess whether there were any associations between neurohormonal response to experimental challenges and neurocognitive measures (Table 6.7). In addition, partial correlations (two-tailed), controlling for BMI, were performed separately on each participant group and results are presented in Tables 6.8 and 6.9. A Bonferroni correction was applied to each analysis to control for multiple correlations.

Table 6.7 Partial Correlations of Neurohormone Response to Experimental Challenge and Neuropsychological Performance
<table>
<thead>
<tr>
<th></th>
<th>AUC Cortisol Stress</th>
<th>AUC Cortisol Food</th>
<th>AUC Leptin Stress</th>
<th>AUC Leptin Food</th>
<th>AUC Ghrelin Stress</th>
<th>AUC Ghrelin Food</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>r</td>
<td>r</td>
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<td>r</td>
<td>r</td>
</tr>
<tr>
<td>Trails A (seconds)</td>
<td>-.15</td>
<td>-.04</td>
<td>.13</td>
<td>.07</td>
<td>-.06</td>
<td>-.28</td>
</tr>
<tr>
<td>Trails B (seconds)</td>
<td>.11</td>
<td>.17</td>
<td>-.14</td>
<td>-.10</td>
<td>.04</td>
<td>-.17</td>
</tr>
<tr>
<td>Stroop- Interference Score</td>
<td>.12</td>
<td>.14</td>
<td>.04</td>
<td>-.20</td>
<td>-.22</td>
<td>-.06</td>
</tr>
<tr>
<td>IGT – Net Total Score</td>
<td>.19</td>
<td>-.11</td>
<td>.18</td>
<td>.05</td>
<td>.06</td>
<td>-.06</td>
</tr>
<tr>
<td>IGT – Total Money Won ($)</td>
<td>.19</td>
<td>-.12</td>
<td>.22</td>
<td>.15</td>
<td>.08</td>
<td>-.22</td>
</tr>
<tr>
<td>CPT – Number of Commissions</td>
<td>-.02</td>
<td>-.02</td>
<td>-.25</td>
<td>-.18</td>
<td>-.17</td>
<td>-.34</td>
</tr>
<tr>
<td>CPT – Number of Omissions</td>
<td>-.09</td>
<td>-.07</td>
<td>-.33</td>
<td>-.24</td>
<td>-.02</td>
<td>.10</td>
</tr>
<tr>
<td>CPT – Hit Reaction Time (ms)</td>
<td>.07</td>
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<td>.12</td>
<td>.38</td>
<td>-.09</td>
<td>.27</td>
</tr>
<tr>
<td>CPT – Perseveration Score</td>
<td>.02</td>
<td>.05</td>
<td>.03</td>
<td>.23</td>
<td>-.35</td>
<td>-.20</td>
</tr>
</tbody>
</table>
Table 6.8 Partial Correlations of Neurohormone Response to Experimental Challenge and Neuropsychological Performance – Depressed Group Only

<table>
<thead>
<tr>
<th>Depressed (n=16)</th>
<th>AUC Cortisol Stress</th>
<th>AUC Cortisol Food</th>
<th>AUC Leptin Stress</th>
<th>AUC Leptin Food</th>
<th>AUC Ghrelin Stress</th>
<th>AUC Ghrelin Food</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>r</td>
<td>r</td>
<td>r</td>
<td>r</td>
<td>r</td>
</tr>
<tr>
<td>Trails A (seconds)</td>
<td>-.01</td>
<td>-.25</td>
<td>.14</td>
<td>.10</td>
<td>-.06</td>
<td>-.31</td>
</tr>
<tr>
<td>Trails B (seconds)</td>
<td>-.17</td>
<td>-.47</td>
<td>.10</td>
<td>.21</td>
<td>.26</td>
<td>-.16</td>
</tr>
<tr>
<td>Stroop- Interference Score</td>
<td>.17</td>
<td>.03</td>
<td>-.12</td>
<td>-.68*</td>
<td>-.31</td>
<td>-.40</td>
</tr>
<tr>
<td>IGT – Net Total Score</td>
<td>.34</td>
<td>-.13</td>
<td>-.03</td>
<td>-.19</td>
<td>.10</td>
<td>-.32</td>
</tr>
<tr>
<td>IGT – Total Money Won ($)</td>
<td>.32</td>
<td>-.28</td>
<td>.20</td>
<td>.14</td>
<td>.16</td>
<td>-.49</td>
</tr>
<tr>
<td>CPT – Number of Commissions</td>
<td>.25</td>
<td>-.81**</td>
<td>-.08</td>
<td>-.19</td>
<td>-.42</td>
<td>-.55</td>
</tr>
<tr>
<td>CPT – Number of Omissions</td>
<td>-.02</td>
<td>-.77*</td>
<td>.24</td>
<td>.01</td>
<td>-.15</td>
<td>-.24</td>
</tr>
<tr>
<td>CPT – Hit Reaction Time (ms)</td>
<td>-.07</td>
<td>.06</td>
<td>-.20</td>
<td>.37</td>
<td>.06</td>
<td>.48</td>
</tr>
<tr>
<td>CPT – Perseveration Score</td>
<td>-.10</td>
<td>-.85**</td>
<td>.25</td>
<td>.11</td>
<td>-.20</td>
<td>-.20</td>
</tr>
</tbody>
</table>

*uncorrected (p<.05); ** remained significant after correction (p<.01)
Table 6.9 Partial Correlations of Neurohormone Response to Experimental Challenge and Neuropsychological Performance –Control Group Only

