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CHAPTER 1

Symptoms, Classification and Measurement of Depression

1.1 Synopsis

This chapter reviews the concept of depression, by providing background information regarding definition, classification, symptomatology and measurement. In particular, consistent with the background and aims of the present thesis, the issue of gender differences in depression, particularly symptomatology and coping styles of men, will be examined.

1.2 Definition

While there are numerous definitions of depression, ranging from: “A mental state of depressed mood characterized by feelings of sadness, despair and discouragement” to: “A state of sadness marked by inactivity and inability to concentrate” the underlying concept common to most definitions is the implication of low, sad mood.

Although the term 'depression' is used in a number of different ways - in non-clinical populations it is used to describe a normal human emotion. Feelings of low, sad mood are common emotions experienced by nearly every human being as a reaction to a number of life events or circumstances. These feelings are usually transitory, generally lasting for minutes, hours or days (Parker, 1979) and they usually respond to circumstantial changes. These feelings are thought to serve several adaptive functions: firstly, behaviour consequential to these feelings prompt the individual to focus on the particular stressor, preventing them from dispersing their energy on other activities, and secondly, these feelings communicate to surrounding others that the individual is undergoing a difficult process and that may be in need of external help.

Transient feelings of depressed mood are distinguished from pathological or clinical depression by a number of distinguishing features: the most common ones being the duration of the depressed mood (two-week period, as defined by the DSM-IV) (APA, 1994), the presence and number of other symptoms related to depression (such as appetite, sleep and energy disturbance) and the degree of impairment in daily functioning resulting from the depressive symptoms.

1.3 Diagnosis and Classification of Depression

The two most widely used diagnostic systems for depression are the DSM-IV (Diagnostic and Statistical Manual, IV, published by The American Psychiatric Association, 1994) and the International Classification of Diseases, ICD 10, endorsed by the World Health Organisation. These systems have been used by mental health professionals to improve

reliability and uniformity of diagnosis. Both of these diagnostic systems have adapted a dimensional approach to the classification of depression. The conceptual framework of this unidimensional approach encompasses a spectrum of severity of depression, ranging from mild to severe form. In addition to the presence of depressed mood and/or loss of interest or pleasure, the DSM-IV requires the presence of at least five of the following symptoms to have been present during the same 2-week period:

1. Depressed mood most of the day, nearly every day.
2. Markedly diminished interest or pleasure in activities
3. Significant weight loss when not dieting or weight gain
4. Insomnia or hypersomnia nearly every day
5. Observable psychomotor agitation or retardation nearly every day
6. Fatigue or loss of energy nearly every day
7. Feelings of worthlessness or excessive or inappropriate guilt
8. Diminished ability to think or concentrate, or indecisiveness
9. Recurrent thoughts of death, plans or suicide attempts.

In addition to diagnosing depression, the DSM-IV includes descriptive specifiers of depression, such as “with melancholic features” and “with atypical features”.

1.4 Depression subtypes

It has long been recognised that depression is a heterogeneous illness. It may take a variety of forms, varying in clinical features, severity, response to treatment and etiological factors. Mental health professionals have recognised that patients tend to

display reasonably distinct clusters of clinical symptoms, and they increasingly regard such clusters as subtypes of depression. The classification of these different depressive symptoms has been a centre of long-standing international debate in psychiatry, most probably since Kraepelin's differentiation of manic-depressive psychosis from dementia praecox in the late 19th century. A detailed examination of this debate is beyond the scope of the current thesis, thus, only the most important and pertinent issues applicable to this thesis will be outlined.

The most widely used subtyping of depression is that differentiating between melancholic (also known as psychotic and endogenous) – with its assumption of neurochemical and/or genetic etiology, and the non-endogenous, also known as reactive and neurotic – with its assumptions of environmental precipitants and/or personality deficits (Gillespie, 1929).

According to DSM-IV criteria, the principal diagnostic feature exhibited by patients with melancholic depression is a loss of pleasure in all, or almost all, activities or a lack of reactivity to usually pleasurable stimuli (APA, 1994). Additionally, further symptoms most frequently observed in patients with melancholic depression include: loss of appetite, insomnia, diurnal variation (with depression at its worst in the morning), psychomotor disturbances and decreased responsiveness to the environment. Patients with melancholic features usually have higher illness morbidity (Parker, Hadzi-Pavlovic, Brodaty et al., 1992) and severity of depression (Zimmerman, Coryell, Pfohl et al., 1986). Estimates of the prevalence of melancholic features among patients diagnosed with depression range from 16% to 53% (Parker, Roussos, Austin et al., 1998; Lafer,

Nierenberg, Rosenbaum et al., 1996) although the prevalence may be as high as 76% among inpatients (Hildebrandt, Stage, Kragh-Soerensen et al., 2003).

Furthermore, considerable body of evidence suggests that biological differences also exist between melancholic and non-melancholic depression. Hypercortisolism as a feature of melancholia has been described as perhaps the best documented finding in biological psychiatry (Carroll, 1981). Thus, depressed patients with melancholic features consistently demonstrate an activation of the hypothalamic-pituitary-adrenocortical (HPA) axis increased cortisol levels (Nemeroff, 1996) resulting in laboratory findings of dexamethasone non-suppression.

In addition, a number of the features exhibited by melancholic patients closely resemble those that occur in non-depressed populations as a response to stressful or threatening situations (Wong, Kling, Munson et al, 2000). Melancholic patients may have diminished activities of the growth hormone and reproductive axes (Gold and Chrousos, 2002).

When compared with non-melancholic depressed patients, patients with melancholia have also been shown to exhibit lower concentration of nighttime serum melatonin (Brown, Kocsis, Caroff et al, 1985), lower plasma serotonin (5-HT) concentrations (Sarrias, Artigas, Martinez et al, 1987) and an impaired *in vivo* immune response (Hickie, Hickie, Lloyd et al 1993). Another biological distinction between patients with melancholic features and those without is that melancholia is strongly predictive of response to ECT (Electroconvulsive Therapy) (Kiloh, 1962; Carney, 1965 from thesis). However, a number of controlled studies comparing real and sham ECT have been

inconsistent regarding the prognostic value of psychomotor symptoms in response to ECT (O'Leary, 1995; Sobin, 1996).

On the other hand, patients with atypical depression present with a syndrome that seems the antithesis of melancholia. They are lethargic, fatigued, hyperphagic, hypersomnic, reactive to the environment, and show diurnal variation of depression that is at its best in the morning (Posternak and Zimmerman, 2002).

Not only has the classification of depression into the above two subtypes served as a topic for a long debate, but so too has the association between these two depressive subgroups, and the ways in which they are related to each other has been the subject of much debate and controversy. There have been two major arguments: one posits that the two types are dimensionally related, arising along a continuum of severity from nonendogenous to endogenous. This view has been called the unitary view. The binary view, on the other hand, has argued that the two types of depression are categorically distinct, positing not only etiological differences but also clinical distinctions, (such as the presence of psychomotor disturbance, pathological guilt and delusions), and response to treatment distinctions (preferential response of endogenous depression to ECT and tricyclic treatment (Carney and Garside, 1965; Kiloh, Andrews, Neilson et al., 1972).

1.5 Measuring Depression

Depression rating scales have been largely developed in response to the psychopharmacological revolution in the 1950's (Demyttenaere and De Fruyt, 2002). As the development of new antidepressants increased, so did the number of depression rating

scales, designed to measure the severity of depression and a change with antidepressant treatment (Moller, 2000). As a result, today there are a large number of depression rating scales –some are used to quantify severity of depression and change of depressive symptoms and severity over time, and some are used as diagnostic tools.

The choice of the rating scales used in a particular study often seems arbitrary. Some of the most commonly used rating scales are: Hamilton Depression Rating Scale (HDRS) (Hamilton, 1967), Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979), Beck Depression Inventory (BDI) (Beck, Ward, Mendelson et al., 1961) and Zung Self-Rating Depression Scale (Zung and Durham, 1965). The majority of these scales have been developed around criteria adapted from the DSM and ICD. While some of these scales are most useful in clinical setting (e.g. HDRS), some are reserved for research purposes. In research, rating scales are commonly used for assessment of therapeutic response, such as in drug trials, whereas in clinical practice they are used to chart the changes in depressive symptoms (Williams, 2001).

1.6 Symptoms

Depression has been recognised as a multi-faceted illness which encompasses a constellation of symptoms. Not all depressed patients experience every symptom. The severity of symptoms varies among individuals and over time. Patients with depression usually display a ‘signature’ of depressive symptoms: this is commonly a term reserved for a collection of depressive symptoms which tend to recur on subsequent episodes.

One of the key symptoms of depression is the presence of low, depressed mood, where the patient feels worthless, hopeless and sad. This mood can often be distinguished (by the clinician and the patient themselves) from the normal emotion of sadness, as the depressed mood is usually described as one of agonizing pain.

Another one of the most common symptoms of depression is lack of energy which results in impairment of daily activities and creates difficulties in finishing tasks and undertaking new projects in the future. Loss of interest and pleasure in activities is another very common symptom of depression.

In addition, depression is usually accompanied by a number of somatic complaints.

Disturbance in sleep patterns is a frequent somatic symptom of depression, experienced by approximately eighty percent of patients (Kaplan and Sadock, 1988). Sleep disturbance may manifest in several kinds, such as delay in falling asleep, intermittent awakening during the night and early morning awakening. Some patients typically experience only one kind of the above sleep disturbances, whereas others may experience two or even all three. Nevertheless, sleep disturbance in depression usually causes the patient to lose on average three to four hours of sleep per night.

In addition, disturbances in sleep architecture, particularly in Rapid Eye Movement (REM) frequently been reported to accompany depression. These abnormalities usually consist of an earlier onset of this sleep stage, a greater amount of REMS in the beginning of the night and an increase in the actual rapid eye movements (REM activity and REM

density) during this sleep stage (Reynolds and Kupfer, 1987; Buysse and Kupfer, 1990). Some of these abnormalities seem to relate to the severity (Kupfer, 1984; Hubain, Van Veeren, Staner et al., 1996) as well as to the symptomatological profile of the acute depressive state (Hubain, Souery, Joonck et al., 1995; Stefos, Staner, Kerkhofs et al., 1998).

Weight loss is another common symptom of depression. This symptom is usually due to loss of appetite in the majority of patients, where they feel they have lost interest in food, and sometimes find food to be tasteless and unrewarding.

Decrease in sexual interest, problems with erections and orgasms, and overall decrease in sexual function is another frequent symptom of depression. The prevalence and severity of this symptom is exceptionally difficult to assess because many patients are unwilling or unable to readily offer information, discuss sexual dysfunction and have difficulty acknowledging decreased libido and many clinicians do not prompt for it.

1.7 Prevalence, Onset and Course

Depression is one of the most common psychiatric disorders in the primary health service (Katon, 1992). A recent epidemiological study has found that the prevalence of major depressive disorder for lifetime is 16.2% (32.6 – 35.1 million US adults) and for 12-month it is 6.6% (13.1 – 14.2 million US adults) (Kessler, Berglund, Demler, Jin, et al., 2003). Epidemiological studies from Canada have closely reflected this figure, reporting a lifetime prevalence of 12.2% in Canadian population (Patten, Wang, Williams, Currie,



et al, 2006). Likewise, a national survey on mental health and well-being published in 1998 by the Australian Bureau of Statistics found that approximately 5.1% of the country's adult population reported suffering from depression (ABS 1998).

While depression can occur at any age, the average age of onset is from early 20s to mid 30s (Weissman, Bland, Canino, 1996; Spaner, Bland, Newman, 1994). Depression is increasingly being recognised as a chronic illness due to its high episode recurrence rate. As such, high recurrence is one of the most challenging aspects for treatment of depression, with as many as 85 percent of patients experiencing a recurrence within 15 years (Mueller, Leon, Keller et al, 1999). Varying degree of residual symptoms can be present in between depressive episodes, and subsequent episodes may occur months and even years following effective treatment for previous episodes (Judd, Aksisal, Maser et al., 1998).

The predictors for recurrence of depressive episodes are difficult to specify, however, evidence suggests that factors such as: female gender, unmarried status, severity and duration of previous episodes and the number of previous episodes are risk factors for recurrence as they positively correlate with the likelihood of experiencing a recurrence. Similarly, the number of previous admissions into hospital seems to carry a risk for readmission, where patients who have been hospitalised for first episode of depression have a 50% risk of being readmitted, while patients with previous readmissions have a 50% risk of readmission within the next 3 years (Lee and Murray, 1988). The literature is inconsistent regarding the effect of age on recurrence risk (Mueller et al., 1999). Lower

age has been positively associated with greater risk in some studies of clinical samples (Gonzales, Lewinsohn, Clarke, 1985; Giles, Jarrett, Biggs et al., 1989) and a nonclinical sample (Coryell, Endicott & Keller, 1991) and negatively associated with greater risk in other clinical samples (Keller, Lavori, Lewis et al., 1983).

1.8 Depression as a health problem

Depression is one of the commonest psychiatric disorders and one of the leading causes of disability worldwide. The World Health Organisation (WHO) Collaborative Project noted that disability levels among patients with depression were greater than in patients suffering other chronic/recurrent illnesses, such as asthma, chronic back pain or diabetes (Davidson & Meltzer, 1999). Indeed, the WHO predicted that by 2020, major depression will be second on the list of as a cause of disability (following ischaemic heart diseases), ahead of such common health problems as cancer, infectious diseases, road-traffic accidents and AIDS (Murray and Lopez, 1997).



Depression not only causes mental anguish, but also impairs some of the most fundamental biological functions, such as sleep, appetite, sexual activity and metabolic and immune functions. As a consequence, depression is related to, and is seen as a risk factor for a number of chronic and critical illnesses, such as diabetes, cardiovascular disease and cancer (Patten, 1999). Depression also frequently accompanies other psychiatric disorders, such as schizophrenia, anxiety disorders, drug and alcohol dependence and personality disorders (Enns, Swenson, McIntyre et al. 2001).

As well as being a risk factor for a number of chronic illnesses, depression also increases patient's susceptibility to suicidal ideations and actual acts of suicides. Suicide rates among patients with depression are disproportionately higher than the general population, higher even than rates for other psychiatric conditions (Bostwick and Pankratz, 2000). About two thirds of patients with depression show suicidal ideation, and 10 -15 percent complete suicide. Although depression seems to be more common in women, and although women have more suicide attempts than men, the rates of completed suicide are about 4 times higher in men (NSW Dept Health, 1999). The higher rates of completed suicide in men have usually been attributed to the differences in suicide methods used by men and women: men usually tend to use more fatal and effective methods, such as a gun or incarceration.

1.9 Gender Differences in Depression

The investigation of gender differences in the prevalence of depression has received extensive attention, particularly over the past two decades (Wilhelm, Roy, Mitchell et al., 2002). Epidemiological studies in large community samples consistently demonstrate higher prevalence of depression in women than in men. Kessler, McGonagle and Zhao (1994) reported that women in the United States are about two-thirds more likely than men to be diagnosed with depression. Similarly, a national psychiatric morbidity survey in Britain showed a similar greater risk of depression for women (Meltzer, Gill, Petticrew et al., 1995). Existence of gender differences in prevalence of depression have also been reported in Australian context - recent studies have shown rates of depression of 2.4

percent in men, compared to 3.9 percent in women (Australian Bureau of Statistics, 1998; Andrews, Henderson and Hall, 2001).

1.9.1 Factors influencing reported depression

There has been considerable interest in differences in depression rates between men and women, but the explanations remain elusive. Nevertheless, a number of factors have been proposed, including: symptomatology, previous psychiatric illnesses, coping styles, expression of symptoms as well as medical practitioner expectations.

1. Symptoms: Features associated with depression in men include: somatic symptoms, the inability to cry, sense of guilt and failure, and social withdrawal (Nolen-Hoeksema, 1987). Women, on the other hand show more emotional arousability (Wilhelm et al., 2002), such that they are more prone to crying, and have low self-esteem, and report feelings of hopelessness and helplessness.
2. Previous psychiatric problems: Females are at a greater risk of developing depression and anxiety disorders at earlier ages than males. This may partially account for the higher incidence of depression in females in adulthood, as there is ample evidence which suggests that depressive episodes in childhood and adolescence predict more episodes and longer duration of depression in adult life.
3. Coping styles: Personality attributes and coping styles have also been thought to contribute to gender differences in depression. For example: Women are more likely

than men to have established social and emotional support networks, to which they turn to upon the appearance of depressive symptoms. Furthermore, women have been shown to score higher on scales of neuroticism, be more dependent and engage in self-consoling behaviour, whereas recklessness as a coping style is likely to be practiced more by men (Wilhem and Parker, 1993). Thus, men are more likely to engage in risky and antisocial behaviour, such as drug and alcohol abuse (Tyssen, Vaglum, Aasland et al., 1998), speeding and roadrage (Fong, Frost, Stansfeld et al., 2000) as a response to their depression.

4. Differing clinical manifestations of depression: women are able to verbalise their symptoms, whereas men are more likely to have difficulty in verbally expressing their feelings. Men are also more likely than women to be hypoemotional (minimising or downplaying the strength of their emotions) or alexythymic (unable to identify and describe their feelings) (Heesacker, 1999). In addition, men are also more prone to attribute their symptoms to other causes.
5. Differing recall of depression: A number of studies have demonstrated that men are more likely than women to forget to report their depressive symptoms, which leads to under-representation. By the same token, women are more likely to be more focused on their feelings and have a tendency to remember and recall more symptoms than men, thereby leading to greater likelihood of meeting criteria for a depressive disorder (Wilhelm and Parker, 1994).

6. Possible lower threshold levels for diagnosing depression in women: medical practitioners can also be influenced to diagnose depression more readily in women than in men due to socially embedded stereotypes and expectations that depression occurs more frequently in women (Potts, Burnam, Wells et al. 1991). Furthermore, men are more likely to manifest somatic symptoms of depression, such as loss of appetite, decrease in energy, muscular aches, gastrointestinal problems etc (Goldberd and Bridges, 1988). These in turn might be mistaken for physical symptoms by doctors.

The above observations are some of the factors which might be involved in the gender differences in the prevalence in depression. Consideration of these variables has initiated further interest in and contributed to the knowledge base concerning men's experience of depression, particularly regarding symptoms and coping styles (Wilhelm et al 2002). Current research has postulated the need for specifically designed diagnostic criteria and measurement questionnaires which reflect specific gender differences in symptom manifestation and coping styles (Brownhill, 2003).

CHAPTER 2

The HPG axis

Section 1 – Physiology and Mechanisms of Action

This section presents an introduction to the physiology of the Hypothalamic-Pituitary-Gonadal (HPG) axis in the human male, as well as an overview of the production, secretion, transportation, metabolism and actions of gonadotropins (LH and FSH) and testosterone.

The reproductive hormonal axis in men consists of three main components: the hypothalamus, the pituitary gland and the testis. The male HPG axis is an integrated system whose main constituents: the gonadotropins (LH and FSH), and testosterone are involved in a complex interplay to produce and regulate sex hormones and to produce gametes. These two functions, called steroidogenesis and spermatogenesis, respectively, are the main functions of the HPG unit.

1.1 Testosterone Production and Synthesis

Testosterone is the most important and abundant androgen in the male body (Mooradian, Morley, Korenman et al., 1987). Testosterone in the male is essential for the development and maintenance of specific reproductive tissues such as testis, prostate, epididymis, seminal vesicles and penis, as well as other properties such as: increased muscle strength, hair growth, maintenance of bone density, thickening of the larynx etc. (Shahidi, 2001). In men, more than 95% of testosterone is secreted by the testis, which produces around 6-7mg per day (Coffey, 1988). Very small amounts of testosterone are also produced in the brain as well as the zona reticulosa of the adrenal cortex (Baulieu, 1997).

The metabolic procedure required for testosterone production takes place in approximately 500 million Leydig cells which are located in the testes. Testosterone is secreted under the influence of Lutenizing Hormone (LH), which is the most important factor for regulation of Leydig cell number and function.

The source for synthesis of testosterone is cholesterol, which may be synthesised from acetate but it may also be taken up from plasma lipoproteins (Rommerts, 2004). The transformation of cholesterol into testosterone involves five enzymatic steps, in which the side chain of cholesterol is cleaved through oxidation (Fig.1).

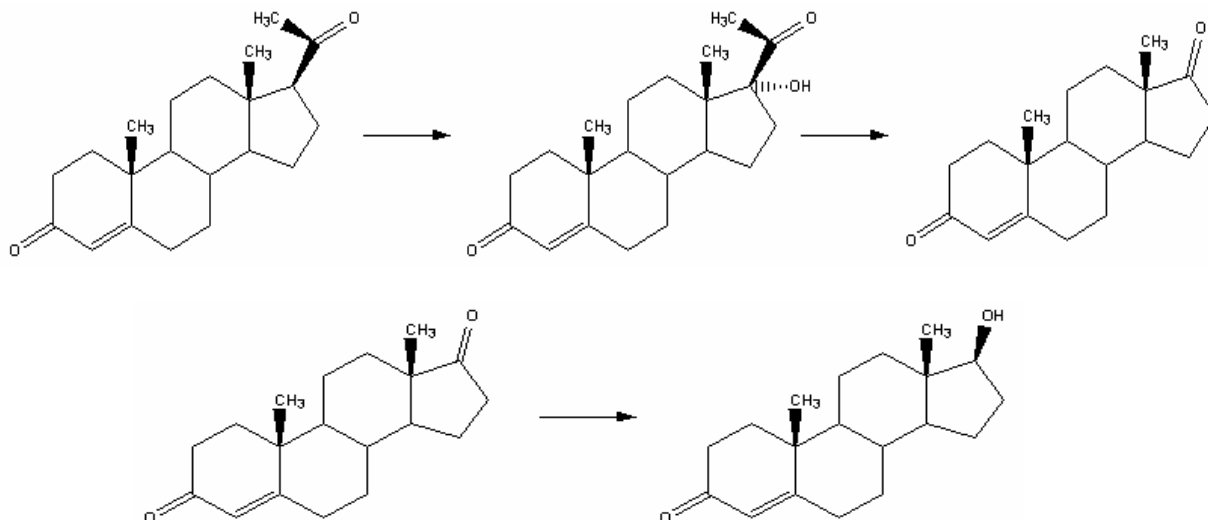


Fig. 1. Testosterone synthesis (with permission from Wikimedia commons, 2005)

The synthesis of all androgens starts with the hydroxylation of C-17 of progesterone, a derivative of cholesterol, to yield 17α -hydroxyprogesterone. The side chain is cleaved to form androstendione. The keto group on C-17 is reduced to an alcohol to yield testosterone (Shahidi, 2001). The various steps in the side chain cleavage process are catalysed by one enzyme, the P450, which is regulated by LH. Under normal physiological conditions the synthesis of androgens from cholesterol mainly depends on the amount of P450 in the mitochondria.

1.2 Testosterone Transport in Blood – The Sex Hormone Binding Globulin

Transportation of testosterone throughout the body is achieved either by lymphatic circulation or venous blood circulation (Rommerts, 2004). While levels of testosterone are very similar for both of these circulatory systems, there are differences in the flow rate and velocity of transportation. The exact mechanism by which testosterone is

transported from the Leydig cells to either of the circulatory systems has not yet been elucidated.

In the blood, testosterone circulates largely bound to plasma proteins. The major binding molecules are albumin and sex-hormone binding globulin (SHBG, also called Testosterone Binding Globulin (TeBG)). In normal men testosterone circulates in plasma in three physical states: 1) albumin bound, b) Sex Hormone Binding Globulin bound and 3) free (Pardridge, 1988). Only 2% of the total testosterone circulates freely in the blood, while the rest is bound to either of the two proteins. Thus, it has generally been regarded that the free portion is biologically active and readily available for uptake by tissues, while the albumin-bound and SHBG-bound hormone is physiologically inert and sequestered in plasma (Westphal, 1971; Sitteri, 1981). A corollary of this, so called “free-hormone hypothesis” is that SHBG acts as a reservoir for testosterone that can be readily made available for metabolism, this being achieved either by rapid dissociation of testosterone in the immediate vicinity of the target tissue surface or entering the cell intact (Pardridge, 1988). In addition, in vivo studies have shown that human SHBG selectively delivers testosterone to tissues in an organ-specific way. These studies have shown that SHBG does not normally deliver testosterone to tissues such as the brain, liver, salivary glands or lymph nodes (Cefalu, Pardridge, Chaudhurri et al.,1986), whereas SHBG-bound testosterone is readily available for uptake by the testis and prostate gland (Sakiyama, Pardridge, Musto, 1988).

In addition to its principal role as a carrier of testosterone, evidence indicates that SHBG plays further roles in determining levels of testosterone: for example, it has been proposed that SHBG reduces the metabolic clearance rate of testosterone (Vermeulen and Ando, 1979; Sitteri, Murai, Hammond et al 1982). SHBG may also modify the rate of conversion of androstenedione to testosterone (Vermeulen and Ando, 1979) and it may also protect testosterone from hepatic metabolism (Selby, 1990).

Similar to several other plasma proteins, human SHBG is produced in the liver (Hammond, Underhill, Rykse et al., 1989; Khan, Knowles, Aden, et al., 1981) and secreted into the blood stream where it serves as a modulator of sex steroid action.

SHBG is under hormonal regulation, and concentrations are regulated by being a part of the negative feedback mechanism (discussed later in this chapter), whereby increase in SHBG is followed by decrease in free testosterone, which in turn stimulates production of testosterone (Nieschlag and Behre, 1997). In healthy men the concentration of SHBG is positively correlated with testosterone, as most factors which affect SHBG lead to parallel changes in testosterone. Male and female children have similar SHBG concentrations until the onset of puberty, when SHBG levels begin to decrease more rapidly in males than in females. By adulthood, SHBG concentration in men is about one-third to one-half the level in women, resulting in a significantly larger amounts of free testosterone in men compared to women.

1.3 Mechanism of testosterone effects

Testosterone has androgenic and anabolic effects. Androgenic effects are changes in primary and secondary sexual characteristics. These include enlargement of the penis and testes, voice changes, hair growth on the face, axilla, and genital areas. The anabolic effects of testosterone include accelerated growth of muscle, bone, and red blood cells, and enhanced neural conduction. Testosterone exerts its biological effect through a single intracellular receptor that is widely distributed in various sites in the body, such as the reproductive system, bone tissues, brain, liver, kidney, skeletal muscle as well as adipocyte tissue.

Free testosterone diffuses out of capillaries and is transported into the cytoplasm of target tissue cells, where two possible outcomes exist: it can either be reduced to DHT by the cytoplasmic enzyme 5α -reductase, or aromatized into estradiol (Santen, 1975). DHT acts on the skin and target cells of the reproductive tract, whereas the bones, brain and adipose tissue are the three most important tissues in humans where the primary effect of testosterone is by way of aromatization to estradiol.

As will be discussed later in this report, there are a variety of environmental and physiological factors which may reduce the levels of testosterone available in the body.

Low levels of testosterone, called hypotestosteronism, results in a range of negative effects: such as decreased bone mineral density, decreased cognitive functions, cardiovascular disease and decreased appetite. Recent evidence from clinical trials



indicates that these conditions are improved by correcting testosterone levels (Behre,

Kliesch, Leifke, et al., 1997; Kenny, Bellantonio, Gruman, et al., 2002; Webb, McNeill, Hayward, et al. 1999; Grinspoon, Corcoran, Stanley, and Baaj, 2000).

1.4 Gonadotropic Hormones

Gonadal function and reproduction in mammals are controlled primarily by pituitary hormones, which bind to specific receptors in the ovary and testis to regulate steroidogenesis and gametogenesis (Jaffe, 1991). These actions are exerted predominantly through the pituitary gonadotropins: follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH acts on the Sertoli cells in the testis to promote germ cell development in the gonads, while LH acts on the steroidogenic cells of the testis to regulate local and peripheral concentrations of the gonadal steroid hormones that are essential for normal sexual development and sexual function.

LH derived its name with reference to the action it serves in the female reproductive system, that is, it facilitates ovulation of mature follicles. Regarding its role in the male system, LH was originally called interstitial cell-stimulating hormone (ICSH), as a consequence of its tropic effect on the Leydig cells (Jaffe, 1991). This observation has been subsequently confirmed, where subnormal levels of this hormone being reported to be associated with Leydig cell hypoplasia (Kremer, Martens, van Reen et al., 1999).

