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# Associations Between Testosterone, Androgen Receptor Polymorphism, And Mood

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# Abstract

Recent findings suggest that insufficient testosterone levels may be associated with depressive affect in men. Genetic variability in the androgen receptor (polyglutamine [CAG] repeat length) may be important for this relationship. However, the relationship between testosterone, androgen receptor CAG repeat length, and depressive affect remains inconclusive. The current thesis examined the association between testosterone concentration, androgen receptor CAG repeat length, and depressive affect in 218 young men with diverse mood patterns. Saliva samples were collected to quantify bioavailable testosterone, cortisol, and CAG repeat length. Participants completed the Profile of Mood States scale. In men with low testosterone concentrations, lower testosterone and longer androgen receptor CAG lengths predicted greater negative affect. Testosterone, CAG length, and negative affect were unrelated in men with average or higher testosterone concentrations. Cortisol concentrations were not related to negative affect. These findings suggest a complex relationship between testosterone and depressive affect in young men.



## Keywords

Testosterone, sex steroid, androgen receptor, AR polymorphism, CAG repeat, cortisol, depression, negative mood, males

# Summary for Lay Audience

The regulation of mood is a complex process, and no single model can explain all aspects of mood, or the emergence of pathological mood states like depression. For example, biological, psychological, and environmental mechanisms can all contribute to different mood states in different individuals, as well as the development of mood disorders. Of the biological mechanisms, little is known with respect to the role of the sex hormone testosterone (T) in mood regulation in men. Recent research suggests that insufficient T activity may increase the risk of depressive affect in men. T achieves its effects in the human body primarily by binding to the androgen receptor (AR). Consequently, genetic variability in the AR which produces differences in AR function may be important for understanding the relationship between T and mood regulation. Specifically, a variable polyglutamine (CAG) repeat length within the AR gene produces differences in AR activity and may contribute to mood states, as well as an increased risk of depression. However, the relationship between T levels, AR CAG repeat length, and depressive affect in men remains inconclusive. Consequently, the current thesis sought to investigate the statistical relationship between T activity and depressive affect in 218 physically healthy young men. Participants attended a single test session in our laboratory. Saliva samples were collected to quantify bioavailable T and cortisol levels, as well as to measure AR CAG repeat length. Participants completed the Profile of Mood States (POMS), a standardized mood scale, to measure recent mood, including depressive affect. Principal components analysis of the POMS identified 7 mood components, including a Negative Affect (NA) component. For men with low circulating T concentrations, lower bioavailable T and longer AR CAG repeat lengths were predictive of greater NA. A relationship between T activity and NA was not found in individuals with average or higher T concentrations. Circulating cortisol concentrations were unrelated to NA. The findings of the current thesis suggest a complex relationship exists between circulating T and negative mood in young men, and that levels of bioavailable T may influence the intensity of depressive affect experienced.

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# 1 Introduction

## 1.1 Depression

### 1.1.1 *Prevalence of Depression*

Depression is one of the most common mood disorders in the world, affecting individuals across all groups within society. The one-year prevalence of major depressive disorder (MDD) in North America is approximately 6% overall (Kessler & Bromet, 2013). The lifetime risk of developing depression is significantly higher, at approximately 15-18% (Bromet et al., 2011). Therefore, nearly one in five individuals will experience at least one episode of depression over the course of their lives. With such a high prevalence, this mental health disorder is a huge individual and economic burden on society (Wells et al., 1989; Wang, Simon, & Kessler, 2003). Consequently, greater research is required to inform the development of more effective treatment and prevention programs.

Although depression is a widespread disorder, it does not affect all groups within society equally. For example, a sex difference in the prevalence of depression is well established. Specifically, females consistently report higher rates of depression compared to males (Ibrahim et al., 2013; Kessler & Bromet, 2013; Lim et al., 2018). However, despite their lower lifetime risk of major depression, males have greater difficulty with seeking and accepting support for their depressive symptoms than females (Liang & George, 2012; NIH, 2018). Consequently, while there are many complex factors regulating mood (Malhi & Mann, 2018), the existence of a sex difference in the prevalence of depression suggests a possible role of sex hormones, such as androgens and estrogens, in regulating mood.

In addition to a sex difference, another population that is affected by disproportionately higher rates of depression and negative mood than the general population are university students and young adults. A recent meta-analysis by Ibrahim and colleagues analyzed 24 articles published between 1990 and 2010 that assessed the prevalence of depression among university students across North America, Europe, Asia, and the Middle East. They reported

a weighted mean prevalence rate of depression in university students of 30.6% (Ibrahim et al., 2013). This figure is considerably higher than the roughly 6% one-year prevalence rate of major depressive disorder in the general adult population (Kessler & Bromet, 2013). Several biological, psychological, and sociological explanations have been proposed to account for this high prevalence, including the possibility that university students perceive experiences related to university life as threatening, which negatively impacts their mental health and increases their risk of depressive symptoms (Ramón-Arbués et al., 2020). Among university students, a sex difference in prevalence is maintained, as female university students have been found to be significantly more depressed than their male counterparts (Young, Fang, & Zisook, 2010; Ghodasara et al., 2011; Kessler et al., 2015; Ramón-Arbués et al., 2020). Although the high prevalence of depression in university students is very concerning in general, the high rate in male students is especially worrisome given that they are less likely to seek out or accept support. Specifically, completed suicide rates, for which mood disorders such as depression are typically implicated, are significantly higher in males than females (Möller-Leimkühler, 2003).

### 1.1.2 *Symptomatology of Depression*

For most individuals, depression has a gradual onset and episodic course. However, depression can also have a sudden onset in some, and its pattern throughout one's life can vary significantly. Previous research has found that individuals are most likely to experience their first episode of MDD between adolescence and middle age (Malhi & Mann, 2018). However, almost half of all individuals experience their first depressive episode before age 20, and the mean onset for MDD is consistently found to be in the mid-20s (Kessler et al., 2005). To identify cases of depression, diagnostic systems such as the *Diagnostic and Statistical Manual of Mental Disorders, 5$^{th}$ edition* (DSM-5; APA, 2013) and *Beck Depression Inventory-II* (BDI-II; Beck, Steer, & Brown, 1996) measure important hallmark symptoms.

The most well-known symptom of depression is negative affect, such as feelings of worthlessness, hopelessness, and helplessness (NIH, 2018). However, depression can also manifest in a wide array of additional symptoms. One of the most commonly reported

symptoms associated with depression is disturbances in sleep (Yates et al., 2004; Benca & Peterson, 2008; Kaplan & Harvey, 2009), which can take the form of either insomnia or hypersomnia (Yates et al., 2004; Benca & Peterson, 2008), resulting in fragmented sleep and poor sleep quality. Researchers have found the relationship between depressed mood and disturbed sleep to be bidirectional, as poor sleep can be antecedent to depressed mood, and depressed mood can disrupt typical patterns of sleep (Murphy & Peterson, 2015). In addition to disturbed sleep, anhedonia, defined as the loss of interest, pleasure, and initiative (Cooper, Arulpragasam, & Treadway, 2018), is also a core feature of depression (Treadway & Zald, 2011). Furthermore, it is common for individuals suffering from depression to experience increases and/or decreases in appetite, as well as corresponding changes in weight (Nierenberg et al., 1996; Maxwell & Cole, 2009). Anxiety is also often co-morbid with depression, as previous research has found that nearly 70% of individuals with MDD have clinically significant levels of anxiety (Goldberg & Fawcett, 2012). Finally, patients with depression often exhibit cognitive dysfunction (Lam et al., 2014), including impairments in facial emotion recognition, memory, learning, and concentration (Murrough et al., 2011; Halvorsen et al., 2012).

In general, the core symptoms of depression typically fall into 1 of 3 categories: affective, such as feelings of worthlessness; neurovegetative, such as insomnia; and cognitive, such as impaired facial emotion recognition (Malhi & Mann, 2018). Factor analytic studies of different depressive measures, such as the BDI-II (Beck, Steer, & Brown, 1996) and *Center for Epidemiologic Studies Depression Scale* (CES-D; Radloff, 1977), have confirmed that such measures not only assess the affective symptoms of depression, but that they also assess neurovegetative and cognitive symptoms (Brown, Kaplan, & Jason, 2012; Sankar & Hampson, 2012). However, despite the existence of a wide array of depressive symptoms, no single symptom is pathognomonic. Furthermore, since many symptoms of depression are commonly reported in other mental and physical health disorders (Malhi et al., 2014; Malhi & Mann, 2018), conclusive diagnosis can be difficult.

While depressive symptomatology can be highly variable, a sex difference in depressive symptom profiles between women and men is frequently found. Specifically, females are

more likely to report fatigue, hypersomnia, suicide attempts, and reduced psychomotor activity. In contrast, men are more likely to report reduced libido and sexual dysfunction (Marcus et al., 2005; Angst et al., 2006; Smith et al., 2008; Kim et al., 2015). Furthermore, completed suicide rates are significantly higher in males than females (Möller-Leimkühler, 2003). Consequently, the existence of such a wide array of depressive symptoms, and the variability of such symptoms across individuals, depressive episodes, time, and sex, potentially alludes to the fact that many divergent mechanisms have been found to contribute to the regulation of one's mood, including the pathogenesis of negative mood states such as depression.

## 1.2 Regulation of Mood

Regulation of mood is a highly complex enterprise. Although our understanding has advanced significantly over the last several decades, a single biological or psychosocial model is not sufficient to explain all features of mood and its regulation. Accordingly, a single model cannot adequately be used to describe the emergence of pathological mood states, such as depression (Malhi & Mann, 2018). Biological, psychological, and social/environmental mechanisms can all differentially contribute to different mood states in different individuals at different times.

A variety of biological mechanisms have been identified as contributing to mood. For example, previous research has found that monoamine neurotransmitter systems, including serotonergic, dopaminergic, and noradrenergic systems, play a large role in the neurobiology of mood (Segal, Kuczenski, & Mandell, 1974; Delgado et al., 1990). The effects of serotonin, or 5-HT, on mood are particularly well known. Specifically, experimental manipulation of 5-HT levels, including use of acute tryptophan depletion to lower brain 5-HT levels, has been found to alter mood and emotional processing (Shopsin et al., 1975; Concu et al., 1977; Young & Leyton, 2002). However, much of what is known regarding the serotonergic regulation of mood comes from antidepressants. Specifically, most currently used antidepressants are either selective serotonin reuptake inhibitors (SSRIs) or selective serotonin/norepinephrine reuptake inhibitors (SNRIs) (Cleare et al., 2015). These medications are thought to improve mood by elevating synaptic 5-HT and norepinephrine

levels, resulting in increased activation of postsynaptic neurotransmitter receptors (Delgado et al., 1990; Young, 2013). Although monoamines have a well-documented effect on mood, a recent review of the neurobiology of depression found that dysregulation in monoamine neurotransmitter systems alone could not explain the widespread variability in symptoms experienced both across and within patients with depression (Willner, Scheel-Krüger, & Belzung, 2013). Furthermore, given that serotonergic-based antidepressants have been found to have limited efficacy for improving mood and other symptoms in adults with moderate to severe depression (Melander et al., 2008; Hengartner & Plöderl, 2018), it suggests that monoamine neurotransmitter systems only partially contribute to the neurobiology of mood.

Another well-established biological determinant of mood is the hypothalamic–pituitary–adrenal (HPA) axis. The HPA axis is a neuroendocrine regulatory system that consists of cells in the hypothalamus, anterior pituitary gland, and adrenal cortex above the kidney. Stimulation of the HPA axis ultimately results in the release of cortisol into the bloodstream (Watson & Mackin, 2009). In humans and other primates, cortisol is the body's primary stress hormone, and it has been found to have a broad array of effects, encompassing the stress response, sleep-wake cycle, metabolism, immune function, learning, memory, and mood (Miller, 2018). Abnormal function of the HPA axis has been identified in multiple mood disorders, such as bipolar disorder (Daban et al., 2005) and major depression (Keller et al., 2017). For example, elevated levels of basal plasma cortisol are commonly found in many individuals with severe depression (Rubin et al. 1987; Vreeberg et al., 2009). Furthermore, depressed individuals have also been found to display abnormally high secretion of cortisol in response to both psychological (Burke et al., 2005) or chemical stressors (Holsboer, 2000; Young et al., 2003). Such elevations in basal and stress-related cortisol in individuals with depression may be related to disturbances in glucocorticoid (GR) receptor-mediated negative feedback (Malhi & Mann, 2018). However, treatments that target the HPA axis and lower cortisol levels, such as antagonists of the glucocorticoid receptor (GR), have not been found to be effective at improving mood and other symptoms in major depression (Stetler & Miller, 2011). Thus, similar to monoamines, the HPA axis appears to only partially contribute to the neurobiology of mood. In addition to the HPA axis, a variety of other biological systems and processes have been linked to mood regulation, including

particular structures of the nervous system, such as the amygdala (Hamilton et al., 2012); neuroplasticity (Egeland, Zunszain, & Pariante 2015); and neuroinflammation (Bauer & Teixeira, 2021).

While a significant amount of literature exists regarding the neurobiology of mood, psychological, social, and environmental factors also have a role to play in mood regulation (Eckenrode, 1984; Tamir & Mauss, 2011; Malhi & Mann, 2018). For example, stress can have a large effect on mood and can trigger the onset of mood disorders. Specifically, previous research has identified a strong association between stressful life events and depressive affect (Kendler, Karkowski, & Prescott, 1999). Furthermore, social cognitive factors, such as beliefs regarding one's emotion regulation self-efficacy and the ability to control one's emotions, have been found to exert an important role (Tamir & Mauss, 2011). However, similar to the biological determinants of mood, psychological, social, and environmental determinants only partially explain the pathogenesis of negative mood states such as major depression.

Current understanding of mood, and the factors underlying mood regulation remains incomplete. While several factors, such as the monoamine neurotransmitters, have been identified as playing a contributing role, these mechanisms alone cannot explain all aspects of mood, mood regulation, and the pathogenesis of mood disorders. Consequently, further research regarding novel, less explored mechanisms is required to elucidate a greater understanding of mood regulation. The existence of a sex difference in the prevalence and symptomatology of depression, as well as the onset of the sex difference during puberty (Steiner & Young, 2008), suggests a possible role of the neuroendocrine system and sex hormones, such as androgens and estrogens, in the regulation of mood. In support of this, disruptions in hypothalamus-pituitary-gonad (HPG) axis function, a neuroendocrine regulatory system responsible for regulating reproductive function and the release of reproductive hormones, are commonly observed in individuals with major depression (Rupprecht et al., 1988; Schweiger et al., 1999). However, little is known with respect to the specific role of sex hormones such as testosterone (T) in mood regulation or their contribution to depressive affect in particular.

## 1.3 Testosterone

### 1.3.1 *Testosterone the Sex Steroid Hormone*

Testosterone (T) is the primary sex hormone in men. It is typically conceptualized as a ‘male’ hormone, as circulating serum T levels are many times higher in men than women. In men, the Leydig cells in the testes are the primary source of T and other androgens. Besides acting on its own, T may be converted into at least two other steroid hormones, estradiol or dihydrotestosterone (DHT). Synthesis of T is tightly regulated by many factors, including the release of luteinizing hormone (LH) from the pituitary gland as part of the hypothalamus-pituitary-gonad (HPG) axis (Melmed et al., 2016; Aladamat & Tadi, 2021).

In male adults, normal circulating serum T levels range from 300 to 1000 ng/dL (Rey & Josso, 2016). In addition to the serum, T is also found in saliva, where normal levels range from approximately 50 to 300 pg/mL (Clifton et al., 2016; Melmed et al., 2016). Many factors influence the amount of circulating T. For example, T is secreted in a pulsatile pattern of release, with pulses occurring approximately every 90 minutes (Veldhuis et al., 1987). Furthermore, serum T displays a circadian rhythm, whereby serum T levels reach their highest concentrations during the early morning hours, fall to their lowest concentrations in the early-to-mid evening, and remain most stable in the afternoon and early evening (Diver et al., 2003). In addition to a circadian rhythm, average T concentrations display a circannual rhythm (i.e., T production and release follows a cycle which is approximately one-year in length). Specifically, for adult males, T concentrations are highest in autumn months, such as October and November, and lowest in spring months, such as April and May (Moffat & Hampson, 2000; Perry et al., 2000; Svartberg et al., 2003).

When T levels fall below 300 ng/dL in male adults, individuals are usually diagnosed with ‘low T’ (Mulhall et al., 2018). Hypogonadism, where the testes produce little-to-no T, is the typical cause (Seidman, 2006), although hypogonadism can also be of central origin (e.g. hypogonado-tropic hypogonadism). Low T and hypogonadism are most frequently observed in older adults, as circulating levels of T are age dependent (Amiaz & Seidmen, 2008). At the onset of puberty, T becomes the primary circulating sex hormone in males, and serum T

levels reach their apex during early adulthood (Kelsey et al., 2014). However, HPG axis function declines with age. After reaching their apex, serum T levels marginally decline to the population average by approximately 40 years of age, after which bioavailable T declines by roughly 2% per year of increasing age (Feldman et al., 2002). Consequently, men over age 70 have been found to have serum T levels that are approximately 35% lower than young men, and a significant number of men over the age of 50 have T levels below the criterion of 300 ng/dL and would be considered to have low T (Vermeulen & Kaufman, 1995).

Importantly, individuals with low T often report psychological symptoms, including mild depression, difficulties with sleep, and increased irritability (Morales, Heaton, & Carson, 2000; Zitzmann & Nieschlag, 2001; Zitzmann, 2006). Such psychological symptoms of low T suggest a role for T in the regulation of mood, as described further below.