<table>
<thead>
<tr>
<th>Control (n=15)</th>
<th>AUC Cortisol Stress</th>
<th>AUC Cortisol Food</th>
<th>AUC Leptin Stress</th>
<th>AUC Leptin Food</th>
<th>AUC Ghrelin Stress</th>
<th>AUC Ghrelin Food</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>r</td>
<td>r</td>
<td>r</td>
<td>r</td>
<td>r</td>
</tr>
<tr>
<td>Trails A (seconds)</td>
<td>-.32</td>
<td>-.03</td>
<td>-.05</td>
<td>-.06</td>
<td>-.03</td>
<td>-.34</td>
</tr>
<tr>
<td>Trails B (seconds)</td>
<td>.01</td>
<td>.32</td>
<td>-.38</td>
<td>-.17</td>
<td>-.03</td>
<td>-.22</td>
</tr>
<tr>
<td>Stroop- Interference Score</td>
<td>.35</td>
<td>.22</td>
<td>.14</td>
<td>-.07</td>
<td>-.22</td>
<td>.28</td>
</tr>
<tr>
<td>IGT – Net Total Score</td>
<td>-.39</td>
<td>-.25</td>
<td>-.12</td>
<td>.03</td>
<td>.24</td>
<td>.11</td>
</tr>
<tr>
<td>IGT – Total Money Won ($)</td>
<td>-.23</td>
<td>-.20</td>
<td>-.07</td>
<td>.10</td>
<td>.15</td>
<td>.03</td>
</tr>
<tr>
<td>CPT – Number of Commissions</td>
<td>-.01</td>
<td>.20</td>
<td>-.35</td>
<td>.25</td>
<td>-.03</td>
<td>-.08</td>
</tr>
<tr>
<td>CPT – Number of Omissions</td>
<td>.09</td>
<td>.08</td>
<td>-.48</td>
<td>-.02</td>
<td>-.07</td>
<td>.09</td>
</tr>
<tr>
<td>CPT – Hit Reaction Time (ms)</td>
<td>-.08</td>
<td>.02</td>
<td>-.03</td>
<td>-.10</td>
<td>-.15</td>
<td>-.18</td>
</tr>
<tr>
<td>CPT – Perseveration Score</td>
<td>-.15</td>
<td>.06</td>
<td>-.12</td>
<td>.31</td>
<td>-.46</td>
<td>-.40</td>
</tr>
</tbody>
</table>

No significant associations were noted when the entire sample was included in the analysis. However, a number of significant associations were noted when the depressed sample was analyzed alone. First a negative association between leptin response during the food...
challenge, and Stroop performance, was noted, but it did not remain significant after the correction for multiple correlations was applied. In addition, a number of scores from the CPT-II task were significantly associated with total cortisol response to the food challenge. Specifically, higher levels of cortisol were associated with lower scores of commissions and perseveration, which remained significant after correction was applied. This suggests that higher levels of cortisol were associated with better performance on a sustained attention task in depressed patients. However, the correlation found with respect to perseveration scores can be explained by the dichotomous nature of the results and given the small sample size, are not considered of value and are disregarded.

When control participants were analyzed separately, no significant associations were noted between total levels of hormones in response to experimental conditions and performance on cognitive tests. Results are presented in Table 6.9.

6.4 Discussion

Group Differences in Neuropsychological Performance

In the current investigation, no significant group differences in neuropsychological measures were noted between depressed and healthy control participants. This is inconsistent with evidence from a large body of literature describing cognitive impairment across a number of domains in patients with major depressive disorder. Evidence from meta-analytic investigations and systematic reviews has documented moderate effects sizes of impairment in executive function, attention, psychomotor speed, as well as verbal and non-verbal memory (Zakzanis et al., 1998; Synder, 2013; McIntyre et al., 2013; Rock et al., 2013). The neuropsychological
battery used in the current investigation focused on tests that have shown impairment in both obese and depressed populations (e.g. decision making and sustained attention tasks), and therefore it is possible that it was too limited to capture all existing deficits. In addition, studies using the specific tests used in this investigation (e.g. IGT) have had conflicting results, with both superior and inferior performance by depressed samples reported compared to controls (Cella et al., 2010; Smoski et al., 2008). Further, the depressed group in the current investigation was also considered to be in the mild-moderate range in terms of severity of depression, and thus, any cognitive impairment may not have been pronounced. Previous investigations of depressed patients of low severity (e.g. partially remitted) have reported unimpaired performance on similar tasks of executive functioning (Westheid et al., 2007). A number of participants in this study were also receiving antidepressant treatment, which could have influenced their performance. However, there are reports documenting cognitive deficits in depressed patients receiving antidepressant treatment (Cella et al., 2010, McIntyre et al., 2013). Lastly, it is possible that the lack of findings may be attributable to the small sample size, though a previous report did note distinctions in similar measures (e.g. IGT) between depressed and control participants in a sample of similar size (Cella et al., 2010).

Given the large body of literature documenting cognitive deficits in individuals with depression (e.g. Snyder, 2013), it is not clear why no differences in neuropsychological performance were found between depressed and control participants in this current investigation.

Neuropsychological Performance by BMI Category

The association between cognitive functioning and BMI has been well documented, and findings have been relatively consistent that individuals with elevated BMI exhibit impairment in cognitive functioning (Verdejo-Garcia et al., 2010). Recent systematic reviews have indicated
that poor performance on assessments of executive functioning, specifically cognitive tasks that require inhibition of responses, like the Stroop task, has been most consistently associated with elevated BMI (Vainik et al., 2013). Further, decision-making tasks are among the most impaired in eating disordered patients with binge eating symptoms (bulimia nervosa) (Zakzanis et al., 2010), which is relevant to both depressed and obese populations with hyperphagia and binge eating behaviours. In addition, distinctions based on weight categories have been noted in decision making tasks, such as the IGT, suggesting that those with elevated BMI display impaired decision making abilities (Davis et al., 2004). Consistent with the latter report, overweight/obese participants in this current investigation performed worse on the IGT in comparison to participants of normal weight. However, when correlational analyses were performed, significant associations between BMI and IGT performance were noted in the control group only, and not in the depressed group. Specifically, a significant inverse association between BMI and IGT performance (Total Money Won) was noted, suggesting that control subjects with a higher BMI performed worse on this decision making task. The lack of such association for the depressed group is somewhat unexpected, given the positive findings in other investigations of depressed samples as well as the documented association between obesity and depressive illness. It is possible this finding could be related to impulsive tendencies, as those high in impulsive behavior (e.g. substance abusers, problem gamblers) (Verdejo-Garcia et al., 2008) perform poorly on this task and measures of impulsivity have been linked to obesity (Kelly et al., 2009). While some depressed individuals may exhibit some impulsive tendencies, they tend to exhibit high degrees of behavioural inhibition, which may dampen the expression or reduce overt behaviours related to impulsive tendencies (McFarland et al., 2006; Pinto-Meza et al., 2005). Indeed, some have made distinctions between impulsive choices versus impulsive action, which some have suggested to be distinct processes (e.g. Broos et al., 2012), the former
being associated with decision-making process related to perceived rewards, while the latter relates to ability to inhibit inappropriate responses (Reynolds et al., 2006). Given that the IGT is also a reward-based task that involves a certain level of risk, overweight or obese depressed participants who may be aversive to risk, may not share the same decision-making processes as non-depressed obese individuals (Smoski et al., 2012).