Secretion of both LH and FSH is stimulated by the Gonadotropin Releasing Hormone, (GnRH), also known as LH-releasing hormone (LHRH). While originally thought to stimulate the secretion of LH only, subsequent evidence has shown that GnRH is also

responsible for the hypothalamic regulation of FSH secretion. To date, there is no known evidence that there exists a separate releasing factor for FSH.

Non-Steroidal control of FSH

Recent observations have shown that testosterone is not the only hormone controlling FSH: for example: in oligozoospermia there is often an increased FSH, whereas testosterone is normal. Current state of knowledge postulates that the most likely nonsteroidal factor able to control FSH is inhibin B, secreted from Sertoli cells (Hayes, Pitteloud, DeCruz et al., 2001). This tentative postulation is derived from scarce reports from a relatively young body of evidence, where a negative feedback loop between FSH and inhibin has been reported, whereby inhibin has been shown to suppress FSH secretion in experimental animals, and production of inhibin is, in turn, partially dependent on FSH (Hayes et al., 2001). However, the exact role of inhibin, regarding its connection with FSH in humans, is as yet unknown. Recent development of sensitive and specific assays for inhibin B measurement have been instrumental in the quest for quantifying the relationship between FSH and inhibin (Groome, Illingworth, O'Brian et al., 1996).

1.5 Regulation of HPG Activity

The HGP axis, as other endocrine systems, is self-regulated by a feedback mechanism. GnRH is released in a pulsatile manner into the hypophyseal portal blood, a process that is regulated by a pulse generator located in the arcuate region of the medial basal hypothalamus. Since GnRH release is pulsatile, secretion of LH and FSH also occurs in a

pulsatile pattern. Evidence shows a more prominent peaks for LH due to its shorter half-life in circulation than that of FSH.

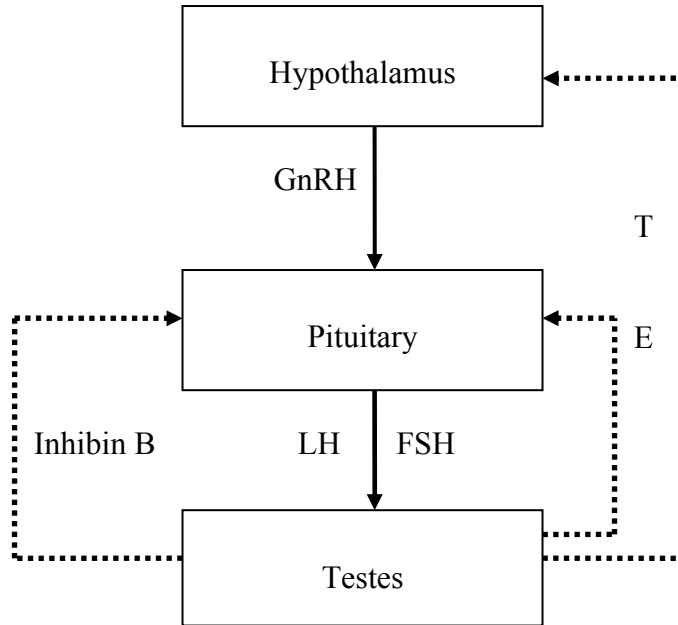


Fig. 2 Negative feedback mechanism of the HPG axis

GnRH stimulates the production and release of LH and FSH in the pituitary (solid lines). LH acts on Leydig cells and stimulates the synthesis and release of testosterone (T). T (and its derivative, estradiol (E)) exert an inhibitory feedback effect on the hypothalamic GnRH secretion to reduce secretion of LH and FSH (dashed lines). FSH is in addition under the inhibitory control of inhibin, which acts directly on the pituitary.

The function of LH, FSH and testosterone is regulated by the hypothalamus through a negative feedback mechanism (Fig.2). In man, the major hormone controlling GnRH secretion is testosterone, which inhibits gonadotropin secretion at both the hypothalamic (GnRH) and pituitary levels (LH and FSH). In other words, testosterone has a specific

ability to control and modify its own concentration: when levels of testosterone in the serum reach the upper limit, testosterone acts either on the hypothalamus to control GnRH levels or on the pituitary to directly modify LH and FSH levels. This mechanism of control thus ensures an adequate circulating levels of testosterone are present at all times.

Section 2. Factors affecting normal gonadal function

Introduction

Due to its functional and physiological complexity, particularly concerning spermatogenesis, the HPG is rendered susceptible to endogenous and exogenous factors which have the potential of affecting its function. While there exist few particular factors, such as age, whose relationship to testosterone has been well-evidenced, there is a large inconsistency and controversy concerning other factors, such as smoking and circannual variation. The following section focuses on some of the more recognised factors that have been thoroughly studied and examines the evidence regarding the effect of the factors on the HPG axis.

2.1 Hypogonadism

The term hypogonadism is used to refer to an umbrella of symptoms associated with disrupted functioning at any level of the HPG axis. While there has been some debate regarding the definition and diagnostic criteria of hypogonadism (Loughlin, 2004), it is a widely accepted norm that free testosterone levels of less than 50 ng/L (174 pmol/L) or total testosterone levels of less than 260 ng/dL (9.02 nmol/L), accompanied with consistent clinical symptoms are diagnostic of androgen deficiency (Tenover, 1994; Sih, Morley, Kaiser, 1996).

Although the ensuing physiological and psychological symptoms are the same, hypogonadism occurs as a consequence of either of two main causes, and thus it is

divided into primary (hypergonadotropic hypogonadism) and secondary (hypogonadotropic hypogonadism).

Primary hypogonadism results from primary failure of the testes to produce adequate amounts of testosterone, thus disrupting the negative feedback and leading to elevated levels of LH and FSH (Jaffe, 1991). The most common cause of primary hypogonadism is associated with sex chromosome abnormalities, as seen, most frequently in Klinefelter's Syndrome (Behre, Yeung, Holstein et al., 2000). Primary gonadal failure can also occur as a consequence of chemotherapy and radiotherapy (Beck, Schwarz, Heidemann et al., 1982). The hormonal profile of patients with this condition usually consists of low levels of testosterone accompanied by elevated levels of gonadotropins (Ghusn, and Cunningham, 1991).

Secondary hypogonadism, occurring more frequently than primary hypogonadism, may result from hypothalamic or pituitary defects. The range of causes is varied, and it includes: hypothalamic damage, syndromes such as Prader-Willi, Bardet-Biedl and Kallmann syndrome, particular treatments, such as radiation therapy, environmental stressors, such as malnutrition, excessive psychological stress and marijuana use result in deficient GnRH secretion and action. Testosterone levels in patients with secondary hypogonadism is usually low, whereas LH and FSH, unlike in primary hypogonadism, are within the lower end of the normal range (Ghusn, and Cunningham, 1991).

The symptoms of hypogonadism vary depending on the part of the life-cycle at which they occur. Thus, prepubertal onset of hypogonadism results in decreased or absent body hair, small prostate, high pitched voice and testicular and penile hypoplasia or atrophy, whereas post pubertal onset is evidenced by decreased/diminished libido, slow facial and body hair growth, decreased muscle and bone mass, decrease appetite, decline in sexual function and interest, depressed mood, lethargy and amotivation (Wang, Alexander, Berman et al., 1996).

2.2 Age

The topic of age-related decline in testosterone was first assessed in light of observed physical and behavioural changes which frequently accompany aging, such as frailty, sexual dysfunction, loss of muscle mass, decrease of appetite and decrease in energy. These changes also constitute the core symptoms of hypogonadism (Ghusn, and Cunningham, 1991).

The initial evidence for an age associated decrease of testosterone secretion was provided by Hollander and Hollander, who, in 1958 reported that testosterone was lower in elderly than in young men. Ever since, numerous authors have studied plasma testosterone levels in elderly men, yielding somewhat inconsistent results. The debate continues today, with most authors claiming that the increased presence of medical conditions and related pharmacotherapy in older men are the steering factors for the supposedly lower testosterone levels observed in older men.

Although intraindividual variability has been frequently noted, large, well-controlled epidemiological studies indicate that there is a gradual decline in levels of testosterone, beginning around the fifth or sixth decade of life (Gray, Feldman, McKinlay et al., 1991). Compared to the alterations in total testosterone, free testosterone and SHBG, which usually begins around the 5th or 6th decade of life, the changes observed in LH and FSH are a compensatory increase around this time, with drastic increase after the 6th decade (Morley, Kaiser, Perry et al., 1997). Table 1 outlines some of the alterations in hormone properties that have been reported to be age-related. Evidence suggests that the decline of testosterone and free testosterone and increase in SHBG generally occur more rapidly in men with coexisting chronic medical illness (Handelsman and Staraj, 1985), such that at any age the level for a patient with chronic illness is about 10 - 15 % lower than the level in healthy, age-matched men (Plymate, Tenover, Bremner, 1998).

Table 1. Age-related changes of the HPG axis

Increase	Decrease
SHBG	Total T
LH	Free T
FSH	GnRH
Increased irregularity in LH pulsatile pattern	Leydig cell function
	Circadian rhythm
	Mean LH pulse amplitude

There have been three recent longitudinal studies examining age-related changes in the HPG axis: The Massachusetts Male Aging Study (Feldman, Longcope, Derby et al., 2002) enrolled 1156 men who were followed-up for 7-10 years. The results showed an annual decrease of 0.8% in total T, 2% decrease in free T and a parallel increase in SHBG of

1.6% per year. In a sample of 77 patients between the ages of 61 and 87, Morley, Kaiser, Perry et al. (1997) also found an age-related decline in testosterone of approximately 1% per year, as well as increase in SHBG, LH and FSH as a function of age. In addition, Harman, Metter, Tobin et al. (2001), analyzing the data from the Baltimore Longitudinal Study on Aging which involved 890 men (22 – 91 years old), found a decrease in Total T levels.

Large cross-sectional studies have also reported lower total and free testosterone, increased SHBG and compensatory increase in FSH and LH in older patients: in a large cross-sectional study of 1709 men aged between 39 and 70 years, Gray et al. (1991) reported an annual decrease of 1.2% in Free T accompanied by a decrease of 0.4% in Total T. SHBG was found to increase at a factor of 1.2% per year. Controlling for factors affecting SHBG, such as alcohol intake and smoking, (Erfurth and Hagmar, 1995) found a significant decrease in FT and T in middle-aged compared to young men. Ukkola, Gagnon, Rankinen et al. (2001) performed a large, cross-sectional, multi-centre study, involving 295 medication-free men (age range 17-65 years), and found that age was a major predictor for testosterone and SHBG. In addition, another large cross-sectional study of 1563 men (age range 25-84 years), Svartberg, Midtby, Bonnaa et al. (2003) found inverse relationship of free and total testosterone with age, and an increase in SHBG with age. Moreover, regression analyses revealed a more pronounced decline of free testosterone (standardised Beta = -0.41) compared to the decline in total testosterone (standardised Beta = -0.08).

However, these reports have been challenged by the failure of other authors to find changes in testosterone and related HPG parameters as a function of age. Harman and Tsitouras (1980), studying a sample of 69 healthy men aged 25-89 years, found increased LH, but no change in testosterone with age. Similarly, there was no association between testosterone or free testosterone in a sample of 86 healthy men (aged 31-88 years) as reported by Sparrow, Bosse, Rowe et al. (1980).

2.2.1 Mechanisms of age-related effects on the HPG axis

Age-related reduction of testosterone is believed to be a result of an overall decline in the functional capacity of the HPG axis (Erfurth & Hagmar, 1995). Namely, evidence suggests that there are three main aspects of the physiopathological mechanism that is responsible for the age-related decline in HPG functioning. Firstly, a large body of evidence indicates that the decreased testosterone levels as a function of age has a primary testicular origin. This has been supported by studies which have shown decreased response of testosterone to human choriogonadotropin (hCG) (Harman & Tsitouras, 1980; Landcope, 1973) or to prolonged stimulation of LH using GnRH (Mulligan, Iranmanesh, Kerzner et al., 1999). Moreover, there is also evidence of changes in testicular steroid mechanism induced by decreased oxygen supply (Pirke, Sintermann, Vogt et al., 1980) as well as a decrease in the number of Leydig cells (Rubens, Dhont, Vermeulen et al., 1984).

Secondly, the ageing of the endocrine testicular function may involve alterations in central mechanisms, namely, the hypothalamic GnRH-secreting system. There are several

lines of evidence which suggest that neuroendocrine changes occur at this level: firstly, the responsiveness of LH to GnRH administration is not impaired in the elderly (Kaufman, Giri, Deyslipere et al 1991). Secondly, in an intact pituitary or hypothalamic functioning, Free T levels would be expected to be normalised via the feedback mechanism, however, given the well-evidenced low levels of Free T, and observations of preserved pituitary secretory capacity in the elderly (Kaufman, et al 1991; Mulligan, et al 1999), it is most likely that the lower levels of testosterone are observed in older men due to alterations at the hypothalamic, as well as the testicular level.

The third aspect of the physiopathological mechanism involved in age-related decline in HPG measures is the increase in the capacity of SHBG to bind testosterone, as well as increases in levels of SHBG. This hypothesis has been the primary explanatory factor in the age-related decline of testosterone levels, particularly in light of the more predominant decrease in free testosterone. The reason for increase in the level and binding capacity of SHBG with age is not been completely understood, however, evidence has implicated Growth Hormone (GH) as a mediating factor in the age-related changes in SHBG. For example: there is a wealth of evidence which suggests that GH decreases SHBG levels (Vermeulen, Rubens and Verdonck, 1972; De Moor, Heyns and Bouillon, 1972). In addition, it is also an established finding that GH decreases with age (Vermeulen, 1987; Iranmanesh, Lizarralde and Veldhuis, 1991). Thus, it is most likely that decreases in GH, as a consequence, might facilitate the increase of SHBG with age.

2.2.2 Methodological Difficulties in measuring age-related decline of testosterone

As discussed in a recent meta-analysis on the relationship between testosterone and age (Gray, Berlin, McKinley et al., 1991), a number of methodological issues need to be considered when interpreting results from age-related decline studies. Firstly, type of sample and subject characteristics have varied greatly among studies: many studies are confounded by selection bias, hormonal levels being assessed in young male hospital staff or blood donor clinics and compared with levels of older men who are hospital patients or nursing home residents. In addition, understanding of the natural biologic changes that affect the gonadal axis in the aged has been confounded by higher prevalence of chronic medical conditions and medication in this age group and many studies have failed to control for presence of illness and medication usage in their sample. Particularly problematic are conditions such as diabetes and obesity, both of which have a high prevalence in older men and both of which are known to be inversely correlated with SHBG (Glass, Swerdloff, Bray et al., 1977; Toscano, Balducci, Bianchi et al., 1992).

The second, and potentially relatively more significant problem, has been researchers sampling blood in the afternoon: diurnal variation in testosterone is minimal or even abolished with aging (Diver, Imtiaz, Aftab et al., 2003), whereas testosterone levels decrease in the afternoon in young men, therefore, differences in ages is underestimated when afternoon samples are taken.

A further methodological issue which may contribute to the explanation of the differences is the differences in types and quality of assays used in different studies, which could also produce great variability between studies and preclude interpretation of the findings.

2.3 Stress

Studies in the area of stress and the HPG system indicate that suppression of the pituitary-gonadal activity may commonly accompany emotional arousal or distress, as an integral part of a broad pattern of hormonal response. The literature in this field is generally convergent on the conclusion that testosterone is decreased as a response to a stressful situation or state. A wide range of experimental conditions have been designed to study the effects of stress on testosterone. These range from combat training to school examinations. For example, one of the earliest studies in this area studied soldiers in basic training and special force personnel anticipating imminent combat in Vietnam: these men showed lower urinary excretion of testosterone than normal population of medical center personnel (Rose, Bourne, Poe et al. 1969). Also with military recruits, Bernton, Hoover, Galloway, et al (1995) showed decreased total T and free T in male soldiers during 8 weeks of extremely stressful training. A rapid recovery of these hormones was observed 72 hrs after the training period. Similarly, Opstad (1992) studied military cadets during a five-day training period involving strenuous exercise as well as limited food and sleep. This author also found significantly reduced levels of testosterone.

Studies investigating the effects of stress on testosterone under less extreme and more naturalistic conditions have also shown a decline of testosterone in stressful conditions: For example, watching a movie containing stressful events, university exams, financial difficulties and confrontations have all been associated with decreased testosterone levels (Christiansen and Hars, 1995; Nilsson, Moller, Solstad, 1995; Hellhammer, Hubert, Schurmeyer, 1985). Moreover, the outcome of a competition has also been shown to have an effect on testosterone levels, with increases of testosterone found in winners, and decreases in losers (Booth, Shelley, Mazur et al. 1989). This finding has also been observed in primates, whereby lowered testosterone levels have been observed upon the loss of a high position in the social hierarchy following defeat by the dominant male (Rose, Bernstein, Gordon, 1975).

In addition, correlational studies have reported that certain behaviours and several life-style changes, such as marriage and a change in socioeconomic status may be associated with changes in testosterone levels. For example, testosterone has been shown to be higher in men engaging in antisocial behaviour (Booth, Johnson and Granger, 1999) and risk-taking (Daltzman and Zuckerman, 1980), and lower in men experiencing low occupational success and socio-economic class (Dabbs and Morris, 1990). In addition, (Booth and Dabbs, 1993) have reported that men with lower testosterone have tendency to have a single marital status or to have high rates of divorce. Likewise, Francis (1981) reported lower testosterone levels in men who were classified as high psychological stress than their low stress counterparts.

Interventional studies have also showed a relationship between stress and testosterone: MacLean, Walton, Wenneberg et al., (1997) showed an increase of testosterone levels following meditation, while Cruess, Antoni, Schneiderman, et al., (2000) reported an increase in free T levels following Cognitive Behavioural Therapy (CBT).

Lower levels of T and FT have frequently been associated with increase in stress and/or the presence of a chronic medical condition. In the Massachusetts Male Aging Study, Gray, et al. (1991) observed 10% lower levels of T and FT in their group of subjects with one or more chronic disease present.

Additionally, there exists a large body of primate studies which provide evidence that social parameters (e.g. failure, submissiveness, losing a competition, social defeat) are associated with reduced testosterone levels. Animal models show lowered testosterone levels with losing a high position in the social hierarchy following defeat by the dominant male (Rose, et al. 1975). Examining the effect of subordination stress in rats, Blanchard, Sakai, McEwen et al. (1993) concluded that male rats that have subordinate position show lower testosterone levels compared to dominant male rats or control rats. Increased levels of testosterone in dominant males compared to subordinate males have also been reported in other animals, for example: mouse lemurs (Perret, 1992), Verreaux's sifakas (Brockman, Whitten, Richard and Benander, 2001) and talapoin monkeys (Eberhart, Keverne, Meller, 1980).

An extensively studied hypothesis in the field of animal behaviour and hormones is the challenge hypothesis (Wingfield, Hegner, Dufty et al, 1990). The challenge hypothesis has been developed to explain fluctuations in testosterone concentration with respect to competition and parental care. The challenge hypothesis states that a positive correlation between plasma testosterone and aggression occurs only under conditions of social instability, such as when access to food and water is restricted or during competitions for one's rank in the hierarchy. As there are risks and costs of having increased testosterone levels, such as increased energy expenditure due to increased activity levels, the hypothesis postulates that at all other times testosterone levels should be kept constant and only be elevated when needed. While this hypothesis has been supported in many species (Beletsky, Orians and Wingfield, 1992; Vleck and Brown, 1999), contradictory findings have also been reported (Moore, Wada, Perfito et al, 2004).

2.4 Medical Illness

Critical illness is defined as a condition requiring support of failing vital organ systems without which survival would not be possible (Van den Berghe, 2003). Low serum testosterone, abnormal Leydig cell function, increased SHBG, decreased LH and abolition of pulsatile LH secretion have been well-documented as common endocrine abnormalities accompanying chronic or critical illnesses, such as cancer, cystic fibrosis, chronic pulmonary disease, chronic liver disease (Galvao-Teles, Burke, Anderson, et al., 1973), spinal cord injury (Naftchi, Viau, Sell et al., 1980), renal failure (Gupta and Bundschu, 1972), burn injury, prostatitis (Yunda & Imshinetskaya, 1977) and diabetes. It appears that these changes in HPG axis function in chronic disease are non-specific

effects of the illness, as similar changes have been reported in men who had recently undergone major surgery (Carstensen, 1973), in myocardial infarction (Deslypere and Vermeulen, 1984), as well as severe burns (Vogel, Peake, Rada, 1985).


The suppression of the HPG axis as a result of illness may be seen as a categorical as well as continuous relationship, that is, there is a rapid decrease of testosterone during the critical stage, and upon the prolongation of critical illness testosterone levels drop below the normal range and hypogonadism develops. By the same token, there are numerous reports which suggest that the magnitude of HPG axis suppression is related to the degree of illness: Spratt, Cox, Orav et al. (1993) compared testosterone levels between men with severe illness and healthy controls and found that testosterone levels were higher in healthy controls. These authors also observed that testosterone levels in men with severe illness were lower than in men with relatively mild or moderate illness, and that these differences accentuated as hospitalisation progressed. Similarly, Luppia, Munker, Nagel et al. (1991), studying groups of normal, acutely ill and chronically ill male patients found that, compared to normal controls, acutely ill men had a moderate, whereas chronically ill men had a markedly lower levels of testosterone. Finally, in a sample of 41 men with severe burns, Vogel et al. (1985) found a significant inverse relationship between severity of burns and testosterone levels.

2.5 Psychotropic Medication

Research investigating the effects of psychotropic medication on testosterone has been relatively inactive. Overwhelming majority of the studies performed in this field have assessed testosterone levels in relation to sexual function and have examined whether

sexual function is affected by medication. Thus, beside few reports of clinical trials which have assessing testosterone levels in relation to medication as a secondary analyses, and few studies conducted with animal models, there exist little data on the association of testosterone and psychotropic medication.

Studies on rats indicate that citalopram and mianserin can increase testosterone levels by up to 60% (Prezegalinski, 1987), whereas serotonin reuptake inhibitors minimally suppress testosterone levels (Rehavi, Attali, Gil-Ad et al., 2000). In human patients, it has been reported that administration of antipsychotics such a haloperidol and thioridazine (Brown, Laughren and Williams, 1981) and mood stabilisers, such as lithium and epilim (Whalley, Kutcher, Blackwood et al., 1987) may decrease testosterone and LH levels.

The serotonergic (5-HT) system has long been implicated in the neurobiology of mood disorders because numerous evidence shows that antidepressant treatments result in an enhanced 5-HT neurotransmission (Deakin, 1988; Owens, 1996). Recent studies show that testosterone and estrogen affect regions of the rat brain which in the human brain parallel regions controlling cognition and mood state. Fink, Sumner, Rosie et al. (1999)  showed that testosterone increased the content of 5-HT_{2A} receptor and serotonin transporter mRNA in the dorsal raphe nucleus and the density of 5-HT_{2A}R and serotonin transporter binding sites in the higher centers of the brain. Tinajero (1992) reported that 5-HT inhibits testosterone production in adult rat Leydig cells. Few years later, Frungieri, Gonzalez-Calvar, Rubio et al. (1999) demonstrated the presence of 5-HT in Leydig cells of the testis of the golden hamster, and they too showed that 5-HT has inhibitory function

on testosterone. In addition, Dufau, Tinajero, Fabbri (1993) found that while 5-HT stimulates the secretion of corticotrophin-releasing hormone (CRH) in rat Leydig cells, it acted as a negative regulator of gonadotropin-induced cyclic adenosine monophosphate (cAMP) generation and androgen production. Frungieri, Zitta, Pignataro et al. (2002) reported similar results: the inhibitory action of 5-HT on testosterone production can be prevented by exposure to CRH antibodies or antagonists. This observation suggests that the effects exerted by 5-HT are modulated by CRH. Furthermore, Rehavi (2000) showed that chronic treatment of rats with dopamine and serotonin reuptake inhibitors resulted in lowered serum estradiol and progesterone levels in female rats. An additional finding was that the dopamine transporter blockers suppressed testosterone levels in male rats, whereas serotonin reuptake inhibitors induced a reduction of 30% of this hormone.

2.6 Prolactin

Prolactin is a protein hormone secreted by the anterior pituitary gland, secretion occurring in a pulsatile matter (Molitch, 1992). Pituitary prolactin secretion is regulated by neuroendocrine neurons in the hypothalamus, acting both at the level of the median eminence and pituitary to inhibit prolactin secretion. Prolactin has broad physiologic functions, acting on various kinds of cells and bodily systems, such as the reproductive and neurophysiologic systems. The function of prolactin on the reproductive system is dual: firstly, it acts as a growth-promoting hormone in the development of the genital tract (Dombrowicz, Sente, Closset, Hennen, 1992), and secondly, prolactin has inhibitory effects on the secretion of sex hormones. Indeed, high levels of circulating prolactin result in dysfunction of the hypothalamus, with decreased serum LH and testosterone

production as a consequence (Bartke, Klemcke, Matt, 1986). Hyperprolactinaemia-induced hypogonadism has been attributed to a dysfunction in GnRH-producing neurons, whereby secretion and release of GnRH is decreased. As a consequence, the gonads are exposed to a lesser extent of stimulation from the gonadotropins, resulting in decreased release of testosterone by the testes (deGreef, Ooms, Vreeburg, Weber, 1995). Besides the impact on the gonadotropins, a more direct effect of prolactin on testosterone levels has been proposed, due to the discovery of widely distributed prolactin receptors located on the testicular cells (Jabbour and Lincoln, 1999).

2.7 Smoking

A review of the epidemiological literature suggests that smoking is associated with modest reductions in semen quality, including sperm concentration, motility and morphology (Vine, 1996). On the contrary, the results emanating from studies of the association between smoking and male reproductive hormones have been conflicting, largely owing to variability in methodology. The majority of the studies performed in this field are cross-sectional and frequently involve male patients recruited from infertility clinics. Possible factors contributing to the observed discrepancies include: inadequate control of potential confounding factors, such as age, BMI, diet and exercise, as well as the presence of cardiovascular disease; methodological problems, such as inadequate power as a result of small sample sizes in most studies, subject selection bias and varying methods of hormone assay used in different studies.

Most studies performed in populations with normal men have incorporated a cross-sectional approach, comparing smokers to non-smokers. The results have been conflicting, some studies reporting increased levels of total testosterone (Handa, Ishii, Kono et al., 1997; Allen, Appleby, Davey et al., 2002), Free Testosterone (Handa et al., 1997; Trummer, Habermann, Haas et al., 2002), FSH (Shaarawy and Mahmoud, 1982; Sherins, Patterson, Brigtwell, 1982), LH (Mendelson, 2003; Allen et al., 2002) and SHBG (English, Pugh, Parry et al., 2001; Allen, et al., 2002) in smokers compared to non-smokers. However, other studies have found decreased levels in smokers or no difference in testosterone (Barret-Connor and Khaw, 1987; Klaiber, Broverman, Pokoly et al., 1987; Krause 2000), LH (Shaarawy and Mahmoud, 1982; Seyler, Pomerleau, Fertig et al., 1986) and FSH (Anersen, Semezuk, Tabor, 1984).

The results derived from large epidemiological studies are similarly conflicting: Simon, Preziosi, Barrett-Connor et al. (1992) studied hormone levels in 1408 healthy men aged 20-60 years and found no difference in testosterone between smokers and non-smokers. Tsitouras (1982) found no relationship between smoking and testosterone, while Field, Coldits, Willett et al. (1994) in a population of 1241 randomly-sampled men aged 38-70 years found increased levels of testosterone in smokers. Both of these studies controlled for BMI and age. The only longitudinal study in this area found a more prominent age-related decline of testosterone in smokers, this decline being positively correlated with number of cigarettes smoked (Zmuda, Cauley, Kriska et al., 1997).

Thus, while the majority of current literature does support a view of altered gonadal hormones, the association is by no means well characterised and as outlined above, changes are as likely to include increases as much as decreases (Vine 1996).