### 1.3.2 *Testosterone Actions in the CNS*

Like all steroids, circulating T can move across the blood-brain barrier (BBB) and exert effects on the brain. T has been found to directly affect both the excitability of neurons and the activity of various neurotransmitters (Dubrovsky, 2005; Eser et al., 2006). Specifically, T can bind to intracellular steroid hormone receptors, in particular the androgen receptor (AR; Rupprecht et al., 2001); or can act at ligand-gated ion channels, such as the TRPM8 receptor (Asuthkar et al., 2015). Through its nuclear or membrane-effects, T can also directly modulate the effects of various neurotransmitters, including 5-HT, dopamine, norepinephrine, and GABA, as well as their respective neurotransmitter receptors (Amiaz & Seidman, 2008). In addition to the direct effects of T in the CNS, T can also undergo enzymatic conversion within neurons or glia into either dihydrotestosterone (DHT) or estradiol. The latter conversion from T occurs via the enzyme aromatase, and subsequent binding of estradiol to estrogen receptors. However, only a small fraction of circulating T is thought to act via conversion to estradiol (Longcope, Kato, & Horton, 1969; Lakshman et al., 2010).

Through its wide array of actions in the CNS during early brain development (prenatal or early postnatal period), and again in adulthood, T has been associated in animal studies with

a broad range of neuroanatomical and neurobehavioural effects. However, in humans T has also been implicated as a potential moderating factor in several mood disorders, such as major depression, bipolar depression, and anxiety (Ebinger et al., 2009). Hypothetically, T might be capable of influencing the pathogenesis of these mood disorders through its interaction with serotonergic (Rupprecht & Holsboer, 1999), dopaminergic (Weltzien et al., 2006), and/or noradrenergic (Hernandez-Rauda & Aldegunde, 2002) neurotransmitter systems. For example, electrical recordings of serotonergic neurons in the dorsal raphe nucleus found that administration of T increased the serotonergic neurons' firing rate (Robichaud & Debonnel, 2005). T has also been found to promote the release of dopamine from neurons in the mesolimbic system (Alderson & Baum, 1981; Tobiansky et al., 2018). This system has a well-established role in motivated behaviour and has been found to display abnormal function in depression (Russo & Nestler, 2013). Furthermore, specific metabolites of T may antagonize serotonergic receptors, such as the 5-$HT_3$ receptor, which has been found to contribute to the pathophysiology of depression (Rupprecht, 2003; Sankar & Hampson, 2012). In addition to the monoamine neurotransmitter systems, T may alternatively influence the pathogenesis of depression or other mood disorders via the HPA axis and cortisol. Previous research has shown that androgens suppress the activity of the HPA axis during the stress response (Viau & Meaney, 1996; Williamson & Viau, 2008), but also that high levels of HPA activity caused by exposure to chronic stress are associated with an inhibition of testosterone production (Cumming et al., 1983; Chen et al., 2012; Kheirabad et al., 2016). However, the specific mechanisms underlying the interactions between T and the monoamine neurotransmitter systems, as well as between T and the HPA axis, and the contribution of such interactions to mood regulation, are not well understood. Such a gap in our understanding exists, in part, because the fundamental relationship between T and different mood states in men remains unclear and poorly mapped out. Thus, further research regarding the precise relationship between T and mood states, including pathological mood states such as depression, is needed (McHenry et al., 2014).

## 1.4 Testosterone and Depressive Affect

### 1.4.1 *Seminal Work in Hypogonadal Men*

The potential contribution of T to mood regulation in men was first widely entertained in the early-to-mid 20th century based on anecdotal reports of depression and negative mood from hypogonadal men who had low levels of T (McHenry et al., 2014). In addition to symptoms such as increased fat mass and erectile dysfunction, males with hypogonadism frequently report disturbances in mood such as negative affect, exhaustion, and low libido (Morales, Heaton, & Carson, 2000). Accordingly, males with hypogonadism have been found to display significantly higher rates of clinically significant mood disorders, such as MDD, compared to individuals with levels of T within the normal range (Shores et al., 2004; Almeida et al., 2008; Zarrouf et al., 2009). The apparent association between low T and depressive symptoms among hypogonadal men led researchers to begin to systematically explore the relationship of T to pathological mood states.

A large body of literature has emerged regarding the relationship between T and mood. Evidence has been derived primarily from 2 sources: experimental studies and observational studies. Observational studies typically involve measuring circulating T concentrations via saliva or serum sampling, collecting scores from the same participants on a mood or depression measure, such as the BDI-II (Beck, Steer, & Brown, 1996), and analyzing the association between the measures using statistical methods such as multiple regression. Conversely, experimental studies typically involve measuring baseline scores on a mood or depression measure, followed by the planned manipulation of participants' T concentrations by using the exogenous administration of T or by using androgen deprivation therapy (with or without a placebo condition as a control), and then observing any resulting changes in scores on the mood or depression measure. Many different populations have been sampled including hypogonadal men, psychiatric in- or out-patients, elderly men, adolescents, and community-based samples (Amiaz & Seidman, 2008). However, despite the large, broad array of literature, current evidence regarding a contribution of T to mood regulation and depression is inconsistent and still inconclusive.

### 1.4.2 *Apparent Inverse Relationship Between Testosterone and Mood*

In general, most current literature is suggestive of an inverse relationship between T and negative mood, such that the presence of lower T may increase negative affect and/or increase the risk of experiencing depressive symptoms (Rupprecht, et al., 1988; Barrett-Connor, Von Muhlen, & Kritz-Silverstein, 1999; Almeida, et al., 2004; Shores, et al., 2004; Seidman, Miyazaki, & Roose, 2005; McIntyre et al., 2006; Sankar & Hampson, 2012). Much of the evidence for an inverse relationship comes from hypogonadal men and men over 40 years of age. While clinically hypogonadal men represent a naturally occurring population with low circulating T levels, older men as a group have also been found to have lower circulating T concentrations and to display greater variation in T levels than younger men under age 40 (Kelsey et al., 2014). For example, in community-based samples of middle age and older adult males, researchers have reported a significant inverse correlation between bioavailable T levels and depression severity on the *Beck Depression Inventory* (BDI; Beck et al., 1961), a widely accepted rating scale used to detect symptoms of depression (Barrett-Connor et al., 1999; Chen et al., 2020). Furthermore, in another community-based study of older men by Morsink et al. (2007), researchers found that men in the lowest T quartile reported significantly more depressive symptoms than men in all other T quartiles. In addition to community samples of older men, several studies of hypogonadal and borderline hypogonadal men have found that these men, who have low circulating T concentrations, exhibit rates of depression and depressive symptoms that are significantly higher than the general population (Rabkin et al., 2000; Shores et al., 2004; Zarrouf et al., 2009; Westley et al., 2015).

The contribution of T to mood regulation has been under-studied in healthy, eugonadal, younger men between ages 18-40 compared to older men. In this population, T levels are at their highest point during the lifespan, and it is possible that such high T levels may exert a ‘protective’ effect on mood (McHenry et al., 2014). However, several studies in this age range have found evidence indicative of an inverse relationship between T and negative mood. Specifically, in healthy adolescent males, circadian-related declines in salivary T concentrations throughout the day have been associated with concurrent elevations in

depressive and anxious behaviours (Granger et al., 2003). Furthermore, a recent cohort study of healthy adolescents reported an inverse association between T and MDD, as well as between T and MDD without any comorbid anxiety disorders, in males (Kische et al., 2022). Another study of young adult men by Sankar & Hampson (2012) found that lower T concentrations predicted a more severe degree of negative affect among a subgroup of unmedicated men who were experiencing moderate to severe depressive symptoms.

Evidence for an inverse relationship between T and negative mood has also been reported in various patient samples, particularly those diagnosed with MDD. For example, in a study of men with MDD by Giltay et al. (2017), researchers found that both free and total T levels were lower in the MDD patients compared to healthy controls. Furthermore, daytime, nighttime, and 24-hour T secretion have also been found to be significantly lower in male MDD inpatients compared to controls (Rupprecht et al., 1988; Schweiger, et al., 1999). However, it is unclear whether the use of antidepressant medications might account for the lower T levels observed in the MDD samples. Furthermore, in a two-year study that followed a community sample of older men without prior diagnosed MDD, low T levels ($\leq 2.5$ ng/mL) were associated with a greater prevalence of depression and a shorter time to the onset of depressive symptoms over the assessment period (Shores et al., 2005). Finally, older men with dysthymic disorder, which shares depressed mood as a primary feature with MDD but involves fewer and longer-lasting symptoms, have also been found to have lower total T levels compared to men without depression (Seidman et al., 2002).

In addition to observational research, a few studies involving experimental manipulations of T levels have also produced evidence supporting a contribution of T to mood regulation, including an inverse relationship between T and negative mood. For example, in hypogonadal men, T replacement therapy has been found to improve affect, reduce irritability and fatigue, and alleviate depressive moods (Rabkin et al., 2000; Wang et al., 2000; Wang et al., 2003; Daniell et al., 2006; Snyder et al., 2016). Many of the studies, however, did not include a control group. This is problematic because studies involving T supplementation are often subject to a large placebo effect (Seidman & Roose, 2006). In male patients with prostate cancer, androgen-deprivation therapy is associated with an

increased risk of developing depressive or anxiety disorders compared to prostate cancer patients who do not receive androgen deprivation therapy (DiBlasio et al., 2008). Furthermore, in healthy eugonadal young men, administration of supraphysiological doses of T has been related to increases in mania and aggression (Pope et al., 2000). The finding of an increase in aggression following administration of supraphysiological doses of T has been replicated in at least one other study (Perry et al., 2003). However, in Perry and colleagues' study, alterations in mania were not detected. Conversely, in healthy men, following 1 month of T suppression using leuprolide, the 'T Deficient State' was associated with negative affect, reduced libido, and apathy, and a small portion of the men developed clinically significant depressive symptoms. Furthermore, the clinically significant depressive symptoms were reversed following T replacement (Schmidt et al., 2004). This finding regarding the effect of pharmacologically induced hypogonadism and T restoration in young men has been replicated in at least one other study (Schmidt et al., 2009). Together, findings from these clinical studies converge to suggest an antidepressant effect of T.

To summarize, findings from a wide array of observational and experimental studies across different populations of men are suggestive of an inverse relationship between T and negative mood, such that lower T levels may be associated with depressive affect. However, as is true for many areas of study in psychiatry, discrepancies and conflicting evidence in the literature are not uncommon. Accordingly, studies suggestive of no relationship between T and negative mood will be considered next to provide a balanced perspective. These contradictory studies require consideration when assembling a complete understanding of the present state of the literature pertaining to T and negative affect.

### 1.4.3 *Conflicting Evidence*

As mentioned, a body of current literature points to an inverse relationship between T and negative mood, and suggests that lower levels of T may be associated with depressive affect (Rupprecht et al., 1988; Barrett-Connor et al., 1999; Almeida et al., 2004; Shores et al., 2004; Seidman, Miyazaki, & Roose, 2005; McIntyre et al., 2006; Sankar & Hampson, 2012). However, many other researchers have disputed the existence of any relationship between T and negative mood. Across several observational and experimental studies in different

participant populations, some researchers have failed to find evidence of an association between low T and depressive affect (Levitt & Joffe, 1988; Rubin et al., 1989; Davies et al., 1992; Feldman et al., 2002; Gray et al., 2005). Here too, most of the refutational evidence is derived from studies of hypogonadal men and men over 40 years of age. For example, using data from the Massachusetts Male Aging Study (MMAS), a large community-based sample of middle aged and older men, researchers failed to find an association between serum T levels and the *Center for Epidemiological Studies-Depression* (CES-D) depression severity score (Feldman et al., 2002). Furthermore, in a study of older men by T'Sjoen et al., (2005), no correlation was found between T levels and scores on the *Geriatric Depression Scale* (GDS). Refutational evidence has also been obtained from experimental studies. Specifically, in several clinical trials of testosterone-replacement therapy, researchers have failed to find evidence for an antidepressant or mood-elevating effect of T in either hypogonadal men (Steidle, 2003) or men diagnosed with MDD (Seidman et al., 2005). Furthermore, in a study of healthy eugonadal young men, administration of exogenous T produced no change in hostility, anger, or mood (Tricker et al., 1996).

In general, although most research demonstrates an inverse relationship between T and depressive affect, a noteworthy amount of contradictory evidence exists. Such discrepancies in the literature may arise for a variety of different reasons, including methodological considerations. Specifically, discrepancies may reflect the frequent reliance on small sample sizes, particularly in experimental studies involving the exogenous administration of T (e,g., Tricker et al., 1996). Furthermore, discrepancies may also reflect the use of widely different scales across studies to assess negative mood and depressive symptoms, including scales that measure general mood and well-being rather than depressive symptom severity (Ebinger et al., 2009). Heterogeneity in the nature and severity of depressive symptoms in different samples may contribute to some of the variance in the literature. The relatively common failure to account for circadian, circannual, and age-related variability in T production may also account for some notable discrepancies across different studies. Finally, a failure to examine the relationship between T and other neuroendocrine correlates of mood, such as cortisol, which is anomalous in a subset of individuals with mood disorders (Lopez-Duran, Kovacs, & George, 2009), may also result in inconsistencies. Specifically, there is an inverse

relationship between circulating cortisol and T levels (Bambino & Hsueh, 1981; Cumming et al., 1983). Under chronic levels of stress, the high concentrations of circulating cortisol have been found to promote apoptosis of the Leydig cells, which ultimately results in suppressed levels of T (Chen et al., 2012; Kheirabad et al., 2016). This mechanism is thought to decrease the chances of reproduction under adverse conditions in the environment. Consequently, it is possible that circulating levels of cortisol, which are not typically analyzed concurrently with T, may underlie the observed relationship between T and depressive affect (Hirokawa et al., 2016; Ludwig, Roy, & Dwivedi, 2019).

In addition to methodological considerations, inconsistencies regarding the relationship between T and depressive affect may potentially arise due to the existence of a more complex, non-linear relationship between T and depressive affect. Several studies have suggested that the relationship between T and negative mood might, in fact, be curvilinear rather than linear. In a study by Booth, Johnson, & Granger (1999), researchers found that, in those with below average T levels, the relationship between T and depressive affect was negative, but in those with above average T levels, the relationship between T and depressive affect was positive. They suggested that T might have a positive effect on mood in general, but at high levels, T may be associated with behaviours that constitute risk factors for depression. Similarly, in a more recent study, researchers also observed a quadratic relationship between bioavailable T levels and BDI scores, such that the risk of depressive affect was greater in men with low and high T levels. However, they found this quadratic relationship existed only in underweight and obese men, and not in men with ‘normal’ bodyweights, in which they observed an inverse, linear relationship between T and depressive affect (Kratzik et al., 2007). Consequently, if the relation between T and depressive affect is, in fact, curvilinear in nature rather than linear, and this is not accounted for or examined in statistical analyses, it could potentially produce some of the refutational, inconsistent findings observed in the literature.

In summary, while much research is demonstrative of an inverse relationship between T and depressive affect, little work exists regarding this relationship in eugonadal *young* men, and several inconsistencies are also seen in the current literature. Such inconsistencies may exist

for several reasons, including failures to account for the relationship between T and other neural correlates of mood such as cortisol. However, if a relationship between T and depressive affect does exist, it is also possible that T's contribution to depressive affect may be influenced by moderators such as genetic variation in the receptors through which it mediates its effects. Consequently, an understanding of the relationship between T and depressive affect requires consideration of the androgen receptor (AR), T's primary bodily target (Davey & Grossmann, 2016).

## 1.5 Androgen Receptor

### 1.5.1 *Androgen Receptor Structure and Function*

The actions of testosterone (T) and its metabolites, such as dihydrotestosterone (DHT), are achieved via the binding to androgen-specific steroid hormone receptors, which are expressed in a diverse array of tissues throughout the body, including the brain (Zitzmann & Nieschlag, 2003; McHenry et al., 2014). T and its metabolites are hydrophobic, lipid-soluble molecules and can diffuse passively across the cell membrane and interact with intracellular steroid receptors (Wierman, 2007). Therefore, upon reaching its target site, T may not only bind to membrane-bound androgen-specific receptors, but it can also diffuse across the cell membrane and bind to intracellular androgen-specific steroid hormone receptors. The latter is considered its classical mode of action. Consequently, T may exert its effect via rapid non-genomic processes, such as altering signaling pathways or cell membrane excitability, which can occur within seconds or minutes of receptor binding (Falkenstein et al., 2000). However, T typically exerts its effect through slower, genomic processes, such as activating or inhibiting the expression of certain target genes, via intracellular nuclear receptors (Rupprecht et al., 2001; McHenry et al., 2014).

The effects of T, as well as its metabolite DHT, are primarily mediated by the androgen receptor (AR). The AR is a ligand-dependent transcription factor, which are proteins involved in initiating and regulating the transcription of DNA to RNA. It is classified as a member of the steroid hormone nuclear receptor superfamily, which also includes receptors specific to other steroids (e.g., estrogen, thyroid, or adrenal hormones) (Quigley et al., 1995;

Davey & Grossmann, 2016). The AR is a homodimer receptor protein (Zitzmann & Nieschlag, 2003; McHenry et al., 2014), as upon binding T, two AR bind together to form a protein complex called a dimer to attach to DNA and regulate transcription. When T diffuses across the cell membrane, it binds to an unbound AR in the cell's cytosol. This binding triggers a conformational change in the AR, leading to the release of chaperone proteins and translocation of the T-AR complex to the nucleus. The T-AR complex then dimerizes with another T-AR complex, and the homodimer receptor protein binds to specific genomic DNA sequences. These sequences are known as "androgen-response elements", and are located in the promoter region of androgen-dependent target genes. Consequently, the binding of the T-AR dimer to genomic androgen-response elements leads to the activation or inhibition of the expression of specific proteins (Choong & Wilson, 1998). Accordingly, circulating T serves as a key molecule in the regulation of the transcription of many androgen-dependent proteins throughout the lifespan (Zitzmann & Nieschlag, 2003).