**Neurohormone Measures and Cognitive Performance**

**Baseline Neurohormone Measures and Cognitive Performance**

Previous reports have noted some evidence of effects of both leptin and ghrelin in animal models (McNay, 2007; Harvey et al., 2007; Morrison, 2009). However, this is the first study to examine associations between neuropsychological measures and energy homeostatic hormones in a sample of clinically depressed individuals.

There were no significant associations found for ghrelin, leptin or cortisol for any of neuropsychological measures, when the entire study sample was included in the analysis. With respect to ghrelin and cognitive function, there are very few published investigations in human samples, though animal studies indicate that ghrelin administration improve a number of memory and object recognition tasks (Harvey, 2007). One previous report in humans noted a significant inverse association between ghrelin levels and performance on a number of neuropsychological tests, but it was conducted in an elderly sample (Spitznagel et al., 2010). Other investigations that studied younger populations have noted increases in memory for food stimuli after ghrelin administration during neuroimaging (Malik et al., 2008), though they focused primarily on memory and not other cognitive domains. However, given the lack of
current human studies, it may be premature to speculate and additional investigations with a larger sample size are required.

Interestingly, when the depressed group was analyzed alone, a number of associations between cognitive performance and both leptin and cortisol were noted, though none survived the correction for multiple correlations. Though it did not remain statistically significant after correction, a fairly strong correlation between leptin levels and Stroop performance was noted. Specifically, leptin levels were associated with better performance (less interference) on the Stroop task in depressed patients. While there have not been any published studies investigating associations between leptin and cognitive measures in depressed samples, a small number of studies in other adult populations have had conflicting results. For example, higher leptin levels were associated with less of cognitive decline in elderly patients (Holden et al., 2009) and lower incidence of Alzheimer Disease (Lieb et al., 2009); however, higher levels were associated with worse performance on cognitive measures (Trails B) in other elderly samples (Gunstad et al., 2008). Further, in studies of HIV infected males, low leptin levels were associated with poorer performance on memory and learning tasks (Huang et al., 2007). However, in samples of patients with type II diabetes, lower leptin levels were associated with better performance on executive functioning task (Labad et al., 2012). Given the few published studies in human samples, it is unclear at this point what underlies such inconsistencies in the findings. It is possible that BMI may play a role, given that these studies have been conducted in both lean and obese populations, but only one research group (Gunstad et al., 2008) statistically controlled for BMI. While the plethora of evidence from animal investigations suggests that leptin exerts procognitive effects (Harvey, 2007; Lu, 2007; Morrison, 2008), it has not yet been established in human populations. Further investigations in human samples are warranted to determine what
contribution, if any, leptin plays in cognitive functioning in both healthy and depressed individuals.

Among other correlations, a positive association between cortisol levels and Stroop performance was noted in depressed participants, in that higher levels of cortisol were correlated with higher interference (worse performance). Though this association did not remain significant after correction, it is consistent with previous findings which suggest that higher cortisol levels are significantly correlated with worse performance on tests across a number of cognitive domains in depressed populations (Egeland et al., 2005; Gomez et al., 2009; Hinkelmann et al., 2009). Further, successful antidepressant treatment has been associated with both improvements in cognitive functioning and decreased cortisol secretion (Hinkelmann et al., 2012), suggesting the potential contribution cortisol to the cognitive dysfunction found in depression. However, since this association did not survive the correction for multiple correlations, it cannot be concluded whether current results support previous findings.

*Neurohormone Response to Experimental Conditions and Cognitive Performance*

No significant associations between neurohormonal response to experimental conditions and cognitive performance were noted when the entire study sample was included in the analysis. Similar negative results were found when the control group was analyzed separately. However, when the depressed sample was analyzed alone, a significant association between AUC leptin in response to food consumption and Stroop performance was found, though this did not remain after correction for multiple tests was applied. As previously discussed, higher leptin levels have been associated with better cognitive performance in some reports, and worse performance in others. Based on available evidence, leptin levels remain fairly stable immediately following food ingestion (Korbonits et al., 1997; Havel et al., 1999); therefore it is
possible that AUC measures were a reflection of basal circulating levels, which were found to be elevated in depressed patients in this current investigation. To our knowledge, this is the first report of a relationship between hormone response to food consumption and cognitive performance, and as such, future investigations are warranted.

Of note, an association between performance on a sustained attention task and cortisol levels was found in the depressed group and remained significant after the correction for multiple comparisons. Specifically, higher levels of cortisol were correlated with lower number of commission errors, indicating better performance on a sustained attention task. This result is somewhat inconsistent with published findings, which suggest that higher cortisol levels are associated with worse performance on cognitive measures in depressed samples (Egeland et al., 2005; Gomez et al., 2009; Hinkelmann et al., 2009), as discussed in the previous section. However, a number of extreme scores in the depressed sample could have influenced this result. Given the small sample size and the heterogeneous presentation of depression, it is not clear if these individuals are true outliers per se or are representative of particular depressive symptom presentation. As such, these results should be interpreted with caution. Both hyperactive and hypoactive of HPA axis have been documented in depressed populations (e.g. Gold and Chrousos, 2002), but it is debatable whether these differences can be attributed to subtype or chronicity of illness (O’Keane et al., 2013). In this sample, mean levels of cortisol did not differ between depressed and control participants; however, given the heterogeneity of depression, it is possible that some depressed participants may exhibit a different pattern. Due to the small sample size, conclusions cannot be drawn from the current study. Future investigations that include a larger sample are necessary to investigate possible differences in cortisol levels based on the varying characteristics of depressive illness.
Limitations