However, it seems that even if testosterone levels are indeed affected by smoking, this effect is not direct, as most studies have failed to establish a dose-dependent relationship between testosterone and the amount of tobacco used. Furthermore, if testosterone is affected by smoking, the effect seems transient, as (Briggs, 1973) reported that although testosterone was lower in smokers compared to non-smokers, this increased following a week of abstinence from smoking, indicating that any effect of smoking on testosterone is transient.

2.8 Diet and Nutrition

Much of the research activity on the interaction between diet and sex hormones was carried out in the 1970's and 1980's, mainly as a result of epidemiological evidence that the risk of prostate and breast cancer is related to dietary factors, particularly to a high consumption of fat (Hamalainen, Adlercreutz, Puska et al., 1984).

Notwithstanding the methodological difficulties inherent in conducting controlled fasting and food deprivation studies, such studies in men have demonstrated an increase in SHBG with a consequential decrease in free testosterone and unmodified levels of serum total testosterone, LH and FSH (Tegelman, Lindeskog, Carlstom et al., 1996; Hoffer, Beitins, Kyung et al., 1986). The effect of extreme fasting on SHBG has also been

observed in pathological malnutrition: for example, in women with anorexia nervosa infusion of calories is related to a decrease of SHBG concentrations (Estour, Pugeat, Lang, et al., 1986).

The effects of specific nutrients and dietary constituents on androgens have predominantly been studied in women or experimental animals (e.g. Vawda and Mandlwana, 1990), which show a large reduction of testosterone (up to 50%) during protein deficiency. There are very few studies in the literature systematically examining the effects of malnutrition on testosterone. Owing to the relative simplicity in methodology, a number of studies have been conducted comparing androgen levels between vegetarians and omnivores. Evidence of the literature on this topic is relatively unequivocal: Belanger, Locong, Noel et al., (1989) reported that vegetarians have significantly higher SHBG concentrations than omnivores, whereas no difference in total testosterone was observed. Comparable results were demonstrated by Field et al., (1994), who showed that consumption of animal fat is inversely related to SHBG, but not associated with total testosterone. Similarly, Reed, Cheng and Simmonds (1986) demonstrated that a low fat diet administered to normal men induces an increase of SHBG levels with a subsequent decline in free testosterone. In addition, protein deficiency seems to be associated with a reduced level of total testosterone (Reed, Cheng and Simmonds, 1986).

However, these results need to be interpreted with caution, due to the large variation in methodology employed by studies in this research area, particularly with respect to

nutritional questionnaires and food intake quantification, as well as the short intervention periods (Allen, Key and Allen, 2000).

Contrary to the paucity in evidence regarding the association between diet/nutrition and hormones, most studies are consistent in their findings relative to the relationship between Body Mass Index (BMI) and hormones. BMI (calculated as the weight divided by height squared) is a universal measure of body mass. Many studies have shown that SHBG levels are reduced in obese men, and some studies have found an inverse relationship between visceral fat and SHBG (Glass et al., 1977). Similarly, the literature on the association between testosterone and BMI is conclusive: lower androgen levels are associated with increasing visceral adiposity and higher BMI (Oh, Barrett-Connor, Wedick et al., 2002). There is also evidence that testosterone replacement decreases visceral fat without decreasing total body fat in obese men with low testosterone levels (Marin, Holmang, Jonsson et al, 1992).

2.9 Exercise

Findings of recent studies suggest that physical activity can potentially have a range of effects on the HPG axis, depending on type, intensity and duration of the activity, as well as fitness level and characteristics of the individual. In general, it appears that, in healthy men, relatively short bouts of exercise increase testosterone levels, while more prolonged exercise (i.e. exceeding 2 hours) tends to reduce testosterone levels, which may be inhibited for considerable time beyond the completion of the activity (Cumming, Wheeler & McColl, 1989).

Similarly, Kraemer, Häkkinen, Newton et al. (1998) observed that vigorous exercise of 3 hours or more per week is associated with higher testosterone levels, while Fahner and Hackney (1997) as well as Hurel, Koppiker, Newkirk et al. (1999), reported an increase in FT following endurance exercise.

In addition, a wealth of results from recent clinical trials of testosterone administration support the relationship between testosterone and energy by providing evidence that testosterone administration increases energy levels and decreases fatigue and tiredness in hypogonadal men (Burriss, Banks, Carter et al., 1992; O'Connor, Archer, Hair et al. 2002; Wang et al., 1996; Wang, Swedloff, Iranmanesh et al., 2000) and HIV-infected men (Rabkin Wagner and Rabkin, 1999 and 2000).

While literature reports have somewhat converged onto a general consensus that gonadal hormones are positively affected by short-term exercise (up to duration of approximately 2 hours), and negatively affected by long-term exercise, there is little agreement as to the mechanisms of the specific changes.

2.10 Circadian and circannual variation of hormones

2.10.1 Circadian variation

As applicable to other endocrine systems, it is a well-supported finding that the HPG system exhibits circadian variation. Circadian variation of testosterone is characterised

with higher levels early in the morning (approx. 5 to 8 am), then decreasing gradually during the day. While variations of about 25 to 43% between morning and night testosterone levels are usually shown in healthy young men, (e.g. Winters, Brufsky, Weissfeld et al., 2001; Diver et al., 2003), these differences are not usually observed in older men (Bremner, Vitiello & Prinz, 1983). That is, circadian variation of testosterone often starts to diminish in men past the age of about 50 years (Plymate et al., 1989). The decrease or loss of diurnal rhythm in serum total testosterone in older men is reported to be in partly due to low concentrations in the morning when compared to concentrations found in young men (Diver et al., 2003). Although the phenomenon of circadian variation of hormones has been well-established, there exists a high intraindividual consistency: this is especially applicable regarding specifying the age when circadian variations start to diminish.

2.10.2 Circannual variation

The concept of an existence of circannual rhythm of gonadotropins and androgens in human males has been invoked from records on frequency of sexual intercourse, reports of rapes, sexually transmitted disease and the sale of contraceptives (Smolenski, Reinberg, Bocakova-Rocher, 1981). While there is a general agreement about diurnal variations of testosterone in men, studies of seasonal testosterone variation have shown contradictory reports. Seasonal variation has been reported in cross-sectional studies by Bellastella, Esposito, Mango et al., (1982), Nicolau, Haus, Lakatua et al. (1985) and Dabbs and Morris (1990), but not by Dai, Kuller, LaPorte et al., (1981) and Abbaticcio, de Fini, Giagulli et al (1987). Longitudinal studies have also shown inconsistent reports (Merrigiola, Noonan, Paulsen, 1990). One of the factors most commonly implicated in

the HPG variation as a function of season is the geographical area where the study was performed. Majority of researchers have recently recognised that seasonal variation in HPG parameters is more likely to be present in men living in geographical areas with extreme seasonal variation in sunlight and temperature, such as Scandinavian countries (Svartberg, Barrett-Connor, 2004).

A number of additional factors, such as body weight, fat distribution, diet, exercise and alcohol consumption could also be viewed as potential confounders in the examination of seasonal variability of gonadotropins and testosterone. The study of circannual variation in testosterone is a relatively young field and future studies will need to consider the above, and other factors in their investigation. While circannual variation of testosterone is obviously a favourable survival phenomenon in seasonally breeding animals (Mann & Mann 1981), its existence (and reproductive significance) in the human male has yet to be confirmed.

Section 3. Sexual Function, testosterone and depression

Sexual function is a central feature of the HPG axis, and frequent symptom of depressive illness. Pertinent to the current thesis, erectile function (and dysfunction) and the association of this with testosterone and depression is outlined.

3.1 Background

Erectile dysfunction, (ED), is commonly defined as: “the inability to attain and/or maintain erection satisfactory for sexual intercourse or other sexual expression” (Nicolosi, Moreira, Shirai et al., 2003). ED is a common problem for a large proportion of men, particularly in middle-age and older men (Mulligan, Retchin, Chinchilli et al., 1988). In an American survey conducted with 1410 men and 1749 women between the ages of 18 and 59 years, 43 percent of women and 31 percent of men reported sexual disinterest (Laumann, Paik, Rosen, 1999).

3.2 Factors associated with Erectile Dysfunction

Age is a well-established factor associated with ED. An early study by Kinsley, Pomeroy and Martin (1948) reported that ED was an age-dependent disorder with a prevalence of 0.1% at 20 years of age, 6.7% at 50 and 75% at 80 years. Furlow (1985) reported prevalence of 2% at age 40 and 25 -30% at 65 years, while in geriatric patients, Mulligan et al. (1988) reported prevalence of 26% ED at age 60-65 and 50% at 75-80 years.

Although all published data agree that prevalence of ED increases with age, it is not an inevitable outcome of the aging process, as the majority of studies demonstrate substantial intra-individual variability. In addition, while age is probably the most strongly associated variable, a number of other factors have been implicated to be closely associated with ED. The fact that older men are more likely to be affected by medical conditions and/or receiving pharmacological treatments that can impair erectile function, precludes the distinction of the physiological effects of aging on the endocrine system from the consequences of pathology or its treatment.

Not surprisingly, patients affected by a chronic disease have a higher prevalence of sexual dysfunction, including lowered libido, than the general population (Segraves, 1989). Two studies of diabetic patients reported prevalence of ED ranging from 4.5% in younger patients to 52% in elderly patients with diabetes (McCulloch, Campbell, Wu et al., 1980; Fedele, Bortolotti, Coscelli et al., 2000), while others have found that the risk of ED increased with the duration of illness (Nicolosi et al., 2003). Similarly, the percentage of patients with ED has often been reported to be higher among patients with vascular diseases, neurogenic disorders, hepatic and renal failure as well as patients undergoing surgery, or receiving head or pelvic trauma (Krane, Goldstein, Saenz, 1989).

Furthermore, ED has often been reported to accompany depressive episodes: clinical surveys consistently document reduced interest in sexual activity in at least 50 – 90% of depressed patients (Casper, Redmond, Katz et al., 1985). Despite the significant impact of depression on sexual function, the nature of these disturbances remains poorly

understood. For example: Depression-induced ED may derive from a disturbance or alteration in the underlying sexual neurophysiology during depression (Thase, Reynolds, Jennings et al., 1992) or it may be secondary to non-sexually-specific symptoms of depression, such as low self-esteem, social withdrawal, diminished interest and motivation and decreased energy.

Medications can affect sexual function at a variety of points along the erection pathway, such as inhibition of ejaculation or sedation leading to reduced libido (Brock and Lue, 1993). Antihypertensive drugs have been most commonly associated with impotence, with sexual dysfunction among men receiving diuretics the most frequent cause for withdrawal from therapy (Bansal, 1988). Psychotropics have also been strongly associated with ED: sexual dysfunction has been reported in 25% of all patients receiving antipsychotics, and there are case reports of sexual dysfunction with almost every class of antipsychotic (Segraves, 1989).

Similarly, antidepressants have often been reported to cause a range of sexual dysfunction side effects: In general, tricyclic antidepressants tend to cause reduced libido and loss of motivation (Meston, 2000), while the SSRIs have been associated with inducing painful, delayed or retrograde ejaculation (Fava and Randin, 2002; Rosen, Lane, Menza, 2003). Estimates of the incidence of ED during antidepressant treatment range from 20 to 40% (Balon, Yeragani, Pohl et al., 1993), although it is highly likely that the prevalence is under-reported due to failure of clinicians to explicitly inquire about sexual dysfunction and the patients' reluctance to voluntarily offer details about their sexuality.

In many cases, however, it remains difficult to determine from the available literature the effect a particular drug has on sexual function from the effect of the disease that precipitated the use of the medication. In addition, patients often take multiple medications concurrently, causing complex interactions which add to the difficulty in determining the adverse effect profile of a particular medication.

3.3 The role of testosterone in sexual function

Normal male sexual function depends on a complex relationship between psychological, neurological, vascular and endocrine factors (Jain, Rademaker and McVary, 2000). There is considerable controversy on the relative importance of each factor in the initiation and maintenance of erection, this being particularly true for the role of testosterone. The majority of studies and clinical reports (Kwan, Greenleaf, Mann et al, 1983) indicate that sexual behaviour in the male is impaired by hypogonadism. It is widely accepted that nocturnal erections are androgen-dependent (Baumgartner, Graaf, Kuurten et al., 1990). Nocturnal tumescence is also frequently impaired in hypogonadism, and improved when androgen replacement therapy is commenced (Carani, Granata, Fustini et al., 1995). Similarly, withdrawal of exogenous testosterone in hypogonadal or castrated men results in a rapid decrease in sexual interest and behaviour, whereas replacement therapy re-establishes sexual desire within a few weeks of therapy (Schiavi and Seagraves, 1995). Furthermore, there is also growing, although not entirely consistent, evidence indicating a

decline in testosterone levels as well as sexual function with age (Panser, Rhodes, Girman, et al., 1995).

The above behavioural and hormonal observations have led to the hypothesis that testosterone is necessary to sustain sexual drive and behaviour. However, studies of the relationship between testosterone levels and psychological aspects of sexual function, such as drive and libido, have generally yielded inconsistent, and at best weak positive correlation (Christiansen and Hars, 1995). This is due to the complexity and number of social, psychological, physiological and methodological factors associated with sexual function, as well as testosterone.

However, although there is no consensus in the literature regarding the relationship between sexual function and testosterone levels per se, researchers and clinicians agree that the presence of testosterone is an essential factor in sexual function. Literature results suggest that sexual function - including psychological (interest, motivation) and physiological (erection, orgasm) factors - is dependent on testosterone levels only in the lower range, approximately 300 ng/dL (Bagatell, Heiman, Rivier et al., 1994; Buena, Swerdloff, Steiner et al., 1993; Nieschlag, 1979) i.e. once a threshold of T at the lower range is achieved, normalisation of sexual function occurs. Increasing serum levels of T higher in the normal range, even past the normal range does not further improve sexual function. (Wang et al., 2000).

CHAPTER 3

Neuroendocrinology of Depression

Synopsis

The purpose of this chapter is to briefly introduce the physiological and behavioural connection between depression and some of the most researched endocrine systems. Psychoneuroendocrinology is playing an increasing role in the diagnosis and treatment of the mood and anxiety disorders. While the study of the hypothalamic-pituitary adrenal axis has become a fertile area of investigation in studying psychiatric disorders, investigations of the thyroidal and growth hormone axes and their relationships with depression have also received a great deal of attention.

3.1 Introduction

Advances such as the development of sensitive hormonal assays, have enabled psychoneuroendocrinology to emerge as a significant clinical and research discipline (Brambilla, 2000). The concept of an interrelationship between mood, behaviour and

hormones was first conceived in the 19th century with the description of endocrine disorders, such as Cushing's disease, Addison's disease and hypothyroidism (Beard, 1869; Gull, 1874; Ord, 1878) all of which are usually accompanied with profound mood effects. Clinical observations such as these, coupled with ongoing advances in hormone synthesis and hormone analysis have set the scene for further systematic, empirical studies in psychoneuroendocrinology.



Recent neuroendocrine research interest has focused on the changes in endocrine function that may accompany various psychiatric disorders, the most extensive area being endocrine dysfunction in depression. Research in this area has mainly focused on the three primary hypothalamic systems: hypothalamic-pituitary-adrenal (HPA), hypothalamic-pituitary thyroid (HPT) and hypothalamic-pituitary growth-hormone (HPGH) axis.

3.2 HPA axis

The HPA axis is an integrated hormonal system whereby the three main components, hypothalamus, pituitary and adrenal gland, influence and regulate each other via neurohumeral signals to regulate a range of bodily functions and behaviours.

Cortisol is produced in and secreted by the adrenal glands in response to control signals from the pituitary gland and hypothalamus. Cortisol secretion is increased in response to physical and psychological stress (Martin and Reichlin, 1987). While cortisol itself has

many important functions, including regulation of metabolism and blood pressure, its major role in the bodily stress response has earned it the nickname of "stress hormone."

The physiological and behavioural response of HPA activation has been well documented, for example: intraventricular administration of CRH (corticotrophin releasing hormone) in rats results in increase in heart rate, blood pressure and blood glucose, as well as behavioural changes, such as anorexia, increased vigilance and arousal and decrease in libido (Sutton, Koob, LeMoal et al., 1982).

In healthy individuals, the hypothalamus produces corticotropin-releasing factor (CRF), which stimulates the production of adrenocorticotropin-releasing hormone (ACTH) in the pituitary gland, which in turn stimulates the production of glucocorticoids (cortisol in humans and corticosterone in animals) in the adrenal gland. As is the case for the HPG system, the HPA axis has an autoregulated system mediated by glucocorticoids, whereby the production of ACTH is regulated via feedback inhibition from within the pituitary corticotropic cells that produce cortisol. Activation of glucocorticoids receptors (GRs) at the level of the paraventricular nucleus (PVN) reduces CRF release from the PVN and thus reduces HPA activity (Steckler and Holsboer, 1999). Feedback regulation of the HPA axis by glucocorticoids is regulated by two distinct corticosteroid receptors, the mineralcorticoid receptor (MR) and the glucocorticoids receptor (GR) (De Kloet, Vreugdenhil, Oitzl and Joels, 1998). The MR has a 10-fold higher affinity for corticosteroids (Reul and De Kloet, 1985) and is believed to play a role in the maintenance of the activity of the stress system, while GRs, in conjunction with MRs, are

involved in recovery from stress (De Kloet, Vreugdenhil et al, 1998). Evidence suggests that activation of GR is necessary for the HPA feedback regulation when levels of glucocorticoids are high, and that MR in addition plays a crucial role by modulating GR-dependent regulation (Spencer, 1998; De Kloet, Vreugdenhil et al, 1998). The hyperactivation of the HPA axis (see below) is thought to be related, in part, to altered feedback inhibition by glucocorticoids.

The changes in HPA axis has been well implicated in depression. It has become apparent and well-accepted observation that melancholic depression represents an exaggerated and prolonged form of the hyperarousal seen with stress system activation, and patients with this illness show behavioural patterns similar to the ones observed in the rat following CRH administration (Gold, Goodwin & Chrousos, 1988). The most consistent finding by researchers in this area has been that of hypersecretion of cortisol, found in approximately 50% of patients with depression (Osran 1993).

Another frequently observed finding in patients with depression is an abnormal cortisol response to the dexamethasone suppression test (DST). This test was first elucidated by Sachar, Carroll and Stokes, among others, in the 1970's and early 1980's. The dexamethasone suppression test measures the response of the adrenal glands to ACTH. In normal subjects cortisol levels decrease (are suppressed) in response to the administration of dexamethasone, whereas patients with depression frequently do not exhibit cortisol suppression following administration of dexamethasone. This observation has subsequently been replicated in studies by numerous investigators, e.g: Johnson, Hunt &

Caterson, 1988; Glassman, 1987. In most patients, the hypersecretion of cortisol reverts to normal with clinical remission of depression (Linkowski, 1987). However, it should be noted that there is considerable individual variability in the degree of HPA axis activation. The increased plasma cortisol level is found in approximately 50% of patients with depression (Osran 1993), while 66% show non-suppression of cortisol to dexamethasone (Young, Carlson, Brown 2001).

Although adaptive in an acute crisis, chronic hypersecretion of CRH could lead to dysregulation of the HPA axis and account for the many symptoms seen in melancholic depression, such as dysphoria, hyperarousal, poor concentration, insomnia, anorexia, and decreased libido. Because infusion of CRH into laboratory animals has been shown to induce each of these symptoms, CRH is thought to play an etiologic role in depression rather than merely representing an epiphenomenon.

3.3 HPT axis

The HPT is a complex and integrated network whereby the hypothalamus releases thyroid releasing hormone (TRH) to stimulate the pituitary hormone thyrotropin (TSH) which regulates the production of thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). The thyroid hormones have an effect on a number of organism functions, which range from behavioural changes, growth effects, muscle myopathy and gastrointestinal function.

The relation of the HPT axis to depression has been supported by numerous reports, owing to observations that symptoms common to depression, such as weakness, poor appetite, slowed speech and impaired memory also occur in hypothyroidism. Indeed, a frequent finding in most, but not all, recent studies is that a high number (up to 45% in one review (Arana, Zarzar, Baker, 1990)) of patients with depression exhibit a blunted response to TRH challenge tests. This characteristic has been reported to be associated with the chronicity of depression (Prange, Garbutt, Loosen, 1987). Another frequent and widely replicated finding is that of lower TSH and higher free T₄ levels in depressed patients compared to healthy controls (Unden, Ljunggren, Beck-Friis et al., 1988). Moreover, there is a suggestion from existing literature reports of a correlation between severity of depression and levels of T₄ (Baumgartner et al., 1988). Turnover studies using radiolabeled T₄ in a small group of depressed patients have also demonstrated that the daily production rate of T₄ is significantly increased compared to normal controls, by 30% (Kirkegaard, Kørner & Faber, 1990). These elevated levels of T₄ seem to follow clinical outcome of depression, with reduction of T₄ observed following recovery from depression (Kirkegaard & Faber, 1991).

3.4 HPGH

The HPGH axis is the third hormonal system which has attracted much attention in relation to depression, however, relative to the above two hormonal systems, where literature findings are quite consistent, evidence regarding the relation of HPGH axis to depression is not as clear.

Growth hormone (GH) release is controlled by two hypothalamic peptides, one being a releasing hormone and the other a release inhibiting factor (somatostatin). GH is secreted episodically throughout the 24 hours and shows a major secretory burst early at the onset of sleep, usually in association with the first non-REM phase or slow-wave sleep (Boyar, 1978). Growth hormone levels are affected by a variety of physiological and environmental factors, such as physical exercise, stress and hypoglycemia.

Neuropharmacological studies have demonstrated the complexity of the neural control of growth hormone secretion: evidence indicates that a number of pathways, such as the dopaminergic, noradrenergic and serotonergic, all increase growth hormone secretion either by increasing the hormone release factors or inhibiting somatostatin secretion.

There are a number of reports which indicate a normal basal growth hormone level in depression (Marchesi, Chiodera, DeFiri et al., 1990), however, mean GH levels (measured every 15 mins for 24 hours) have been shown to be reduced in patients with depression. Subsequent studies on this finding have reported that this reduction in mean GH is largely due to a decline of GH release during sleep and elevation of release during daytime in depressed patients, thereby creating a balance not reflected in a single measure of average level. Similarly, GH challenge tests (via the clonidine) have shown a decreased GH response among depressed patients (Krishnan, Manepalli, Ritchie et al., 1988).

CHAPTER 4

The Relationship Between Testosterone and Mood: Empirical studies

4.1 Introduction

While there has been a high level of research activity in how hormones affect women's moods, especially during pre- and post-menopause, relatively little is known about the relation between hormones and mood in men.

Studies of the relationship between depression in men and testosterone have been prompted by several experimental and clinical observations, for example: Animal studies demonstrate the diverse effects of testosterone receptors on several central nervous system neurotransmitters, including serotonin and dopamine (Fink and Sumner, 1997, & 1998). Similarly, there have been numerous observations pointing to similarities in

psychiatric symptomatology between depressed patients and hypogonadal patients. For example, hypogonadal patients have consistently report loss of energy, amotivation, loss of libido, low mood and high scores on depression scales (Burriss et al., 1992). These symptoms, among others, constitute the core symptoms of depression (APA, 1994). Moreover, testosterone replacement therapy is reported to be effective in improving many of these clinical features of androgen deficiency in adult men (Wang, Alexander, Berman, et al 1996).

This chapter will provide a review of the available evidence, exploring possible relationships between depression and testosterone in men, drawing from findings from two major sources of studies: the first type is the clinical trials of the psychiatric effect of various doses of testosterone administration on different populations, such as normal, hypogonadal and depressed. The second source is studies which have used non-interventional methods to investigate the relationship between testosterone and mood in normal men, hypogonadal men and patients with depression.

4. 2 Clinical trials

Research activity in the area of testosterone administration has been especially active in the last decade, particularly as a result of newer synthetic androgen compounds having been released in the market. Upon the realisation that testosterone is responsible for muscle build and reduction of fatigue and lethargy, its use, particularly at supraphysiologic doses, has increased remarkably over the last three decades (Pope and Katz, 1994). Likewise, the many and varied effects of testosterone, as well as recent

advances in synthetic preparations that are more user-friendly, have contributed to an increase of the clinical indications of testosterone, to include not only hypogonadism, but also HIV/AIDS (APA, 2000). With the surge of case studies and anecdotal evidence that testosterone produces feelings of well-being, increases euphoria and produces an overall improvement in mood, there have been a number of clinical studies which have systematically assessed its impact on mood in various populations: normal, hypogonadal and patients with depression. The parameters and brief summary of the outcome of these studies are listed in table 2.

4.2.1 Testosterone Administration – Healthy Men

Much of the data on the relationship of testosterone usage and mood in normal healthy men have been obtained from field, uncontrolled studies of professional athletes using large doses of testosterone. While results are inconsistent, majority of studies performed with healthy men demonstrate a potentially hypomanic-inducing effects of testosterone.

The effect of high dose testosterone administration on mood has been studied in recent placebo-controlled, randomised studies in healthy, normal men. Bhasin, Storer, Berman, et al (1996) randomised 43 male athletes into four groups: placebo, placebo plus exercise, testosterone and testosterone plus exercise. The treatment arms included administration of testosterone, 600 mg for 10 weeks. These authors reported no difference in any mood parameters or behaviour between the placebo and treatment group at the end of the treatment period. Similarly, Anderson, Bancroft and Wu (1992) administered 200 mg of

testosterone to 16 men and found no evidence of alteration of mood or behaviour compared to the placebo group.

In a double-blind, placebo-controlled crossover study of two doses of methyltestosterone (40 mg and 240 mg), Su, Pagliaro, Schmidt et al., (1993) reported increases in euphoria, energy, mood swings and irritability in most of their 20 normal subjects. In addition, these authors reported a testosterone-induced acute manic episode in one of their patients: there were no predictors (e.g. personal and/or family history of psychiatric disorder or substance abuse) related to the symptom changes. The manic episode commenced on the last day of treatment and subsided three days after testosterone administration had ceased.

Yates, Perry, MacIndoe, et al (1999) studied the effects of different doses of testosterone administration (100 mg, 250 mg and 500mg) on mood parameters such as depression, aggression and mania and found no dose-dependent effect of testosterone on any of the mood and behaviour measures studied. Notably, these authors also observed hypomanic symptoms in one of their 18 subjects. Similarly, Pope (2000) found a marked increase in manic scores in 2 out of 50 subjects following a period of 6 weeks of testosterone administration of doses of up to 600 mg.

Pope and Katz (1994) compared psychiatric diagnoses (using the DSM-III) of 88 athletes who were using steroids with 68 nonusers, and found that mood disorders, such as manic and hypomanic episodes as well as depression, were significantly associated with steroid use. Specifically, the presence of a manic episode was diagnosed in 5% of the users and

in none of the non-users, hypomanic episode was detected in 10% of the users and in none of the non-users and major depression was present in 13% of the users compared to 4% of the nonusers.

4.2.2 Testosterone Administration - Hypogonadal Men

Data from studies on androgen treatment of hypogonadal men have suggested mostly positive, or, at worst, neutral effects of testosterone administration on mood and behaviour. Burriss et al. (1992) compared mood parameters (depression, anger, fatigue and confusion) between hypogonadal, infertile and normal men before and after testosterone replacement. They found that untreated hypogonadal men reported significantly higher ratings of depression, anger, fatigue, and confusion than did normal men. During testosterone replacement these mood scores decreased. This observation led the authors to conclude that testosterone treatment had some capacity to improve mood-related symptoms in hypogonadal men.

Wang et al., (1996) studied the effects of testosterone replacement therapy in 51 hypogonadal men. The total treatment period was of 60 days duration. These authors assessed various mood parameters (e.g. energy, anger, nervousness, sadness) before the administration of testosterone, as well as on days 21, 41 and 60 of treatment. The results showed improvement in a number of mood parameters, such as energy and friendliness and decrease in nervousness, anger and irritability. Notably, the improvements in mood took place in the first 21 days of treatment, and prolonged treatment with testosterone maintained, but did not further improve these mood changes. Similarly, a direct

correlation of testosterone levels and ratings on mood parameters was only seen at baseline testing (before testosterone replacement therapy), when serum testosterone levels were subnormal. The authors concluded that in hypogonadal men there is an association between mood and testosterone only when testosterone is below the normal range: once an adequate level of testosterone have been achieved by replacement therapy, subsequent increases of serum testosterone do not further contribute to improvement in mood parameters.