The gene for AR is found on the X chromosome at Xq11-12 (Brown et al., 1989). It is encoded by 8 exons (Lubahn et al., 1988). As a member of the steroid hormone receptor superfamily, the AR has 3 primary functional domains: the ligand-binding domain close to the C-terminus of the protein; the DNA-binding domain in the central region of the protein; and a variable transactivation domain (TAD) close to the N-terminus (Mangelsdorf et al., 1995). The ligand-binding domain is responsible for binding to T and its metabolite DHT, and displays very strong affinity for androgens (Tan et al., 2015). The DNA-binding domain mediates the binding of the AR to the promoter regions of androgen-regulated genes and is very conserved among members of the steroid hormone nuclear receptor superfamily. Conversely, the TAD is the most variable domain of the receptor protein and regulates the transcription of androgen-regulated genes (MacLean, Warne, & Zajac, 1997).

The AR is expressed in many cells and tissues throughout the human body (Davey & Grossmann, 2016). Consequently, AR has been found to exert important functions in a variety of bodily systems including the reproductive (Chang et al., 2013), musculoskeletal (Zajac & Fui, 2012), immune (Lai et al., 2012), cardiovascular (Ikeda et al., 2005), and nervous systems (Mhaouty-Kodja, 2018). In the human central nervous system (CNS), AR

activity has been observed in cells in the amygdala (DonCarlos et al., 2006); cerebellum (Perez-Pouchoulen et al., 2016); hippocampus (DonCarlos et al., 2003); mesocorticolimbic system (Aubele & Kritzer, 2012); and the prefrontal cortex (Finley & Kritzer, 1999). Expression of the AR is particularly high in the hippocampus and amygdala (Ebinger et al., 2009). Accordingly, AR has been found to play an important role in learning and memory (Edinger & Frye, 2004). The AR has also been suggested to contribute to the expression of sexual and aggressive behaviours (Scordalakes & Rissman, 2004; Marie-Luce et al., 2013). Mutations and polymorphisms in AR's sequence have been correlated with a number of medical conditions, such as complete androgen insensitivity syndrome (CAIS; Quigley et al., 1995) and prostate cancer (Denis & Griffiths, 2000). However, given the expression of AR in several brain regions implicated in mood regulation and the pathogenesis of depression, recent research has also begun to suggest that a genetic polymorphism in AR may contribute to depressive affect and negative mood in men (Härkönen et al., 2003; Sankar & Hampson, 2012; Hung et al., 2019).

### 1.5.2 *Major Androgen Receptor Polymorphisms*

A polymorphism is a location in the genome where at least two different sequences, also known as alleles, are found in the same population. Different types of polymorphisms can occur, but the most common polymorphism seen in the genetic sequences of sex steroid hormone receptors, such as AR, are called short tandem repeats (STRs). STRs are short genetic sequences, typically 3 nucleotide bases in length, which are repeated over a variable stretch of a gene. For example, the CAG trinucleotide repeat is a common STR polymorphism, which may be repeated 8 times in one individual and 32 times in another individual (Maney, 2017). STR's typically arise due to errors during the DNA replication process (Ellegren, 2004).

The genetic sequence of the AR's DNA- and ligand-binding domains have been strongly conserved throughout evolution. However, the highly variable N-terminus transactivation domain (TAD) of the AR has been found to contain two major trinucleotide STR polymorphisms which can alter the AR's regulation of the expression of androgen-responsive genes, and consequently, may contribute to the pathophysiology of different medical

conditions (Choong & Wilson, 1998; Zajac & Fui, 2012). The first major trinucleotide STR polymorphism is the $(CAG)_nCAA$, or polyglutamine, repeat. This repeat is referred to as the CAG repeat. The second major trinucleotide STR polymorphism in AR is the $(GGT)_3GGG(GGT)_2(GGC)_n$, or polyglycine, repeat (the GGN repeat; Jiang et al., 2016). The last nucleotide base is referred to as 'N', as the GGT and GGC nucleotide triplets code for the same amino acid, glycine. Both trinucleotide repeat polymorphisms are located in the first exon of the AR gene (Davey & Grossmann, 2016).

The precise number of CAG and GGN repeats in the AR gene varies naturally across the male population. Consequently, variability in the length of these two repeat polymorphisms has been associated with differences in the transcriptional activity and expression of the AR (Choong et al., 1996; Irvine et al., 2000). Limited research exists regarding the GGN repeat polymorphism, or how variability in GGN repeat length may influence the properties of the AR. Most research on the GGN repeat polymorphism concerns its relationship to prostate cancer risk (Correa-Cerro et al., 1999; Hsing et al., 2000). In contrast, a sizable amount of research exists regarding the CAG repeat polymorphism, including the influence of variable CAG repeat length on AR function and expression (Choong et al., 1996; Ding et al., 2004), and, most importantly, the contribution of the CAG repeat polymorphism to mood regulation, including depressive affect and negative mood (Seidman et al., 2001; Colangelo, et al., 2007; Sankar & Hampson, 2012; Hirtz et al., 2021).

### 1.5.3 *Polyglutamine (CAG) Repeat Polymorphism*

The CAG trinucleotide STR polymorphism is located in the transactivation domain on exon 1 of the AR gene (Choong & Wilson, 1998). The CAG polymorphism encodes a polyglutamine segment, the most common amino acid in the human body, in the overall AR protein (Davey & Grossmann, 2016). The fewer the number of CAG repeats in the AR gene, the smaller the polyglutamine region of the AR protein. Conversely, the larger the number of CAG repeats in the AR gene, the larger the polyglutamine region of the AR protein. Across the male population, the number of CAG repeats in the AR polyglutamine segment has been found to range from 8 to 37, with an average repeat length of approximately 20-22 (Edwards et al., 1992; Vermeersch et al., 2010). Mean CAG repeat length has been reported in a few

studies to show ethnic variation (e.g., Edwards et al., 1992; Platz et al., 2000), although the differences reported are small.

Differences in the CAG repeat length (CAG-RL) of the AR gene have been associated with alterations in the transactivational activity and expression of the AR (Zitzmann & Nieschlag, 2003). In the context of the AR's role in regulating the expression of androgen-responsive genes, transactivation activity refers to the rate of gene expression that is initiated through T binding to AR (Lindzey et al., 1994). The transactivation activity of AR has been found to be inversely related to the CAG-RL both *in vitro* (Irvine et al., 2000) and *in vivo* (Gao et al., 1996). Specifically, the shorter the CAG-RL, the stronger the bond between T and AR, and the greater AR's transactivational activity. Conversely, the longer the CAG-RL, the weaker the bond between T and AR, and the poorer AR's transactivational activity (Choong et al., 1996). So, with shorter CAG-RLs, T has a stronger effect on the transcription of androgen-responsive genes, whereas with longer CAG-RLs, T has a weaker effect. The inverse relationship between CAG-RL and AR transactivational activity is likely related to differential strengths in the bond between the AR polyglutamine segment and important coactivator proteins, depending on CAG-RL (Irvine et al., 2000).

In addition to the effect of CAG-RL on transactivational activity, the degree of expression of the AR has also been found to be inversely related to CAG-RL. Shorter CAG-RLs are associated with increased AR mRNA and protein expression, whereas longer CAG-RLs are associated with reduced AR mRNA and protein expression (Choong et al., 1996). However, while the size of the CAG-RL in the AR gene has been found to show an inverse association with the transactivation activity and expression of the AR, literature typically fails to find an association between CAG-RL and circulating T levels. Importantly, this indicates that bioavailable T concentrations do not homeostatically compensate for having a longer or shorter CAG-RL, as might be predicted (Van Pottelbergh et al., 2001; Härkönen et al., 2003; Sankar & Hampson, 2012; Ryan et al., 2017; Hirtz et al., 2021).

Although the contribution of CAG-RL to AR functioning and physiology in the central nervous system (CNS) is not as well characterized as peripheral sites such as the testes and prostate gland (Hirtz et al., 2021), due to the high expression of AR in neurons of the

hippocampus, amygdala, and prefrontal cortex (Ebinger et al., 2009) we might expect AR CAG-RL to be associated with important neurological and psychological effects. Although evidence is still limited, within the typical range of CAG repeats (8-37), research has found evidence of a relationship between CAG-RL and cognition (Yaffe et al., 2003), competitiveness (Eisenegger et al., 2017), and the personality traits extraversion and neuroticism (Westberg et al., 2009). Within the normal scope of CAG repeats observed in the male population, the CAG-RL of the AR is beginning to be studied in mood regulation, including the expression of depressive affect in both adult (Seidman et al., 2001; Härkönen et al., 2003; Colangelo et al., 2007) and adolescent males (Vermeersch et al., 2010; Hirtz et al., 2021). Unfortunately, current literature is quite limited. Preliminary findings are suggestive of a relationship between circulating T levels, CAG-RL, and depressive affect. However, inconsistencies, caveats, and limitations are not uncommon in this literature.

## 1.6 Testosterone Activity and Depressive Affect

The contribution of T to depressive affect and negative mood in men may be influenced by many moderating variables, including genetic polymorphism in AR due to individual differences in the number of CAG repeats (CAG-RL). Since current literature on testosterone itself suggests an inverse relationship between T availability in the bloodstream and depressive affect, we might predict that men with longer CAG-RL are at an increased risk of experiencing depressive affect. However, research regarding the relation between circulating T, CAG-RL, and depressive affect in men is limited, especially in eugonadal young men. The limited past research supports some sort of association between circulating T, CAG-RL, and depressive affect (Seidman et al., 2001; Colangelo et al., 2007; Vermeersch et al., 2010; Sankar & Hampson, 2012). However, literature remains mixed, as several studies have found evidence suggestive of either: (1) no relationship between circulating T and CAG-RL (defined hereafter as ‘T activity’) and depressive affect, or (2) a complex association between circulating T and depressive affect that is not true in all men, but only in those falling within a particular range of AR CAG-RL or particular depressive symptom severity.

Most of the limited literature regarding T activity and depressive affect is based on older-aged men. In a study of 213 men between ages 41 and 70, a positive association was found

between CAG-RL and depressive symptom severity, including depressive affect, suicidal ideation, and poor general well-being (Härkönen et al., 2003). Furthermore, researchers have also found evidence of a positive correlation between CAG-RL and *Patient Health Questionnaire-9* (PHQ-9; Spitzer, Kroenke, & Williams, 1999) depression scores in psychiatric outpatients and andro-logic patients over age 50 (Schneider et al., 2011). As mentioned in Section 1.4.3, a study by Feldman et al. (2002) failed to find any association between circulating T levels and depression scores based on the *Center for Epidemiological Studies-Depression Scale* (CES-D) in older men participating in the Massachusetts Male Aging Study (MMAS). However, Seidman et al. (2001) analyzed the same MMAS dataset and included individuals' AR CAG-RL as a covariate. In a logistic regression, while the individual T and CAG-RL terms both failed to predict CES-D-diagnosed depression, the interaction term between T and CAG-RL emerged as a significant predictor of CES-D-diagnosed depression. Specifically, depression was inversely associated with circulating T levels in older men with shorter CAG-RLs, but not in older men with average or long CAG-RLs (Seidman et al., 2001). However, negative findings suggestive of no association between T activity and depressive affect in older men have also been reported (T'Sjoen et al., 2005; Schneider et al., 2013).

In young men little research on the association between T activity and depressive affect currently exists. However, the few studies that have been conducted have found evidence that circulating T concentrations may not be associated with depressive affect in all men, but only in those falling into particular AR CAG-RL subcategories. For example, using longitudinal data from young black and white men under age 40 that participated in the Coronary Artery Risk Development in Young Adults (CARDIA) Male Hormone Study, Colangelo et al. (2007) found a significant interaction between circulating T and CAG-RL on CES-D-diagnosed depression. A significant association was found between circulating T and depressive symptoms among young men with shorter CAG-RL, but not in those with average or longer CAG-RLs (cf. Seidman et al., 2001). Opposite to the above findings, in a more recent study of adolescent males, Vermeersch et al. (2010) reported an inverse association between free T and depressive symptoms in males with longer CAG-RLs, but not in those with short CAG-RLs.

The relationships observed between T activity and depressive affect might also depend upon the severity of depression. Specifically, in a recent study of adolescents with mild-to-moderate depressive symptoms, researchers found an inverse relationship between free T and BDI-II score in individuals with short CAG-RLs (< 19 repeats), a positive relationship between free T and BDI-II score in individuals with long CAG-RLs (> 28 repeats), and no relationship between free T and BDI-II score in individuals who had moderate CAG-RLs (Hirtz et al., 2021). Finally, in previous study of young males (ages 17-27) conducted in our laboratory (Sankar & Hampson, 2012), lower bioavailable T concentrations and shorter CAG-RL predicted more severe sleep symptoms of depression across the entire sample, which included both depressed and non-depressed men. However, in participants reporting moderate-to-severe depressive symptoms on the CES-D and PHQ-9, two standardized measures of depressive symptom severity, lower T concentrations and longer CAG-RL both predicted more severe depressive affect. Sankar and Hampson's findings not only suggest that the relationship between T activity and depressive affect might depend on depressive symptom severity, but also that CAG-RL might differentially contribute to different symptoms of depression. However, like other studies that currently exist, Sankar and Hampson did not investigate the possible contribution of other neuroendocrine correlates of mood, such as cortisol, and whether such correlates might contribute to the relationship between T activity and depressive affect. Furthermore, no studies have attempted to replicate Sankar and Hampson's sleep finding, and there has been little subsequent research since 2012 with respect to the hypothesized relationship between T concentration and depressive affect in young men.

In summary, the relation between T, AR CAG length, and depressive affect remains inconclusive (Tirabassi et al., 2015). Furthermore, several studies have suggested that a relationship between T activity and depressive affect may not be present across all men, but rather only in specific subgroups such as those with extreme AR CAG-RLs. The association between T activity and depressive affect may also depend on the severity of depressive affect. However, in general, there has been little work investigating the role of T activity in mood regulation, especially in young men (Sankar & Hampson, 2012). Consequently, further

research regarding the precise relationships between circulating T, AR CAG-RL, and depressive affect in healthy young men is needed.

## 1.7 Gaps in the Literature

The literature described in Section 1.4.2 and 1.6 is suggestive of a possible contribution of T to mood regulation, including the pathogenesis of negative mood states like depression. However, the current body of research is limited, especially for eugonadal young men, who report higher rates of depression than the general population (Ibrahim et al., 2013; Ettman et al., 2020) and whose T levels are at their highest point during the lifespan (McHenry et al., 2014). Furthermore, notable inconsistencies exist in the research literature, which may arise due to failures to appropriately account for T-related sources of variability, such as T's circadian and circannual rhythms which are well-established (Moffat & Hampson, 2000; Diver et al., 2003; Svartberg et al., 2003) and may influence the quality of the T measurements. The existing literature has not yet addressed whether the adrenal steroid cortisol may contribute to the variations in study findings, even though cortisol has been implicated in depression and found to have an inverse relationship with circulating T levels (Bambino & Hsueh, 1981; Cumming et al., 1983; Kheirabad et al., 2016). Most past studies have looked only for linear associations with T levels, but have not entertained the existence of a more complex non-linear or conditional relationship between T and depressive affect (Amiaz & Seidman, 2008). Hence, the true nature of the relationship between T activity and depressive affect in young men remains elusive.

The current thesis aimed to expand our theoretical knowledge regarding the importance of T activity in mood regulation by investigating the following questions in a sample of physically healthy young men: (1) What is the statistical relationship between bioavailable T levels, AR CAG-RL, and depressive affect in young men, (2) Are longer AR CAG-RLs associated with increased depressive affect in young men, and (3) What is the statistical relationship between bioavailable basal cortisol levels and depressive affect in young men? The following specific hypotheses were tested: (1) Greater depressive affect would be associated with lower bioavailable T concentration and/or longer AR CAG-RL, (2) Longer AR CAG-RLs would be associated with greater self-reported depressive symptoms, and (3) Depressive affect would

be associated with higher mean cortisol, but cortisol would not mediate associations between T levels and depressive affect.

# Chapter 2

# 2 Methods

## 2.1 Participants

Two hundred and eighteen participants were enrolled to participate in a study of attachment behaviour and T performed from 2011 to 2015 (Sankar & Hampson, unpublished data). The present study involved the statistical analysis of testosterone variables that were collected as part of the archival dataset. All participants were physical healthy heterosexual men, ranging in age from 18-35 years old ($M$ = 20.70, $SD$ = 2.98). Participants were recruited from the University of Western Ontario via poster advertisements placed around the university campus (see Appendix A for a copy of the poster). Participants were also recruited through the PSYCHOL 1000 Research Participation Pool using the SONA portal. The Research Participation Pool is an online platform that lists dozens of ongoing research studies by the Department of Psychology, which are currently seeking participants. Individuals recruited through poster advertisements received monetary compensation ($15 CAD) for their participation. Individuals recruited through the Research Participation Pool received one research credit for their participation.