There are several limitations in this exploratory investigation that should be noted. First is the small sample size. Given the small sample size and conservativeness of the statistical correction, it is possible that observed associations would become more evident in a larger sample size. However, such conclusions cannot be made in this current investigation and future investigations are warranted. In addition, the small sample size did not allow for the control of additional variables such as age and gender, which may impact results. Second is the correlational nature of, many of the results. Some of the significant findings in the correlational analysis, as noted in previous sections, are likely due to some extreme scores that may be considered outliers, and due to the small sample size, these extreme scores may have had more of an impact. Third is the lack of a true baseline measure of circulating hormones, as it does not allow for control of potential contributing factors such as diurnal variation. Future investigations should include a more appropriate measure of basal levels of circulating hormones.

Clinical Implications And Future Directions

In summary, no significant differences in cognitive performance were noted between depressed and control participants. Worse performance on decision-making task was documented in overweight/obese participants. It is interesting that some of the associations between hunger satiety hormones were more pronounced in depressed patients, even though these associations did not remain statistically significant after a correction was applied. Published studies have had conflicting findings in regard to differences in ghrelin and leptin concentrations between depressed samples and controls, suggesting that while disturbed concentrations of leptin and ghrelin may be a result of depressive illness itself, and may contribute to cognitive impairments found to accompany this mood disorder, this may not be true
for all depressed participants given the heterogeneity of depressive symptom presentation. Given
the infancy of the literature, and the exploratory nature of this current investigation, it is
premature to speculate. It may also be somewhat counterintuitive to consider that leptin may be
associated with better cognitive functioning, given that elevated leptin levels are associated with
higher BMI. Elevated BMI and obesity have been associated with poorer performance on a
number of cognitive measures, specifically ones assessing executive functioning and sustained
attention (Vainui et al., 2013). It is unclear at this point whether leptin and ghrelin are
associated with cognitive functioning in human samples, let alone whether there are distinctions
in depressed populations. Additional investigations with larger sample size are warranted.
7 Key Findings and Synthesis: General Discussion

7.1 Key Findings

The following table summarizes the main findings described in this thesis in relation to specific study aims stated in section 2.2.

<table>
<thead>
<tr>
<th>HYPOTHESES</th>
<th>RESULTS</th>
</tr>
</thead>
</table>
| **Primary Hypothesis (1.1):** It is hypothesized that participants with depression will experience altered HPA axis reactivity following stress and food consumption compared to control participants. | **Study #1**

No difference in HPA reactivity in response to stress or food consumption between depressed and control participants was noted.

Inverse association between duration of illness (years) and peak percent change in cortisol in response to an acute stressor, suggesting that the longer the duration of illness, the smaller the cortisol response to stress.

No difference in total cortisol (AUC) response to either stress or meal consumption based on length of depressive episode was noted.

Due to small sample size, no comparisons including subjects with melancholic subtype of depression could be made. No difference in any of the neurohormonal responses to either stress induction or food consumption between typical and atypical types of depression was observed.

<table>
<thead>
<tr>
<th>Secondary Hypotheses</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i): Participants with a chronic course or atypical subtype of depression will experience blunted HPA axis reactivity in response to experimental challenges in comparison to control participants.</td>
</tr>
</tbody>
</table>

No difference in total cortisol (AUC) response to either stress or meal consumption based on length of depressive episode was noted.

Due to small sample size, no comparisons including subjects with melancholic subtype of depression could be made. No difference in any of the neurohormonal responses to either stress induction or food consumption between typical and atypical types of depression was observed.

(ii) HPA axis reactivity will vary by emotional eating status. Thus participants categorized as emotional eaters will experience blunted HPA axis reactivity in response to...
experimental challenges compared to non-emotional eaters.

(iii) Participants with depression and elevated body weight will exhibit the greatest oxidative stress damage.

No significant differences in cortisol secretion (AUC, peak percent change) between individuals categorized as emotional and non-emotional eaters in response to an acute stressor were found.

Those categorized as an emotional eater exhibited significantly lower cortisol secretion (AUC) in comparison to non-emotional eaters in response to meal consumption.

**Primary Hypothesis 2.1:** It is hypothesized that subjects with depression will exhibit greater leptin and ghrelin secretory responses following stress induction and food consumption compared to healthy control participants.

**Secondary Hypotheses:**

(i) Secretion of leptin and ghrelin in response to food challenge will vary by chronicity and subtype of depressive episode with greater response among participants with chronic depression.

(ii): Altered secretion of ghrelin in response to food consumption will vary by emotional

Overall, elevated levels of leptin were noted in depressed patients in response to stress induction. However, leptin secretion pattern did not differ in depressed compared to control participants in response to an acute stressor.

Greater BMI/leptin ratio was noted in depressed participants in comparison to control participants.

Leptin: Individuals with a shorter episode of depression exhibited greater levels of leptin than individuals with an episode of depression > 2 years in response to a stress induction.

Depressed participants also exhibited higher leptin levels in response to food consumption, though this difference
eating status. Participants categorized as emotional eaters will exhibit less of decline in post-prandial ghrelin levels in comparison to non-emotional eaters. approached statistical significance. Leptin secretion pattern did not differ in depressed compared to control participants in response to meal consumption. Both groups exhibited a significant decline in ghrelin secretion in 60 minutes of meal consumption. No difference in levels or pattern of ghrelin secretion between depressed and control participants were noted. No differences in ghrelin levels (AUC) in response to an acute stressor or meal consumption between those with chronic depression and those without. No difference in any of the neurohormonal responses to either stress induction or food consumption between typical and atypical types of depression was noted. No significant differences in leptin levels in response to both an acute stressor and meal consumption between emotional and non-emotional eaters were noted. However, for individuals categorized as an emotional eater, higher levels of negative affect were associated with a greater magnitude of change (decrease) in leptin levels in response to stress. This association was specific to emotional eaters, and was not found for non-emotional eaters.
Emotional eaters exhibited lower total ghrelin secretion (AUC) in response to meal consumption in comparison to non-emotional eaters.