Similar results were reported by the same group of authors in a more recent study (Wang, et al., 2000). Namely, it was found that testosterone administration in 227 hypogonadal men increased positive mood parameters, such as sense of well being and energy, and decreased aspects of negative mood, such as irritability and sadness. Concordant to their previous findings, the improvements in mood reached maximum by day 30 and maintained a steady level thereafter.

In addition, the relationship of testosterone use with depression measures is demonstrated by another study: Arver, Dobs, Meikle et al. (1997) conducted an open-label study involving 37 male hypogonadal subjects (age range: 21 – 61 yrs). These authors administered a battery of psychological scales on various phases of the study: at baseline, to evaluate patients' current testosterone replacement therapy, at washout from patients' current therapy, and after commencement with the study testosterone. The results suggested that depressive symptoms, as measured by BDI (higher scores indicate higher severity of depression), changed in relation to testosterone treatment, that is, increasing

during withdrawal from testosterone treatment and decreasing with re-commencement of study testosterone treatment.

O'Connor et al. (2002) compared the efficacy of testosterone in improving mood in 30 normal and 8 hypogonadal men. The normal men were randomised to receive either 200 mg of testosterone weekly for 8 weeks or 200 mg sodium chloride weekly for 8 weeks. All of the hypogonadal men received 200 mg of testosterone biweekly for 8 weeks. The results showed significant reductions in negative mood by week 2 in response to testosterone treatment in the hypogonadal group.

In summary, findings from testosterone administration studies in hypogonadal men demonstrate a mostly positive effect of testosterone on mood. In particular, it appears that maximal mood elevating effects are observed within two to three weeks following commencement of therapy, coinciding with the time it takes to correct testosterone deficiency and achieve levels that are in the low to normal in the normal range. These observations thus provide reinforcement for the hypotheses that mood is closely dependent on testosterone.

Table 2. Main Testosterone administration studies (evaluating psychiatric symptoms)

Study	N	Population	Age	Results	Compound Type & dosage	Study type
Anderson, et al 1992	31	Healthy	21 – 41	<ul style="list-style-type: none"> ▪ No change in mood ▪ Improvement of sexual function 	T enanthate 200 mg	Randomised, placebo controlled, single blind
Arver, et al 1997	37	Hypogonadal	21 – 65	<ul style="list-style-type: none"> ▪ Improvement of sexual function ▪ Improvement of depressive symptoms 	T enanthate 200 mg	Open Label
Burris, et al 1992	28	Hypogonadal vs. infertile vs. normal	25 - 54	<ul style="list-style-type: none"> ▪ Improvement in mood parameters: depression, anger and fatigue ▪ Increase in sexual interest 	T enanthate 200 mg	Open label
Grinspoon et al. (1998)	51	HIV	42	<ul style="list-style-type: none"> ▪ Increase in weight ▪ Improvement of mood and quality of life 	T enanthate, 300 mg	Randomized, placebo-controlled, double-blind

Table 2. (cont.)

Study	N	Population	Age	Results	Compound Type & dosage	Study type
Grinspoon et al. (2000)	52	HIV	41.6	<ul style="list-style-type: none"> ▪ Increase in weight ▪ Improvement in mood 	T enanthate, 300 mg	Randomized, placebo-controlled, double-blind
Itil, et al (1984)	52	Depression	42.7	<ul style="list-style-type: none"> ▪ Improvement of depression, ▪ No difference between T and placebo 	Mesterolone 300 – 450 mg	Randomised, placebo controlled, double blind
Itil, (1978)	17	Depression	24 - 60	<ul style="list-style-type: none"> ▪ Improvement of anxiety ▪ Increase in libido and sexual desire 	Mesterolone 75 mg	Randomised, placebo controlled, double blind
O'Connor et al. (2002)	38	Healthy vs. Hypogonadal	19 - 45	<ul style="list-style-type: none"> ▪ Reductions in negative mood parameters (tension, anger, fatigue) 	T enanthate 200 mg	Randomised, placebo controlled, double blind
Orengo et al. (2005)	18	Hypogonadal vs. depressed		<ul style="list-style-type: none"> ▪ Improvement in depressive symptoms 	T gel 5 mg	Randomised, placebo, cross-over
Pope et al. (1994)	160	Athletes (users/non-users)	25.5/28.3	<ul style="list-style-type: none"> ▪ Higher percentage of psychiatric problems among T users 	T enanthate	Observational

Table 2. (cont.)

Study	N	Population	Age	Results	Compound Type & dosage	Study type
Pope et al. (2000)	56	Healthy	46.5	<ul style="list-style-type: none"> ▪ Increase in ratings of manic scores 	T cypionate 150 – 600 mg	Randomised, placebo, cross-over
Pope et al. (2003)	22	Depression	49	<ul style="list-style-type: none"> ▪ Improvement of depression 	T gel 10 g	Randomised, placebo
Rabkin et al. (1999)	124	HIV	41/41	<ul style="list-style-type: none"> ▪ Improvement of depression ▪ Improvement in energy 	400 mg	Open-label
Rabkin et al. (2000)	74	HIV	38.1/40.1	<ul style="list-style-type: none"> ▪ Improvement of depression ▪ Improvement in energy ▪ Weight increase 	400 mg	Randomised double-blind
Seidman et al. (1998)	5	Hypogonadal with depression	40	<ul style="list-style-type: none"> ▪ Improvement of depression 	T enanthate 400 mg	Open label, followed by single-blind discontinuation phase
Seidman et al (2001)	40	Hypogonadal with depression	52	<ul style="list-style-type: none"> ▪ Improvement of depression, but no difference between T and placebo ▪ Improvement in sexual function 	T enanthate 200 mg	Randomised, placebo controlled, double blind

Table 2. (cont.)

Study	N	Population	Age	Results	Compound Type & dosage	Study type
Su et al. (1993)	20	Healthy	18 - 42	<ul style="list-style-type: none"> Increases in energy, euphoria, sexual arousal & irritability 	Methyltestosterone 40/240 mg	Double-blind, placebo controlled, cross-over
Wang et al. (1996)	51	Hypogonadal	22 - 60	<ul style="list-style-type: none"> Improvement of mood: increase of sense of well-being, decrease in nervousness, tiredness and irritability 	T cyclodextrin 2.5/5 mg	Randomised, placebo, double blind (dose)
Wang et al. (2000)	227	Hypogonadal	19 - 68	<ul style="list-style-type: none"> Improvement in mood parameters: depression, anger and fatigue Increase in sexual function 	T gel or T patch 50/100 mg (gel)/5 mg (patch)	Randomised, placebo, double blind (dose)
Yates et al. (1999)	42	Healthy	21 - 40	<ul style="list-style-type: none"> Minimal effects on mood No change in sexual functioning 	T cypionate 100/250/500 mg	Randomised, placebo controlled, double blind

A number of studies had not reported on the mean age of the participants, therefore, the age range only is reported in the above table

4.2.3 Testosterone Administration - HIV infected men

The assessment of the efficacy of testosterone in treating clinical symptoms in patients with HIV is a relatively new area of clinical inquiry, which has dramatically increased over the last decade. Endocrine dysfunctions are usually common in HIV patients, with testosterone deficiency being the most prevalent (Rabkin et al., 1999). Testosterone levels are generally normal in asymptomatic men, however, the prevalence rate of hypogonadism in men who are symptomatic ranges from 29 to 50% (Dobs, Dempsey, Ladenson et al., 1988; Raffi, Brisseau, Planchon et al., 1991). Thus, the principal reason which has prompted clinical trials of testosterone administration in HIV infected patients has been the high prevalence of hypogonadism in this population, as well as the treatment of wasting and weight loss.

There have been several clinical trials of testosterone administration in hypogonadal, HIV infected men which have also included change in mood as an outcome measure, these are summarised in Table 2.

Rabkin et al. (1999) conducted a clinical trial of 8-week, open treatment with 400 mg of biweekly intramuscular testosterone in 124 HIV-infected men (mean age 41 yrs). These authors reported remarkable improvement in depression: of the 34 patients who were diagnosed with depression, 79% reported significant improvement in mood (as assessed by the HDRS) and energy levels at the end of the trial.

In a later study, the same authors performed a 6-week, double-blind, placebo controlled trial of testosterone in 74 HIV infected men (Rabkin, Wagner, Rabkin, 2000). This study found significant improvement in mood: of the 26 patients who were diagnosed with depression, significantly improved mood was found in 58% patients in the testosterone-treated, compared to 14% of the patients in the placebo group. Additionally, these authors reported significant improvement in energy and increase in weight at the end of the trial.

Similarly, in a randomized, placebo-controlled trial with 51 HIV-infected men, (Grinspoon, Corcoran, Askari et al. 1998) administered 300 mg testosterone intramuscularly every three weeks for six months. These authors found that patients reported subjective benefits with respect to improved overall quality of life, appearance, and well being. Similarly, muscle and lean body mass increased significantly in testosterone-treated relative to placebo-treated patients, by approximately 2 to 3 kg.

Grinspoon, Corkoran, Stanley et al. (2000) performed another study of 300 mg testosterone administration every three weeks for six months in 52 hypogonadal HIV-infected men. The results showed a negative relationship between free testosterone and depression scores (as measured by the BDI) on baseline. At the end of the trial, it was reported that mood improved significantly in the patients receiving testosterone, but not in the group receiving placebo. Moreover, based on results of the regression analysis, these authors concluded that the improvement in mood was mostly attributable to increase in weight, with this factor contributing 35% in the variability of BDI scores in the regression model.

4.2.4 Testosterone Administration - Male patients with Depression

Largely based on findings that testosterone has mood-elevating in normal men and antidepressant properties in hypogonadal and HIV patients, attempts have been made to evaluate testosterone's antidepressant effects in patients with depression. However, owing to ethical and methodological reasons, the number of these studies has been relatively small. Although the results are somewhat inconsistent, the majority of the studies do seem to demonstrate an efficacious result when testosterone is administered in conjunction with a standard antidepressant.

In a 6-week, randomized, placebo-controlled trial, Seidman, Spatz, Rizzo et al., (2001) studied the efficacy of testosterone enanthate (200 mg) as antidepressant monotherapy in male patients with moderate (HDRS = 21) depression and low testosterone levels, defined as serum levels of 350 ng/dL or less. Thirteen patients were randomised into the treatment group and seventeen in the placebo group. The average age of the patients was 52 years. At the end of the treatment period, it was found that the depression score had improved for both groups, in fact, the response rate observed for patients who received placebo was slightly greater than the response rate for those receiving testosterone (41.2% versus 38.5%). Similarly, reduction in group mean HDRS score from baseline to endpoint was 10.1 in the testosterone group and 10.5 in the placebo group. These results suggest that testosterone supplementation may not be an efficacious treatment for moderate to severe depression in hypogonadal men in the absence of standard antidepressants. -

In an earlier study, Itil (1978) treated eleven male patients with depression with low doses of mesterolone (a synthetic androgen) (2-6 mg) and six additional depressed men with high doses (25 – 200 mg) of mesterolone. In both groups remission of depressive symptomatology was found in the first two weeks of treatment, whereas no other adverse effects were observed. In a later study with 52 patients (mean age = 42.7 yrs.) the same group of authors reported improvement of depressive symptoms with higher doses of mesterolone (300 – 450 mg), however, no statistically significant difference was observed between placebo and testosterone group (Itil, Michael, Shapiro et al., 1984). Similar results were reported in one of the most recent studies: in a cross-over design, randomising 18 treatment-resistant, depressed, hypogonadal men to either 5 mg gel or placebo, Orengo, Fullerton & Kunik (2005) reported a significant improvement in depressive symptoms from baseline to 12 weeks of testosterone treatment. However, these authors also reported that there were no significant differences between the placebo and testosterone treatment phases.

Vogel, Klaiber, Broverman (1985) compared the antidepressant effects of mesterolone and amitriptyline, (a standard antidepressant) in 34 male outpatients with chronic depression and found that after 12 weeks of treatment, mesterolone was as equally effective as amitriptyline in reducing depressive symptoms, but while amitriptyline caused a number of side effects (e.g. dizziness, and blurred vision), mesterolone did not induce any side effects.

Testosterone treatment has also been trialed as augmentative treatment for depression. In an uncontrolled clinical trial, Seidman and Rabkin (1998) treated five depressed men who had not responded to an adequate SSRI trial. Patients were maintained on their current SSRI regimen and treated in addition with intramuscular injections of testosterone enanthate, 400 mg, every 2 weeks for 8 weeks. Responders were offered 6 additional weeks of treatment, followed by a single-blind placebo discontinuation, in which they continued taking SSRI but received placebo instead of testosterone. At the end of the treatment period (initial 8 weeks) it was found that the mean depression score was significantly decreased from 19.2 to 4 (HDRS-21) and at the end of the optional treatment period three of four subjects who underwent discontinuation of testosterone began to relapse. Although the small sample size and the open-label design are major limitations of this study, these results support the use of testosterone supplementation therapy as augmentation therapy with SSRIs in men with treatment-resistant depression.

More recently, Pope-Cohan, Kanyama et al. (2003) conducted a 8-week randomized, placebo-controlled trial of testosterone transdermal gel with ten men aged 30–65 (mean age 46.9 yrs.) with refractory depression and low or borderline testosterone levels (350 ng/dL or less). Patients were allowed to continue with their existing antidepressant regimen. The results showed that patients receiving testosterone had significantly greater improvement in scores on the HDRS (mean baseline score = 22, mean post-treatment score = 13) than the placebo group. Moreover, testosterone seemed to be superior over placebo in reducing affective aspects of depression (such as depressed mood, guilt and anxiety) to nearly the same degree as the somatic symptoms (sleep, appetite and libido).

The authors concluded that testosterone gel may produce antidepressant effects in patients with depressive illness who have low testosterone levels.

4.2.5 Conclusions from Testosterone administration studies

The above studies demonstrate the effect of testosterone administration on improving various mood parameters, such as depressed mood, quality of life, energy levels, appetite and weight gain in hypogonadal and HIV-infected men. In addition, the mood-elevating properties of testosterone is reflected in studies in normal men which show the emergence of hypomanic symptoms.

4.3 Observational studies

There exist only few psychiatric studies which have assessed HPG axis functioning in men with depression. The main studies are summarised in Table 3. The majority of these studies employ a cross-sectional design, usually comparing hormone levels in men with depression to normal healthy controls. There are only few studies which employ a longitudinal approach, whereby comparisons are made between two times points: during illness and following recovery.

4.3.1 Cross-sectional studies

Levitt and Joffe (1988) compared total testosterone and free testosterone between 12 medication-free patients with depression (mean age = 31.9 yrs.) and 12 age-matched normal volunteers and found that while there was a trend in the depressed group for a

lower mean T (10% lower) and lower mean free T (20% lower) compared to controls, the trend was not statistically significant.

An earlier study, (Rubin, 1981) of nine patients with depression found 18 % lower circulating testosterone levels and 15-30 % lower circulating levels of LH and FSH as compared with six healthy controls. The results were not statistically significant. These authors reported the same finding nearly a decade later: there was no statistically significant difference in any of the gonadal hormones measured using a 24 hour sampling technique between 16 males with depression and 16 matched normal controls (Rubin, Polland and Lesser, 1989).

Similarly, Kaneda and Fujii (2002) reported no significant difference in levels of T, FSH and LH between 11 patients with depression (mean age = 61.9 yrs.) and 11 age-matched healthy controls. These authors also reported no correlation between levels of testosterone and measures of depression severity (as assessed by the Zung self-rating depression scale).

However, there a number of studies which have reported a relationship between testosterone and depression: Using a 24 hr multiple sampling method, (Schweiger, Deuschle, Weber et al., 1999) compared levels of T, FSH and LH between 15 patients with moderate to severe depression (mean age = 48 yrs) and 22 control subjects (mean age = 53 yrs) and found that, after adjustment for age, daytime testosterone, nighttime

testosterone as well as 24-hr mean testosterone secretion were significantly lower in the depressed group. These authors also reported a trend for a decreased LH pulse frequency in their patients with depression.

In an earlier study, the first study with a control group, Vogel, Klaiver, Broverman (1978) reported lower Total testosterone (4.48 vs 6.82 $\mu\text{g/L}$) in 27 outpatients with depression compared with 13 normal controls of similar age. Seidman, Araujo, Roose (2002) compared total testosterone in an elderly (mean age = 70.8 years) group of subjects: 32 with dysthymia, 13 with depression and 175 with no depression. Median testosterone levels were significantly lower in the group of patients with dysthymic disorder (295 ng/dl) compared to patients with depression (425 ng/dl) and no depression (423 ng/dl). In addition, the majority of the elderly men with dysthymic disorder had total testosterone levels in the hypogonadal range (≤ 300 ng/dl). As demonstrated by the observation that the dysthymic men had a duration of depressive illness of almost 15 years, compared with less than one year for the men with major depression, these authors propose that the chronicity of depressive illness leads to low testosterone levels, as one explanation of their finding.

Heuser (1998) compared 18 male inpatients with depression (mean age = 47) to 22 normal subjects who were matched for BMI (mean age = 53). Blood was collected every 30 minutes and comparisons performed for total testosterone levels and LH pulse frequency. These authors found that, after controlling for age, the depressed group had a

significantly lower mean total testosterone level, particularly during nighttime hours, as well as a (nonsignificant) trend toward lower LH pulse frequency ($p < 0.07$).

Comparably, Yesavage, Davidson, Widrow et al., (1985) found a strong negative correlation ($r = -0.67$) between severity of depression and total testosterone levels in 18 medication-free men with depression. Likewise, Davies, Harris, Thomas et al., (1992) reported a significant negative correlation between FT and depression severity in eleven patients with depression.

Seidman, Araujo, Roose et al., (2001) studied the relationship between total testosterone, androgen receptor (AR) CAG repeat length (CAG RL) and depression in an older group (mean age = 62.6 yrs.) of 1000 subjects. They identified 110 men with depression as defined by a cut-off score of 16 or more on the Centre for Epidemiologic Studies – Depression scale. While it was reported that neither total testosterone nor CAG RL were associated with depression, subsequent stratification of data showed that depression was significantly and inversely associated with total T in men with shorter CAG RLs but not in men with moderate and longer CAG RLs. These authors concluded that CAG isotype may mediate the expression of the central nervous system effects of testosterone deficiency in men.

Table 3. Observational studies assessing the relationship between depression and testosterone

Study	Number P/C*	Mean age P/C	Depression scale (mean score)	Med. Free	Results	Hormones studied	Sampling period
Cross-sectional studies							
Barret-Connor, et al, (1999)	856/0	70.2	BDI (= 4.5)	No	Negative correlation between BDI and T and BDI and bioavailable T	T, bioav. T	Once (am)
Davies et al. (1992)	11/10	52.2	HDRS (= 29.5)	No	No difference in T between control and depressed group Negative correlation between T and HDRS	Salivary T	7 x (pm)
Delhez et al. (2003)	153/0	59.9	Caroll Rating Scale (= 7.8)	Yes	Negative correlation between free T and depression severity	T, Free T, SHBG	Once (am)
Kaneda et al. (2002)	11/11	61.6/61.9	Zung (= 46.2)	Yes	No difference	T, LH, FSH	Once (midday)

Table 3. (cont.)

Study	Number P/C*	Mean age P/C	Depression scale (mean score)	Med. Free	Results	Hormones studied	Sampling period
Levitt et al. (1988)	12/12	31.9/31.4	HDRS (= 22.2)	Yes	No correlation of T with HDRS A trend for lower T and FT in patients	T, FT	Once (pm)
Mason et al. (1987) Table 3. (cont.)	12/23	34.5	HDRS	No	Lower T in depression	T	Once (am)
Rubin et al. (1989)	16/16	39.5	HDRS (= 28)	Yes	No differences in any hormones	T, LH, FSH	24-hour
Schweiger et al. (1999)	15/22	48/53	HDRS (= 30)	Yes	Lower T in depressed patients	T, LH, FSH	24-hour
Seidman et al. (2002)	32/15/175	70.5/67/70.8	HDRS(=14.5 dysthymia, 20.9 depression)	No	Lower T in dysthymic group	T	Once
Tsujimura et al. (2003)	130/0	42	modified scale (?)	No	No correlation	T, FT, SHBG, LH, FSH	Once (am)
Vogel et al. (1978)	27/13	39.5/38	(?)	No	Difference in T between depressed and control	T	Once (am)

Table 3. (cont.)

Study	Number P/C*	Mean age P/C	Depression scale (mean score)	Med. Free	Results	Hormones studied	Sampling period
Yesawage et al. (1985)	18/0	?	HDRS (?)	Yes	Sign. Correlation between T and HDRS (-0.67)	T	3 x (am)

Longitudinal studies

Study	Number P/C*	Mean age P/C	Depression scale (mean score)	Med. Free	Results	Hormones studied	Sampling period
Cooper et al. (1989)	14/0	52.4	HDRS (=31.8)	No	No change in T No correlation with HDRS	T, LH, FSH	Once (am)
Sachar et al. (1973)	15/0	61.5	DSI (?)	Yes	No change	T (serum & saliva)	Once (am)
Steiger et al. (1991)	12/0	46.4	HDRS (= 27.7)	Yes	Increase of T in remission	T	24-hour

* P/C = Patient/Control

(?) = score not reported

HDRS = Hamilton Depression Rating Scale

DSI = Depression Symptom Inventory

CPRS = Comprehensive Psychopathological Rating Scale

The notion of a relationship between testosterone and depression is strengthened by evidence derived from studies examining hypogonadal men: Shores, Sloan, Matsumoto, et al. (2004) compared incidence rates of depression between eugonadal and hypogonadal men in a 2 year follow-up study involving 278 men aged 45 years and older. In this prospective study, Shores, Sloan, Matsumoto, et al. (2004) found that hypogonadal men with total testosterone levels of 200 ng/dL or less showed an approximate 4-fold increase in the risk of incident depression.

Delhez, Hansenne and Legros (2003) compared levels of free testosterone, LH, FSH and SHBG between 107 hypogonadal men (mean age 60.9 yrs.) and eugonadal men (mean age 57.9 yrs.) in relation to severity of depression (as assessed by the Carroll Rating Scale) and found a negative correlation between free testosterone and depression severity in the hypogonadal group. No significant relationships were reported for the other HPG hormones studied.

There have also been clinical case reports that patients treated with leuprolide (Gonadotropin-releasing hormone analog - inhibiting the production of testosterone) have developed depression (Rosenblatt and Mellow, 1995). In addition, in the first of its kind, Schmidt, Berlin, Danaceau et al. (2004) studied the effects of a pharmacologically-induced hypogonadism in healthy men by administering Lupron (GnRH antagonist). Their results indicated that short-term hypogonadism is sufficient to precipitate depressive symptoms in a small minority of younger men.

4.3.2 Longitudinal studies

Most of the studies discussed above have been cross sectional, studying depressed patients only during an episode of major depression. However, it is reasonable to ask whether the observed differences persist throughout an episode or into clinical remission. Such data can provide valuable information regarding HPG dysfunction in depression, and particularly, whether this dysfunction changes with clinical state.

However, owing to the methodological issues of these studies, their number has been relatively low. Steiger, von Bardeleben, Wiedemann et al., 1991, investigated nocturnal total testosterone levels in 12 medication-free men with depression during hospitalisation and again after stable remission from depression had been achieved. It was found that testosterone concentration was higher upon remission compared to while patients were hospitalised.

Other longitudinal studies have found no change in testosterone as a function of remission from depression: While Mason, Giller & Kosten, (1988) found lower testosterone levels in patients with depression compared to patients with other psychiatric diagnoses, these authors reported no change in total testosterone between illness and recovery in their seven patients with depression. In the same study, these authors also compared patients with depression to patients with schizophrenia, and reported significantly higher levels of testosterone in schizophrenia compared to patients with depression.

An earlier study by Sachar, Halpern, Rosenfeld et al. (1973) compared total testosterone in 15 elderly (mean age = 63 yrs), medication-free men during depression and after recovery and found no difference in testosterone levels as a function of the change in severity of depression as assessed by the Depression Symptom Inventory.

Finally, Cooper, Finlayson, Velamoor et al., (1989) examined total testosterone, LH and FSH in 14 men with depression (mean age 52.4 yrs) before and after receiving a course of ECT. It was reported that LH levels were significantly higher after each individual session of ECT, while changes in testosterone and FSH were not significant. These authors did not find a relationship between the clinical status before and after the ECT course and hormone changes.

4.3.3 Epidemiological Studies

There have been three large epidemiological studies which have assessed testosterone levels and depressive symptoms: The Massachusetts male Aging Study (MMAS) (Araujo, Durante, Feldman et al., 1998), the Rancho Bernardo Study (Barret-Connor, Von Muhlen, Kritz-Silverstein, 1999) and the Veterans Experience Study (VES) (Booth et al., 1999).

The MMAS was a cross-sectional, population-based survey of 1709 men aged between 40 and 70 years. Depressive symptoms, as measured by the Centre for Epidemiological Studies-Depression (CES-D) scale were not correlated with testosterone levels (Araujo, et al., 1998).

Barret-Connor et al., (1999) conducted the Rancho Bernardo study, which examined the association between fasting bioavailable and total testosterone levels and depressed mood in 856 older men (age range 50 – 89, mean age 70.2 years). These authors found that bioavailable testosterone levels were significantly and inversely correlated with BDI scores, independent of age, weight change or physical activity. Furthermore, these authors noted that bioavailable testosterone levels were 17% lower in the patients who were categorically categorized as ‘depressed’ (as defined as BDI score of 13 or more).

In the largest published study to date, Booth et al. (1999) studied the relationship between testosterone levels and depression in 4393 Vietnam veterans aged 33 – 42 years. The measure of depression was from the Diagnostic Interview Schedule items designed to assess depression as defined by the Diagnostic and Statistical Manual of Mental Disorders (3rd edition). Measures of other psychosocial variables, such as antisocial behaviour, risk behaviour and protective factors (marriage and employment) were also conducted. The results showed a curvilinear relationship between testosterone and depression. Testosterone level was negatively correlated with depression in men with testosterone levels below 590 ng/dL. Measures of psychosocial factors did not affect this relationship. Among men with testosterone levels above 590 ng/dL, these authors observed a positive relationship between testosterone and depression. However, this relationship disappeared after adjusting for psychosocial variables (antisocial behaviour, risk behaviour and protective factors). The authors speculate that the practice of antisocial

and risk behaviour, unemployment and single marital status increases the likelihood of depression in men with above average or high testosterone levels.

4.3 General conclusion and aims and objectives of the current section:

Examination of a relationship between the HPG axis and depression is a difficult process, particularly regarding the complexity of the HPG axis and the multifaceted nature of depression. Inherent in the difficulty of pursuing this examination are the realisation that a number of factors are potentially involved in affecting both the HPG axis and depression and that disagreements between the small number of studies performed in this field are prominent. Methodological differences among studies may partly explain the inconsistencies in results. For example:

1. Definition and measurement scale used for depression has varied greatly among prior studies. Although the HDRS is used often to quantify depressive symptoms, some studies decide on the use of other measurement scales, thus rendering it difficult to conceptualise the degree of severity in depression. This is particularly true in testosterone administration studies, where the categories of mood are poorly defined, as well as in longitudinal studies, where it is difficult to obtain an insight into the degree of change in mood.
2. Similarly, the choice of recruitment method of patients in most studies is questionable, as some methods used in previous studies are susceptible to self-selection bias.
3. In addition, as most studies have been cross-sectional, they can not provide answers as to whether changes in the HPG axis measures are paralleled by changes in depression outcome.

4. Furthermore, the number of patients studied in most observational studies is frequently small (in the order of 10 to 20), thereby increasing variability and increasing the chance of committing a Type II error rate, i.e. not having enough power to detect a significant difference.

5. Finally, most studies have only measured testosterone levels (free, total or both) and have largely neglected to examine other measures of the HPG axis, namely LH, FSH, as well as free T and SHBG. Neglect to do so has resulted in even more scarce evidence regarding the interrelationships between hormones and depression. Given the close connection and interplay between testosterone and gonadotropins as well as testosterone and SHBG, such studies are not as informative and as descriptive as studies which have examined the HPG axis as a unit and measured LH, FSH and SHBG in conjunction to testosterone. In particular, obtaining measurements of these additional parameters frequently provides indication regarding which component of the HPG axis might be particularly affected in depression.