The sample that was included in the final statistical analyses consisted almost entirely of undergraduate students. While university samples can be expected to differ from the general population in several key areas (e.g. SES, IQ), university samples are commonly used in research regarding depression (e.g. Sankar & Hampson, 2012; Beiter et al., 2015; Cooper et al., 2020). Furthermore, rates of self-reported depression are typically found to be greater in university student samples compared to the general population (Garlow et al., 2008; Ibrahim et al., 2013). Consequently, a sample of male university students was deemed as both acceptable and desirable to assess the associations between testosterone (T) activity and depressive affect in young men.

Eligibility for the study required that participants meet several inclusionary criteria. First, participants were required to be between ages 18-35. This range of ages was chosen based on an attachment variable of interest that was assessed as part of the broader purpose of the

study (Sankar, 2015), but not included in the current analyses. However, the restriction of participants to this age range was desirable in the context of the current study for several reasons. Male total T levels peak at approximately 19 years of age and decline slightly to the population average by approximately 40 years of age (Kelsey et al., 2014). After age 40, variation in total testosterone levels becomes significantly greater with advancing age compared to individuals under age 40 (2014). In addition to exhibiting increased variation, both the serum total T concentration and bioavailable T concentration (consisting of free T and albumin-bound T) also decrease with advancing age in men (Vermeulen, Rubens, & Verdonck, 1972; Harman et al., 2001). The age-related decline in bioavailable T is especially profound, as cross-sectional research with a sample of men between ages 40-70 found that bioavailable T declines at a rate of 2% per year of age, whereas total T declines at a rate of only 0.8% per year of age (Feldman et al., 2002). This age-related decline is associated with a variety of factors, including age-related increases in serum sex-hormone binding globulin (SHBG), social and behavioural factors such as nutritional deficiencies, and onset of chronic illnesses associated with aging such as cancer. Consequently, young men between ages 18-35 represented a pristine sample for investigating individual differences in T concentrations, and their associations with mood.

Saliva was used to quantify T in the present study because it affords a direct measurement of the bioavailable component of circulating T (Pardridge & Demers, 1991; Keevil et al., 2014). Only the bioavailable fraction is able to interact with tissues in the body, whereas SHBG-bound T is considered inert and inactive. To ensure the saliva specimens reflected participants' normal, endogenous T levels, individuals that reported any history of endocrine pathology were excluded. Several commonly used medications may have significant effects on the endocrine and/or reproductive systems, causing disturbances in T levels (Smith, 1982; Ilgin, 2020). Accordingly, 2 questions were included on the Health and Demographics questionnaire administered to all participants (see Section 2.2 for questionnaire description) to assess if participants either had a medical condition that could alter their T metabolism or were currently using any prescription or non-prescription medications (e.g. selective serotonin reuptake inhibitors; Hansen et al., 2017) known to disturb T levels. Participants

whose responses indicated a potentially problematic medical condition and/or medication use were excluded from the statistical analyses.

## 2.2 Study Design

The present study was part of a larger investigation that assessed (i) the role of attachment in male mating and reproductive strategies; and (ii) the relation of testosterone (T) to individual differences in attachment style (Sankar, 2015). Participants were scheduled to attend a single one-hour test session at the Laboratory of Neuroendocrinology on the university campus. Test sessions were individual-based and took place in a quiet testing room, in which the biological specimens were collected and paper-and-pencil questionnaires were completed. To control for T's well-established circadian rhythm (Gall, Glowania, & Fischer, 1979; Gupta, Lindemulder, & Sathyan, 2000), all sessions were held between 1300 and 1800 hrs. Specifically, serum and salivary T levels achieve maximal concentrations in the early morning, fall to minimal concentrations in the early-to-mid evening, and remain most stable between the afternoon and early evening hours (Diver et al., 2003). Accordingly, T sampling during the afternoon and early evening is recommended for studies interested in assessing individual differences in T uncontaminated by time-of-day variations (Yang et al., 2007).

Upon arrival to the laboratory, participants provided written informed consent. Participants then collected a salivary DNA sample, according to the procedure outlined in Section 2.3.2. While blood samples are recognized as the gold standard for DNA genotyping, saliva has been found to be a reliable and adequate source of DNA (Hu et al., 2012), and produces a greater DNA yield than cheek swabs (Rogers et al., 2007). DNA samples were subjected to genotyping to characterize each participant's CAG repeat length in their AR gene, using the procedures described in Section 2.3.2. Participants then collected 2 saliva specimens for the measurement of bioavailable T and basal cortisol levels, according to the procedure outlined in Section 2.3.3. One specimen was collected at the beginning of the one-hour test session immediately after the DNA sample was obtained, and the second specimen was collected at the end of the test session. Two specimens were collected at each end of the test session to be averaged, as the release of T in men is pulsatile, with a burst approximately every 90 minutes (Veldhuis et al., 1987). The two saliva specimens were each subjected independently to

immunoassays (using the procedures described in Section 2.3.3 and 2.3.4 below) to produce two single-timepoint T concentrations and two single-timepoint basal cortisol concentration values, each based on duplicate measurements from the same vials. These two timepoints were then averaged for greater reliability to produce a mean T and mean cortisol concentration representing each participant. T measurement for each participant was limited to a single randomly chosen day. However, research has found a strong correlation ($r$ = .85) between single timepoint measurements of T and the mean of several samples subsequently taken over the span of one year (Vermeulen & Verdonck, 1992) as well as over the span of several years (Mazur & Michalek, 1998), providing proper controls over the sampling methods are used.

Besides the biological samples, participants also completed a set of self-report paper-pencil questionnaires during the test session. These included a standardized self-report measure of mood, the *Profile of Mood States* (POMS; McNair et al., 2003). This mood scale served as the primary measure of interest in the current thesis (see Section 2.3.1 for a detailed description of the mood scale).

In addition, all participants completed a paper-pencil health and demographics (H&D) questionnaire during the test session (shown in Appendix B). The H&D questionnaire was given at the beginning of the test session, as participants collected their first saliva specimen. The H&D questionnaire contained items regarding personal health and environmental factors that could potentially affect measured levels of T and/or mood, such as history of endocrine pathology, current prescription and non-prescription medication use, usual sleep-wake time, height and weight (to calculate body mass index, BMI), recent illness or recent stressors, and cigarette and alcohol use. Given that some past research has found CAG repeat length to differ according to ethnicity, one questionnaire item asked participants to self-report their ethnic background. Questionnaire items also assessed demographic variables such as first language, level of education, and occupation. Finally, the questionnaire contained a series of questions that were related to variables, such as attachment patterns, that were assessed simultaneously as part of the broader study, but were not of interest to the current study and its statistical analyses (Sankar, 2015).

## 2.3 Measures

### 2.3.1 *Profile of Mood States (POMS)*

To quantify individual differences in mood status, participants completed a self-report mood inventory that inquired about their recent mood states over the past two weeks, including the extent to which they had experienced symptoms of depressive affect. A two-week timeframe was chosen because a two-week period is considered relevant when evaluating depression for clinical purposes (Kroenke, Spitzer, & Williams, 2001; APA, 2013). A substantial body of research has demonstrated that self-report measures of depressive symptoms, such the *Profile of Mood States* or the *Quick Inventory of Depressive Symptomology-Self-Report* (QIDS-SR; Rush et al., 2003), have acceptable validity and reliability across a variety of populations (APA, 2019). For scales assessing depressive symptoms, the correlation between detection of depression based on self-report measures and clinician-rated scales is moderate-to-strong, providing well-established questionnaires are used (Tondo et al., 1988; Rush et al., 2006). Consequently, although discrepancies between self-report measures and structured interview formats and/or clinician-rated measures can arise (Cameron et al., 2011), literature currently supports the use of self-report measures to assess depressive affect.

In the current study, the *Profile of Mood States* (POMS; McNair et al., 2003) was administered to assess recent mood, including depressive affect. The POMS has been found to be an effective measure that retains its factor analytic structure in a diverse range of different subject samples, such as college students (Nyenhuis et al., 1999) and psychiatric outpatients (Lorr, McNair, & Weinstein, 1963), and in many different research settings, such as in drug development (McNair et al., 1965) or psychotherapy studies (Lorr et al., 1961). It is comprised of 65 mood-related adjectives. Individuals were asked to rate the extent to which each adjective described their typical mood within the past 2 weeks (including the date of assessment) on a 5-point Likert scale, ranging from 0 (not at all) to 4 (extremely). Example items included: “Miserable”, “Peeved”, “Carefree”, and “Restless”. Responses are summed to produce separate scores on six subscales, which have been empirically derived via factor analysis: Depression-Dejection (D), Tension-Anxiety (T), Anger-Hostility (A), Fatigue-Inertia (F), Vigour-Activity (V), and Confusion-Bewilderment (C) (McNair & Lorr,

1964; Lorr, McNair, & Weinstein, 1963; Boyle, 1987). A Total Mood Disturbance (TMD) score can also be calculated by summing individual scores on each of the 6 subscales but weighing the Vigour-Activity factor negatively. In normative samples of adult male outpatients, the POMS subscales have been found to show high internal consistency, including .95 for the Depression-Dejection factor (McNair, Lorr, & Droppleman, 1992). Similar to previous findings, in the current study Cronbach's alpha values for the POMS subscales ranged from .71 to .92, including $\alpha = .92$ for the Depression-Dejection component (see Table 1, below). Previous research has identified high test-retest reliability for scores on each of the 6 POMS subscales (McNair, Lorr, & Droppleman, 1992; Gibson, 1997).

While scores on each of the 6 subscales were calculated for each participant, the POMS Depression-Dejection factor (POMS-D) served as the primary factor of interest. The POMS-D is defined by items that assess depressive affect and feelings of personal inadequacy, such as "Sad", "Miserable", "Helpless", "Unworthy", "Hopeless", "Guilty", and "Lonely" (McNair et al., 2003). Across factor analytic studies of the POMS, the factor structure of the POMS-D has been consistently replicated (Boyle, 1987; Bourgeois, LeUnes, & Meyers, 2010). While the POMS was designed to assess typical mood states more broadly in respondents, many studies have utilized the POMS-D specifically to measure depressive mood, as well as changes in depressive mood following a given treatment (Szaflarski et al., 2003; Szaflarski & Szaflarski, 2004). The POMS-D score exhibits a strong correlation ($r \geq .75$) with the *Beck Depression Inventory-II* (BDI-II; Beck, Steer, & Brown, 1996), a widely validated scale that is commonly used to measure the severity of depressive symptoms (Griffith et al., 2005). Furthermore, in individuals with HIV infection, the POMS-D has been found to perform similarly to the BDI and display a high degree of accuracy for identifying non-depressed patients and MDD-diagnosed patients according to the *Structured Clinical Interview for DSM-IV* (Patterson et al., 2006). Consequently, the POMS-D can be considered an appropriate measure of depressive affect.

**Table 1.**

*Reliability of the Profile of Mood States (POMS) Subscales in the Present Dataset.*

| POMS Subscale | *Cronbach's α* |
|---|---|
| Depression-Dejection (D) | .92 |
| Anger-Hostility (A) | .90 |
| Tension-Anxiety (T) | .86 |
| Fatigue-Inertia (F) | .85 |
| Vigour-Activity (V) | .79 |
| Confusion-Bewilderment (C) | .71 |

Although several revised versions of the POMS have been developed since its inception, current literature does not indicate any significant differences in internal consistency or effect size between the original POMS and its later revised versions (McNair et al., 2003). Furthermore, use of the original, 65-item POMS in the present study allowed for an in-depth analysis of the Depression-Dejection factor (POMS-D), the primary factor of interest. For these reasons, the original 65-item POMS was used, and was deemed a satisfactory means to quantify mood, including depressive affect, for the current thesis.

### 2.3.2 *AR Genotyping*

Participants were instructed in advance not to eat, drink liquids other than plain water, chew gum, brush their teeth, or smoke for at least 30 minutes before their test session to minimize impurities in the saliva specimens. Prior to collection of the first saliva sample, participants rinsed their mouths using plain water. Participants then used a sterile OG Oragene·DNA tube (DNA Genotek Inc., Ottawa, Ontario, Canada) to collect approximately 2 mL of saliva. After

collection was completed, 2 mL of Oragene·DNA stabilizing solution was mixed into the saliva sample by the researchers. The samples were then stored at room temperature until analysis.

Salivary DNA is predominantly derived from buccal epithelial cells and white blood cells, the latter of which is a good source of genomic DNA (Thiede et al., 2000). While bacterial DNA is found alongside human DNA in saliva specimens, saliva specimens collected with the Oragene·DNA collection kit contain significantly less bacterial DNA compared to saliva obtained from mouthwash or cytobrush methods of collection (James, Iwasiow, & Birnboim, 2011). The median DNA yield from a 2 mL saliva sample with an Oragene kit is 110 µg, and previous research suggests that saliva collection with an Oragene kit produces a greater quantity and quality of DNA than buccal swab or cytobrush methods (Rogers et al., 2007).

Genotyping of the CAG repeat polymorphism in the AR was performed at The Center for Applied Genomics at The Hospital for Sick Children in Toronto (Ontario, Canada), using previously established methods (e.g., Sankar & Hampson, 2012). Specifically, 50 ng of genomic DNA was isolated from each saliva sample. Polymerase chain reaction (PCR) utilizing one unlabeled primer (5’- GAAGGTTGCTGTTCCTCATC-3’) and one primer labeled with 6-FAM dye for visualization (5’- CTTTCCAGAATCTGTTCCAG-3’) was then used to amplify the CAG repeat segment of the AR gene. To separate the amplified CAG repeat fragments according to size (i.e. repeat length), following several cycles of PCR, the fragments were subjected to capillary electrophoresis. Fragments were detected using an ABI3730XL DNA Analyzer (Applied Biosystems Inc., Waltham, Massachusetts). GeneMapper software (Version 3.5, Applied Biosystems Inc.) was used to quantify the length of the CAG repeat segment from each saliva sample. A subset of saliva samples with different CAG repeat lengths was sequenced to verify the repeat sizes that were obtained following capillary electrophoresis.

### 2.3.3 *Testosterone Measurement*

To quantify bioavailable testosterone (T), participants used a plain sterile polystyrene culture tube to collect 3 mL of whole saliva. Separate polystyrene tubes were provided for the collection of the first and second saliva specimens. To collect the saliva, participants used the 'passive drool' method, in which the head and chin are tilted down, allowing saliva to accumulate passively near the front of the mouth over a period of a few minutes. Once a sufficient amount of saliva had accumulated, participants were instructed to expel the saliva into the collection tube provided. The passive drool method does not use any artificial reagents to stimulate saliva flow (e.g. commercial gums), which sometimes contain ingredients that can interfere with the accuracy of immunoassay measurements including, rarely, radioimmunoassays (RIA) (Granger et al., 2004). Participants repeated the passive drool procedure until they reached the 3 mL symbol on the collection tube. All saliva specimens were frozen at -20 degrees Celsius until they were analyzed. The assays were performed by the Laboratory of Neuroendocrinology at the University of Western Ontario.

In human serum, T typically exists in 3 forms: a majority is bound to sex hormone binding globulin (SHBG), a fraction is bound non-specifically to albumin, and a smaller fraction of the hormone is free, unbound, T. However, only the free and albumin-bound forms of T are bioavailable and thus biologically active (Vermeulen, 1973; McCann & Kirkish, 1985). Conversely, SHBG-bound T is considered to lack biological activity, as the protein complex dissociates very slowly (Baird et al., 1969). Saliva consists only of free hormone, and does not contain any SHBG-bound T (Vining, McGinley, & Symons, 1983). Previous research has found free T to exist in equilibrium between the serum and saliva (Lood et al., 2017). Furthermore, high correlations, ranging from $r = 0.83$ (Johnson, Joplin, & Burrin, 1987) to $r = 0.97$ (Vittek et al., 1985), between serum and saliva free T concentrations have been found. Consequently, the concentration of T in saliva is considered to be highly representative of the fraction of free T circulating in the bloodstream which is able to bind to AR in various target tissues, including the CNS (Keevil et al., 2014).

In the present study, radioimmunoassay (RIA) was used to analyze each saliva specimen in duplicate, following centrifuging and without extraction. A well-established laboratory

protocol developed locally (Moffat & Hampson, 1996) was used to modify a $^{125}$I Coat-A-Count kit for T (Siemens Healthcare Diagnostics Inc., Deerfield, Illinois) for use with saliva. The $^{125}$I Coat-A-Count antiserum has a cross-reactivity with dihydrotestosterone (DHT) of less than 5% and negligible cross-reactivity with other steroids and is thus highly specific for T. The intra-assay coefficient of variation (CV) averaged 5%. The lower limit of detection of the assay was 6 pg/mL.

### 2.3.4 *Cortisol Measurement*

In addition to quantifying bioavailable T, each of the two 3 mL saliva samples collected by participants (see Section 2.3.3) were also used to quantify bioavailable basal cortisol (CORT) concentration.

Like testosterone, CORT typically exists in one of 3 forms in human serum: either bound to corticosteroid binding globulin (CBG or transcortin), non-specifically bound to albumin, or in free form (not bound to a carrier protein, i.e. unbound CORT). In non-stressful situations, the unbound form accounts for only about 5% of total serum CORT. Conversely, the CBG-bound and albumin-bound forms account for 80-90% and 10-15% of total serum CORT, respectively (Lewis et al., 2005). However, only the free, unbound hormone can enter tissues, diffuse across cell membranes, and bind with high affinity to the glucocorticoid receptor (Mendel, 1989). Therefore, only the unbound form is considered bioavailable, and thus, biologically active (Perogamvros, Ray, & Trainer, 2012). Saliva consists only of free, unbound CORT (Katz & Shannon, 1969), and free CORT exists in equilibrium between the serum and saliva (Umeda et al., 1981). Similar to salivary T, previous research has found that measures of salivary CORT are reliable indicators of the bioavailable portion of CORT in the bloodstream that is able to interact with the CNS (Vining et al., 1983).