No differences between emotional and non-emotional eaters in ghrelin levels in response to an acute stressor were noted.

**Hypothesis 3.1:** Greater deficits in neurocognitive functioning will be seen in depressed participants in comparison to controls. The greatest deficits in cognitive functioning will be seen in individuals with lower levels of leptin and ghrelin and higher levels of cortisol and in individuals with elevated BMI.

**Study #3**

No difference in neuropsychological performance were noted between depressed and control participants.

In the depressed group, higher baseline levels of leptin were associated with lower scores of interference on the Stroop task. In addition, higher levels of cortisol were associated with worse performance on the Stroop task.

However, neither of these associations remained significant after the correction for multiple correlations was applied.

In response to experimental challenges, higher levels of total leptin (AUCg) in response to food ingestion were associated with better performance (less interference) on the Stroop task in depressed patient, however, it did not remain significant after the correction for multiple correlations was applied.

Higher levels of total cortisol (AUCg) were associated
with fewer errors on a sustained attention task in the depressed group. Though given the small sample size, this may be due to a number of extreme scores and should be interpreted with caution.

When the entire sample was included, comparisons between overweight/obese vs. normal weight participants indicated worse performance on decision-making task in overweight/obese participants. Further, higher BMI was associated with worse performance on a decision-making task but for healthy control participants only.
7.2 General Discussion

Summary of Research Program

The review of scientific literature provides a clear rationale for the systemic evaluation of the relationship between depression and altered eating behaviour to clarify the pathophysiological mechanisms responsible. The three sets of experiments conducted as part of this research program provide a significant early step towards this goal.

Figure 7-1 depicts an integrated model developed from published literature which incorporates physiological, psychological/behavioural and cognitive components.
As previously noted, a very large number of pathways may contribute to the association between depressive illness and weight disturbances. This research program focused on three major systems and themes: 1. HPA axis (cortisol) response to psychosocial stress and food consumption in patients with depressive disorders; 2. Appetite and satiety hormone (leptin and ghrelin) response to stress and stress induced eating behaviours, such as emotional eating (e.g. ghrelin secretion and emotional eating); 3. The inter-relationship between cognition, appetite regulation, and stress reactivity in participants with depression.

The first study assessed the impact of cortisol reactivity to an acute social stressor on eating behaviours, i.e., emotional eating in individuals with depression in comparison to healthy control participants. It has been previously reported those with emotional eating tendencies exhibit a blunted cortisol response to acute stressors (Tomiyama et al. 2011; van Strien et al., 2013). We sought to determine if the cortisol reactivity following stress induction and food challenge was influenced by the presence of emotional eating behaviour. Given that depressed individuals report higher levels of both perceived stress and emotional eating, this question may provide new insight into why depressed patients often experience appetite and weight changes. Dallman et al. (2010), based on animal models of chronic stress and comfort eating, have proposed that duration of illness (and by proxy, chronicity of stress), is an important contributor to this relationship. Our findings of significantly lower cortisol secretion (AUC) in emotional vs. non-emotional eaters in response to meal consumption will add to the above report. As well, depressed individuals with higher BMI and longer duration of illness (years) exhibited a smaller peak percent change in cortisol in response to an acute stressor, suggesting that the longer the duration of illness, the less pronounced the cortisol response to stress. There has been some suggestion (Dallman et al., 2010) that individuals that exhibit a blunted response to stress tend to eat more “comfort foods”, i.e., those high in fat and carbohydrates, and as such, emotional eating
and duration of illness may represent vulnerabilities towards weight gain. However, HPA reactivity to both stress and food consumption did not differ between depressed and control participants in the current study. Thus there is evidence to suggest that reduced HPA axis reactivity associated with emotional eating, and chronicity of depression, may represent independent obesogenic vulnerabilities.

The second study assessed the role of appetite and satiety hormones, leptin and ghrelin, in mediating appetite and weight disturbances in depressive illness. Both leptin and ghrelin are known to interact with the HPA axis, and is thought to contribute to stress-induced eating behaviours (Lu, 2007). However, the pathophysiological mechanism of this interaction has not been fully elucidated. Results of investigations of leptin and ghrelin concentrations in depressed samples have been mixed or inconclusive, while other studies suggest that the presence of emotional eating may influence appetite hormones, and vice versa (Raspapow et al., 2010). This led to our attempt to evaluate the role of emotional eating in ghrelin and leptin response to both stress induction and food challenge in depressed patients. Our findings that leptin levels were elevated in depressed participants compared to healthy controls and the observation that stress exaggerates this response in acute depression, have strong clinical relevance. The additional observation that emotional eaters tend to have lower levels of ghrelin compared to non-emotional eaters may also have clinical implications. However, no differences were observed in degree of decline in post-prandial ghrelin levels between depressed and control participants, or between emotional and non-emotional eaters.

The third study evaluated the inter-relationship between cognition and appetite and weight changes. It is well accepted that impaired cognition occurs in both depression and obesity. Interestingly both leptin and ghrelin appear to have neuroprotective actions and positive
impact on cognition, but these are recent findings that have not been well replicated. Their exact roles in modulating cognitive function are not fully elucidated even in normal populations, and even less so in subjects with depression. Our findings are consistent with previous reports of individuals with higher BMI performing poorly on decision-making tasks. However, in our study, this was only noted in control participants and not in the depressed subjects. In addition, although we found higher leptin levels to correlate with better cognitive performance in the depressed subjects, the significance was lost when controlling for multiple correlations. It is also of note that no significant differences in overall cognitive function were noted between depressed patients and controls. As such, our results did not support an association to between levels of appetite/satiety hormones and vulnerability to cognitive impairment.