By the same token, researchers have largely used only limited parameters of depression, i.e either the presence or absence of depression, or severity of depression, as measured by conventional depression scales. Other aspects of depression, such as duration of episode, and their relationship with the HPG axis have been largely unresearched. It is not clear when (if at all) the abnormalities in HPG axis described above develop in the course of

depressive illness. One indicator of this might be to assess the relationship between duration of episode and HPG parameters.

Nevertheless, the available evidence, albeit inconsistent to a degree, suggests a connection between depression and the HPG axis which needs to be further examined.

We performed the following study, taking into account the above-mentioned methodological considerations. The aim was threefold:

- 1) To assess the difference in HPG axis hormones between patients with depression and patients with other psychiatric diagnoses,
- 2) To investigate the interrelationships between hormones and other clinical and demographic variables in patients with depression, and
- 3) To compare HPG parameter measures between remitters and non-remitters and to examine whether any changes in clinical outcome of depression is accompanied by a change in any of the HPG measures.

CHAPTER 5

Method

5.1 Study setting

The study was conducted at The Northside Clinic, a private psychiatric hospital located centrally on the lower north shore of Sydney. The hospital is a 94 bed (98% occupancy rate) tertiary referral center and teaching hospital of The University of Sydney, which provides specialist diagnosis and hospital-based treatment for psychiatric disorders, specialising in mood disorders and eating disorders. The Northside Clinic receives referrals from all areas of Sydney as well as metropolitan and rural areas of NSW.

5.2 Participants

Participants were 77 male patients admitted to The Northside Clinic for psychiatric care during the period on June 2001 to January 2003. Fifty two were diagnosed with depression and twenty five with other psychiatric disorder. Diagnostic information of the subjects is listed in Appendix I. While we did not include a group of participants comprising of healthy, unhospitalised males, the choice for including a group of males diagnosed with psychiatric disorder other than Major Depressive Disorder was based on the rationale that our comparison group of subjects controls for a number of important variables which may influence testosterone levels. These include: administration of

psychotropic medication, environmental surroundings (being admitted to hospital and being away from home) and the presence of mental illness.

Patients' admission records (and previous hospital files – if applicable) were checked upon their admission into the hospital to ascertain suitability for participation. Patients had no current or past history of major medical disorder as determined by a physical examination and routine blood examination. Other criteria for selection included: no self-reported chronic illness (cancer, coronary heart disease, hypertension or diabetes); no self-reported prostatic hypertrophy or history of prostate surgery. If needed, patients were allowed to settle in the hospital environment for a few days, before being approached by the investigator. All testing was performed within 5 days of admission. Demographic and clinical data are summarized in Table 6.

5.3 Psychiatric assessment

Following enrollment into the study, patients completed the relevant questionnaires. Some demographic and clinical information (such as admission date, marital status, number of children, smoking behaviour, previous medications) was collected from self-reports and hospital files. Patients were classified into two major diagnostic groups according to criteria of DSM-IV using the Composite International Diagnostic Interview (CIDI) (WHO, 1997), i.e. into depressed (Group 1) or other psychiatric illness (Group 2).

The following modules of the CIDI were applied: depression, somatoform disorders, anxiety disorders, mania and psychosis. Each session took approximately 40 minutes to complete. Detailed role of the author's contribution to the methodology of this project is provided in Appendix II.

5.4 Medications

The patients remained on their scheduled medication regimen at the time of testing. All were taking either one or a combination of psychotropic medications. Thirty patients (57%) from Group 1 and eleven patients (44%) from Group 2 were receiving a combination of two or more medications (combination also includes prn hypnotics and other agents). A list of the medications with which the patients were treated is included in Appendix III.

The decision to enroll patients while on medication was determined by the design of our study: we chose to perform a naturalistic study whereby patient's outcome is observed without any modification of their treatment. The decision to enroll patients while on medication was also based on ethical reasons: being a tertiary referral clinic, The Northside Clinic admits patients with very severe depression, thus it is deemed unethical to modify or cease patient treatment for entirely research purposes.

5.5 Activities and Meals

All patients were ambulatory during their hospital stay. The Northside Clinic is equipped with a gymnasium, which patients had access to for up to 2 hours a day. While inpatients, most study participants also chose to take part in other activities that the clinic had to offer, such as watching movies, playing board games or participating in art classes. Furthermore, most patients consumed standard meals prepared by the hospital. The

patients ate three meals per day, with breakfast at about 8.30 am, lunch about 1pm and dinner at 7pm.

5.6 Longitudinal section: Follow-up

Follow-up visits were repeated in patients with depression at 3 – 6 months following discharge. At this visit clinical assessments and measurement (HDRS, BSFI), as well as blood sampling was repeated. As depressed men, in contrast to depressed women, are more likely to lose weight (Frank, Carpenter and Kupfer, 1988) weight measurements were repeated on this visit. There were no differences in weight on follow-up compared to admission. In order to minimise the rate of lost to follow-up, two methods were employed: 1) patients received a courtesy telephone call approximately 2 months following discharge and reminded of their upcoming follow-up visit; 2) patients were sent a reminder letter approximately 2 weeks before their follow-up visit. Thirty-nine out of the 52 (75%) patients with depression returned to the clinic for the follow-up visit. This rate is relatively high for psychiatric studies involving phlebotomy and a restricted window for interview (morning blood sampling). Regarding the reasons which contributed to patients not attending the follow-up visit, seven patients were unable to be contacted, four indicated that they no longer wished to participate in the study and two had been admitted to another hospital.

The assessments which were used in the follow-up visit were: HDRS, BSFI and blood sampling. At the follow-up visit, based on their HDRS score, patients were categorised into remitters (defined as a HDRS score of 7 or below) or non-remitters (HDRS of 8 or

above). A cut-off score of 7 on the HDRS was used as a defining characteristic of remitters as this has been a standard procedure in the majority of basic and clinical research studies in psychiatry.

There were no statistically significant differences in age, BMI and basal hormone levels between patients who agreed to be seen at follow-up and those who declined. The average time between discharge and follow-up was 123 days (SD = 34.89). There was no relationship between the length of time following discharge and the follow-up clinical and hormonal assessment.

5.7 Instruments

The following questionnaires were used in the study:

1. Hamilton Depression Rating Scale
2. Brief Sexual Function Inventory

1. Hamilton Depression Rating Scale

The 21-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1967), used to measure severity of depressive symptoms, is one of the most widely used instrument in psychiatric clinical research studies (a copy of this questionnaire is included as Appendix IV). It is a clinician-rated questionnaire, consisting of 21 items, where higher scores indicate higher severity in symptoms. Some of the questions are rated on the basis of the observable behaviour during the interview and some on the basis of direct questioning of the patient.

Questions are designed to reflect the symptoms related to depression, such as depressed mood, guilty feelings, suicide, sleep disturbances, anxiety levels and weight loss. Since its initial publication, it has generally been used as an outcome measure in clinical studies as well as serving as the criterion for validating and standardising new instruments. Most of the items are rated on a five-point scale reflecting symptom severity, with 0 indicating absence of symptoms and 4 being generally reserved for extreme manifestation of symptoms. Inter-rater reliability is very high, ranging from 0.87 to 0.98 (Thompson 1989). As well as being adapted for use across many different languages and cultures (Fava, 1982; Furukawa, Streiner, Azuma et al., 2005), the HDRS has been shown to be reliable instrument for measuring depressive symptoms across broad age ranges (Moberg, Lazarus, Mesholam et al., 2001) and clinical diagnoses, such as alcoholism, Parkinson's disease and schizophrenia (Hedlung and Vieweg, 1979).

Since its initial publication, the HDRS has been used extensively in clinical and research practice, particularly in clinical trials. Although the HDRS serves a primary function of measuring severity of depression, it is also frequently used as a guideline to define clinical outcome of depression. In practice, majority of researchers have adopted a score of 7 or less as a defining criterion of remission. In addition, total scores of the HDRS are frequently used to categorically define severity of depression, such that: a score of 10-13 indicates mild depression; a score of 14-17 indicates moderate depression; a score of 17 or higher indicates moderate to severe depression.

2. Brief Sexual Function Inventory

The Brief Sexual Function Inventory (BSFI) (O’Leary, Fowler, Lenderking et al., 1995) was used to measure sexual function (a copy of this questionnaire is included as Appendix V). The BSFI is a self-report questionnaire consisting of 11 items, each rated on a Likert scale from 0 to 4 (lower score indicating higher dysfunction). The BSFI covers the key dimensions of male sexual function: sexual drive, erection, ejaculation, perception of problem in each area and overall satisfaction. The authors have specifically recommended that due to the multi-dimensional nature of sexual function, a total score on this instrument is not viable. Therefore, each of the dimensions need to be considered separately. The BSFI consists of questions selected from a number of sexual function questionnaires, including one for depression (Reynolds, Frank, Thase et al, 1988). It is especially useful for patients with depression in a hospital setting as it is parsimonious and takes on average 10 mins to complete.

5.8 Blood Sampling

Blood samples were taken to measure Total T, Free T, LH, FSH and SHBG. In order to standardise blood collection times, blood sampling was performed by the investigator or a nurse between the hours of 8.30 and 10.30 am. Blood was drawn from the antecubital vein into a SSD tube, allowed to stand vertically for about 30 mins at room temperature and centrifuged at 2920 rpm for 10 mins (Spintron, Pty. Ltd.). Aliquots of serum were pipetted and stored in a freezer at -20°C. Serum was then transported to Douglas Hanly Moir Pathology for assaying.

5.9 Hormone Assays

Total testosterone, LH and FSH were measured by Bayer Centaur automated chemiluminescence methodology; SHBG was measured by Immulite 2000 chemiluminescence, while free T was measured by radioimmunoassay kits. The intra- and interassay coefficients of variation are listed in Table 4.

Table 4 Intra- and interassay coefficients of variation for hormones*

ASSAY	Mean	Within run CV	Total CV
LH	(IU/L)	%	%
	5.2	3.1	4.1
	40.1	2.1	3.3
	76.5	2.5	3.4
FSH	(IU/L)		
	5.6	4.2	4.6
	25	2.9	3.6
	78.1	3.3	4.1
Total T	Nmol/L		
	2.9	4.5	8
	16.9	2.4	4.3
	34.5	2.1	3.6
Free T	Pmol/L		
	1.2	8.3	12
	8.9	8.5	6
	20	8	5.5
SHBG	Nmol/L		
	4.7	2.3	4
	21	2.5	5.2
	63	2.7	5.2

* source: Douglas Hanly Moir Pathology; coefficients, Feb. 2003.

5.10 Statistical Analysis

Shapiro-Wilks's W test was used to analyze hormone data for normality. The W statistics was significant for SHBG, LH and FSH, therefore these variables needed to be transformed before parametric statistical analyses could be applied (Hutcheson and Sofroniou, 1999). Logarithmic transformation of SHBG and LH, and inverse transformation for FSH produced normally distributed curves and non-significant W statistic. For these variables, transformed values were used in statistical procedures, while raw values were used for graphical and descriptive purposes.

For continuous analyses of comparisons, Levene's test for homogeneity of variance was used to test whether groups had equal variances. As results from these comparisons may be unreliable when the two samples are unequal in size and also have unequal variances (Toothacker, 1993), significant F statistic obtained from the Levene's test of equality of variance indicates that the variances are unequal. As homogeneity of variance is a primary assumption of parametric tests, violation of this assumption would prompt the application of comparable non-parametric analyses.

Comparisons between Group 1 and Group 2

Comparisons between the two main diagnostic groups were made using one-way Analysis of Variance (ANOVA) for continuous variables and χ^2 for categorical variables. Analysis of covariance (ANCOVA) was used to separate the effects of potentially confounding variables such as age and BMI.

Interrelationships of hormonal, clinical and demographic data

Bivariate Pearson's coefficient of correlation was used to evaluate interrelationships between variables, while partial correlation coefficients were calculated in order to control for appropriate variables as needed. To examine the correlation between dichotomous and continuous variables Point Biserial correlation coefficient was used (with the presence of a particular factor coded as "1" and the absence coded as "0").

Due to the high number of correlation coefficients calculated, maintaining a significance level at 0.05 would have inflated the Type I error rate. Statisticians have argued that on the rationale that the more coefficients to be tested, the more conservative the test should be, significance cut-off level should be set at $.05/C$ (where C is the number of coefficients to be tested) (Bobko, 2001). Thus, the significance level should be set at .01 if five coefficients are to be tested. However, this method of approach has been seen as a too conservative rule and it is very stringent when testing a large number of coefficients. Since in medical research relationships are often not strong and obtaining a large sample size is always a challenge, such a test implicates a high risk of a Type II error. In addition, medical research studies are often exploratory and require the testing of a large number of coefficients (the present study included). A common practice in exploratory research is to set the significance level at 0.05 but acknowledge that the results are preliminary and confidence in the observed associations requires replication of the results.

Continuous (ANOVA, paired and independent t-tests) and categorical (χ^2 , with Yate's correction, where appropriate) analytical methods were used to examine the relationships and differences between HPG measures and depressive features, such as duration and severity of episode, presence of melancholia, psychotic features, as well as behavioural variables, such as age and BMI.

To further investigate interrelationships between independent and dependent variables, multiple regression analysis was performed. Two main models were examined: 1) assessing which demographic and clinical factors accounted most for hormone measures, and 2) identifying whether there exists a particular cluster of depressive symptoms which is particularly associated with hormonal measures. In the first model, the application of hierarchical multiple regression analysis was deemed most suitable, as out of the range of independent factors to be entered in the model, a particular factor, i.e. age, has been most consistently found to be associated with hormonal measures, and thus was viewed as a confounder which needed to be controlled in the analysis. For the second model, i.e. assessing symptom clusters and their association with hormone measures, a simultaneous multiple regression analysis was chosen, as this is the most suitable form of analysis to be used in exploratory stages of research, i.e. in research studies where there is little theoretical or empirical knowledge regarding the association of a particular independent variable(s) to a dependent variable (Leech, Gliner, Morgan et al., 2003). In this method, all independent variables are entered together in the model, as one block.

Data were screened to ensure adequacy for analysis. The assumptions for multiple regression were tested prior to conducting the analyses: i.e. homoscedasticity and linearity. All models were screened for the presence of multicollinearity (Tabachnick and Fidell, 2001). Multicollinearity, or the degree of correlation between independent variables, was assessed through the use of collinearity diagnostics in SPSS. Data was screened graphically using scatterplots to determine linearity between independent and dependent variables. The distribution of residual error terms was inspected via a histogram of standardized residuals for each independent variable. Each histogram showed a normal distribution of residual error.

Factor analysis and multiple regression of HDRS scale

To investigate a possibility of a relationship between hormonal measures and specific depressive symptoms, simultaneous multiple regression analysis models were performed using each of the hormone measures (Total and free Testosterone, LH, FSH and SHBG) as dependent variables and factor scores of the HDRS as the independent variables (see below). An important consideration in the conduct of multiple regression analysis is keeping the number of independent variables small relative to the number of dependent variables in order to achieve stable regression coefficients (Tabachnick and Fidell, 2001). Thus, it would not have been statistically sound had all of the 17 individual items of the HDRS been entered in the multiple regression models separately. Therefore, a factor analysis of the HDRS scores obtained from the present study was performed.

As there are conceptual as well as clinical reasons to expect a significant correlation between factors, the orthogonal solution was not an appropriate rotation method in this study. Therefore, an oblique method of rotation, using the Promax method, which allows for correlation between factors, was applied. The number of factors to be extracted was determined according to the scree-plot method (Cattell, 1966) and an eigenvalue >1 . The screeplot (or root curve criterion) graphs the components (on X axis) and their corresponding eigenvalues (on Y axis) from largest to smallest. The plot identifies the point in the graph where the drop in eigenvalues ceases and the curve makes an elbow toward a less steep decline. The decision as to how many factors to retain reflects the number of factors before this point. Thus, as illustrated in Fig. 3, the first seven factors (all of which have eigenvalues of > 1) correspond to the point.

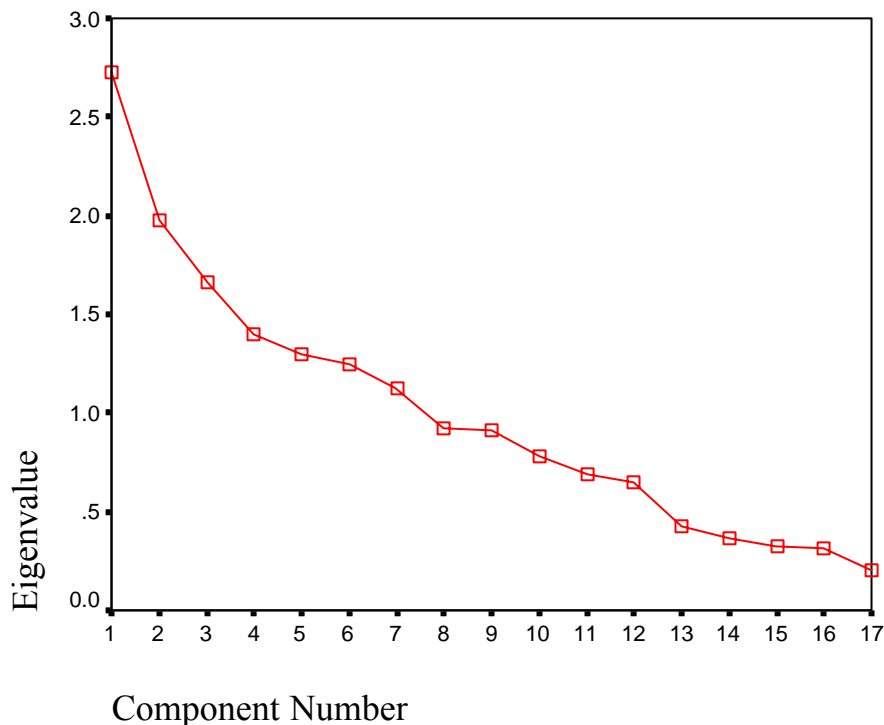


Fig. 3 Scree-plot illustrating the eigenvalue of each component of the factor analysis

The factor solution were checked by means of the Kaiser–Meyer–Olkin measure of sample adequacy and the Bartlett’s test of sphericity ($p = 0.19$). A significant p-value on the Bartlett’s test of sphericity indicates that one can reject the null hypothesis that the non-zero correlations in the sample matrix are due to sampling error.

Factors were extracted using principal component analysis. The cutoff for size of loading to be interpreted was set at 0.32. This cutoff corresponds to 10% of common variance between a variable and a factor, and it is widely used in factor analysis (Tabachnick and Fidell, 2001). The factor structure of the HDRS identified accounted for 67.2% of the total variance in HDRS. The rotated factor matrix is reported in Table 5 where variables are ordered by size of loading to facilitate interpretation. Blank spaces indicate a loading of 0.32 or under.

The seven factor scores identified were:

1. anorexia (energy and interest (Item 7), loss of appetite (Item 12) and weight decrease (Item 15)).
2. sleeping disturbances (middle insomnia (Item 5) and late insomnia (Item 6)).
3. psychic anxiety (psychic anxiety (Item 10), agitation (Item 9), early insomnia (Item 4)).
4. suicidal ideation (Item 3), genital symptoms and retardation (Item 14 and 8)
5. somatic symptoms (somatic anxiety (Item 11) and general somatic symptoms (Item 12)).
6. depressed mood (Item 1) and feelings of guilt (Item 2)

7. Loss of insight and hypochondrias (Item 16 and 14)

Table 5. Rotated factor solution

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Energy	.70						
Apetite	.78						
Weight loss	.64						
Middle insomnia		.79					
Late insomnia		.73					
Initial insomnia			.56				
Agitation			.83				
Psychic anxiety			.78				
Suicide				-.67			
Retardation				.54			
Genital symptoms				.63			
Somatic anxiety					.43		
General somatic symptoms					.83		
Depression						.87	
Guilt						.57	
Hypochondria							.58
Loss of insight							.85
Eigenvalue	2.72	1.98	1.67	1.4	1.30	1.24	1.13
Variance Explained	16.02%	11.65%	9.81%	8.20%	7.62%	7.31%	6.64%

Extraction Method: Principal Component Analysis. Rotation Method: Promax with Kaiser Normalization.

Factor analysis sample size consideration

Unfortunately, there are few sample size guidelines for performing Principal Component Analysis and many of these have minimal empirical evidence (Guadagnoli & Velicer, 1988). In the pursuit of clarifying the criteria essential for a stable solution of a factor analysis, some authors have suggested the ratio of sample size to number of variables as a criteria: the recommendations range from 2:1 through 20:1, while other researchers have suggested that absolute sample size is more important for stable solutions than functions

of sample size, with minimum sample size of 100 (Hatcher, 1994), 200 (Gorsuch, 1983) or 300 (Tabachnick and Fidell, 2001) observations. In particular, Guadagnoli & Velicer, (1988) performed a Monte Carlo study to systematically examine the effects of varying sample size, number of variables, number of components and component saturation on the production of stable factor solution. Using a statistic, g^2 to compare these variables, they concluded that, contrary to the more readily accepted rules, sample size as a function of the number of variables is not an important factor in determining stability, rather, component saturation and absolute sample size were the most important factors. Moreover, these authors showed that solutions with several high loading marker variables (i.e. > 0.8) do not require as many cases, such that with high component saturation (i.e. factor loadings of greater than .80) solutions were stable across replicated samples regardless of the number of indicators, even with as few as 50 participants. In the present solution four (out of 17, i.e. 24%) of the components had factor loadings of more than 0.8 and a high number of the remaining components had factor loadings of just below 0.8, and the current sample size was 52, indicating a compliance of the present factor solution with factor analysis parameters set by Guadagnoli & Velicer, (1988).

Nonetheless, owing to concerns of a resulting unstable factor structure as a consequence of controversially low number of factors (despite their high loadings), in addition to the present factor solution, we sought to utilise previously reported HDRS factor structure which had been obtained from a larger population of patient with comparable clinical and demographic parameters, such as age and depression severity. Using scores from 186 medication-free, medically healthy patients with depression, Pancheri, Picardi, Gaetano et al., (2002) identified four HDRS factor scores using Principal Component factor analysis

with oblique rotation. The factor structure identified by these authors accounted for 43.8% of the total variance in HDRS.

The four factor scores identified were:

- 1) somatic factors (saturated by somatic anxiety, hypochondriasis, general somatic symptoms, gastrointestinal symptoms and the three items concerned with insomnia)
- 2) psychic factors (saturated by psychic anxiety, agitation, feelings of guilt and loss of insight)
- 3) core depressive symptoms (depressed mood, decrease in work and interests and retardation), and
- 4) anorexia (loss of appetite and decrease in body weight).

In separate models, we then applied simultaneous multiple regression analyses to establish whether any of the factors obtained from the present HDRS factor analysis as well as the factors from Pancheri et al.'s (2002) solution significantly accounted most for the variance in hormone levels.

Longitudinal analysis

The difference in admission and follow-up hormone levels was assessed using Paired sample t-tests for each group, i.e. remitters and non-remitters. The age span of the patients in this study was relatively large (18 – 68), therefore employing the repeated measures t-test is particularly useful in separating effects of illness from those of age

(Klockars and Sax, 1986). Similarly, as the number of patients in each of the depression outcome groups was relatively low (13 in the remitters and 26 in the nonremitters group), paired samples t-test is useful as it economises numbers needed in each group, as well as eliminating between-subject differences (Tabachnic and Fidell, 2001). Independent samples t-tests were used to examine differences in hormone levels between the groups on admission as well as follow-up.

All tests used were two-tailed and significance level was set at 0.05. Data were analyzed using SPSS for Windows, Version 11.0.1 (SPSS, 2001).

5.11 Ethical consideration

This study was approved by The Ramsay Sydney Psychiatric Hospitals Ethics Committee as well as The University of Sydney Ethics Committee (Ethics approval is attached as Appendix VI). Both of these committees are governed by guidelines set out by the NH&MRC (National Health and Medical Research Council). All patients read the Patient Information Statement and signed the consent form before entering the study. Patients were offered a summary of the results of the study and copies of their hormonal results.

CHAPTER 6

Results

Introduction

The following Results chapter has been organised into three main sections:

1. The first section analyses the differences in hormones between the two main diagnostic groups (depression and other psychiatric diagnoses).
2. The second section focuses on the interrelationships between hormones and other clinical and demographic variables in patients with depression.
3. The third section assesses the difference in hormones between remitters and non-remitters and to examine whether any changes in clinical outcome of depression is accompanied by a change in any of the HPG measures.

6.1 Comparison between main diagnoses

Basic demographic data for each diagnostic group are presented in Table 6. There were no differences between the groups in any of the demographic and behavioural variables studied. Within the depressed sample HDRS scores averaged above 25.7, which indicates moderate to severe depression. The mean HDRS score in Group 2, was 5.1,

and it was significantly lower than the mean for Group 1. Patients with depression had a relatively longer admission compared to patients with other diagnoses, although this difference was not statistically significant.

Table 6. Basic demographic and clinical data for each diagnostic group, results presented as mean and SD (in parentheses).

	Group 1 (n = 52)		Group 2 (n = 25)		Significance value
Age (years)	42.04	(14.1)	40.72	(13.8)	0.7
HDRS	25.67	(4.8)	7.50	(5.1)	0.001
BMI (kg/m ²)	28.02	(4.3)	27.49	(4.5)	0.62
Length of hospital inpatient stay (days)	22.85	(16.3)	16.13	(9.3)	0.06
Marital status (%)					
Single	35.8		56		0.11 ^a
Married/De-facto	49.1		28		0.13 ^a
Divorced/separated/widowed	15.1		8		0.31 ^a
Children					
% yes	39		23		0.23 ^a
Employment status					
% employed	32.1		28		0.49 ^a
% Student/Unemployed	67.9		72		0.61
Smoking					
% Yes	35.8		40		0.54 ^a
mean number of cigarettes/day	25		30		0.36

a. from chi-squared analysis

6.2 Cross-sectional HPG profile of Group 1 and Group 2

Analysis of variance revealed significant differences in levels of Free T ($F = 8.69$; $p = 0.004$) and Total T ($F = 7.87$; $p = 0.006$) between Group 1 and Group 2 (Table 7).

ANCOVA revealed that controlling for age resulted in no change in the significance of the differences: both, free T ($F = 9.51$; $p = 0.003$) and Total T ($F = 7.84$, $p = 0.007$)

were significantly lower in the depressed group (Group 1). Comparison of LH, FSH or SHBG revealed no significant differences between the groups.

Table 7. Hormonal results for main diagnostic groups (mean, SD in parentheses)

	Group 1 (n = 52)		Group 2 (n = 25)		significance value
Total Testosterone (nmol/L) normal range (8 – 38)	14.82	(4.8)	18.49	(6.4)	0.006
Free Testosterone (pmol/L) normal range (43 – 138)	41.43	(12.2)	52.45	(19.5)	0.004
LH (IU/L) normal range (2 – 12)	5.76	(3.1)	5.59	(1.5)	0.55
FSH (IU/L) normal range (1 – 10)	5.89	(4.1)	4.80	(2.2)	0.45
SHBG (nmol/L) normal range (10 – 50)	27.27	(13.2)	26.72	(10.59)	0.92

As illustrated in Table 7, the means for all hormones, from both groups were within the normal range (as set out by the pathology laboratory). However, closer inspection of the hormonal distribution for each group revealed that thirty patients (57.7 %) from Group 1 and 5 (20%) from Group 2 were below normal limits for free T (i.e. < 43 pmol/L). This is illustrated in Fig. 4. A Pearson Chi-square test was used to analyse whether the number of patients who were below normal levels for Free T was independent of diagnostic group. The analysis (with Yates' correction, as there were 5 cases in one cell), produced a statistically significant result of $\chi^2 = 8.21$, ($p = 0.003$), indicating that there is a significant difference in the number of patients who were below the Free T threshold between the two diagnostic groups.