To quantify CORT, RIA was performed by the Laboratory of Neuroendocrinology at Western. RIA was used to analyze the saliva specimens, without extraction. A $^{125}$I Coat-A-Count kit for cortisol (Siemens Healthcare Diagnostics Inc., Deerfield, Illinois, United States) was modified according to a well-established and previously used laboratory protocol (Moffat & Hampson, 1996) for use with saliva. Each sample was measured in duplicate.

## 2.4 Statistical Analysis

All data were analyzed with IBM SPSS Statistics 27. To characterize the sample and obtain a preliminary understanding of the relationships among the variables, descriptive statistics, reliability coefficients, scatterplots, and zero-order correlations between the study variables were computed. The criterion for statistical significance was set at $\alpha = .05$ for each test.

To investigate the first and second hypotheses, two sets of mood scores were computed. First, conventional scores on each of the 6 subscales of the Profile of Mood States (POMS) were calculated according to the formulas given in the test manual (McNair et al., 2003). Scores on the Depression-Dejection subscale (POMS-D) served as the primary subscale of interest. Secondly, all 65 individual items of the POMS were entered into a principal components analysis (PCA) to obtain empirically derived, weighted mood components.

Scores on the PCA-extracted depressive affect component, as well as scores on the POMS-D, served as criterion variables in multiple regression (MR) analyses to determine whether individuals' depressive affect component and/or POMS-D scores could be predicted from their bioavailable T concentrations and/or AR CAG-RL. A forced entry regression model was used. In addition to predictors for T concentrations and CAG-RL, 3 control variables were included in the regression models. Two variable selection techniques were employed in the current thesis: theory-driven and model comparisons (Kelley & Maxwell, 2019). Initially, current literature and a priori considerations were used to select a subset of predictors that could potentially affect the measured levels of T or CAG. Model comparisons, specifically hierarchical regressions, were then employed to compare models containing different combinations of potential predictor variables (Kelley & Maxwell, 2019; Maxwell, Delaney, & Kelley, 2018). Several variables which were selected for model comparisons on theoretical grounds, including an interaction term between AVGT and CAG-RL, failed to contribute any predictive power to the regression models and were eliminated on that basis from inclusion in the final regression equation. Ultimately, AGE, or the age of each participant in years, served as a control variable. Although participants were 'young' men between ages 18 and 35, male T levels do decline slightly to the population average until age 40 after reaching their apex at approximately age 19 (Kelsey et al., 2014). DATE, or the month of the year that participants

were tested, served as a second control variable. Male T levels have been found to display a circannual rhythm, in which T concentrations are highest in autumn months and lowest in spring months (Moffat & Hampson, 2000; Perry et al., 2000; Svartberg et al., 2003). Finally, the interaction between bioavailable T concentration and whether or not participants met the established POMS-D cut-point for depressive symptoms (AVGT*DEP_CUTOFF) was included as a predictor in the regression model. Specifically, a score of greater than 20 on the POMS-D was selected as the threshold for identifying mild-to-moderate depression based on previous work using the POMS (Nyenhuis et al., 1999; Patterson et al., 2006; Bay, Hagerty, & Williams, 2007). Because the relationship between T concentration, CAG-RL, and depressive affect has been found by some researchers to depend on the severity of an individual's depression (Colangelo et al., 2007; Sankar & Hampson, 2012; Hirtz et al., 2021), and because dummy-coded depression status predictors have been used in previous literature (Hirtz et al., 2021), AVGT*DEP_CUTOFF was computed and included as a predictor in the regression analyses.

To investigate the third hypothesis, the PCA-derived depressive affect component scores were regressed on basal cortisol and cortisol-influencing control variables. A forced entry regression model was used. Here again, theory-driven and model comparison techniques were used to select the control variables to be included in the regression models (Kelley & Maxwell, 2019). In addition to the inclusion of DATE and AGE as predictor variables, TIMEDIFF, or the difference in hours between the time that participants typically woke up in the morning and the time of day they were tested in our laboratory served as a control variable to account for the effect of such a time difference on the circadian rhythm (Krieger et al., 1971).

# Chapter 3

# 3 Results

## 3.1 Data Processing

Data for all POMS and Health & Demographics (H&D) questionnaire items, as well as the biological measures (bioavailable T and CORT levels, CAG-RL), was inspected prior to any statistical analyses. Univariate outliers and any missing data were checked using the 'Frequencies' function of SPSS. A criterion of ± 3 standard deviations (SD) from the sample mean, as well as Mahalanobis distance, were used to identify outliers (Dunn, 2021).

Analysis of missingness revealed no missing data for bioavailable T, bioavailable CORT, or CAG-RL in the current sample. Given the minimal amount of missing data, a complete case analysis was employed, in which it is assumed that any missing data are missing at random (Liu, 2016). A complete case analysis is the simplest method for handling missing data and is ideal in cases where there is little missingness, such as the current thesis.

## 3.2 Biological Measures

The two bioavailable T concentration measurements for each participant were averaged across the test session to optimize reliability. Average salivary T concentration in the sample ranged from 30.53 pg/mL to 302.98 pg/mL ($M$ = 86.83, $SD$ = 29.35) (Figure 1). These values are in agreement with previous studies of bioavailable T concentrations in young men (e.g., Moffat & Hampson, 1996; Yang et al., 2007). While it is possible that the T concentration of 302.98 pg/mL found for one participant occurred naturally and did not reflect experimental error, this value was far beyond the outlier criterion of ± 3 SD from the sample mean. Consequently, this participant was classified as an extreme outlier and removed from further statistical analyses. Furthermore, one other participant that constituted a multivariate outlier on bioavailable T concentration and Negative Affect component score (see Section 3.3.2) was also removed. Therefore, the final sample size consisted of N = 216 participants.

Androgen receptor (AR) CAG repeat length (CAG-RL) ranged from 13 to 30 repeats ($M$ = 22.09, $SD$ = 2.89). The distribution of CAG-RLs can be seen in Figure 2. These values are in

agreement with other studies of CAG-RL in previous samples of physically healthy men, which typically report a range of CAG-RL between 8 to 37 and an average repeat length of roughly 20-22 repeats (Edwards et al., 1992; Vermeersch et al., 2010; Sankar & Hampson, 2012). A *t*-test revealed that participants who self-identified as East Asian ($n$ = 43) had significantly longer CAG-RL than participants who did not self-identify as East Asian ($t(216) = -2.89$, $p = .004$). This finding is commonly reported in the literature (Hsing et al., 2000; Platz et al., 2000), and further suggests that the current study contained a typical sample of CAG-RLs.

To check whether having short and long CAG-RLs might lead to homeostatic compensation in T levels, a Pearson correlation coefficient between bioavailable T concentration and CAG-RL was computed. In the current sample, the Pearson correlation coefficient was $r = -.02$ ($p = .740$), which suggested that T levels did not compensate for having a longer or shorter CAG-RL, and that bioavailable T levels and CAG-RL varied independently. This finding is in line with several previous studies that have found no association between circulating T levels and CAG-RL (e.g., Sankar & Hampson, 2012; Ryan et al., 2017; Hirtz et al., 2021).

For cortisol, the two bioavailable CORT concentration measurements were likewise averaged across the test session. The average salivary CORT (AVG CORT) concentration ranged from 1.36 pg/mL to 22.34 pg/mL ($M = 6.01$, $SD = 3.20$). This range is consistent with other studies of salivary basal CORT levels in physically healthy men evaluated at a similar time of day (Moffat & Hampson, 1996; Brownlee, Moore, & Hackney, 2005). The distribution for AVG CORT was highly positively skewed and can be seen in Figure 3. Positively skewing in basal cortisol is nearly always seen when cortisol concentrations are monitored (e.g., Kobayashi & Miyazaki, 2015). Consequently, to transform this skewed distribution so that it better approximated normality for subsequent regression analyses (Feng et al., 2014), a logarithmic transformation was applied, generating a new variable: LOG AVG CORT. As seen in Figure 3, the distribution for LOG AVG CORT approximated normality. In men, an inverse association between circulating T and CORT levels is frequently reported in the literature under stress conditions where cortisol levels are raised (Bambino & Hsueh, 1981; Cumming et al., 1983). However, a positive correlation is more routinely seen under basal, non-stress

conditions (e.g. Mehta & Joseph, 2010; Sherman et al., 2016). Consequently, a Pearson correlation coefficient between bioavailable T concentration and LOG AVG CORT was computed. The correlation coefficient was $r = .34$ ($p < .001$), consistent with previous literature.

## 3.3 Measurement of Mood

To quantify mood, including depressive affect, two sets of scores were computed. For the first set, conventional scores on each of the 6 factor analytically derived subscales of the POMS were calculated according to the standard formulas provided in the test manual (McNair et al., 2003). For the second set, a principal components analysis (PCA) was applied to the 65 individual items of the POMS. This allowed empirically-derived, weighted mood scores based on the empirical dimensions underlying the POMS to be calculated. Because the individual items of the POMS are weighted according to their importance, the second set of scores served as the primary outcome variable of interest for subsequent regression analyses.

### 3.3.1 *Profile of Mood States (POMS)*

Based on the standard formulas provided in the test manual (McNair et al., 2003), each participant's individual scores on the 6 POMS subscales (Depression-Dejection (D), Tension-Anxiety (T), Anger-Hostility (A), Fatigue-Inertia (F), Vigour-Activity (V), and Confusion-Bewilderment (C)) were calculated. Descriptive statistics for each subscale can be seen in Table 2. The POMS-D was found to have a mean score of 12.47 ($SD = 9.56$). This score is relatively typical for university samples, who report greater depressive affect than the general adult population. For comparison, a normative community sample of male adults was found to have a mean score of 7.5 ($SD = 9.2$) on the POMS-D (Nyenhuis et al., 1999).

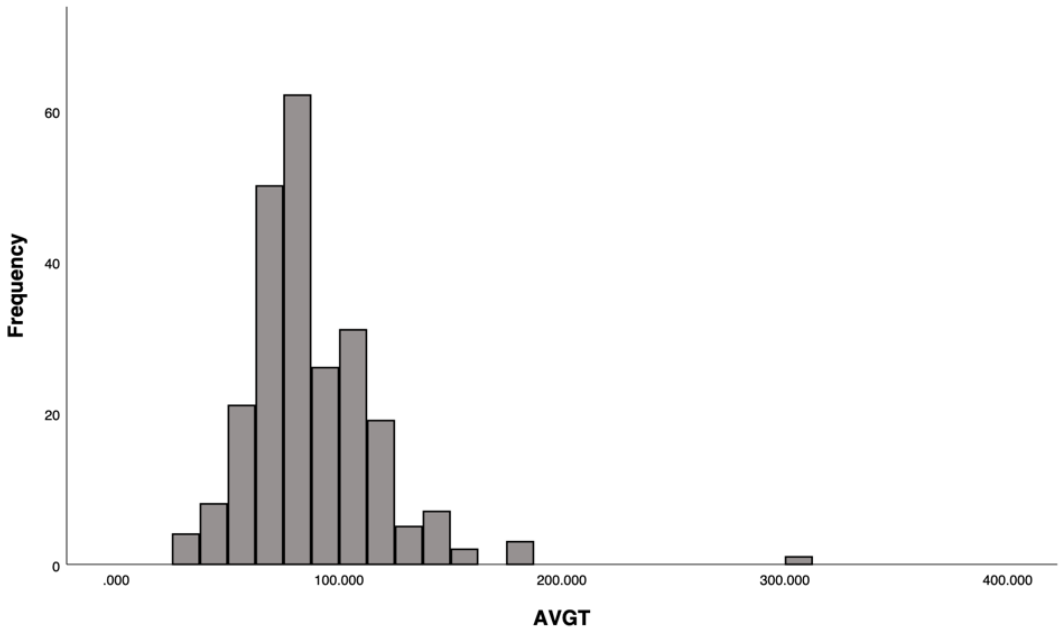


**Figure 1.** *Average Salivary Testosterone Concentration*. Average salivary T concentration ranged from 30.53 pg/mL to 302.98 pg/mL (*M* = 86.83, *SD* = 29.35). These values are consistent with past studies of bioavailable T concentration in healthy young men.

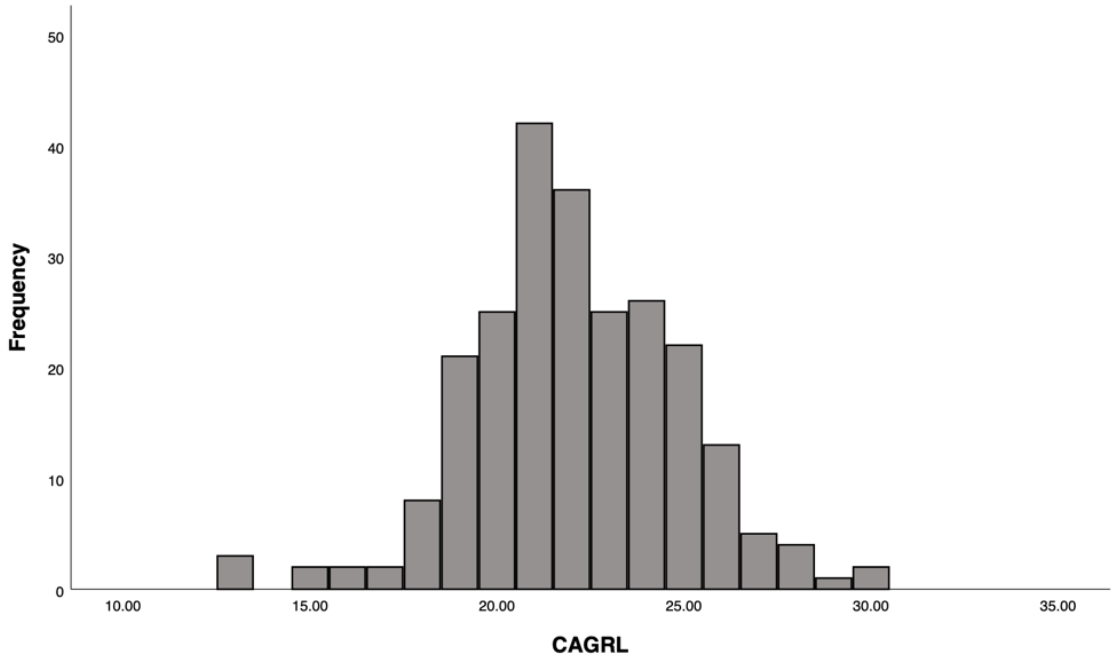


**Figure 2.** *Androgen Receptor CAG Repeat Length*. AR CAG repeat length ranged from 13 to 30 repeats ($M$ = 22.09, $SD$ = 2.89). These values are consistent with past studies of CAG length in samples of physically healthy men.

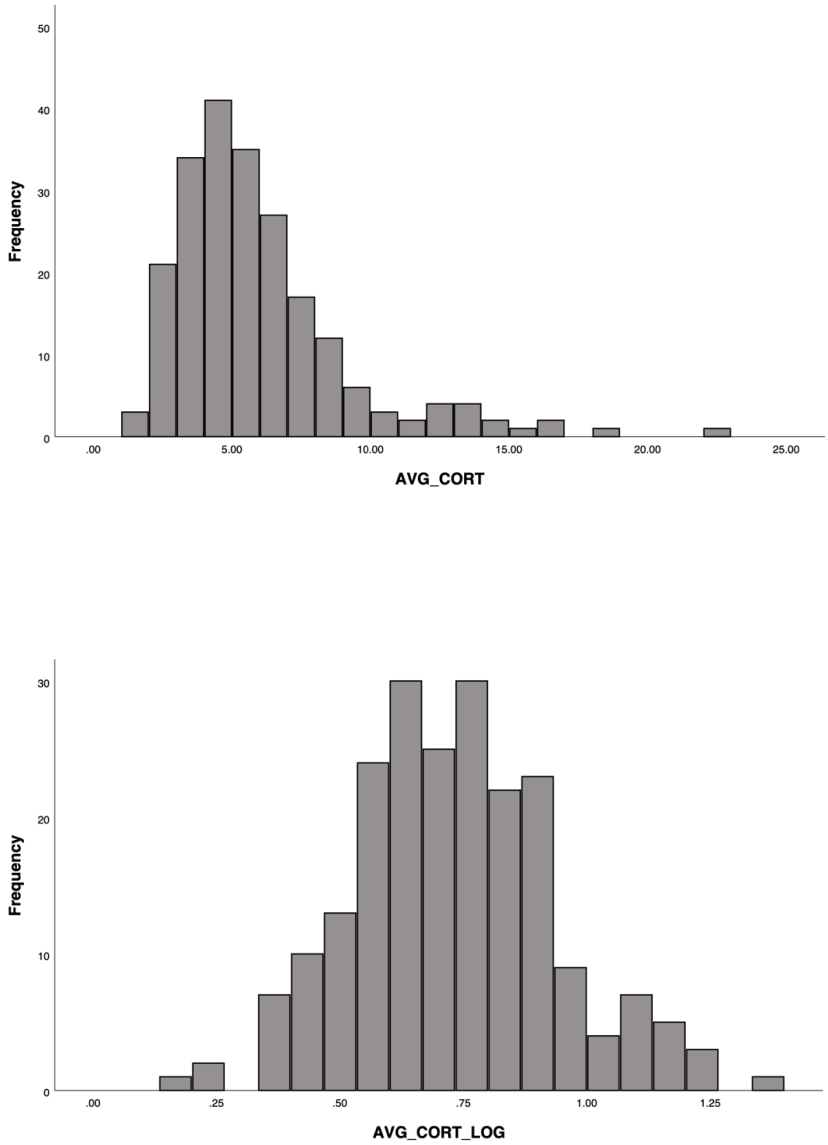


**Figure 3.** *Average Basal Cortisol Concentration*. Average salivary CORT concentration (top) ranged from 1.36 pg/mL to 22.34 pg/mL ($M$ = 6.01, $SD$ = 3.20). These values are consistent with other studies of basal cortisol levels in young men. A logarithmic transformation was applied (AVG CORT LOG) to correct the highly positively skewed distribution (bottom).