**Depression and Obesity: Co occurrence vs. common pathophysiology**

The common co-occurrence of overweight status and depression (in chronic depression in particular) is well documented. At the same time, several pathophysiological mechanisms have been proposed as leading to both conditions. This literature is well reviewed (McIntyre et al., 2009; Soczynska et al., 2011) and discussed in Section 1. One key finding of our investigation was that depressed participants exhibited higher levels of leptin secretion in response to psychosocial stress, and higher leptin to BMI ratio, in comparison to controls. Given that leptin levels are dependent upon adiposity (and by proxy, BMI), higher leptin levels would be expected in individuals with elevated body weight. Interestingly we found that individuals with depression exhibit elevated levels of leptin independent of BMI, suggesting possible dysfunction in leptin regulation as part of the disease condition. High levels of leptin are well documented in obese individuals, yet they continue to consume more calories than necessary, suggesting a reduced anorectic physiological effect of leptin or a reduced sensitivity to it. It may be that a
similar dysregulation in feedback mechanisms may occur in depressed individuals, also favouring a state of leptin resistance. In such state, the physiological systems of satiety do not respond to effects of leptin, consequently making obese and depressed individuals eat more and gain weight.

Another key finding was that, with BMI controlled as a covariate, individuals categorized as emotional eaters exhibited lower levels of ghrelin compared to non-emotional eaters in response to the food challenge. Previous reports found overweight and obese individuals to exhibit low levels of ghrelin (English et al., 2003), but we found emotional eaters to exhibit lower ghrelin levels compared to non-emotional eaters, and this effect was independent of BMI. Emotional eaters, by definition, consume food in response to emotional stimuli rather than physiological cues. If food intake is continued regardless of diurnal rise and fall of ghrelin (e.g. eating in response to negative affect, when ghrelin levels are low), perhaps a ‘flattening’ of the diurnal variation occurs, and this may be reflected as persistent low levels of active ghrelin. Thus it may be proposed that if energy homeostasis can be maintained (likely in a positive balance) without fluctuations in ghrelin levels, then eventually such diurnal variations become unnecessary to initiate food intake, and over time, results in low or more stable diurnal levels, such as those exhibited in emotional eaters.

We also found that emotional eaters with higher levels of negative affect exhibited a greater magnitude of change (decrease) in leptin levels in response to stress. This is the first report of a physiological link between reduction of a satiety signal and negative affect in response to an external stressor in emotional eaters, but it needs replication. This finding suggests the likelihood that leptin levels may be influenced by both emotional and physiological signals.
Taken together, alterations in ghrelin and leptin production represent vulnerabilities to elevated body weight that may result from the experience of a depressive episode or emotional eating tendencies, or both, as presented in Figure 7-2.

As previously noted, depressed individuals may exhibit elevated leptin levels, which may suggest leptin resistance and its reduced effectiveness as a satiety signal. In addition, in depressed individuals with emotional eating tendencies, an altered ghrelin production may
contribute to increased eating. As previously discussed, emotional eaters in the current investigations exhibited lower levels of ghrelin, possibly representing some dysregulation in ghrelin system as a hunger signal. Therefore if a depressed individual is also an emotional eater, they may exhibit impairment of hunger as well as satiety signals. Our data also indicates that reliance on emotional rather than physiological cues is linked with physiological alterations in appetite hormones, though the direction of this association is not yet clear.

Furthermore, our data provides some evidence for interaction between cortisol, ghrelin and leptin. Inverse associations between ghrelin and cortisol, and between leptin and cortisol, were noted in response to stress (Section 3). As previously discussed in Study #2 (Section 5), it is possible that elevated leptin levels may be mediated, at least in part, by the hyperactivity of the HPA axis in depressed subjects.

The chronic course of depression and neurohormonal regulation of appetite and weight

It should be noted that the majority of participants in this current investigations likely had a recurrent or chronic form of depression rather than an acute depressive episode. It is often clinically observed that a chronic course of depression increases vulnerability to weight gain. Indeed, we found that higher BMI and longer duration of illness predicted blunted cortisol reaction to stress induction, which is consistent with the proposition that chronic stress increases the likelihood of comfort eating. Further, we found that patients experiencing an episode of depression that was less than 2 years in duration had significantly higher levels of leptin (AUC) in response to an acute stressor. In other words, while depressed patients as a group exhibited higher leptin levels than healthy controls, patients with a current depressive episode of less than 2 years exhibited the highest levels. While the relationship of leptin levels and duration of
illness has not been well studied, it is of note that similar observations have been made regarding HPA axis activity in acute vs. chronic depression, with distinct but opposite shifts in direction of appetite, sleep and weight changes (O’Keane et al., 2013).

To sum up, the data confirms physiological vulnerabilities that favour weight gain in subgroups of depressed populations. This obesogenic tendency appears to be particularly related to emotional eating tendencies and to chronic forms of the illness.

**Future Research Perspectives**

We would suggest that the findings from the current investigation provide evidence to link appetite and satiety hormones and depression. Given both the novelty of this research program and the importance of delineating factors contributing to the pathophysiology of depression and obesity, further investigations are warranted in order to identify other potential mediating factors. Listed below are two potential areas of research that may be particularly relevant in this respect.

*a) Exploring the interactions of cytokines, inflammatory markers and leptin: relevance to Depression*

Leptin is considered to be part of the class I cytokine receptor superfamily (Tartaglia et al., 1995). Cytokines, as well as leptin, are known to act through the Janus kinase (JAK)-signal transducers and activators of transcription (STAT) intracellular signaling pathway. Leptin signals are thought to regulate food intake via the JAK-STAT pathway (Ladyman and Grattan, 2013). In addition, leptin has been shown to promote production of pro-inflammatory markers such as TNF-α, IL-6, and IL-12 (Carbone et al., 2012). As well, TNF-α is thought to act
synergistically with leptin to increase levels of STAT3 phosphorylation, and has been suggested as a possible modulator of the anorectic effects of leptin (Rizk et al., 2001).