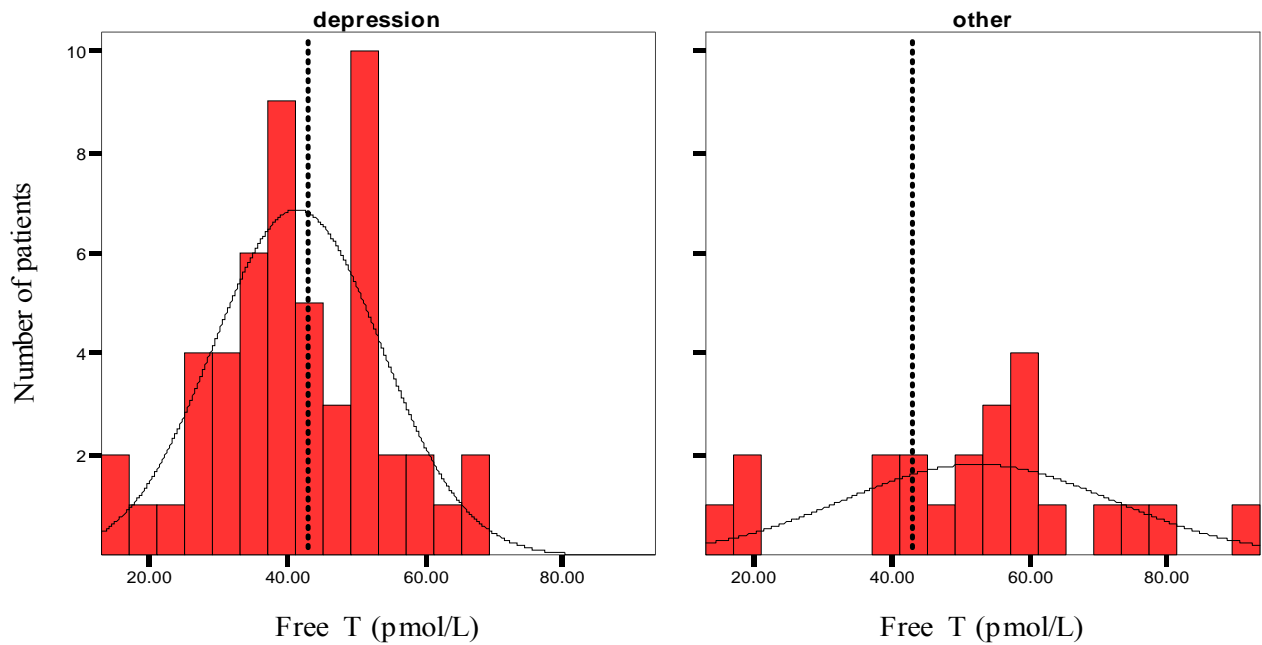


Fig. 4 Distribution of Free T among Group 1 and Group 2 (normal-range cut off represented by a dashed vertical line)

On the other hand, only five (9.6 %) of the patients with depression (Group 1), and one (4 %) of the patients in Group 2 had Total T levels which were below the normal range (< 8 nmol/L). The distribution of Total T levels across the two groups is illustrated in Fig 5. This difference was not statistically significant using the Pearson Chi-square test with Yates' correction ($\chi^2 = 0.17$, $p = 0.68$).

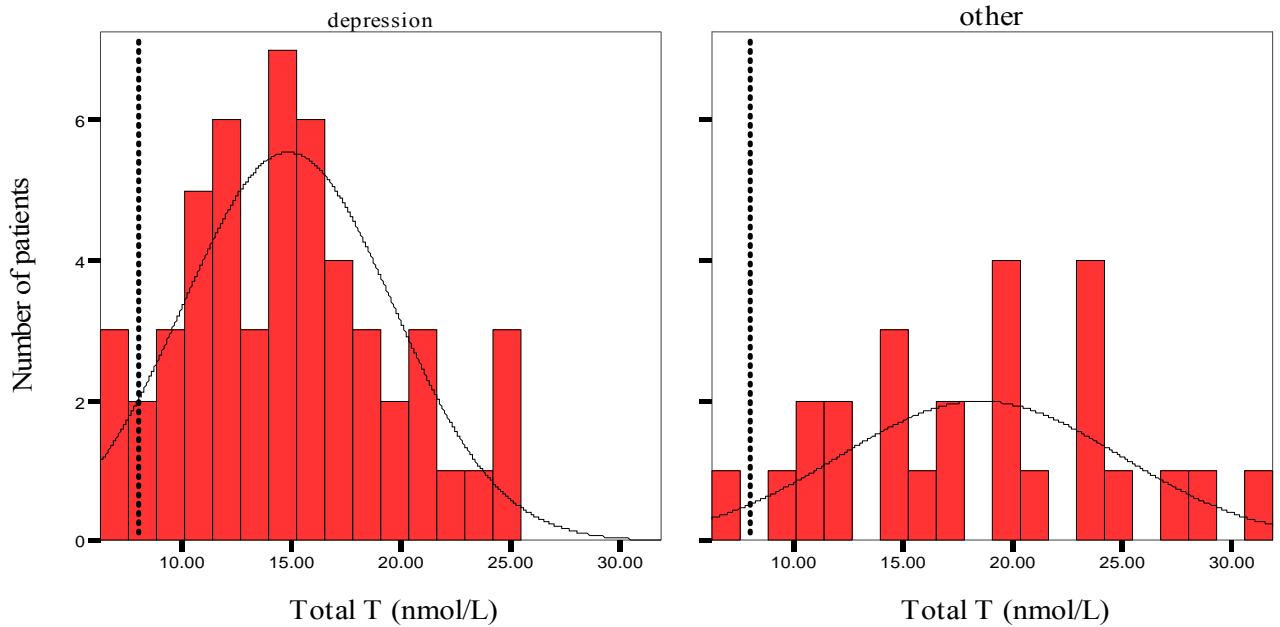


Fig. 5 Distribution of Total T among Group 1 and Group 2 (normal-range cut off represented by a dashed vertical line).

Moreover, four patients (3 from Group 1 and 1 from Group 2) displayed levels of SHBG that were higher than the normal range. A similar pattern of supranormal and disproportionate levels were found for FSH: seven patients (13.5 %) from Group 1 and one patient (4 %) from Group 2 had FSH levels above the normal range. Pearson chi-square test with Yates' correction revealed no significant differences ($\chi^2 = 0.76$, $p = 0.38$). Regarding distribution of LH, four patients from Group 1 displayed levels that were out of the normal range: two had LH levels below the normal and two had levels above the normal range. The difference between the groups regarding LH was not statistically significant ($p = 0.34$).

6.3. Interrelationships of hormonal, clinical and demographic data in patients with depression

The Pearson correlation coefficients of the relationships between the hormones measures and other variables are depicted in Table 8.

Table 8. Pearson Correlation coefficients matrix

	Free T	Total T	SHBG	LH	FSH	Age	BMI	HDRS [#]	Episode [^]
Total T	0.29*	-							
SHBG	-0.2	0.42**	-						
LH	-0.05	0.23	0.13	-					
FSH	-0.2	-0.14	0.19	0.58**	-				
Age	-0.39**	-0.40**	0.34*	-0.01	0.39**	-			
BMI	0.04	-0.40**	-0.31*	-0.11	-0.01	0.02	-		
HDRS	-0.34*	-0.30*	-0.04	0.01	0.07	0.05	0.04	-	
episode	-0.64**	-0.45*	-0.04	0.04	0.14	0.34*	0.14	0.1	-
Means	41.43	14.82	27.27	5.76	5.89	42.04	28.02	25.67	21.85
S.D.	12.2	4.8	13.2	3.1	4.1	14.1	4.3	4.8	15.6

* statistically significant correlation at the 0.05 level

** statistically significant correlation at the 0.01 level

[#] HDRS – Hamilton Depression Rating Scale

[^] Episode – Duration of depressive episode

Total and Free T were significantly and negatively correlated with HDRS score ($r = -0.34$, $p = 0.031$ for free T and $r = -0.30$, $p = 0.033$ for Total T). Similarly, both of these hormones exhibited significant negative correlation with age, the correlation coefficient being almost identical in magnitude and significance for both of the associations

($r = -0.40$, $p = 0.004$ for Free T and $r = -0.41$, $p = 0.004$ for Total T). In addition, the strongest association was evidenced between both Free T and Total T and duration of episode: $r = -0.60$; $p = 0.001$, for Free T, and $r = 0.45$, $p = 0.001$, for Total T. Total T, but not Free T, was also highly positively correlated with SHBG ($r = 0.42$, $p = 0.001$) and negatively correlated with BMI ($r = -0.40$ $p = 0.003$).

6.3.1 Demographic features and hormones

To further explore factors associated with subnormal testosterone levels, patients with depression were grouped into those whose testosterone level was within the normal range and those whose testosterone level was below the normal range. There were thirty (57.7 %) patients whose free testosterone was below the normal range (i.e. below 43 pmol/L) and five (9.6 %) whose total testosterone was below the lower limit (i.e. below 8 nmol/L).

Independent samples t-test showed that when patients were compared based on whether free testosterone was below or within the normal range, significant differences were observed in age, duration of episode and scores on HDRS, such that patients below the lower limit were older and had a longer depressive episode than those patients whose testosterone levels were within the normal range. Numerical results are presented in Table 9 for comparison. This categorical approach reflects some of the above results from the correlation analysis: namely, the correlation between free T and duration of episode and free T and age.

Table 9. Comparison of values on age, duration of episode and HDRS between patients with free testosterone below and within normal range.

	free T below normal range (n = 30)	Free T within normal range (n = 22)	Significance value
Age (years), (mean, (SD))	46.6 (13.8)	35.8 (12.3)	0.005
Duration of episode (months), (mean, (SD))	28.67 (15.01)	11.14 (9.1)	0.001
HDRS, (mean, (SD))	25.70 (4.86)	23.27 (4.33)	0.07
BMI (mean, (SD))	27.91 (4.04)	28.17 (4.76)	0.83
Melancholic (n; (%))	20 (66.6)	10 (33.3)	0.25*
Psychotic (n; (%))	3 (50)	3 (50)	0.99*

* significance value obtained from Chi-square analysis

Categorical analysis using Pearson's Chi-square revealed that there were no differences in the number of patients with melancholic or psychotic features that had below or above normal free T levels.

None of the variables were significantly different between patients whose Total T was within the normal range compared to below the normal range. This is probably attributable to the low number of subjects in the test, i.e. 5 (9.6% of total sample).

6.3.1.1 Age:

Age was negatively correlated with Free T ($r = -0.392$; $p = 0.004$) and Total T ($r = -0.397$; $p = 0.004$) and positively correlated with SHBG ($r = -0.341$; $p = 0.013$) and FSH ($r = -0.385$; $p = 0.005$). Partial correlation controlling for the effects of BMI did not change the significance levels: the relationship between age and total ($r = -0.43$; $p =$

0.002) and age and free testosterone ($r = -0.39$; $p = 0.004$) remained significant after partialling out the effects of BMI.

Linear regression models showed a relatively identical regression slopes for the free T and age association ((standardised) BETA coefficient = -0.392) and the total T and age association ((standardised) BETA coefficient = -0.397). These results are reflected in Figs. 8 and 9 respectively. Similarly, age accounted for a similar percentage of variance in free testosterone ($R^2 = 0.15$) as it did for total testosterone ($R^2 = 0.16$).

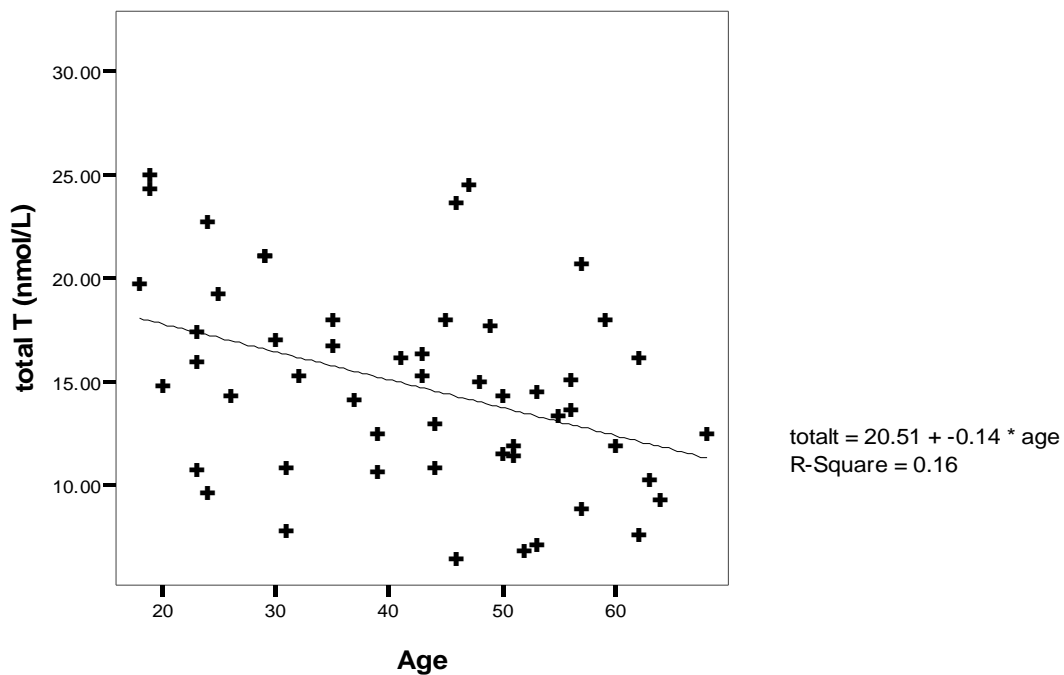


Fig. 6 Relation of total T with age

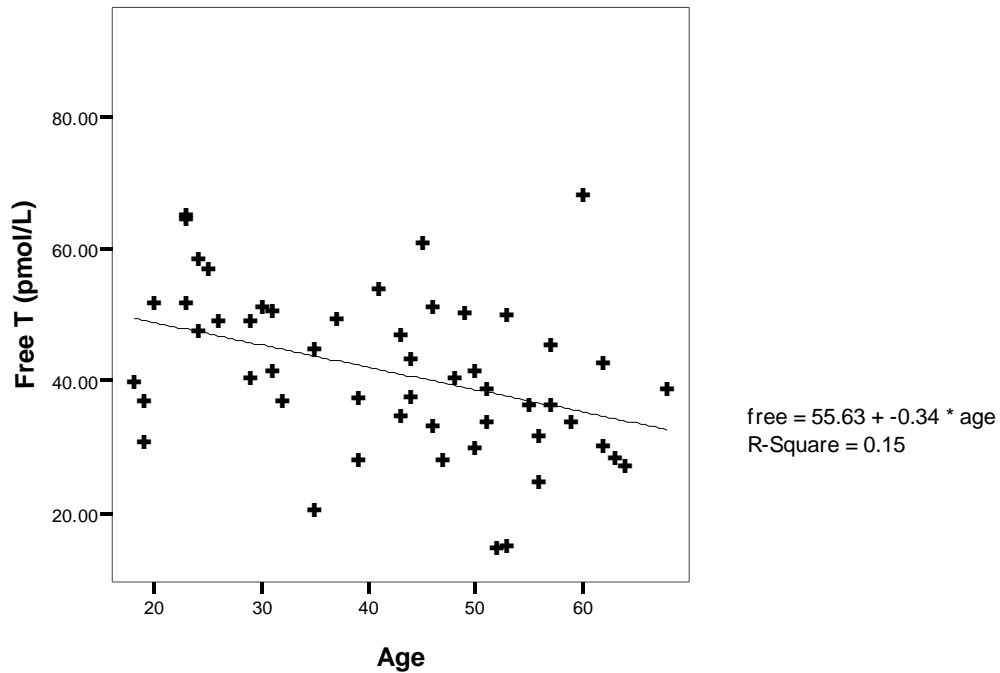


Fig. 7 Relation of free T with age

6.3.1.2 BMI

As indicated in Table 8, there were significant negative correlations between Total T and BMI ($r = -0.4$, $p = 0.003$) and SHBG and BMI ($r = -0.33$; $p = 0.018$). BMI was not associated with other clinical and symptom features, such as duration of episode, HDRS score or the presence of melancholia.

6.3.1.3 Smoking

Approximately thirty six percent of the patients in Group 1 were classified as smokers. The average number of cigarettes consumed was 24 cigarettes per day ($SD = 12.6$).

Categorical univariate analysis revealed that there were no significant differences in any of the HPG measures between current smokers and non-smokers, as illustrated in Table 10.

Table 10. Differences in hormonal measures between smokers and non-smokers (means)

	Smokers (n = 21)	Non-smokers (n = 31)	Significance value
Total T (nmol/L)	15.75	14.20	0.26
Free T (pmol/L)	40.68	41.94	0.72
SHBG (nmol/L)	30.19	25.29	0.19
LH (nmol/L)	4.99	6.28	0.14
FSH (nmol/L)	4.63	6.73	0.07

When only the smokers were analysed, Pearson correlation analysis revealed that levels of HPG measures were not related to the number of cigarettes smoked per day or to the duration of smoking.

6.3.2 Clinical features and hormones

6.3.2.1 Severity of depression

Mean total HDRS scores correlated negatively with total T ($r = -0.30$; $p = 0.03$) and free T ($r = -0.34$; $p = 0.013$) (Table 8). HDRS scores were not associated with the remaining hormonal measures.

Partial correlations controlling for age did not change the significance of the outcome: HDRS was significantly negatively correlated with Free T ($r = -0.35$; $p = 0.01$) and Total T ($r = -0.31$; $p = 0.03$), after the effects of age had been removed.

6.3.2.2 Specific depressive symptoms and hormones

To further examine the relationship of HPG measures to specific aspects of depressive symptomatology, separate simultaneous multiple regression analyses were performed with HPG measures as dependent variables and the four HDRS factor scores obtained from the factor analysis solution by Pancheri et al. (2002) (somatic anxiety, psychic anxiety, core depressive symptoms and anorexia) as independent variables. For comparison, the same analyses were repeated using the seven-factor HDRS structure obtained from the present study.

Multiple regression analysis demonstrated that the set of four factor scores explained 21.1% ($p = 0.02$) of the variance in free testosterone. As can be seen from the regression coefficients, the somatic symptoms factor was the only factor which significantly contributed to the model with free testosterone as the dependent variable ($\beta = 0.36$; $p = 0.021$) (Table 11).

Table 11. Results of multiple regression analysis to predict free testosterone scores from HDRS factor scores- Factor analysis by Panceri et al. (2002)

HDRS Factors	beta	T - value	95% CI		p-value
			Lower	Upper	
Somatic symptoms	-.36	-2.38	-3.80	-0.32	.021
Psychic symptoms	.05	.312	-1.39	1.89	.756
Core depressive symptoms	-.17	-1.22	-3.29	0.81	.230
anorexia	-.13	-.98	-6.28	2.17	.333
constant			48.99	79.73	.0001

Similarly, the multiple regression model with total testosterone as the dependent variable showed a significant contribution of the four HDRS factor scores to the variance in total testosterone, $R^2 = 0.198$; $p = 0.032$. As with free testosterone, the somatic symptoms factor was the only significant factor which explained the largest proportion of variance in total testosterone (Table 12).

Table 12. Results of multiple regression analysis to predict total testosterone scores from HDRS factor scores – Factor analysis by Panceri et al (2002)

HDRS Factors	beta	T - value	95% CI		p-value
			Lower	Upper	
Somatic anxiety	-.36	-2.42	-1.52	-.142	.019
Psychic anxiety	.18	1.21	-.261	1.047	.233
Core depressive symptoms	-.23	-1.57	-1.457	.179	.123
anorexia	.16	1.20	-.675	2.69	.234
constant		7.32	16.14	28.38	

None of the HDRS factor scores showed a consistent relationship to the other HPG measures studied.

When the HDRS factors of the factor analysis of the present study were modeled to predict Free T, multiple regression analysis revealed that 30% of the variance in Free T was explained by this model ($p = 0.02$). As can be seen from Table 13, Factor 5 (comprising of somatic anxiety and general somatic symptoms) contributed most to the equation (Beta = -0.42; $p = 0.002$), followed by Factor 1 (decrease in weight, appetite and energy) (Beta = -0.28; $p = 0.036$).

Table 13. Results of multiple regression analysis to predict free testosterone scores from HDRS factor scores – Current Factor Analysis

HDRS Factors	Beta	T - value	95% CI		p-value
			Lower	Upper	
Factor 1 (decrease in energy, loss of appetite weight)	-.28	-2.17	-3.43	-.13	.036
Factor 2 (middle insomnia and late insomnia)	-.05	-.37	-2.93	2.02	.714
Factor 3 (psychic anxiety, agitation, early insomnia)	.11	.87	-.79	2.02	.387
Factor 4 (suicidal ideation, genital symptoms and retardation)	.03	.20	-2.68	3.28	.839
Factor 5 (somatic anxiety and general somatic symptoms)	-.42	-3.23	-7.19	-1.66	.002
Factor 6 (depressed mood and feelings of guilt)	-.16	-1.15	-3.92	1.08	.258
Factor 7 (Loss of insight and hypochondrias)	-.07	-.51	-6.10	3.65	.616
constant		6.85	49.32	90.45	0.001

A similar pattern emerged when Total T was modeled as the dependent variable in the analysis. The model explained 27.7% of the variance in Total T ($p = 0.036$). However, compared to the model in which Free T was the dependent variable, only Factor 5 significantly contributed to the variance in Total T (Beta = -0.35 , $p = 0.013$).

Table 14. Results of multiple regression analysis to predict total testosterone scores from HDRS factor scores – Current Factor Analysis

HDRS Factors	Beta	T - value	95% CI		p-value
			Lower	Upper	
Factor 1 (decrease in energy, loss of appetite weight)	-.06	-.47	-.82	.51	.644
Factor 2 (middle insomnia and late insomnia)	-.22	-1.50	-1.74	.25	.140
Factor 3 (psychic anxiety, agitation, early insomnia)	-.09	-.72	-.77	.37	.478
Factor 4 (suicidal ideation, genital symptoms and retardation)	-.24	-1.79	-2.26	.14	.081
Factor 5 (somatic anxiety and general somatic symptoms)	-.35	-2.59	-2.54	-.32	.013
Factor 6 (depressed mood and feelings of guilt)	.11	.78	-.62	1.39	.441
Factor 7 (Loss of insight and hypochondrias)	.11	.87	-1.12	2.81	.390
Constant		5.36	13.748	30.30	0.001

As was the case in the prior analysis, using HDRS factor scores obtained from Panceri (2002), none of the seven factors resulting from the present factor analysis showed a consistent relationship to LH, FSH or SHBG.

6.3.2.3 Duration of episode

Significant negative relationship was observed between duration of episode and free testosterone ($r = -0.60$, $p = 0.001$). In addition, a significant negative association was noted between total testosterone and duration of episode ($r = -0.48$, $p = 0.001$).

Separating the effects of age by partial correlation analysis did not alter the significance of the relationships: the correlation coefficient between Free T and duration of episode was -0.54 , ($p = 0.001$) and the correlation coefficient between Total T and duration of episode resulted in -0.36 ($p = 0.009$) after controlling for the effects of age. Duration of episode was not correlated with any other HPG measures, nor with severity of depression (Table 8).

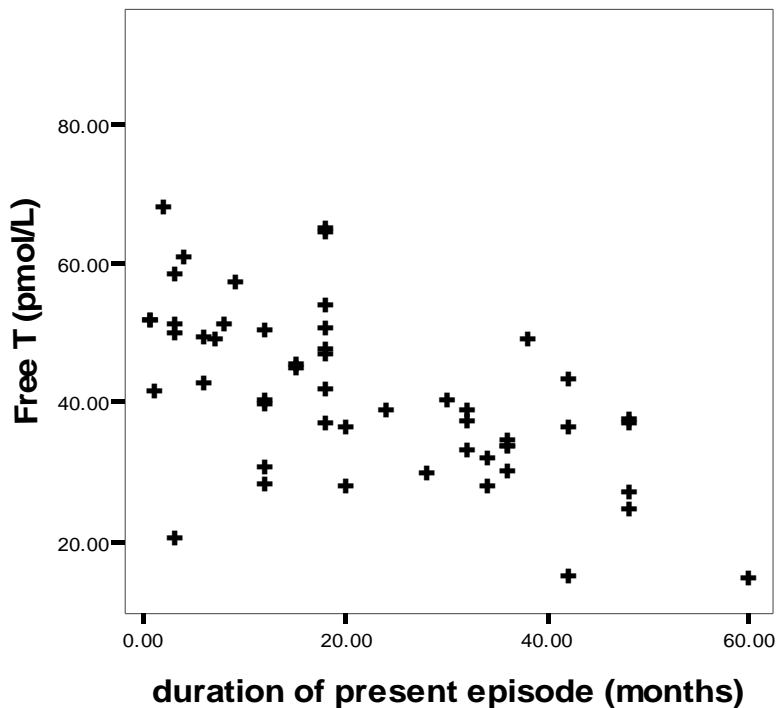


Fig. 8 Association between duration of depressive episode and Free T

Fig 6 illustrates the scatterplot of the association between free T and duration of episode. Although the duration of episode ranged across patients considerably, i.e. from 2 weeks to 60 months (SD = 15.6), visual inspection of the scatterplot indicated that the association of free T and duration of episode might not be even across all values of Free T, more specifically, that the association between Free T and duration of episode might be more prominent for values of duration of episode exceeding approximately 20 months. To investigate this observation further, patients were dichotomised according to duration of episode, i.e. short depressive episode (≤ 20 months) and long depressive episode (> 20 months). The correlation between free T and length of episode was then analysed separately for these groups. The correlation results for each group are presented in Table 15. Analyses revealed that while negative correlations resulted in both groups, only the group with long depressive episode showed statistically significant association between Free T and length of episode. A similar pattern of differing correlation coefficients between the two episode duration groups was also evident in Total T, although the results were not statistically significant.

Table 15. Correlation coefficients between Free T and duration of episode

	Short episode duration (≤ 20 months) (n = 31)	long depressive episode (> 20 months) (n = 21)
Free T	-0.18	-0.42*
Total T	-0.009	-0.27

* significance at the 0.05 level

6.3.2.4 Melancholia

Thirty patients (57.7%) of the current sample were classified as having melancholic features according to the DSM-IV. Point Biserial correlation analysis revealed a significant association between the presence of melancholic features and free T ($r_{pb} = -0.33$; $p = 0.017$) as well as Total T ($r_{pb} = -0.36$; $p = 0.009$) (presence of melancholic features being coded as 1 and absence as 0). Independent samples t-tests revealed that patients with melancholic features had significantly lower levels of free T ($t = 2.47$; $p = 0.017$), total testosterone ($t = 2.71$; $p = 0.009$) and SHBG ($t = 2.2$; $p = 0.03$) compared to patients with no melancholic features. In addition, as summarised in Table 16, patients with melancholic features exhibited significantly higher severity of depression than patients who did not have non-melancholic features ($t = -2.60$; $p = 0.012$).

Table 16. Mean (and SD) of hormonal and other variables for patients with melancholic and patients with no melancholic features

	Melancholic features (n = 30)		No melancholic features (n = 22)		Significance value
Total T	13.36	(4.2)	16.81	(4.9)	0.009
Free T	38.02	(11.5)	46.08	(11.7)	0.017
SHBG	23.57	(8.2)	32.32	(16.9)	0.033
LH	5.40	(2.6)	6.25	(3.7)	0.576
FSH	5.73	(3.2)	6.10	(5.2)	0.752
HDRS	27.07	(4.6)	23.77	(4.4)	0.012
Age	42.93	(14.5)	40.82	(13.8)	0.599

6.3.2.5 Psychotic Features

The presence of psychotic features was identified in six patients (11.6 %). When univariate analysis was conducted, treating hormonal and other variables as dependent and presence/absence of psychotic features as independent variable, none of the results showed significant difference. In addition, patients with psychotic features were no more likely to have a higher severity in depression compared to patients without psychotic features (mean HDRS = 24.83 vs 24.65 respectively; $p = 0.93$).