**Table 2.**

*Descriptive Statistics for the Profile of Mood States (POMS).*

| POMS Subscale | *M* | *SD* |
|---|---|---|
| Depression-Dejection (D) | 12.47 | 9.56 |
| Anger-Hostility (A) | 12.02 | 8.29 |
| Tension-Anxiety (T) | 10.64 | 6.00 |
| Fatigue-Inertia (F) | 9.50 | 5.16 |
| Vigour-Activity (V) | 18.89 | 5.12 |
| Confusion-Bewilderment (C) | 9.65 | 4.42 |

### 3.3.2 *Principal Components Analysis (PCA)*

To create a weighted, empirically-derived measure of mood, responses on the 65 individual items of the POMS were entered into a principal components analysis (PCA). A PCA has commonly been used to study the factor structure of the POMS (Norcross, Guadagnoli, & Prochaska, 1984; Bourgeois, LeUnes, & Meyers, 2010). The extracted dimensions were then subjected to oblimin (oblique) rotation. Oblique rotation was used because the correlations between scores on the 6 theoretical POMS subscales have consistently been found to range from -.12 to .70 in male undergraduate students in past research (e.g., McNair et al., 2003). The number of dimensions identified in past work is reliably about 6 dimensions, but varies from 4 (Reddon et al., 1985), 5 (Lorr, McNair, & Fisher, 1982; Bourgeois, LeUnes, & Meyers, 2010), 6 (McNair & Lorr, 1964), 7 (Norcross, Guadagnoli, & Prochaska, 1984), or even 9 (Boyle, 1987) factor solutions. Consequently, while the visual scree test (Cattell, 1966) was utilized, we evaluated several different component solutions with different numbers of dimensions specified. PCA with oblique rotation and a 7-component structure provided the best solution for the data. While the test developers and other researchers have identified a 6-factor solution for the POMS (McNair et al., 1971), 7-component solutions are not uncommon in analyses of the POMS (Norcross, Guadagnoli, & Prochaska, 1984).

Based on the pattern of items with coefficients of 0.30 or higher for each component, 5 of the 7 components extracted reflected the theoretical subscale structure of the POMS: Depression-Dejection (D), Fatigue-Inertia (F), Anger-Hostility (A), Tension-Anxiety (T), and Vigour-Activity (V) (McNair et al., 2003). Component coefficients that were interpreted for a given component ranged from ± .305 to ± .766. Every POMS item with its respective component coefficient can be seen in Table 3. Notably, the Confusion-Bewilderment (C) component failed to emerge as an independent dimension in our dataset. However, failure to extract a Confusion component is fairly commonly reported in the POMS literature (McNair & Lorr, 1964; Norcross, Guadagnoli, & Prochaska, 1984; Bourgeois, LeUnes, & Meyers, 2010). The remaining 2 out of the 7 extracted components appeared to reflect a splintering of a positive component, which has also been reported previously in at least one study, performed by the original developers of the POMS (Lorr, McNair, & Weinstein, 1963). These two components

accounted for little overall variance. Consequently, these components were labelled Mixed #1 and Mixed #2 and were not analyzed further (Table 3). The eigenvalues for each of the 7 components are shown at the bottom of Table 3.

The component that encompassed the Depression-Dejection (POMS-D) subscale had the largest eigenvalue and accounted for the greatest proportion of variance. As shown in Table 4, while all 15 items from the standard POMS-D weighted on the Depression component, a small number of items from the Anger-Hostility (4), Fatigue-Inertia (2), Tension-Anxiety (2), and Confusion-Bewilderment (4) subscales of the POMS also loaded on this component (see also Norcross Guadagnoli, & Prochaska, 1984; Reddon et al., 1985). Such complexity likely reflects the diverse array of affective symptoms that may be associated with depression (NIH, 2018). In recognition of the fact that the POMS-D component was slightly broader in scope, it was renamed "Negative Affect" for purposes of the present study. Given that the Negative Affect component was empirically extracted, participants' scores on this component served as the primary outcome variable of theoretical interest in subsequent regression analyses.

## 3.4 T Activity and Depressive Affect

To get a preliminary overview of the data, individual scatterplots of the Negative Affect component scores and the standard POMS-D subscale scores were generated, plotted by either T concentration or by CAG repeat length. Scatterplots for T can be seen in Figure 4. The plots suggested a non-linear, quadratic relationship might possibly be present between T concentration (AVGT) and depressive affect in addition to the hypothesized linear trend. A quadratic relationship has been reported previously in the literature in at least one study (Kratzik et al., 2007). To test this possibility, subsequent regression analyses included a linear predictor for T concentration, but also a quadratic predictor for AVGT. Specifically, the squared deviation from the mean was computed as the quadratic term for AVGT (Allison, 1999). In contrast to T, the plots did not suggest any systematic relationship between CAG-RL and depressive affect.

**Table 3.**

*Results of the Principal Components Analysis of the POMS.*

| Depression-Dejection | Vigour-Activity | Fatigue-Inertia | Mixed #1 | Tension-Anxiety | Anger-Hostility | Mixed #2 |
|---|---|---|---|---|---|---|
| Helpless (.704) | Lively (.644) | Fatigued (.766) | Considerate (.759) | Panicky (-.661) | Ready to fight (-.726) | Vigorous (.677) |
| Unworthy (.703) | Friendly (.616) | Exhausted (.717) | Good natured (.652) | Anxious (-.601) | Angry (-.629) | Forgetful (.463) |
| Worthless (.694) | Energetic (.591) | Sluggish (.714) | Helpful (.552) | Nervous (-.569) | Bad-tempered (-.622) | Rebellious (.331) |
| Lonely (.675) | Trusting (.573) | Worn out (.696) | Sympathetic (.439) | Carefree (.549) | Rebellious (-.617) | Carefree (.329) |
| Blue (.658) | Cheerful (.555) | Unable to concentrate (.607) | Efficient (-.394) | Tense (-.525) | Furious (-.544) | Full of pep (.317) |
| Sad (.651) | Full of pep (.506) | Bushed (.537) | Alert (.381) | Uneasy (-.523) | Grouchy (-.482) | |
| Deceived (.649) | Active (.432) | Weary (.464) | Clear-headed (.340) | Relaxed (-.472) | Peeved (-.469) | |
| Miserable (.608) | Sympathetic (.426) | Forgetful (.442) | | On edge (-.398) | Spiteful (-.466) | |
| Discouraged (.596) | Relaxed (-.393) | Active (-.359) | | Shaky (-.392) | Annoyed (-.395) | |
| Sorry for things done (.595) | Unhappy (-.358) | Restless (.347) | | Restless (-.316) | Resentful (-.394) | |
| Hopeless (.550) | | Listless (.342) | | | Trusting (.353) | |
| Bitter (.528) | | Nervous (.323) | | | Active (-.346) | |
| Unhappy (.525) | | Bewildered (.318) | | | Guilty (-.305) | |
| Muddled (.517) | | | | | | |
| Guilty (.503) | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Gloomy (.501) | | | | | | |
| Resentful (.466) | | | | | | |
| Uncertain about things (.447) | | | | | | |
| Terrified (.414) | | | | | | |
| Confused (.412) | | | | | | |
| Bewildered (.380) | | | | | | |
| Weary (.361) | | | | | | |
| On edge (.358) | | | | | | |
| Desperate (.357) | | | | | | |
| Spiteful (.354) | | | | | | |
| Shaky (.352) | | | | | | |
| Listless (.315) | | | | | | |
| **Eigenvalues:** | | | | | | |
| **19.43** | **5.48** | **2.80** | **2.00** | **1.97** | **1.81** | **1.42** |

*Note.* PCA with oblimin rotation was applied to the 65 POMS items. Only items with ±0.30 or higher weights on one or more of the components are shown here. A 7-component structure was extracted. Five of the extracted components reflected the theoretical subscale structure of the POMS: Depression-Dejection, Fatigue-Inertia, Anger-Hostility, Tension-Anxiety, and Vigour-Activity. Component coefficients ranged from ± .300 to ± .766 [POMS Item (Coefficient)]. Eigenvalues for each component are depicted in bold font.

**Table 4.**

*Distribution of Items from the Original POMS Scale That Loaded on the Negative Affect Component in the Present Study.*

| Depression-Dejection | Fatigue-Inertia | Tension-Anxiety | Anger-Hostility | Confusion-Bewilderment |
|---|---|---|---|---|
| Helpless (.704) | Weary (.361) | On edge (.358) | Deceived (.649) | Muddled (.517) |
| Unworthy (.703) | Listless (.315) | Shaky (.352) | Bitter (.528) | Uncertain about things (.447) |
| Worthless (.694) | | | Resentful (.466) | Confused (.412) |
| Lonely (.675) | | | Spiteful (.354) | Bewildered (.380) |
| Blue (.658) | | | | |
| Sad (.651) | | | | |
| Miserable (.608) | | | | |
| Discouraged (.596) | | | | |
| Sorry for things done (.595) | | | | |
| Hopeless (.550) | | | | |
| Unhappy (.525) | | | | |
| Guilty (.503) | | | | |
| Gloomy (.501) | | | | |
| Terrified (.414) | | | | |
| Desperate (.357) | | | | |

*Note*. The Negative Affect component not only contained all 15 items from the original Depression-Dejection subscale of the POMS, but it also contained a small number of items from the standard Anger-Hostility, Fatigue-Inertia, Tension-Anxiety, and Confusion-Bewilderment subscales. Each POMS item with its respective coefficient for the Negative Affect component is listed in the table [POMS Item (Coefficient)].

## 3.5 Prediction of Depressive Affect

Multiple regression analyses were conducted using the Negative Affect component scores and, to demonstrate generalizability, the standard POMS-D subscale scores as criterion variables, and AVGT, CAG-RL, and control variables as predictors to determine whether T activity contributed to the variance in depressive affect. Although regression analyses were conducted for the sample as a whole (N = 216), a median split was also performed (median = 81.79 pg/mL) and separate, identical regression analyses were conducted for the 'Low T' group (AVGT < 81.79 pg/mL; $n$ = 109), and the 'High T' group (AVGT > 81.79 pg/mL; $n$ = 107). To aid interpretation, only standardized regression coefficients ($\beta$, beta weights) were analyzed in the current thesis. The squared semi-partial correlation ($part^2$) was examined to determine the unique proportion of variance in the criterion variable accounted for by a given predictor.

As shown in Table 5, in the sample as a whole (N = 216), AVGT-linear ($\beta$ = .004, $p$ = .941), AVGT-quadratic ($\beta$ = .011, $p$ = .838), and CAG-RL ($\beta$ = .005, $p$ = .908) all failed to emerge as significant predictors of the Negative Affect component, although the overall model was significant ($F(6, 213) = 40.82$; $R^2 = .542$; $p < .001$). The only variable which emerged as a significant predictor of Negative Affect was AVGT*DEP_CUTOFF ($\beta$ = .735, $p$ = < .001), the interaction term between AVGT and whether participants met the POMS-D cut-point for depressive symptoms. This indicated that the slope of AVGT differed depending on whether participants met the cut-point for mild-to-moderate depressive symptoms or instead fell within the non-depressed range (UCLA, 2021). Nearly identical results were obtained if the standard POMS-D subscale score, calculated using the formula in the test manual, was regressed on the predictors in the sample as a whole ($F(6, 213) = 49.09$; $R^2 = .587$; $p < .001$) (Table 5).

Similar to the sample as a whole, in the High T subgroup (AVGT > 81.79 pg/mL) considered on its own ($n$ = 107), AVGT-linear ($\beta$ = .160, $p$ = .266), AVGT-quadratic ($\beta$ = -.139, $p$ = .335), and CAG-RL ($\beta$ = -.076, $p$ = .231) also all failed to emerge as significant predictors of the Negative Affect component ($F(6, 104) = 25.93$; $R^2 = .614$; $p < .001$) (Table 5). Again, the only variable that emerged as a significant predictor in the High T subgroup was

AVGT*DEP_CUTOFF ($\beta$ = .778, $p$ < .001). As shown in Table 5, very similar results were obtained if standard POMS-D subscale scores were regressed on the predictors in the High T group on its own ($F$(6, 104) = 29.73; $R^2$ = .645; $p$ < .001).

As shown in Table 6, in contrast to the sample as a whole and the High T subgroup, in the Low T subgroup (AVGT < 81.79 pg/mL) considered on its own ($n$ = 109), AVGT-linear ($\beta$ = -.561, $p$ = .036), AVGT-quadratic ($\beta$ = -.523, $p$ = .050), and CAG-RL ($\beta$ = .140, $p$ = .050) all emerged as statistically significant predictors of the Negative Affect component ($F$(6, 108) = 16.80; $R^2$ = .497; $p$ < .001). The beta weight for AVGT-linear was negative and of large magnitude according to current conventions. Conversely, the beta weight for CAG-RL was positive and of modest magnitude according to current conventions (Allen, 1999). Therefore, among males with low T availability, lower T concentrations and longer AR CAG-RLs were associated with greater Negative Affect. Based on their $part^2$, AVGT-linear, AVGT-quadratic, and CAG-RL together accounted for 6.11% of the variance in Negative Affect.

In addition to the T activity variables, AVGT*DEP_CUTOFF ($\beta$ = .711, $p$ = < .001) also emerged as a significant predictor of Negative Affect in the Low T subgroup. Together, AVGT*DEP_CUTOFF and the T activity predictor variables accounted for 54.42% of the variance in Negative Affect. Similar values were obtained if, instead of the Negative Affect component, the standard POMS-D subscale score was regressed on the predictors in the Low T subgroup ($F$(6, 108) = 19.68; $R^2$ = .536; $p$ < .001) (Table 6). However, with the POMS-D as a criterion, beta weights for the T activity predictor variables were smaller in magnitude and their $p$ values shifted marginally to become non-significant.

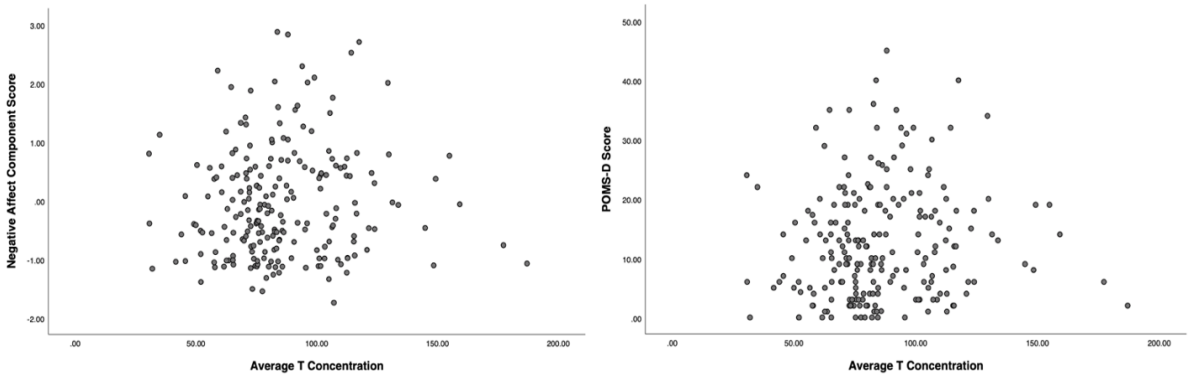


**Figure 4.** *The Relationship Between T Concentration and Depressive Affect.* Individual scatterplots of the Negative Affect component scores (left) or standard POMS-D subscale scores (right) plotted by average T concentration. The scatterplots suggested the possibility of a non-linear, quadratic relationship between AVGT and depressive affect in addition to a linear trend.