This association is particularly relevant to the study of depression and its overlapping presentation with obesity. Literature suggests that depression may itself be a consequence of inflammation (Anisman 2008; Miller, 2009; Hayley et al., 2009), and that obesity may be a state of “low grade” inflammation (Black, 2006). Disruption or dysfunction in the shared JAK-STAT pathway may contribute to the overlapping phenotype of depression and obesity, and should be considered in future investigations of leptin response to acute stress in depressed populations. It is also highly probable, that abnormalities in leptin secretion in response to external acute stressors in depressed and obese populations are mediated by proinflammatory markers, such as TNF-A, IL-6, and CRP, among others.

b) Leptin association with glucose metabolism and insulin

A growing body of literature documenting the role of leptin in metabolic disorders, such as Type 2 Diabetes, is also relevant to the study of depression and obesity. Metabolic syndrome is suggested to occur in approximately 8% of depressed patients (McIntyre et al., 2009). There is a well-documented association between insulin, which is implicated in metabolic disorders, and leptin (McGowan et al., 1992; Obici et al., 2002; Belgardt &Bruning, 2010). In brief, insulin stimulates the secretion of leptin from adipocytes, and in turn, leptin imposes regulatory restraint on insulin secretion, via neural relays, to pancreatic beta cells. Thus, a tight feedback control system sustains an optimal pattern of circulating concentrations of the two hormones to preserve glucose homeostasis (Moran et al., 2004; Karla, 2008). Published reports suggest that leptin resistance likely co-occurs with insulin resistance, and may share some underlying mechanisms (Munzberg &Meyers, 2005). Given the relationship between metabolic syndromes and
depressive disorders, future investigations into the leptin-insulin relationship in depressive disorders would be important. Indeed, it is a limitation of this research program that other satiety signals, like insulin, were not included in the analysis.

**Limitations**

There are a several number of limitations to this series of investigations, some of which have been previously noted and are summarized below.

a) The small sample size is a major limitation. A larger sample would have enabled direct comparisons between different subtypes of depression, specifically, the melancholic and atypical subtypes. As these two subtypes represent bidirectional appetite and weight disturbances in depression, it would be of clinical relevance to determine if differences in appetite hormones may contribute to this distinction in symptom presentation. Indeed, the atypical subtype of depression has been associated with increased risk of obesity (Levitan et al., 2012). Differences in HPA axis reactivity to stress (Gold and Chrousos, 2002), as well in levels of inflammatory markers (Lamers et al., 2013), have been noted between the melancholic and atypical subtypes. Future investigations should include a larger sample size to allow for such evaluations and to strengthen findings.

b) Depressed participants were not specifically selected based on duration of illness and as a result, there were few participants who experienced an acute depression. Together with the small sample size, this limited exploration of differences in hormone response based on duration of illness. Since most patients in the study experienced a recurrent or chronic course of depression, generalizability to individuals with an acute depression may be limited.
c) It was noted that higher levels of leptin were found in female participants in comparison to males. This is a fairly consistent finding in both healthy and depressed populations (Yang et al., 2007; Cizza et al., 2010). However, given the small sample size differences between genders could not be fully explored. This may represent an important vulnerability for females with depression and as such should be the subject of future investigations.

d) Depressed participants were also not specifically recruited based on BMI classification. Many previous reports on the relationship between hormones and weight in the published literature were based on obese populations, and as such, it is possible that there are distinctions in hormone response not only between individuals of normal weight and those with higher BMI, but also between the overweight and obese categories. However, comparisons between overweight and obese subjects could not be made in this series of investigations due to the small sample size.

e) Adipose tissue or distribution was not measured in this current investigation. The finding that the leptin to BMI ratio was higher in those with depression may be reflective of the amount or distribution (e.g. abdominal) of adipose tissue,. This may be an important component to assess in future studies.

f) This study did not include a basal measurement of leptin or ghrelin with 12hr fasting. These hormones were only measured in response to experimental conditions, which may limit generalizability of results.

g) Control of food intakes on experiment days is also a source of potential confounds. Participants were asked to fast 3 hours prior to the food challenge, but they did so outside the hospital, therefore it is possible that not all participants adhered to this requirement. Similarly,
participants were asked not to eat 2 hours prior to the stress induction, but they did not arrive until 10-15 minutes prior to the initiation of the experiment, and as such, again, non-adherence cannot be ruled out. As well, food intake was measured on a separate day without any stress component, and though this was done purposely, it limits inferences about stress-induced food intake in the immediate-term.

**Implications for Patient Care**

Although this research program compartmentalized various aspects that may contribute to the underlying pathophysiology of depression and obesity, it is the patient as a whole entity that is likely to contribute to vulnerabilities to each condition and its inter-relationship, and identification of individual differences in such respects as lifestyle and family history would be important to consider as contributing factors. The study of comorbid depression and obesity is also vital, as it has been associated with poor treatment response and prognosis. Identification of obesogenic vulnerabilities would therefore be an important as well, as both overlapping and independent phenomena in obesity and depression.

Findings from this study can inform treatment in two ways. First is the translational value of these findings to interventions for obese and depressed populations, and for patients with both depression and weight disturbances. The finding that emotional eating tendencies are greater in depressed subjects than in healthy controls suggests that strategies designed to target such behaviours may be useful for preventing weight disturbances in depressed patients. Physiological vulnerabilities could become targets for intervention. For example, if alterations in the leptin feedback system lead to leptin resistance in depressed patients, treatment strategies that increase leptin sensitivity may be helpful in preventing future weight gain. Indeed, evidence
from animal models suggests that increased leptin sensitivity is effective in reducing body weight (Myers et al., 2010).

The second way in which these findings can influence treatment is by informing future research to better understand the pathophysiology of both obesity and depression, which is of high clinical utility given the global public health burden of these overlapping disorders. Our results suggest that both leptin and ghrelin contribute to the physiological underpinnings of emotional eating, and this contribution may occur independent of depression. While emotional eating is particularly relevant to depressed populations, it has been previously linked to obesity in non-depressed samples and identification of how leptin and ghrelin factor into its underpinnings may inform treatment of obesity itself. Our findings may also lead to better understanding of the pathophysiology of depression, since the literature documenting the role of leptin and ghrelin in depressive disorders is relatively small. Further, the current research program is the first to study the inter-relationship between depression, eating behaviour and neurohormonal mediation. Additions to this growing field of research are essential to the better understanding and treatment of depression comorbid with obesity.
8 References


Board F, Wadeson R, Persky H. Depressive affect and endocrine functions: Blood levels of adrenal


leptin receptors, and leptin stimulates the function of the gland in euthyroid non-fasted animals. International Journal of Molecular Medicine, 9, p31–34.