6.3.2.6 Sexual function (BSFI)

The mean scores of the five components of the BSFI questionnaire (sexual drive, erection, ejaculation, perception of problem in each area and overall satisfaction) were separately analysed. Analyses showed that out of the five components, sexual drive was the only factor which was positively correlated with free T ($r = 0.51$; $p = 0.001$). A positive correlation implies that the lower the testosterone level, the higher the impairment in sexual drive (as low scores on the BSFI indicate higher impairment). Additionally, sexual drive was positively correlated with Total T ($r = 0.33$; $p = 0.21$) and negatively correlated with age ($r = -0.37$; $p = 0.009$). The correlation between free T and sexual drive remained significant after partialling out the effects of age ($r = 0.43$; $p = 0.003$). However, partialling the effects of age out of the Total T/sexual drive association resulted in non-significant correlation, suggesting that most of the association observed between Total T and sexual drive was due to the effects of age. Sexual function was not associated with any of the other hormonal measures. Likewise,

none of the parameters of sexual function were associated with BMI, nor were they associated with the duration or severity of depression. The correlation matrix depicting the association between the 6 sexual function components and hormonal and other factors are shown in Table 17.

Table 17. Bivariate correlations between sexual function components and hormonal and other factors

	Total T	Free T	LH	FSH	SHBG	age	HDRS
Sexual drive	0.33*	0.51**	0.05	-0.15	-0.08	-0.37**	-0.14
Erection	0.03	0.15	-0.16	-0.28	0.03	-0.008	0.12
Ejaculation	0.18	0.08	0.02	0.02	-0.09	-0.12	-0.05
Problem perception	-0.25	0.19	0.05	-0.17	-0.45**	-0.18	0.09
Overall satisfaction	0.8	0.24	0.08	-0.05	-0.07	-0.15	0.01

**Correlation is significant at the 0.01 level.

* Correlation is significant at the 0.05 level.

To further investigate the difference in sexual function as a function of testosterone, two separate analyses were performed where patients were dicotomised based on whether their Total T level was below (Group a) or within (Group b) the normal range. The same grouping structure was performed for Free T. Results showed no differences in any of the sexual function measures between Group a and Group b for Total T. However, the Free T analysis showed significantly lower scores in sexual drive ($t = 2.17$; $p = 0.035$) and overall satisfaction ($t = 2.47$; $p = 0.017$) in patients whose Free T was below the normal range.

Four patients refused to complete the BSFI, thus no data was gathered for these patients regarding their sexual functioning. Statistically, these patients were similar to the remaining patients who did complete the BSFI in terms of all demographic and clinical characteristics and hormonal measures.

6.3.2.7 Treatment Effects:

The following comparisons were performed in order to obtain more information whether treatment variables (ECT or antidepressants) were associated with the hormonal factors:

ECT

Nineteen (36.5%) patients were receiving ECT treatment at the time of testing. Independent samples t-test showed that there were no statistical differences in any of the hormones measured between patients who were receiving ECT and those who were not receiving this treatment.

Medication

All patients were undergoing pharmacological antidepressant treatment at the time of testing. Ten patients were concurrently receiving a mood stabiliser (either Lithium or Epilim), and ten were receiving an antipsychotic medication. In order to examine the hormonal profile across the different types of medication, univariate analysis was performed whereby medication category served as the independent variable and hormonal factors served as dependent variables. Four broad classes of antidepressant

medication were included in the analysis: SSRIs, SNRIs, Tricyclics and tetracyclics.

The hormonal profile across the four antidepressant types is presented in Fig 7.

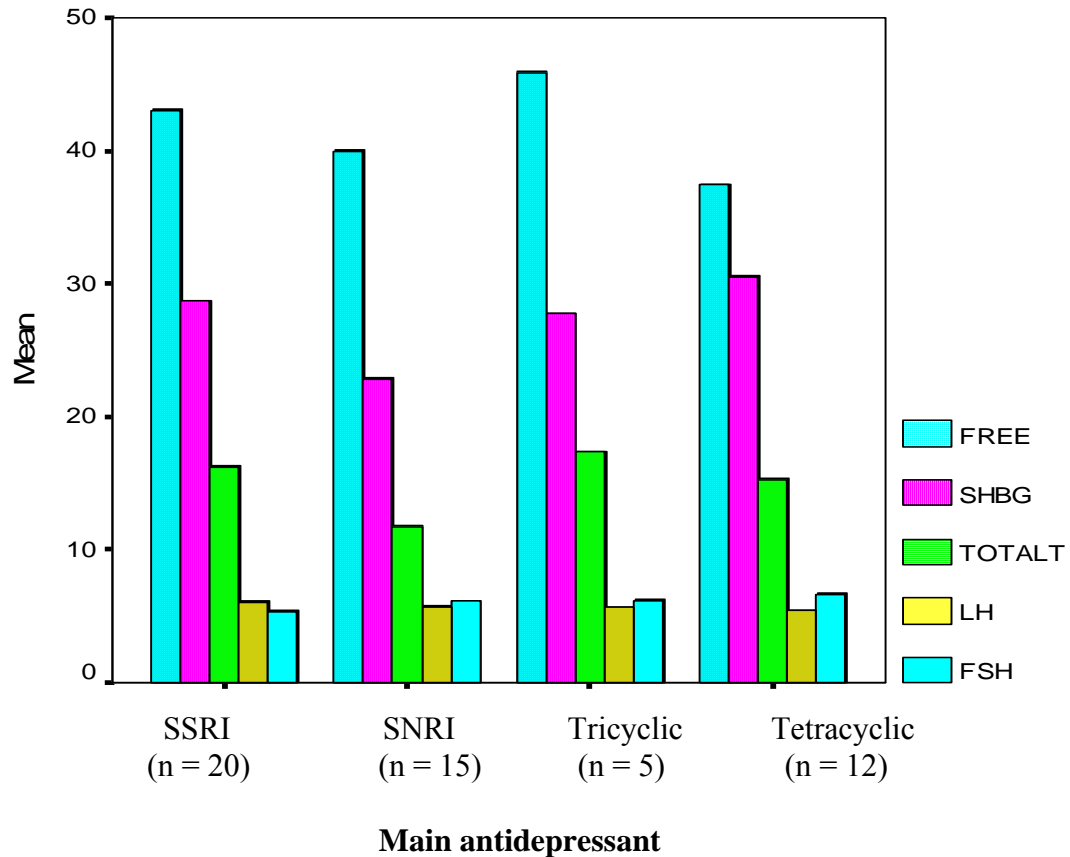


Fig. 9 Hormonal profile as a function of type of antidepressant

Analysis of variance demonstrated no significant difference in any of the hormones between the four classes of antidepressants. Furthermore, there were no differences in any of the hormonal parameters between patients who were also receiving adjunct psychotropic therapy, either in the form of mood stabiliser or antipsychotic.

6.3.3 Hormonal Interrelationships

Statistically significant positive relationship was found between LH and FSH ($r = 0.58$; $p = 0.001$). No significant relationship was observed between severity or duration of episode and levels of LH or FSH (Table 8). In addition, LH and FSH did not correlate with any other factors studied. A slight positive relationship between Total T and LH was observed ($r = 0.23$), however, this correlation coefficient was not statistically significant (0.09).

6.4 Hierarchical Multiple regression Models

To further investigate which demographic and clinical factors accounted most for the variance in hormones, hierarchical multiple regression analysis was performed, with hormonal parameters as dependent variables. Age was entered into the equation first to determine the subsequent contribution of other demographic and clinical variables. In the second step clinical characteristics of depression (i.e. severity and duration) were entered. Melancholic features (dummy coded) was also added in as a predictor variable in this step. BMI and BSFI scores on sexual desire component comprised the third, final step of the equation.

6.4.1 Free T

As summarised in Table 18, When the first block containing age had been entered, 14.6% of the variance in Free T was accounted for ($R^2 = 0.146$, $F = 7.87$, $p = 0.007$).

The addition of HDRS score, duration of episode and melancholia in the model, as block 2, significantly added to the predication of free T: $R^2 = 0.51$, $F_{\text{change}} = 10.77$, $p = 0.001$. Finally, the addition of BMI and BSFI scores on sexual desire resulted in $R^2 = 0.59$, $F_{\text{change}} = 4.33$, $p = 0.02$.

This analysis indicates that collectively, age, HDRS, duration of episode, melancholia, BMI and BSFI scores on sexual desire account for 59.8% of the total variance in free T.

Table 18. Hierarchical multiple regression analysis of Free T, age, duration and severity of depression, melancholia, BMI and sexual desire

	Multiple R	R^2	F	R^2 change	F change
Step 1 Age	0.38	0.146	7.87**		
Step 2 Duration of episode HDRS Melancholia	0.72	0.51	11.29***	0.37	10.77**
Step 3 BMI Sexual desire (BSFI)	0.77	0.60	10.14***	0.08	4.33*

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

6.4.2 Total T

Similar result emerged when a model was designed to predict total T from the above independent variables. Age was entered in Block 1 and on its own this variable accounted for 21.6% of the variance in total T ($R^2 = 0.216$). When scores on HDRS, duration of episode and melancholia were entered as Block 2, variance explained by this model improved significantly: $R^2 = 0.515$. The addition of BMI and BSFI scores on

sexual desire as Block 3 resulted in $R^2 = 0.63$, $F_{\text{change}} = 6.36$, $p = 0.004$, indicating that 63% of the variance in total T is accounted for by the variables in the model. The results are depicted in Table 19

Table 19. Hierarchical multiple regression analysis of Total T, age, duration and severity of depression, melancholia, BMI and sexual desire

	Multiple R	R ²	F	R ² change	F change
Step 1 Age	0.46	0.22	12.653**		
Step 2 Duration of episode HDRS Melancholia	0.72	0.52	11.43***	0.30	8.87***
Step 3 BMI Sexual desire (BSFI)	0.79	0.63	11.64***	0.12	6.36**

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

6.4.3 SHBG

Relative to the results of the Free T and Total T multiple regression analyses, the predictor variables accounted for less variance in SHBG. The results are presented in Table 20. Age accounted for 13% of the variance, ($R^2 = 0.13$). Addition of Block 2 with duration of episode, HDRS and melancholia significantly increased the variance explained in SHBG, $R^2 = 0.34$; $F_{\text{change}} = 4.69$, $p = 0.006$. The addition of BMI and scores on sexual desire only minimally increased the amount of accounted variance – these variables explained a further 9% in the variance of SHBG.

Table 20. Hierarchical multiple regression analysis of SHBG, age, duration and severity of depression, melancholia, BMI and sexual desire

	Multiple R	R ²	F	R ² change	F change
Step 1 Age	0.36	0.12	6.64*		
Step 2 Duration of episode HDRS Melancholia	0.59	0.34	5.58**	0.22	4.69**
Step 3 BMI Sexual desire (BSFI)	0.66	0.43	5.18***	0.09	3.22*

* p<0.05 ** p<0.01 *** p<0.001

6.4.4 FSH and LH

Application of the hierarchical multiple regression model to FSH and LH showed that the variables in the model had only minimal explanatory power of the variance in LH and FSH. Age was the only variable which accounted for a significant proportion of variance in FSH ($R^2=0.18$; $F = 9.78$; $p = 0.01$). Addition of Block 2 and Block 3 into the equation did not contribute significantly to the amount of variance in FSH. Thus, a total of only 18.8% in the variance in FSH was accounted for by the independent variables. Similarly, the regression model did not have any explanatory power for the variance in LH: only 4.5% of the total variance was accounted for by the model, with none of the variables adding significantly to the prediction of LH. The results of the multiple regression analysis for both LH and FSH are showed in Table 21.

Table 21. Hierarchical multiple regression analysis of LH, FSH, age, duration and severity of depression, melancholia, BMI and sexual desire

	Multiple R	R ²	F	R ² change	F change
Dependent variable: FSH					
Step 1					
Age	0.42	0.18	9.78*		
Step 2					
Duration of episode					
HDRS	0.43	0.19	2.48	0.12	0.21
Melancholia					
Step 3					
BMI	0.43	0.19	1.58	0.001	0.009
Sexual desire (BSFI)					
Dependent variable: LH					
Step 1					
Age	0.08	0.007	0.33		
Step 2					
Duration of episode					
HDRS	0.20	0.04	0.43	0.03	0.47
Melancholia					
Step 3					
BMI	0.21	0.05	0.32	0.006	0.13
Sexual desire (BSFI)					

* p<0.05

In summary, the results from the above multiple regression analyses suggest that age, duration and severity of episode, the presence of melancholic features, BMI and sexual desire account for approximately 60% of the variance in Free T and Total T, 40% of the variance in SHBG and for only a small proportion of the variance in LH and FSH.

6.5 Longitudinal analysis

The principal reason for which a longitudinal analysis was performed was to inquire whether there is an association between change in depression and change in testosterone, i.e. whether a testosterone is a state marker of depression. In this section, we also seek to investigate which other factors are associated with changes in depression. As outlined in the Method section, patients were classified as remitters and non-remitters based on their HDRS score on the follow up visit, i.e. those who scored 7 or below were classified as remitters. Outcome was judged in two ways: reduction in depression severity (percentage change in severity score) and by dichotomizing patients according to final outcome of depression score into remitters and non-remitters. Paired samples t-tests were conducted to investigate whether there were any differences between baseline and follow-up hormone levels in the two groups.

Thirty nine patients (75 %) returned for the follow-up visit. Of these, 13 (33.3%) were classified as remitters. Remitters and non-remitters did not differ with respect to baseline clinical, hormonal or demographic data. Table 22 summarises some of the demographic and clinical characteristics of the two groups.

Table 22. Baseline demographic and clinical characteristic comparison between remitters and non-remitters (mean, SD)

	Remitters		Non-remitters		Significance value
HDRS	26.92	(4.33)	24.31	(5.02)	0.12
Age	38.46	(13.33)	45.04	(12.61)	0.14
BMI	27.51	(4.09)	27.59	(3.605)	0.95
Duration of episode	22.69	(16.89)	22.62	(16.05)	0.98

Paired samples t-tests showed significant difference in free T between baseline and follow-up in the remitted group ($t = -4.05$; $p = 0.002$). As outlined in Table 23, the mean Free T on baseline in the remitters group was 38.6 pmol/L and at follow-up this had increased to 59.3 pmol/L. Similarly, there was also significant increase in total T at follow-up in this group ($t = -2.33$; $p = 0.038$), from 14.4 nmol/L at baseline to 20.7 nmol/L at follow-up. Detailed inspection of the follow-up hormone levels showed that free testosterone was increased in all, but one, of the patients who remitted. The average change in free T on follow-up compared to baseline was 68%, whereas the change for Total T was 63%.

There were no significant differences in the other hormones between baseline and follow-up in the remitters, nor in the non-remitters group (Table 23).

Independent samples t-tests between the remitters and non-remitters indicated a significantly higher levels of free T ($t = -2.15$; $p = 0.038$) and total T ($t = -2.67$; $p = 0.011$) in the remitters compared to non-remitters (Table 23). There were no differences in LH, FSH, or SHBG between the remitters and non-remitters.

Analysis of the hormonal measures between baseline and follow-up in the non-remitters group revealed no significant difference. The means are presented in Table 23. Detailed examination of the change in Free T and Total T in this group revealed a mean increase of 10.5% in Free T and a reduction of 1% in Total T.

Table 23. Mean (+- standard deviation) of demographic and hormonal data for remitted and non-remitted patients

	Remitted (n = 13)		Sig. value ^	Non-remitted (n = 26)		Sig. value^	Sig. value #
	baseline	follow-up		baseline	follow-up		
Total T	14.35	20.71	.038	15.23	14.69	.553	.011
Free T	38.62	59.34	.002	42.68	45.33	.391	.038
LH	5.83	7.32	.121	5.63	6.51	.127	.528
FSH	5.35	5.44	.707	6.11	5.51	.288	.963
SHBG	27.23	25.00	.598	27.58	26.92	.578	.667

^ Within-subject analysis (comparing baseline to follow-up)
Between-subject analysis (comparing follow-up parameters between remitters to non-remitters)

CHAPTER 7

Discussion

Introduction

The aims of this thesis were to perform an exploratory analysis which compares the HPG axis between patients with depression and patients with other diagnoses, to investigate in detail the relationship between an array of demographic and clinical factors and HPG functioning in patients with depression and to examine whether any changes in clinical outcome of depression is accompanied by a change in any of the HPG parameter measures. In this chapter the principal findings will be drawn together and a comprehensive interpretation and discussion of the results will be presented in three sections:

- 1) The difference in HPG axis hormones between patients with depression and patients with other psychiatric diagnoses,
- 2) The interrelationships between hormones and other clinical and demographic variables in patients with depression, and
- 3) The differences in HPG functioning between remitters and non-remitters.

In addition, this chapter will discuss the implications of the results, raise conceptual and methodological difficulties of the study and provide directions for future work

7.1. Analysis of differences in HPG measures between patients with depression and patients with other psychiatric diagnoses

The present study found significantly lower levels of free and total testosterone in patients with depression compared to patients with other psychiatric disorders.

Moreover, there were disproportionately larger numbers of patients with below normal free T levels than in the group of other diagnosis. We are aware of only one other study in the literature which has employed a group of patients with schizophrenia as a ‘normal’ control group (Mason et al., 1988). Reflecting on Mason et al.’s (1988) methodological approach, the comparison in this section of the current study was reliant upon the criterion of statistical difference in hormone levels between diagnostic groups, rather than the assumption that a hormonal measure must show abnormal or pathological value (outside the ‘normal range’) in order to bear clinical significance.

Comparable to Mason et al.’s (1988) results, our basal hormone results show lower total and free testosterone in depressed men as compared to patients with other diagnoses (Mason et al., 1988). The two groups did not differ with respect to the other measures of HPG axis, i.e. LH, FSH and SHBG. The results of the present study add to the scarce body of literature comparing HPG functioning in patient populations by supporting the notion that patients with depression have lower levels of total

testosterone. In addition, the findings of the present study expand on the findings of Mason et al., (1988) by adding free testosterone as another parameter of the HPG, beside total testosterone, that is lower in patients with depression as compared to patients with other diagnoses.

A potentially confounding effect in the present study is the use of various medications by the patients for the duration of the study. The present methodological design allowing the use of psychiatric medication in the present study was due to ethical and practical reasons. Namely, we sought to perform a naturalist study in order to assess hormone levels, and in addition, as a tertiary clinical care centre, The Northside Clinic focuses on providing treatment to patients with severe depression, and thus it was deemed ethically unsound to alter patients' antidepressant regimen for research purposes.

However, it is unlikely that the use of medication has played a significant part in affecting the present results as other authors have controlled for medication by enrolling patients who are free from psychotropic medication, and reported similar results (e.g. Schweiger et al., 1999; Yesavage et al., 1985). In addition, various human and animal data suggest that most antidepressants have little or no effect on testosterone levels (Rehavi et al., 2000). Furthermore, as discussed in later section of this thesis, we found no effect of the class of antidepressants on hormone levels in the present study. Few studies, on the other hand, do show an effect of antipsychotic medication on testosterone levels. However, it is unlikely that there was a significant role for

antipsychotic medication in the present study since antipsychotics have been found to decrease (Brown, et al 1981), or have no effect on (Markianos, Hatzimanolis and Lykouras, 1999) rather than increase, testosterone levels. Therefore, since it was the patients with other diagnoses that had higher usage of antipsychotic medication, differences in testosterone levels between the two groups would be expected to be larger than reported here.

7.2. Analysis of variables related to HPG function in depression

7.2.1 Demographic and behavioural factors

7.2.1.1 Age

In the present study a statistically significant negative correlation was observed between free and total testosterone levels and age. This observation is in agreement with the majority of previous studies. The available psychoendocrine literature focusing on depressive illness in men has reported large decreases in total and free testosterone with advancing age, correlation coefficients in the order of -0.40 to -0.70 usually being reported (Sachar, et al, 1973; Yesavage et al., 1985; Levitt & Joffe, 1988, Rubin et al 1989). This finding has also been demonstrated in a large number of longitudinal and cross-sectional studies with normal men (Fieldman, 1995; Morley, 1997), hypogonadal men (Gray, 1999), as well as in men with chronic illnesses such as asthma (Williams, et al 1989) and diabetes (Moore, 1994). The magnitude of the present negative relationship between total T and age ($r = -0.41$) and Free T and age ($r = -0.40$) is in accordance with the above studies. In addition, comparable to prior reports, the present study found a significant positive relationship between SHBG and age ($r = 0.40$), as well as FSH and age ($r = 0.43$).

The physiological mechanisms responsible for the age-related decrease in HPG functioning are complex and are not as yet fully understood (Vermeulen, 2000). Namely, current evidence suggests that the HPG axis is affected on three levels as a function of age.

Firstly, the decreased testosterone secretion and serum levels with advancing age has a primary testicular failure. This proposition has arisen in light of numerous observations of a decrease in the number of Leydig cells in the testes of elderly men, as well as a decrease in responsiveness of testosterone to hCG or LH stimulation. Secondly, in addition to a primary testicular failure, alterations in central mechanisms, namely, the hypothalamic GnRH-secreting system have been implicated. Findings such as decreased LH pulse amplitude (Deslypere, Kaufman, et al 1987) (which is regulated by GnRH signals), and failure of the feedback mechanism to restore decreasing testosterone levels, despite normal functioning of the gonadotropins (Kaufman, Giri, et al 1991; Mulligan, Iranmanesh, et al 1999), have implicated decline in the function of the hypothalamic GnRH- secreting unit as a further explanatory venue for the age-related changes in HPG hormones.

Similarly, several lines of evidence indicate that the decrease in Free T and Total T with age might also be related to changes in SHBG binding affinity and/or increase in SHBG levels. A possible explanation for the age-related increase in SHBG is its relation to GH: it is well established that GH decreases SHBG levels (Vermeulen, Rubens and Verdonck, 1972; De Moor, Heyns & Bouillon, 1972). There is also a wealth of evidence which shows that GH decreases with age (Vermeulen, 1987; Iranmanesh et al., 1991), especially due to the reduction of the nocturnal pulses (Zadik, Chalew, et al 1985). The decrease in GH, as a consequence, might facilitate the increase of SHBG with age. A support for this postulation has been reported by Vermulean, Kaufman and Giagulli (1996), who observed that in adult men with GH deficiency SHBG levels were significantly increased

compared to levels in healthy men of similar age. These authors also reported that treatment with GH resulted in significant decreases in SHBG levels (Vermulean, et al 1996).

In addition, the increase in FSH with age reflects an additional site vulnerable to age-related decline in gonadal functioning. The FSH increase as a function of age demonstrates Sertoli cell failure with advancing age and is likely associated with a decrease in inhibin, which would result in increased FSH (Deslypere, Kaufman, et al, 1987). In addition, the increase in FSH with advancing age might also be as a compensatory increase in response to decreased testosterone.

We did not observe any significant relationships between age and LH. Evidence indicates that the frequency, as well as the amplitude of LH pulsatile secretion decreases with age (Kaufman, 1990). However, this decrease is not reflected in the mean concentration of LH. Thus, the failure to find negative relationship between age and (mean) LH levels in the present study is reflective of these observations.

A number of studies have suggested that the age-associated decline in HPG functioning may often be accentuated by intercurrent disease (Handelsman, 1994; Turner and Walsh, 1997). However, it is difficult to establish whether the current results exemplify a more rapid decrease in testosterone as a function of age as although the magnitude of the correlation is comparable to other studies, suggesting a similar strength of association, previous studies have generally failed to include the regression slope, which is the parameter needed to estimate the degree of change. The standardised Beta coefficients of

Free T and Total T in the current study were -0.30 and -0.31 respectively, indicating a reduction of 0.3 pmol/L per year in Free T and a reduction of 0.31 nmol/L per year in Total T.

A few of the previous studies have reported a more prominent decrease in Free T as compared to Total T. For example, Gray, et al (1991) reported a decrease of 1.2% per year in Free T and a 0.4% decline in Total T. This difference has been largely attributed to an increased binding capacity, as well as increased levels of SHBG with advancing age, thus progressively resulting in diminishing Free T in the circulation. However, the present study did not find any differences in the rate of decline between Free T and Total T. As demonstrated by the standardised Beta coefficients, both Free T and Total T showed a decline of a factor of 0.3. This inconsistency in the findings may be attributed to sample characteristics. Namely, it is possible that the current, relatively small sample of patients did not exhibit the age-range required to detect differences in Total T. As previously outlined by Deslypere & Vermeulen (1985), an age-associated decline in Total T is more likely to be seen in a sample covering a wide age range and including a sufficient number of patients over the age of 60. In the present sample, only five (9.6%) of the men were aged over 60 years.

7.2.1.2 BMI

The present study found significant negative association of BMI with SHBG and total testosterone. In addition, BMI was a significant predictor of total T in a hierarchical

multiple regression model, adding a unique contribution of 13.3% to the variability of total T.

The association of BMI and total T has previously been reported by most studies with physically healthy men (Allen, 2002; Kato, 1992). Although the existence of a relationship between obesity and testosterone is well established (Oh et al., 2002) the mechanisms underlying the hormonal changes that occur as a result of changes in BMI and abdominal obesity are not fully understood (Allen, 2002). Studies have suggested that hyperinsulinemia, associated with increased BMI and obesity, has inhibitory effects on SHBG production (Pasquali, 1995). As a consequence of reduced SHBG, testosterone levels will be decreased, due to the negative feedback mechanism, in order to maintain levels of free T constant. Our observation of no statistically significant correlation between free T and BMI is in agreement with this hypothesis.

No significant relationship was observed between BMI and LH. The lack of an association between BMI and LH is not surprising, since previous studies have suggested that suppressed LH secretion is more likely to be observed in populations of subjects with BMI exceeding 40 kg/m^2 (Glass, 1989), and only one patient in the present study had a BMI larger than 40 kg/m^2 .

Thus, it appears that the relationship between BMI and HPG measures in depression are of the same direction and comparable magnitude as for normal subjects.

7.2.1.3 Smoking

In the present study smoking was not associated with any of the hormones studied. Previous literature has reported some relationships between smoking and gonadal hormone levels in healthy men. For example, Vogt et al (1996) compared plasma testosterone levels in smokers to non-smokers and found an increased level of testosterone in the smokers. Similar results have been reported by other authors, e.g. Dai, et al (1988) and Field, et al (1994). However, other studies are discordant with these reports, some reporting lower levels of testosterone in smokers compared to non-smokers (Barrett-Connor & Khaw, 1987) and yet some reporting no difference (Simon et al, 1992).

While the literature is inconsistent regarding the association between smoking and testosterone, there have been no studies designed to further assess any relationship in a population of patients with depression.

It is important to mention that even if smoking did have an impact on hormone levels, it is unlikely that it would have affected our longitudinal results, as none of our patients who were smokers at admission had ceased or significantly modified their smoking behaviour at discharge from hospital or at follow-up.

7.2.2 Clinical factors

7.2.2.1 Severity of depression

In the present study, mean scores on the HDRS were significantly and negatively correlated with free and total testosterone levels. While the majority of previous studies

have employed a cross-sectional design, typically comparing HPG measures in groups of normal populations and depressed patients, there are few studies which have reported correlations between severity of depression and levels of HPG hormones. As discussed later in this section, the present finding of negative correlation between testosterone levels and severity of depression is consistent with some studies, but not with others.

However, prior to examining the concordance of the present results with previous reports, a concern needs to be raised in relation to the concept of measuring and quantifying severity of depression. A straight forward comparison between studies is precluded by the fact that a range of instruments measuring severity of depression has been used across studies. While relatively high proportion of authors have employed the most standard measurement scales, namely the HDRS and/or BDI, still a considerable number of studies have used other measures, among others: Center for Epidemiologic Studies Depression Scale (CES-D) (Seidman et al., 2001), Comprehensive Psychopathological Rating Scale (CPRS) (Uden, et al, 1988) and Carroll Depression Rating Scale (Delhez, et al., 2003). The obvious repercussion of the usage of various measurements of severity of depression is that a meaningful comparison and interpretation of results between studies is less readily achievable. The main reason for this difficulty is that there exists no strong evidence of a correlation between these instruments. Comparison of results between studies is rendered even more challenging due to the fact that some studies have employed scales which are of questionable validity and reliability, and yet some have also used modified self-rating depression scales (Tsujimura et al., 2003).

Thus, it is important to consider this point when attempting to conceptualise and compare the relationship between testosterone and severity of depression across different studies.