**Table 5.**

*Prediction of Depressive Affect in the Entire Sample and in the High T Subgroup.*

**Entire Sample:**

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| Negative Affect Component | .736 (*p* < .001) | .542 | AVGT (Linear) | .004 | .941 |
| | | | AVGT (Quadratic) | .011 | .838 |
| | | | CAGRL | .005 | .908 |
| | | | AVGT*DEP_CUTOFF | .735 | < .001 |
| | | | DATE | -.016 | .733 |
| | | | AGE | -.012 | .805 |

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| POMS-D | .766 (*p* < .001) | .587 | AVGT (Linear) | .029 | .560 |
| | | | AVGT (Quadratic) | .008 | .879 |
| | | | CAGRL | .02 | .659 |
| | | | AVGT*DEP_CUTOFF | .761 | < .001 |
| | | | DATE | -.013 | .779 |
| | | | AGE | -.034 | .453 |

**High T (AVGT > 81.79 pg/mL) Subgroup:**

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| Negative Affect Component | .783 (*p* < .001) | .614 | AVGT (Linear) | .160 | .266 |
| | | | AVGT (Quadratic) | -.139 | .335 |
| | | | CAGRL | -.076 | .231 |
| | | | AVGT*DEP_CUTOFF | .778 | < .001 |
| | | | DATE | .027 | .684 |
| | | | AGE | -.087 | .172 |

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| POMS-D | .803 (*p* < .001) | .645 | AVGT (Linear) | .121 | .382 |
| | | | AVGT (Quadratic) | -.095 | .490 |
| | | | CAGRL | -.032 | .594 |
| | | | AVGT*DEP_CUTOFF | .800 | < .001 |
| | | | DATE | .019 | .758 |
| | | | AGE | -.090 | .138 |

*Note.* In the sample as a whole, AVGT-linear, AVGT-quadratic and CAGRL all failed to emerge as significant predictors of the Negative Affect component. In the High T subgroup (AVGT > Median) considered on its own, the AVGT-linear, AVGT-quadratic, and CAGRL terms also failed to significantly predict the Negative Affect component. Similar values were obtained if standard POMS-D subscale scores were used as a criterion variable.

**Table 6.**

*Prediction of the Negative Affect Component in the Low T Subgroup.*

**Low T (AVGT < 81.79 pg/mL) Subgroup:**

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| Negative Affect Component | .705 (*p* < .001) | .497 | AVGT (Linear) | -.561 | .036 |
| | | | AVGT (Quadratic) | -.523 | .050 |
| | | | CAGRL | .140 | .050 |
| | | | AVGT*DEP_CUTOFF | .711 | < .001 |
| | | | DATE | -.113 | .121 |
| | | | AGE | .078 | .274 |

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| POMS-D | .732 (*p* < .001) | .536 | AVGT (Linear) | -.470 | .066 |
| | | | AVGT (Quadratic) | -.444 | .083 |
| | | | CAGRL | .112 | .103 |
| | | | AVGT*DEP_CUTOFF | .747 | < .001 |
| | | | DATE | -.097 | .167 |
| | | | AGE | -.029 | .671 |

*Note.* In the Low T subgroup (AVGT < Median) considered on its own, AVGT-linear, AVGT- quadratic, and CAGRL all emerged as significant predictors of the Negative Affect component. Similar values but nonsignificant beta weights were obtained if standard POMS Depression-Dejection (POMS-D) subscale scores were regressed on the T activity predictors in the low T subgroup.

## 3.6 Prediction of Other Mood Components

To determine whether the T activity predictor variables contributed to the variance in any of the other mood components revealed by the principal components analysis (PCA), the PCA-extracted Vigour-Activity, Fatigue-Inertia, Tension-Anxiety, and Anger-Hostility components were each regressed onto the same set of predictors described above. The purpose was to determine whether any predictive relationships seen for the T activity variables were selective to Negative Affect or might also be observed for other negative dimensions of mood. Regressions for each of the non-Negative Affect mood components were only conducted for the Low T subgroup (AVGT < 81.79 pg/mL).

As shown in Table 7, AVGT-linear, AVGT-quadratic, and CAG-RL all failed to emerge as statistically significant predictors of the PCA-extracted Vigour-Activity, Fatigue-Inertia, Tension-Anxiety, or Anger-Hostility mood components. Conversely, the AVGT*DEP_CUTOFF term was a statistically significant predictor for 3 of the 4 mood components. Specifically, AVGT*DEP_CUTOFF was a positive predictor for the Fatigue-Inertia component ($\beta$ = .302, $p$ = .002), but a negative predictor for the Vigour-Activity ($\beta$ = -.207, $p$ = .036) and Anger-Hostility components ($\beta$ = -.405, $p$ < .001). Consequently, the T activity predictor variables failed to predict any other empirically derived aspect of mood besides Negative Affect in the Low T subgroup.

## 3.7 Basal Cortisol and Depressive Affect

To investigate whether changes in cortisol associated with depression might underlie the observed relationship between the T variables and depressive affect, we first performed a one-way ANOVA to test whether mean cortisol levels were higher in individuals with elevated depressive symptoms on the POMS. Using DEP_CUTOFF to classify each participant, we found that mean logged cortisol concentration was 0.75 (*SD* = 0.23) for participants that met the POMS-D cut-point for mild-to-moderate depression and 0.72 (*SD* = 0.20) for non-depressed participants. Consequently, a one-way ANOVA failed to reveal any significant difference in mean logged cortisol concentration between participants that met the

**Table 7.**

*Prediction of the Other PCA-Derived Mood Components in the Low T Subgroup.*

**Low T (AVGT < 81.79 pg/mL) Subgroup:**

| Criterion | *R* | $R^2$ | Predictor | *B* | *p* |
|---|---|---|---|---|---|
| Vigour-Activity Component | .271 (*p* = .241) | .07 | AVGT (Linear) | .219 | .542 |
| | | | AVGT (Quadratic) | .262 | .466 |
| | | | CAGRL | .125 | .196 |
| | | | AVGT*DEP_CUTOFF | -.207 | .036 |
| | | | DATE | .084 | .397 |
| | | | AGE | -.062 | .520 |

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| Fatigue-Inertia Component | .399 (*p* = .006) | .159 | AVGT (Linear) | -.019 | .955 |
| | | | AVGT (Quadratic) | .016 | .963 |
| | | | CAGRL | .127 | .167 |
| | | | AVGT*DEP_CUTOFF | .302 | .002 |
| | | | DATE | .203 | .032 |
| | | | AGE | -.107 | .245 |

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| Tension-Anxiety Component | .225 (*p* = .495) | .051 | AVGT (Linear) | .095 | .793 |
| | | | AVGT (Quadratic) | .050 | .890 |
| | | | CAGRL | -.061 | .532 |
| | | | AVGT*DEP_CUTOFF | -.177 | .076 |
| | | | DATE | -.065 | .516 |
| | | | AGE | -.101 | .301 |

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| Anger-Hostility Component | .423 (*p* = .002) | .179 | AVGT (Linear) | -.029 | .932 |
| | | | AVGT (Quadratic) | -.087 | .796 |
| | | | CAGRL | .007 | .936 |
| | | | AVGT*DEP_CUTOFF | -.405 | < .001 |
| | | | DATE | .014 | .881 |
| | | | AGE | .126 | .167 |

*Note.* In the Low T subgroup (AVGT < Median) considered on its own, the linear T concentration (AVGT-linear), quadratic T concentration (AVGT-quadratic), and CAG repeat length (CAGRL) all failed to emerge as significant predictors of any of the non-Negative Affect components derived from the PCA.

POMS-D cut-point for mild-to-moderate depression ($n$ = 40) and non-depressed participants ($n$ = 176) ($F$(1, 214) = .56, $p$ = .453).

As a preliminary inspection of the data, scatterplots of the Negative Affect component score and the standard POMS-D subscale score were generated, showing how these scores varied as a function of each individual's basal cortisol concentration (AVG CORT LOG). As shown in Figure 5, inspection of the plots did not suggest any systematic relationship between AVG CORT LOG and depressive affect.

Multiple regression analyses were conducted using the Negative Affect component scores as the criterion variable. AVG CORT LOG and control variables were entered as predictors to determine whether basal CORT concentrations contributed to the variance in depressive affect. The analyses were performed for the sample as a whole (N = 216), the Low T subgroup ($n$ = 109), and the High T subgroup ($n$ = 107). As noted earlier, the T activity variables had emerged as significant predictors only for the Low T subgroup.

As shown in Table 8, in the sample as a whole (N = 216), AVG CORT LOG ($\beta$ = .034, $p$ = .641) failed to emerge as a significant predictor of Negative Affect ($F$(4, 212) = 0.57; $R^2$ = .011; $p$ = .684). In the Low T subgroup, AVG CORT LOG ($\beta$ = -.029, $p$ = .782) also failed to emerge as a significant predictor of Negative Affect ($F$(4, 107) = 0.158; $R^2$ = .006; $p$ = .959). Finally, in the High T subgroup, AVG CORT LOG ($\beta$ = .018, $p$ = .861) failed to emerge as a significant predictor of Negative Affect ($F$(4, 104) = 1.48; $R^2$ = .055; $p$ = .224). Consequently, in the current study basal CORT concentrations failed to predict depressive affect.

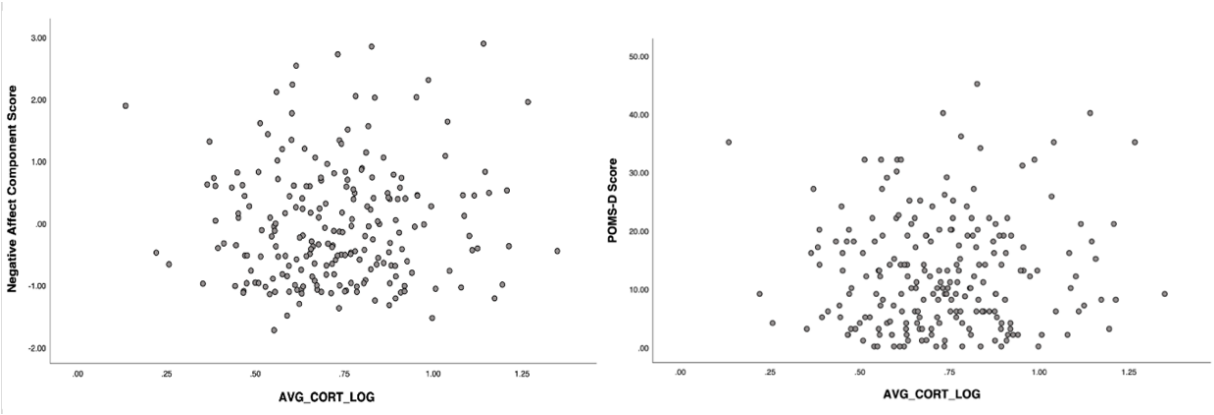


**Figure 5.** *The Relationship Between Cortisol and Depressive Affect.* Shown here are individual scatterplots of the logarithmic cortisol concentration (AVG CORT LOG) plotted as a function of the Negative Affect component score (left) and POMS-D score (right). The plots did not suggest any relationship between AVG CORT LOG and depressive affect.

**Table 8.**

*Regression of Negative Affect on Basal Cortisol Concentration.*

**Entire Sample:**

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| Negative Affect Component | .104 (*p* = .684) | .011 | AVG CORT LOG | .034 | .641 |
| | | | DATE | -.082 | .235 |
| | | | AGE | -.037 | .606 |
| | | | TIMEDIFF | .046 | .537 |

**High T (AVGT > 81.79 pg/mL) Subgroup:**

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| Negative Affect Component | .234 (*p* = .224) | .055 | AVG CORT LOG | .018 | .861 |
| | | | DATE | -.148 | .131 |
| | | | AGE | -.142 | .166 |
| | | | TIMEDIFF | .144 | .170 |

**Low T (AVGT < 81.79 pg/mL) Subgroup:**

| Criterion | *R* | $R^2$ | Predictor | *β* | *p* |
|---|---|---|---|---|---|
| Negative Affect Component | .078 (*p* = .959) | .006 | AVG CORT LOG | -.029 | .782 |
| | | | DATE | -.021 | .835 |
| | | | AGE | .069 | .495 |
| | | | TIMEDIFF | -.043 | .676 |

*Note.* For the sample as a whole, the Low T subgroup, and the High T subgroup, Negative Affect component score was regressed on the log-transformed basal cortisol concentration (AVG CORT LOG) and relevant control variables. AVG CORT LOG failed to emerge as a significant predictor of Negative Affect across any of the three groups.

# Chapter 4

# 4 Discussion

The current thesis aimed to explore the relationship between bioavailable T levels, AR CAG-RL, and depressive affect in young men, and to determine whether alterations in cortisol availability related to depression might underlie any relationships observed. Specifically, it was predicted that: (1) Greater depressive affect would be associated with lower T activity and/or longer AR CAG-RL, (2) Longer AR CAG-RLs would be associated with greater depressive symptoms, and (3) Depressive affect would be associated with higher cortisol. Our results showed partial support for these hypotheses. A relationship between bioavailable T levels, AR CAG-RL, and depressive affect was found, but only in men with low circulating T concentrations, not in men with average or high T concentrations. In contrast to our a priori hypothesis, depressive affect was not associated with higher mean basal cortisol levels and there was no evidence that cortisol mediated the association between T levels and depressive affect.

## 4.1 T Activity and Depressive Affect

In the subgroup of participants who had "low" T concentrations (AVGT < 81.79 pg/mL, the median T level in our sample of young men), the T activity predictor variables, bioavailable T concentration and AR CAG-RL, both emerged as significant predictors of Negative Affect, an empirically derived, weighted depressive affect component extracted from the POMS. Specifically, lower bioavailable T concentrations and longer CAG-RL, which is indicative of lower AR transactivational activity (Hsiao et al., 1999) and lower AR expression (Choong et al., 1996), predicted higher Negative Affect component scores in the subgroup of participants with low circulating T concentrations. Therefore, among the low T subgroup, low T activity was associated with a greater intensity of depressive affect. However, a relationship between circulating bioavailable T levels, AR CAG-RL, and depressive scores was not detected in participants who had average or higher T concentrations. Consequently, our first a priori hypothesis was only partially supported. Accordingly, we also found partial support for our second a priori hypothesis, as longer AR CAG-RL, which is indicative of a less efficacious

AR, predicted greater Negative Affect in the men with low circulating T concentrations, but not in those with average or high T concentrations. Such findings suggest that longer AR CAG-RL may not be associated with greater depressive symptoms in all men, rather only in those who also had lower bioavailable T concentrations.

The mechanism by which T may contribute to the manifestation of depressive affect is poorly understood. As mentioned in Section 1.3.2, testosterone has been shown in laboratory animals to interact with monoamine neurotransmitter systems, including the serotonergic (Rupprecht & Holsboer, 1999), dopaminergic (Weltzien et al., 2006), and noradrenergic (Hernandez-Rauda & Aldegunde, 2002) systems. Interactions between T and the serotonergic system are especially interesting because serotonin (5-HT) is well-known to contribute to mood and plays an important role in the neurobiology of depression (Malhi & Mann, 2018). For example, previous research has found T to antagonize the $5\text{-HT}_3$ receptor (Wetzel et al., 1998), a serotonin-gated ion channel which contributes to the pathophysiology of depression and has recently been recognized as an attractive target for novel antidepressants (Rajkumar & Mahesh, 2010). Therefore, it is possible that T may contribute to mood regulation and the manifestation of depressive affect via its interactions with the serotonergic system (possibly combined with modulation of other neurotransmitter or neuroendocrine systems). However, further research is needed to investigate the precise mechanism(s) through which T might contribute to mood. Furthermore, the present finding that T predicted depressive affect only in men with low circulating T concentrations is not completely unexpected. Specifically, in our sample of physically healthy young men between ages 18 and 35, circulating T levels are at their highest point during the male lifespan. Such high T levels may function to exert a protective effect on mood. Consequently, detection of an association between circulating T levels, AR CAG-RL, and depressive affect may be seen only in those with low bioavailable T levels, as there is less circulating T to afford a protective effect.

The sample in the present study was composed primarily of men that had only normal range variation in their mood scores. As mentioned above, the relatively high levels of circulating T observed in most participants in our sample may have prevented a relationship between T activity and depressive affect from being detected in our sample as a whole. However, very

little research investigating the relationship between T and mood among males who have normal ranges in mood exists. Most literature regarding the relationship between T and mood is dedicated towards (1) investigating the relationship between T and depression specifically, and (2) investigating this relationship in hypogonadal men with low circulating levels of T or in depressed men. Consequently, interpretation of our null findings for participants with average and high circulating T concentrations is limited by the lack of available literature. Therefore, further research is needed to explore the relationship between T activity and mood in young males who have normal ranges in mood.

While current literature is limited, our findings are in agreement with several observational studies that have previously found evidence of a relationship between T concentration, AR CAG-RL, and negative mood (Amiaz & Seidman, 2008). For example, our current findings agree with and extend a previous study conducted in our lab which found that lower salivary T levels ($\beta$ = -.150) and longer AR CAG-RL ($\beta$ = .480) predicted greater depressive affect in young men who reported moderate to severe depressive symptoms on two standardized measures of depression, the CES-D and PHQ-9 (Sankar & Hampson, 2012). However, just as in the present study, no association was seen between T activity and depressive affect across their sample as a whole, when large numbers of nondepressed men were included. Furthermore, while Sankar and Hampson (2012) also found evidence of a relationship between T activity and depression-related sleep disturbances in their sample, they did not find any relationship between T activity and either Concentration, Social/Evaluative, or Appetitive symptoms of depression. Similarly, the current study reported a selective relationship between T activity and Negative Affect only, and not any other aspect of mood that is commonly reported as disturbed in men with depression, including anger and anxiety (NIH, 2017). The latter were extracted from a principal components analysis of the POMS.

While a relationship between T activity and our Fatigue-Inertia component was not detected, the current study might have better replicated Sankar and Hampson's sleep finding if a sleep-centric measure, such as the *Pittsburgh Sleep Quality Index* (PSQI; Buysse et al., 1989) or polysomnography, had been utilized in conjunction with the POMS during data collection. This conjunctive approach should be employed in future studies to help better understand the

contribution of CAG-RL to depression-related sleep disturbances. However, at the moment, our current findings suggest a relationship between T and feelings of depressive affect specifically.