Appendix 1: Supplemental Data

Age was included as a covariate in 2 (participant group) x 6 (time over experiment) repeated measures ANCOVA analysis of cortisol, ghrelin and leptin response to both stress induction and food consumption. As well, gender was included as a covariate in 2 (participant group) x 6 (time over experiment) repeated measures ANOVA analysis of cortisol, ghrelin and leptin response to both stress induction and food consumption. The results are presented below.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td><strong>Cortisol Response to Stress</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time x Gender</td>
<td>(5, 21)</td>
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</tr>
<tr>
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<td></td>
</tr>
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<td>Time x Gender</td>
<td>(3, 26)</td>
<td>1.24</td>
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<td>(3, 26)</td>
<td>.10</td>
<td>.96</td>
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<td><strong>Leptin Response to Stress</strong></td>
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<td><strong>Leptin Response to Food Consumption</strong></td>
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<td>.44</td>
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<td><strong>Ghrelin Response to Stress</strong></td>
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<td></td>
</tr>
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<td>Time x Gender</td>
<td>(5, 21)</td>
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One-way ANOVAs were employed to assess differences in cortisol, leptin and ghrelin response to psychosocial stress and food consumption between depressed individuals receiving medication vs. those who were not. No significant differences in AUC<sub>G</sub>, cortisol, leptin, ghrelin, or LHP in response to both stress induction and food consumption were noted between depressed participants receiving or not receiving antidepressant medication. The results are presented below.

<table>
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<th>No Antidepressant Medication t (n = 6)</th>
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<td>M (SD)</td>
<td></td>
<td></td>
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<td>181.50 (30.03)</td>
<td>170.29 (19.70)</td>
<td>(1, 14)</td>
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<td>.43</td>
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<td>AUC&lt;sub&gt;I&lt;/sub&gt;</td>
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<td>-22.43 (23.22)</td>
<td>(1, 14)</td>
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<td>Peak Percent Change</td>
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<td>57.13 (47.16)</td>
<td>(1, 14)</td>
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<td>.24</td>
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<tr>
<td><strong>Cortisol Response to Food Consumption</strong></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>AUC&lt;sub&gt;G&lt;/sub&gt;</td>
<td>179.58 (18.21)</td>
<td>173.28 (18.67)</td>
<td>(1, 14)</td>
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<td>.51</td>
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<td>AUC&lt;sub&gt;I&lt;/sub&gt;</td>
<td>-5.94 (17.90)</td>
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<td>(1, 14)</td>
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<td><strong>Leptin Response to Stress</strong></td>
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<td>AUC&lt;sub&gt;G&lt;/sub&gt;</td>
<td>718.09 (94.43)</td>
<td>628.31 (72.34)</td>
<td>(1, 14)</td>
<td>3.97</td>
<td>.07</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;I&lt;/sub&gt;</td>
<td>-130.57 (31.85)</td>
<td>-105.04 (27.64)</td>
<td>(1, 14)</td>
<td>2.64</td>
<td>.13</td>
</tr>
<tr>
<td><strong>Leptin Response to Food Consumption</strong></td>
<td></td>
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</tr>
<tr>
<td>AUC&lt;sub&gt;G&lt;/sub&gt;</td>
<td>679.98 (87.62)</td>
<td>639.54 (73.43)</td>
<td>(1, 14)</td>
<td>.96</td>
<td>.34</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;I&lt;/sub&gt;</td>
<td>-10.34 (18.87)</td>
<td>-2.13 (18.13)</td>
<td>(1, 14)</td>
<td>.77</td>
<td>.39</td>
</tr>
<tr>
<td><strong>Ghrelin Response to Stress</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;G&lt;/sub&gt;</td>
<td>191.86 (53.72)</td>
<td>207.59 (68.66)</td>
<td>(1, 14)</td>
<td>.26</td>
<td>.62</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;I&lt;/sub&gt;</td>
<td>-33.55 (53.15)</td>
<td>-54.01 (25.37)</td>
<td>(1, 14)</td>
<td>.77</td>
<td>.40</td>
</tr>
<tr>
<td><strong>Ghrelin Response</strong></td>
<td></td>
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</tbody>
</table>
Univariate analysis of variance was employed to determine if any differences could be attributed to time of administration in the study. No significant differences based on administration visit were found for any of the neuropsychological scores. Results are presented below.

<table>
<thead>
<tr>
<th>Neuropsychological Measure</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trails A (seconds)</td>
<td>(2, 35)</td>
<td>1.24</td>
<td>.30</td>
</tr>
<tr>
<td>Trails B (seconds)</td>
<td>(2, 35)</td>
<td>.01</td>
<td>.99</td>
</tr>
<tr>
<td>Stroop- Interference Score</td>
<td>(2, 35)</td>
<td>.03</td>
<td>.99</td>
</tr>
<tr>
<td>IGT – Net Total Score</td>
<td>(2, 31)</td>
<td>.06</td>
<td>.94</td>
</tr>
<tr>
<td>IGT – Total Money Won ($)</td>
<td>(2, 31)</td>
<td>.07</td>
<td>.93</td>
</tr>
<tr>
<td>CPT – Number of Commissions</td>
<td>(2, 28)</td>
<td>.35</td>
<td>.71</td>
</tr>
<tr>
<td>CPT – Number of Omissions</td>
<td>(2, 28)</td>
<td>1.85</td>
<td>.18</td>
</tr>
<tr>
<td>CPT – Hit Reaction Time (ms)</td>
<td>(2, 28)</td>
<td>.34</td>
<td>.72</td>
</tr>
<tr>
<td>CPT – Perseveration Score</td>
<td>(2, 28)</td>
<td>2.13</td>
<td>.14</td>
</tr>
</tbody>
</table>