Although our finding of a relationship between CAG-RL and depressive affect is in agreement with Sankar and Hampson (2012), the beta weight for CAG-RL ($\beta$ = .140) in the current study was much smaller than the beta weight for CAG-RL reported in Sankar and Hampson's study ($\beta$ = .480). This discrepancy may relate to our use of the POMS to assess participants mood, as Sankar and Hampson utilized two standardized measures of depression: the CES-D and PHQ-9. Consequently, differences in the depressive affect components that were extracted following principal components analyses across both studies may have contributed to the different magnitudes in the beta weights for CAG-RL.

Our finding of an association between bioavailable T and depressive affect in young men with low circulating T concentrations agrees with several observational studies of hypo-gonadal men, a natural population of men with low T levels, that have reported an inverse relationship between circulating T levels and depressive symptom severity (Barrett-Connor et al., 1999; Kratzik et al., 2007; Morsink et al., 2007). The present study's finding that the relationship between T activity and depressive affect might depend on circulating T levels also partially agrees with that of Booth, Johnson, and Granger (1999), who found that in men with below average T levels, the relationship between T and depressive affect was negative; but in those with above average levels of T, the relation between T and depressive affect was positive. In response to this conditional relationship, Booth et al. hypothesized an inverse relationship between T and depressive affect might exist in general, but proposed that at very high levels, T might be associated with behaviours that serve as risk factors for developing depressive affect, such as antisocial and risk behaviours (Booth et al., 1999). Although an inverse relationship between T activity and depressive affect was detected in the current sample in men with low circulating T levels, it is possible that our sample did not contain enough men with 'high' circulating T to detect a significant positive relationship between T activity and depressive affect.

Although the current study found a relationship between T activity and depressive affect in young men with low T concentrations, the correlational nature of the study design prevents any causality from being extrapolated. Nevertheless, as mentioned in Section 1.4.2, several studies involving the experimental manipulation of T, including exogenous T administration and androgen-deprivation therapy, have produced evidence that supports a causal role for T in mood regulation (Rabkin et al., 2000; Schmidt et al., 2004; Wang et al., 2004; Daniell et al., 2006; DiBlasio et al., 2008; Snyder et al., 2016). The existence of a relationship with CAG-RL too suggests a pre-existing risk factor. However, many experimental studies of T have methodological problems, such as small sample sizes and/or no control group, and several other experimental studies have found no evidence of a contribution of T to mood regulation (Tricker et al., 1996; Seidman et al., 2005). Consequently, additional experimental and quasi-experimental work is needed to investigate the direction of causality in the relationship between T and depressive affect, including whether low circulating T levels lead to increased depressive affect or whether depressive affect leads to a reduction in circulating T concentrations.

## 4.2 Cortisol and Depressive Affect

Basal CORT levels did not predict Negative Affect scores across our sample as a whole or in either of our T subgroups. Furthermore, basal CORT levels were not found to be significantly different between participants who met the established POMS-D cut-point for the presence of clinical depression and non-depressed participants. These findings stand in contrast to our a priori hypothesis. Previous research has reported elevated levels of basal cortisol in many but not all individuals with major depression (Rubin et al., 1987; Vreeburg et al., 2009). It is likely that the majority of individuals in our sample did not have depression of a sufficient severity for dysregulation of the HPA axis to be seen. In our sample, basal CORT also did not mediate the observed association between T levels and depressive affect as shown by the absence of any evident relationship between cortisol levels and scores on the Negative Affect component. Researchers have hypothesized that disruptions in the hypothalamic-pituitary-adrenal (HPA) axis, from which CORT is ultimately released into circulation, plays a role in the pathogenesis of depression (Holsboer & Barden, 1996). Additionally, high levels of T in

the supra-physiological range have been found to suppress the activity of the HPA axis and the release of CORT during the stress response (Viau & Meaney, 1996; Williamson & Viau, 2008), whereas prolonged high levels of CORT due to exposure to chronic stress are also associated with suppression in T production (Cumming et al., 1983; Kheirabad et al., 2016). In previous studies of circulating T and depressive symptoms, circulating levels of CORT are not typically analyzed concurrently with T. The present study is among the first to do so. However, when circulating levels of basal CORT were analyzed in the present study, we did not find evidence of any relationship between basal CORT and severity of depressive affect, nor evidence that CORT mediated the observed associations between T levels and depressive affect.

The absence of any significant relationship between cortisol levels and depressive affect in the current study may possibly relate to our measurement of basal cortisol only, rather than stress-induced cortisol levels. Specifically, in addition to elevated levels of basal cortisol, many but not all individuals with depression display abnormally high secretion of cortisol in response to psychological (Burke et al., 2005) and physical stressors (Holsboer, 2000). It is possible that if stress-induced CORT levels were measured, we might find a relationship between CORT and depressive affect. Our inability to detect any significant association between Negative Affect and CORT may also be related to the low number of individuals with severe depression in the current sample, as our dataset consisted predominantly of males with normal-range depressive scores who did not have evidence of HPA axis dysregulation. Accordingly, it is possible that if the current study had been able to include more participants with moderate-to-severe depression, we might have observed a stronger relationship between basal CORT levels and depressive affect scores.

## 4.3 Strengths, Limitations, Future Directions

Although the association we observed for CAG-RL was weak, we obtained partial support for a relationship between T activity and depressive affect. The current study has several strengths. First, a quadratic in addition to a linear relationship between circulating T levels and depressive affect was considered. Several past studies have reported a complex, non-linear, and/or conditional relationship between circulating T levels and depressive symptom

severity (Booth et al., 1999; Kratzik et al., 2007). However, relatively few researchers have considered the existence of a non-linear relationship between T and depressive affect a priori, and even fewer studies, if any, have included non-linear T concentration predictor terms in their regression analyses. Consequently, the current study provided a more comprehensive investigation of the statistical relationship between circulating T levels and depressive affect. Secondly, the current study not only investigated depressive affect, but through its utilization of the *Profile of Mood States* (POMS; McNair et al., 2003), it also had the capacity to investigate the relationship between T activity and other aspects of mood, including anger, anxiety, fatigue, vigour, and confusion. In addition to depressive affect, such as feelings of sadness and hopelessness, men with depression have been found to display a variety of other affective symptoms, including increases in anger, irritability, and aggressiveness (Kim et al., 2015); non-verbal and trait hostility (Fava et al., 1995), and anhedonia and anxiety (NIH, 2017). Therefore, by investigating other facets of mood besides depressive affect, the current study provided a more expansive investigation of the contribution of T to mood regulation and was able to demonstrate a selective relationship between T activity and depressive affect that did not extend to other aspects of negative mood. Another strength is our examination of basal CORT levels. Recall that previous research has found elevated levels of basal CORT in many individuals with depression (Rubin et al. 1987), and an inverse relationship between elevated CORT and T levels (Cumming et al., 1983). Our study is potentially the first in the literature to analyze the concurrent contribution of basal CORT and T to depressive affect. Additionally, the use of antidepressant medications was not a confound in the current study. Previous research has found that some antidepressant medications can alter the production of T (Hansen et al., 2017; Lupu et al., 2017; Munkboel et al., 2018). Consequently, participant antidepressant use represents a confound that is present in some clinical studies on T and depression which have been published previously. Therefore, in the present sample participants were removed from the analyses if they reported use of an antidepressant drug on the health and demographics questionnaire. A final strength of the current study is that it investigated the relationship between T activity and depressive affect in physically healthy young men. Current literature regarding the relationship between T levels, AR CAG-RL, and depressive affect is limited, but especially so for *young* men. Therefore, the current study has

addressed an under-studied age cohort that customarily reports higher rates of depression and depressive affect than the general population (Nyenhuis et al., 1999; Ibrahim et al., 2013).

Although the current study is strong in several respects, multiple limitations should also be noted. For example, the current sample size was relatively limited compared to other studies in the literature, particularly when the sample was divided based on median T concentration. Sample limitations also prevented us from being able to extract a depressed subgroup from the sample as a whole who displayed scores within the range on the POMS-D characteristic of people suffering from clinical depression (POMS-D > 20; see Nyenhuis et al., 1999; Griffith et al., 2005; Patterson et al., 2006; Bay et al., 2007). Only $n = 40$ males scored above the threshold of 20 in the current sample. A larger subgroup of individuals with likely depression would have allowed us to investigate associations between T activity and depressive symptoms in this distinct subgroup, and further extend the findings of Sankar & Hampson (2012). Furthermore, our sample contained few men reporting severe depressive symptoms in which a relationship between T activity and depressive affect might be more robust and easier to detect. Future work may seek to recruit a separate subgroup of demographically matched males who have received, or are currently receiving, treatment for depression (e.g. individuals receiving antidepressants and/or psychotherapy) to help establish whether having a longer AR CAG-RL might confer an increased risk.

Another limitation of the current study is the absence of a depression-centric measure. While the POMS had the important benefit of allowing us to investigate other aspects of mood besides Negative Affect, and several previous studies have employed the POMS-D to measure depressive mood (Szaflarski et al., 2003; Szaflarski & Szaflarski, 2004), the POMS-D was not specifically created to measure depressive affect or assess its severity. Consequently, it is possible that our findings might have been slightly different, or potentially stronger, had we utilized a self-report instrument more specifically designed to measure depressive symptoms, such as the *Beck Depression Inventory-II* (BDI-II; Beck, Steer, & Brown, 1996). Future work may seek to employ multiple self-report scales simultaneously to quantify participants' mood, including scales that assess mood broadly,

such as the POMS, as well as scales explicitly designed to detect depression, such as the BDI-II.

The current study also only assessed individuals' depressive affect at a single timepoint during their lifespan and thus could not evaluate lifetime risk. Depression is episodic for most individuals, and the occurrence or timing of the first depressive episode, as well as the occurrence of further episodes, can vary significantly across the lifetime (Malhi & Mann, 2018). A longitudinal approach would allow researchers to compare changes in depressive affect over time with age-related changes in T levels, and to explore whether age-related changes in circulating T concentrations affect the observed relationship between T activity and depressive affect. Finally, further work should be conducted to investigate the precise mechanisms through which T may contribute to mood regulation, including a potential direct mechanism through binding the AR, through enzymatic conversion to another steroid hormone, or through interactions with other neuronal correlates of mood, such as central monoamines or the HPA axis.

## 4.4 Implications and Conclusions

The current thesis found that bioavailable T concentration and the CAG trinucleotide repeat polymorphism of the AR significantly predicted ratings of depressive affect derived from the POMS. These relationships were observed among men with low circulating bioavailable T concentrations, but not among men with average or higher T. The results suggest that low T activity, specifically low bioavailable T concentrations and longer CAG-RL of the AR, may increase the risk of depressive symptoms in certain groups of men. Importantly, these results were found in *young* males, an under-studied age band in which T concentrations are at their highest point during the lifespan when T might ordinarily be protective. Our data suggest that T, via its actions in the CNS, may modulate levels of depressive affect in men. Ultimately, these data may help researchers to develop a clearer picture of precisely how T contributes to depressive affect and mood regulation more generally.

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# Appendices

## Appendix A: Poster for Recruitment from Western Campus

**MALES Needed**

**for a study of**
***Testosterone, Personality, and Experiences in Close Relationships***

Eligibility:

- Heterosexual **MALE** age 18 - 35
- Open to undergraduates and staff at UWO
- Must have had **at least** one romantic or sexual partner, either currently or in the past 1 year
- Fluent in English

The experiment will last approximately **1 hour**.

You will be compensated **$15.00** for your participation.

You will be asked to:

- Fill out questionnaires about your personality and relationships with romantic or sexual partners
- Fill out questionnaires about childhood experiences with parents/caregivers
- Provide saliva to measure testosterone levels
- Provide saliva to determine the genetic makeup of your hormone receptor

For further information or to make an appointment, please e-mail:

# Appendix B: Health and Demographics Questionnaire

## Demographics Questionnaire

Please provide the information requested below. If, for any reason, you wish not to answer a particular item, you are free to leave it blank. All information provided is strictly confidential and will be used for research purposes only. Your name or other identifying information will not appear anywhere on this questionnaire.

Subject Number: _____ Date: _____

Age: _____ Testing time: _____

1. In the 30 minute period before your appointment today, did you:

| | | |
|---|---|---|
| (a) have anything to eat? | YES | NO |
| (b) have a beverage other than water? | YES | NO |
| (c) have a cigarette? | YES | NO |
| (d) brush your teeth? | YES | NO |
| (e) chew gum? | YES | NO |

2. In the past week before your appointment today, did you:

(a) have an acute illness (e.g., cold or flu)? YES NO

3. What time do you normally wake up in the morning?

On weekdays: _____
On weekends: _____

4. What time did you wake up today? _____

5. Has anything happened in your life over the past few days that made you feel unusually stressed? YES NO

6. What is your height? _____ (feet & inches) OR _____ (cm)

7. What is your weight? _____ (pounds) OR _____ (kg)

8. Do you attend/work at Western or Fanshawe? WESTERN FANSHAWE

9. Are you a smoker, non-smoker, or an occasional smoker?

SMOKER NON-SMOKER OCCASIONAL SMOKER

10. How often do you normally consume alcohol? (Circle one number from 0 to 4).

| 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Never | 1-2 times a **month** | 1-2 times a **week** | 3-4 times a week | Almost every day |

11. What is the average number of drinks you have when/if you drink? (Circle one number).

| 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| None | one to three | four to seven | eight to twelve | more than 12 |

12. How long has it been since you last consumed an alcoholic beverage (e.g., beer, wine, spirit) of any kind? _____

13. Have you ever had any accidents with either of your hands that could affect the growth of your fingers?

_____
_____

14. Are you currently taking any prescription or non-prescription medications? YES NO

If so, please list the medications you are taking.

_____
_____

15. Do you currently have any physical condition(s) that might cause your hormone levels to be unusual? (e.g., diabetes, thyroid, etc.). YES NO

16. What is your ethnicity?

| | | |
|---|---|---|
| _____ White | _____ East Asian | _____ Middle Eastern |
| _____ Black | _____ South Asian | _____ Pacific Islander |
| _____ First Nations | _____ Hispanic | _____ Other |

17. How many brothers do you have? _____ What are their ages? _____

18. How many sisters do you have? _____ What are their ages? _____

19. Up to age **16**, I was raised by (check all applicable answers and indicate ages):

| | |
|---|---|
| _____ Both biological parents living together | From ages ___ to ___ |
| _____ Both biological parents, living separately | From ages ___ to ___ |
| _____ A biological parent and a step-parent | From ages ___ to ___ |
| _____ One biological parent living alone | From ages ___ to ___ |
| _____ Another family member (not a parent) | From ages ___ to ___ |
| _____ Adoptive parents | From ages ___ to ___ |
| _____ Foster parents | From ages ___ to ___ |
| _____ Other (please specify: _____ ) | From ages ___ to ___ |

20. Is English your first language? YES NO

If no, what is your first language? _____

21. The following information should be filled out for the parents who raised you. If you grew up in a single-parent home, fill out the information only for the parent you lived with:

***MOTHER***

Mother's Usual Occupation or Job
(be very specific; e.g., Insurance adjuster): _____

Mother's Highest Education (check one):

_____ Grade 6 or less
_____ Grade 7 to 9
_____ Some high school
_____ High school graduate
_____ At least one year of college or other specialized training
_____ College or university graduate
_____ Master's degree, Ph.D., M.D., or other professional degree

Province or country where your mother went to elementary and high school: _____

***FATHER***

Father's Usual Occupation or Job:
(be very specific; e.g., Insurance adjuster): _____

Father's Highest Education (check one):

_____ Grade 6 or less
_____ Grade 7 to 9
_____ Some high school
_____ High school graduate
_____ At least one year of college or other specialized training
_____ College or university graduate
_____ Master's degree, Ph.D., M.D., or other professional degree

Province or country where your father went to elementary and high school: _____

22. What is your highest level of education (check one):

_____ Grade 6 or less
_____ Grade 7 to 9
_____ Some high school
_____ High school graduate
_____ At least one year of college or other specialized training
_____ College or university graduate
_____ Master's degree, Ph.D., M.D., or other professional degree

23. If not a student, what is your usual occupation? _____

# Curriculum Vitae

**Name:** Christopher Purkis

**Post-secondary Education and Degrees:**
The University of Western Ontario
London, Ontario, Canada
2016-2020 B.Sc., Honors Specialization in Neuroscience

**Honours and Awards:**
Oral Presentation Winner (Human Cognition & Behaviour)
Western University's Neuroscience Research Day
February 25, 2022

Canada Graduate Scholarship – Masters
Natural Sciences and Engineering Research Council (NSERC)
September 2021 – June 2022

Graduate Research Scholarship
The University of Western Ontario
September 2020 – June 2022

Queen Elizabeth II Graduate Scholarship in Science & Technology
Province of Ontario Graduate Scholarship (OGS)
June 2021

Western Gold Medal
The University of Western Ontario
June 2020

Academic Scholarship
Phi Delta Theta Canadian Scholarship Foundation
January 2020

**Related Work Experience:**
Graduate Teaching Assistant
The University of Western Ontario
September 2020 – April 2021

Writer
Kinect MD
November 2021 – April 2021

Honors Thesis Research Student
The University of Western Ontario
September 2019 – April 2020

Research Assistant
The University of Western Ontario
October 2018 – August 2019

Research Assistant
The University of Western Ontario
May 2017 – July 2017

**Publications:**

Purkis, C., & Hampson, E. (2021). Individual differences in tasks that recruit the frontal lobe. *Society for Behavioral Neuroendocrinology Abstracts*, P1.61.