Literature findings

There exist a number of reports which suggest an inverse relationship between depression severity and testosterone levels. These reports emanate from various sources such as epidemiological studies, clinical trials and observational studies.

Epidemiological studies:

Three large epidemiological studies have assessed the relationship between testosterone levels and severity of depression: The Massachusetts Male Aging Study (MMAS) (Araujo et al., 1998); The Rancho Bernardo Study (Barrett-Connor et al., 1999) and the Veterans Experience Study (VES) (Booth et al., 1999).

The MMAS was a cross-sectional, population-based survey of 1709 men aged between 40 and 70 years. Depressive symptoms, as measured by the Centre for Epidemiological Studies-Depression (CES-D) scale were not correlated with testosterone levels (Araujo et al., 1998).

The Rancho Bernardo study (Barrett-Connor et al., 1999) examined the association between bioavailable and total testosterone levels and depressed mood in 856 older men (mean age 70.2 years). The BDI score was used as a measure of severity of depression. The mean BDI for the sample was 4.5. The authors note that this score is relatively low

compared with other studies, and the authors indicate that the low score most likely reflects the inverse association between socioeconomic status and depression (Rabbitt, Donlan, Watson et al., 1995), as more than 80% of the participants in the Rancho Bernardo Study were middle or upper-middle class. Nevertheless, applying linear trends across quartiles of BDI scores, these authors found a graded stepwise decrease in total testosterone and bioavailable testosterone levels with increasing level of depressed mood. Furthermore, these authors noted that bioavailable testosterone levels were 17% lower in the patients who were categorically categorized as 'depressed' (as defined as BDI score of 13 or more).

The Veterans Experience Study, the largest published study to date, investigated the relationship between testosterone levels and depression in 4393 Vietnam veterans aged 33 – 42 years (median age = 37 years). The measure of depression was the Diagnostic Interview Schedule designed to assess depression as defined by the Diagnostic and Statistical Manual of Mental Disorders (3rd edition). While a modest relationship was reported between testosterone and behavioural measures, such as gambling and substance abuse, and between testosterone and antisocial personality measures, no significant relationship was found between testosterone levels and severity of depression.

Booth et al. (1999) re-examined the data from The Veterans Experience Study and generated the hypothesis that the relationship between depression and testosterone is non-linear. Therefore, these authors applied the Lowess smooth curves analysis, which uses a nonparametric algorithm that yields a nonlinear curve to provide an accurate fit to the

data. The results were surprising and did show a curvilinear relationship between testosterone and depression: such that testosterone level was negatively correlated with depression in men with testosterone levels below 590 ng/dL (approx 20 nmol/L) and positively correlated with depression among men with testosterone levels above 590 ng/dL.

Clinical Trials

As outlined in the literature review of the present study, there are numerous clinical trials, conducted in a variety of populations (e.g. healthy, hypogonadal, HIV-infected, depressed) which support the notion of improvement in mood with the administration of testosterone. For example, Pope & Katz (1994) compared psychiatric diagnoses (using the DSM-III) of 88 athletes who were using steroids with 68 nonusers, and found that mood disorders, such as manic and hypomanic episodes as well as depression, were significantly associated with steroid use. Wang et al. (1996) studied the effects of testosterone replacement therapy in 51 hypogonadal men. These authors assessed various mood parameters, such as energy, anger, nervousness, sadness. The results showed improvement in a number of mood parameters, such as energy and friendliness and decrease in nervousness, anger and irritability.

Furthermore, the improvement of mood with testosterone administration has also been demonstrated in HIV patients, for example: in a cohort of 112 hypogonadal men with HIV, Rabkin et al. (1999) found that 79% of the patients reported significant improvement in mood. In a later study, the same authors verified these findings, reporting

a response rate in depressive symptoms of 59% in testosterone-treated patients, and 14% in the placebo group (Rabkin et al., 2000). In addition, extending the administration of testosterone in a new clinical area, depression, has shown beneficial effects of treatment of depression with testosterone, although the results, compared to testosterone administration in other clinical areas, are relatively inconsistent. For example, in a 6-week, randomized, placebo-controlled trial, Seidman et al. (2001) studied the efficacy of testosterone enanthate (200 mg) as antidepressant monotherapy in male patients with moderate (HDRS = 21) depression and low testosterone levels, defined as serum levels of 350 ng/dL or less. Thirteen patients were randomised into the treatment group and seventeen in the placebo group. The average age of the patients was 52 years. At the end of the treatment period, it was found that the depression score had improved for both groups, in fact, the response rate observed for patients who received placebo was slightly greater than the response rate for those receiving testosterone (41.2% versus 38.5%).

Numerous clinical trials of testosterone in a variety of patient populations also support the notion of inverse relationship between severity of depression and levels of testosterone. For example: among a combined group of 62 hypogonadal and eugonadal subjects, Grinspoon, et al (2000) reported a significant inverse correlation between baseline BDI score and both free ($r = -0.41$) and total serum testosterone levels ($r = -0.43$). In addition, O'Connor et al. (2002) found significant negative relationship between mood (specifically tension, anger and fatigue) and testosterone levels.

Observational studies

Observational studies at a smaller scale with respect to sample size have also reported inverse relationships between severity of depression and testosterone. In a sample of eleven men (mean age 52.4 years) with severe depression (HDRS = 29.5), Davies et al. (1992) found that testosterone was significantly and inversely correlated with several measures of depression severity, namely, the HDRS ($p = 0.008$), the MADRS ($p = 0.006$) and depression subscale of the Leeds questionnaire ($p = 0.006$). In an earlier study, Yesavage et al. (1985), controlling for age, reported a statistically significant correlation of -0.67 between testosterone and HDRS in a sample of 18 patients with depression. Similarly, Delhez et al (2003) in their assessment of HPG function in 153 reported an inverse correlation between scores on the CRS and free testosterone levels.

However, the present results of a negative correlation between testosterone and depression severity are not in accordance with results previously reported by other authors: in a sample of eleven patients with depression, Kaneda and Fujii (2002) demonstrated no correlation between total T and depression scores (as measured by the Zung self-rating depression scale). In addition, Levitt and Joffe (1988), reported a non-significant relationship between testosterone and scores on the HDRS in a sample of 12 patients with depression.

Assessment of discrepancies: methodological factors

The discrepancies between the various studies are complicated by methodological problems concerning the entire spectrum of the research methodology used between different studies and particularly by the poor definition of depression severity.

Firstly, as outlined earlier, a number of authors have employed various and sometimes questionable diagnostic and psychometric tools of depression. This practice is problematic as it does not define the population accurately and therefore contributes to the heterogeneity of the sample.

Secondly, most studies have used very low samples of patients, usually in the order of 10 to 20. While the practical and economical reasons for these choices are understandable, using a small sample of patients undoubtedly increases the heterogeneity of the group and introduces higher variability as well as increasing the probability of committing a Type I error rate. In addition, it is a known statistical fact that small sample sizes are more likely to result in a restricted range of values, the repercussion of this being a decreased correlation coefficient.

Thirdly, testosterone measurement techniques vary across different laboratories. The degree of variability between commercially available kits has been demonstrated to be as high as 40% (Boots, Potter, Downing et al., 1998). Thus, comparison between studies using different assays introduces further error and difficulty in cross-study comparison.

Fourthly, although it is a well-supported finding that testosterone exhibits a diurnal variation, with levels being highest early in the morning, some authors have chosen to measure testosterone in the afternoon. This is particularly problematic when studying the relationship between testosterone and depression: while diurnal variation has been frequently observed in normal populations (Winters et al., 2001), and it is known that testosterone levels are 25 to 43% higher in the morning compared to afternoon levels (Winters, 2001), the extent to which testosterone varies during the day has not been systematically studied in populations of depressed patients. Thus, the failure to control for the time of day of serum sampling will contribute to variability in results.

Finally, it is important to realise that differences in studies might also be caused, or attenuated by situational factors. It is likely that the experimental situation itself, particularly anxiety associated with venepuncture, and other experimental investigations, as well as being asked intimate questions, produces stress in individuals, and this may well influence testosterone levels. There are several lines of evidence that indicate that testosterone levels are altered as a function of stress (e.g. Christiansen and Hars, 1995). This aspect will be discussed later in this section. As different studies have employed varied experimental protocols and instruments, and as the situational setting of the experimental procedure has varied (e.g. at an inpatient or outpatient status), it is difficult to predict the degree to which the stress of the experimental procedure has had an effect on testosterone levels.

7.2.2.2 Specific depression dimensions

Most studies performed to date in this area have been correlational, and as outlined in the literature review, most studies have compared testosterone levels between patients with depression and normal controls, and only a relatively small number have obtained measures of association between severity of depression and testosterone.

While some previous reports as well as the current finding of a negative correlation between HDRS and testosterone implies that patients with low testosterone tend to display higher severity of depressive symptoms, the question arises as to which, if any, depressive symptoms in particular are mostly associated with testosterone.

In order to gain more information regarding this inquiry, multiple regression analyses were performed in which the contribution of the HDRS factor scores - from the factor analysis performed in the present study, as well as the factor analysis performed by Pancerri et al. (2001) - on explaining each HPG measure was assessed.

By examining in detail the different symptoms of depression, via factor scores of HDRS, we were able to obtain more details as to the individual relationship of each depressive symptom cluster with testosterone. Against a background of very inconsistent and scarce evidence of a relationship between HPG and depression, this analysis provides a starting point in the quest of examining this relationship in further detail. With the exception of one study (discussed below), the most thorough level of analysis applied by previous studies attempting to elucidate the relationship between particular depressive symptoms

and testosterone, has been the attempt to correlate few of the individual HDRS scores with testosterone. These results have largely amounted to non-significant findings: For example, in their study with 16 men with depression, Rubin et al. (1989) reported no correlation between HDRS score of libido and testosterone, and as well as between the HDRS item of energy and testosterone.

To further assess the relationship between HPG measures with specific dimensions of depressive symptomatology, multiple regression analyses were performed by which the seven factor scores of the present factor analysis as well as Panceri et al. (2002) were modeled as predictors of HPG levels. The results of the present study indicate that somatic symptoms (characterized by general somatic symptoms and somatic anxiety symptoms from the present factor analysis; and somatic anxiety, hypochondriasis, general somatic symptoms, gastrointestinal symptoms and the three items concerned with insomnia from Panceri et al., 2000, factor analysis) are the best predictors of testosterone levels.

It is very difficult to compare our results to previous findings, as literature in this area, especially concerning depressive factors, is scarce. We are aware of only one prior study which has used multiple regression analysis to investigate specific HDRS factor scores in relation to testosterone (Rubin, 1989). These authors modeled seven factor scores from a previously reported factor analysis of the HDRS (Rhoades and Overall, 1983). The factors were: somatisation, diurnal variation, sleep disturbance, weight loss, reality disturbance, mood depression and agitation/anxiety. In their subject population of 16 men

with depression, Rubin et al. (1989) found that none of the depression factor scores showed a significant relationship to total testosterone, LH or FSH.

However, other authors have reported results which do implicate the notion that testosterone is mostly correlated with somatic symptoms of depression: for example, in a sample of 153 men with depression, Delhez et al. (2003) found a trend between testosterone and the somatic symptoms subscale, and a significant correlation between the somatic symptoms and free T. Another line of evidence that testosterone is associated with somatic factors emerges from testosterone administration studies: Rabkin et al. (2000) investigated the efficacy of testosterone therapy (200 – 400 mg) in treating depressed mood in 74 HIV-positive men with hypogonadal symptoms. The results of this 6-week double-blind, placebo-controlled trial indicated that there were no significant effects of testosterone observed on the affective subscale of the HDRS. Rather, significance was identified by the somatic symptom subscale in both the total group and in men fulfilling the criteria for depression. The results of Rabkin et al's (2000) study indicate that testosterone's association with mood might be secondary to its effects on somatic symptoms, such as general somatic symptoms, fatigue, weight loss and sleep.

In a similar study design as Rabkin et al (2000), Pope et al (2003) investigated the efficacy of testosterone supplementation in 12 male patients with refractory depression. The results showed that although improvement was achieved on both the somatic as well as the affective subscales of the HDRS, multiple regression analysis showed that

improvement on the somatic subscale was greater than improvement on the affective subscale.

The results of another randomised, placebo-controlled trial of a 6-month testosterone administration in HIV-infected men further support the findings of an association between somatic symptoms of depression and testosterone (Grinspoon et al., 2000).

Although these authors did not assess depressive symptoms using the HDRS, they found that overall improvement in mood (as assessed by BDI) was mostly attributable to improvement in weight and appetite. In this study, the change in weight was the most significant single predictor of change in Beck score, accounting for 35% of the variation in Beck score.

A number of observational studies also demonstrate the association between testosterone and somatic symptoms. The connection between low testosterone and disturbed night sleep is supported by the observations that night work and being on-call at night suppresses morning testosterone (Chatterton and Dooley, 1999; Touitou, 1990), and that more severe sleep disturbances (long term sleep deprivation, sleep fragmentation and sleep apnea) in the laboratory suppress or disrupt testosterone regulation (Luboshitzky, Aviv, Hefetz et al., 2002; Luboshitzky, Zabari, Shen-Orr et al., 2000; Torsvall and Åkerstedt, 1983). In addition, inverse correlations have been reported by levels of testosterone and need for sleep disturbed sleep, and sleepiness problems in shift workers (Axelsson, Åkerstedt, Kecklund et al., 2003). Moreover, the degree of testosterone suppression has been found to be related to the degree of sleep disruption (Schiavi,

White, Mandelli, 1992). Furthermore, in a sample of 14 men with depression, Baumgartner, Graf, Kurten et al. (1988) demonstrated a significant decrease in testosterone following a night of sleep deprivation.

In addition, there exists a substantial body of evidence which suggests that testosterone is associated with appetite, weight loss, as well as nutritional components. There have been reports of vegetarian diets decreasing testosterone levels (Howie & Shultz, 1985) and decreases in both total and free testosterone due to reduction of dietary fat content (Hamalainen, et al. 1984). Similarly, there is evidence that restricted calorie intake suppresses GnRH activity (Seidman and Roose, 2000), and due to the actions of the HPG feedback mechanism, this decrease in GnRH results in lower testosterone levels. In addition, prior evidence stemming from clinical trials of testosterone administration in hypogonadal and HIV populations illustrate that testosterone increases appetite and weight gain (Rabkin et al., 2000; Grinspoon et al. 1998; Grinspoon et al., 2000). Indeed, testosterone is endorsed as a treatment for wasting disease in a number of Clinical Practice Guidelines (e.g. APA, 2000), and it has been widely used today as an appetite stimulating agents in wasting diseases, such as AIDS (Fisher and Abbatiola, 1998).

In addition, findings of recent observational studies and clinical trials studies illustrate the relationship between testosterone and physical activity. Namely, methodological differences notwithstanding, there is a general consensus that physical activity of up to 2 hours duration increases testosterone levels (Hurel et al., 1999; Fahner and Hackney, 1997).

The notion that testosterone is more likely to be associated with somatic symptoms than any other types of symptoms of depression may partly be explained by bias in symptom reporting and description by men. Although the literature regarding this topic is inconsistent, there is some evidence which indicates that during a depressive illness, men, compared to women, are more likely to verbally and/or non-verbally report somatic symptoms. If this were the case in the present study, one would expect an overrepresentation and overestimation of somatic symptoms over and above symptoms which are more likely to represent psychological symptoms of depression, such as depressed mood. However, this proposition is unlikely, as results of an association between testosterone and somatic features of depression have been obtained from studies in normal subjects (e.g. Fahner and Hackney, 1997; Touitou, 1990), as well as HIV-infected subjects (e.g. Grinspoon, et al., 2000; Rabkin et al., 2000).

7.2.2.3 Duration of Episode

The relationship between the onset of depression and hormonal levels is clearly an important area for investigation, though this area is problematic since the onset of depression, particularly in men is often difficult to define. This difficulty is caused by factors that characterise male depression (discussed in the previous chapter). Namely, men are less likely than women to recognise their depressive symptoms, admit that they are depressed, or seek timely treatment for depression.

The present study found a significant negative relationship between duration of episode and free, as well as total testosterone. Of the few studies examining the relationship between testosterone and depression, only one included duration of episode as a variable in the analysis (Rubin, et al 1989). These authors did not find a significant correlation between testosterone and length of depressive episode. The most obvious explanation for the discrepancies between the current findings and those of Rubin, et al. (1989) might be provided by the observation that the patients in Rubin et al.'s study had an average length of episode of 7.3 months, whereas the patients in the present study had an average of 17.7 months duration of depressive episode. Despite these clinical differences, our patients were comparable in age to those in Rubin et al's study (average age 42.5 yrs and 39.5 respectively).

The discrepancies then, might be explained by the proposition that associations between duration of depression and testosterone are not present in patients with a shorter duration of episode, and that the chronicity of depressive illness is associated with low testosterone levels. Indeed, investigating the plot of the association between duration of episode and testosterone of the present study, it can be observed that a more prominent relationship emerges at a duration of episode of approximately 20 months and beyond. In addition, dichotomising patients into shorter and longer duration revealed a significant inverse relationship between free T and duration of episode in patients with longer depressive episode.

The proposition that the association between testosterone and the length of depressive episode might be more readily observed in patients who have longer duration of depression is supported by findings by Seidman (2002). These authors compared testosterone levels between men with dysthymic disorder (depressive disorder characterised by long-term, chronic duration), age-matched men with major depression and age-matched healthy, non-depressed men, and found that testosterone levels were, on average, lower in the dysthymic group than in the depressed or healthy group. The results of the present study, together with the findings of Seidman (2002) support the notion that the relationship between testosterone and depression is more prominent in patients with chronic depression.

7.2.2.4 Melancholic features

Thirty patients (57.7 %) of the patients in Group 1 were melancholic (according to DSM-IV criteria). In previous studies, the prevalence of melancholic features in populations of depressed outpatients has ranged from 16% to 53%. The substantial variation in the reported rates of melancholia may be a result of the numerous criteria used to define melancholic features over the past two decades, including DSM-III, DSM-III-R, DSM-IV, and the Newcastle 1 Depression Rating Scale (N1). In the present study of depressed inpatients, the prevalence of melancholic features was relatively similar to other previous studies.

Patients with melancholic features in the present study exhibited lower levels of free and total testosterone as compared to non-melancholic patients. This was confirmed by results

from application of correlation analyses as well as categorical analyses. In the only one other observational study incorporating melancholia as a variable, Rubin et al (1989) failed to show any connection between melancholia and depression. The discrepancies are difficult to explain, since the patient population in both, the present study and Rubin et al (1989) were comparable regarding severity of depression. One factor which could have precluded the observation of an association between melancholia and testosterone in Rubin et al's study could be the small sample size. There were 6 out of 16 (38%) patients in Rubin's study diagnosed with melancholic features, and this number might not have been large enough to detect a significant difference in their analysis.

The present observation of an association between the presence of melancholic features and testosterone is not surprising, since melancholia has usually been reported to be reflective of a high severity of depression (Zimmerman, Coryell, Pfohl et al., 1986), and in this study there was a significant negative correlation between testosterone and severity of depression. In addition, the observation provides further support of an association of testosterone and somatic features, as melancholia is characterised by the presence of somatic symptoms, such as decreased sleep, appetite and psychomotor disturbance (APA, 1994).

7.2.2.5 Sexual function

The present study found a positive relationship between sexual drive and overall satisfaction (as assessed by the Brief Sexual Function Inventory) and testosterone levels. The role of testosterone in sexual behaviour, particularly in men, has long been

recognised. In addition, the multifaceted nature of sexual function and behaviour has been widely acknowledged and it has also been an area of study as to which aspects of sexual function, i.e. psychological factors (such as sexual drive, interest and motivation) or physiological factors (such as erection and ejaculation) are mostly associated with testosterone.

Male sexual function has been shown to be testosterone-dependent in many animal species, including man (Davidson, 1982; Kwan, 1983). Some of the most overt observations implicating testosterone as an important factor in sexual function originate from studying men's lifecycle. The general rise and fall in testosterone with age corresponds to average level of sexual behaviour throughout men's lifecycle. For example: while prepubescent boys rarely engage in sexual activity, after puberty, correlating with the dramatic rise in testosterone levels and growth of sexual organs, sexual behaviour is overtly expressed (Christiansen, 2004). Similarly, while there are inconsistencies regarding whether sexual function is age-related, elderly men are more likely to have a decrease in the frequency of sexual behaviour, to a lesser extent a diminution in sexual interest and an increased prevalence of sexual dysfunction, such as impotence and problems with ejaculation (Mulligan et al., 1988). The literature regarding this relationship is complex and inconsistent, with some authors reporting no relationship (Cohen, Hollingsworth & Rubin, 1989), or positive relationship, Sachar (1973) and still others reporting negative relationship (Yesavage et al 1995).

As mentioned above, the available literature is conflicting regarding the relationship between testosterone and sexual function. This subject is even further complicated in patient populations, where difficulties in interpretation are further introduced by concurrent medication and lifestyle factors. Indeed, problems with libido and erection, and sexual function in general, are the most common and probably most severe symptoms of depression (Thase et al., 1992) and may also be affected by antidepressants, particularly Selective Serotonin Reuptake Inhibitors (Gitlin, 1994). Thus, interpretation of associations of sexual function measures and other factors, particularly in chronic medical illnesses such as depression, should be conducted with caution, and should consider the multi-faceted nature of sexual function, as well as a wide array of biological and social factors associated with sexual function. The wide range of variability in methodological approaches in previous studies can perhaps explain the inconsistencies in findings:

Although there is a general disagreement in the literature regarding the correlation of testosterone and sexual activity, a more consistent finding is that patients with abnormally low levels of testosterone experience significant sexual problems – these difficulties improve with testosterone supplementation to restore normal levels (Wang et al. 2000). It has previously been repeatedly shown that once a normal level of testosterone has been achieved, via exogenous testosterone administration, increase of dosage does not further improve sexual function (e.g. Bagatell et al., 1994; Buena, et al., 1993)). Indeed, it is a common finding that the levels of testosterone crucial for sexual function in men lie around 3 ng/ml (8.56 nmol/L) (Nieschlag, 1979; Buena, 1993) Thus, it appears that the

main aspect of the relationship between testosterone and sexual function is that only low levels of testosterone are essential in initiating and maintaining sexual function, increasing the levels past this point does not result in further increase in sexual behaviour.

The present findings provide further support to the above hypothesis: patients whose testosterone level was below the normal range exhibited significantly lower sexual drive and overall satisfaction compared to patients whose testosterone levels were in the normal range. Thus, it appears that a similar threshold level for sexual drive exists in patients with depression.

7.2.2.6 Treatment variables

ECT

In the present study, we did not observe a difference in any of the hormones between patients who were treated with ECT and those who did not receive this treatment. We are aware of only one study examining the effects of ECT on the relationship between testosterone and depression. Cooper (1989) studied the effects of ECT on HPG hormones in 14 patients with depression, by measuring hormones before and after a clinical course of ECT had been administered. These authors concluded that post-ECT levels of LH were significantly higher than pre-ECT levels. No differences in testosterone or FSH were found.

Medication

No relationship between type of medication and levels of hormones was evident in the present study. This finding probably reflects the small number of patients in each antidepressant group. Most of the previous studies in this field have controlled for medication usage by enrolling patients who have been medication-free for at least two weeks. The present findings of an inverse correlation between testosterone and depression, as well as of increased testosterone following remission in a medicated population are consistent with these studies (Schweiger et al., 1999; Yesavage et al., 1985), indicating that antidepressant usage may not play an important role as a mediator of HPG functioning.

We also did not find any differences between patients who were taking only one antidepressant and those who were taking more than one. Nevertheless, many of our patients were taking more than one medication concurrently and while the present study did not find any difference in HPG hormones between patients on monotherapy as opposed to patients on polytherapy, the possibility of a combined effect of medications on gonadal hormones can not be excluded. Undoubtedly, future research should focus on controlling for the effects of various medications, and their combinations, on gonadal hormones.

7.2.3 Hormonal interrelationships

LH

The present study did not find the expected positive correlation between LH and testosterone. This observation is a puzzling finding, given the well-supported evidence that LH stimulates the production of testosterone. This observation does not necessarily carry any significant implications to suggest that there are any abnormalities during a depressive episode: due to fluctuations of LH the demonstration of the positive LH-T relationship would require serial, 10-20 min interval blood samplings and the usage of sophisticated statistical methodology (Delhez, et al 2003). Thus, it is likely that a positive relationship between testosterone and LH might be observed in studies which employ frequent serum sampling. This finding has indeed been reported in studies with patients with depression: using 30-min samples, Rubin et al. (1989) reported correlation of 0.36 between total testosterone and LH. The trend of an increase in LH levels with increase in total testosterone levels reflects the compensatory rise in LH mediated by the feedback mechanism.

The present study did not find a relationship between severity of depression and levels of LH. Previous studies in this area have not investigated levels of LH in relation to depression, thus, there are no results to compare the current results to. This is definitely an area of further investigation.

LH and FSH

The present study reports significant positive correlation between FSH and LH in the depressed group. This is consistent with other previous reports of high correlation between LH and FSH in patients with depression: for example, Rubin, et al (1989) reported a correlation coefficient of 0.78 in 16 male depressives. In addition, the present high correlation between FSH and LH has been previously demonstrated in a population of healthy, older men (Morley, Kaiser, Perry, et al. 1997) as well as hypogonadal men (Delhez, et al. 2003) .

The high positive correlation between LH and FSH reported in this study is not surprising, since the production of both hormones is primarily stimulated by a common releasing hormone, the GnRH. In addition, FSH is also regulated by nonsteroidal factors comprising a negative endocrine feedback loop mediated by inhibin B secretion from Sertoli cells (Hayes). This would explain why the correlation is not even higher than presently observed.

The current results of: 1) comparable correlation coefficient magnitude between LH and FSH with studies in normal populations, and 2) a trend for an increased LH levels with increase in total testosterone indicate that the mechanism responsible for governing LH and FSH is intact in depressive populations and comparable to normal population.

7. 3 Testosterone In Relation To Change In Clinical State

The present study is one of few as it employs a follow-up method to examine hormone levels. As mentioned previously, the majority of studies in this area have employed a cross-sectional method. Longitudinal approach is very valuable as it provides further information regarding the stability of any relationship between depression and testosterone observed in cross-sectional studies.

Thirty nine (75%) of the patients returned for a follow-up visit within 3 to 6 months of discharge from hospital. Generally, given the nature of the study and the fact that phlebotomy is involved, this is considered as a high rate of follow-up for psychiatric studies. A high proportion (54%) of the patients who did not attend a follow-up visit were unable to be contacted. The remaining patients who did not attend a follow-up visit indicated that they did not wish to attend the follow-up visit or were re-admitted to another hospital.

We are aware of only three previously reported studies which have employed a longitudinal approach to study the relationship between testosterone and depression (Steiger et al. 1991; Cooper et al, 1989; Sachar, et al 1973). Our research approach is different to the other three previous studies employing longitudinal methods to study the hormonal change as a function of clinical state: while we have defined our serum sampling times at fixed time points, i.e. admission, and three to six months following discharge, other studies have collected blood samples only when actual remission from

depression had been achieved (e.g. Steiger et al., 1991). Although the latter approach might reflect a more standardised definition of remittance, it fails to include non-remitters in its analysis, and thereby eliminates vital comparisons between groups. That is, by comparing remitters to non-remitters at the follow up visit, we have the opportunity to perform within as well as between comparisons of variables, which, understandably, yields further information regarding the difference between the groups and factors associated with remittance.

Thirteen (33.3%) of the patients were classified as remitters. The observed remission rate is expected given the initial severity and duration of episode. Baseline level of symptomatology or other characteristics did not differentiate outcome.

The present study found significant increases in free T as well as Total T on remission from depression as compared to during illness. In addition, free testosterone was increased in all, but one, of the patients who remitted. The average change in free T on follow-up compared to baseline was 68%, and the change in Total T was 63%. On the other hand, there were no differences in levels of free T, nor total T between baseline and follow-up visit in the nonremitters.

Insofar as the results of the present study can be compared to previous studies, the present observation of increased testosterone levels on remission is consistent with the findings of Steiger et al (1991). Steiger et al (1991) investigated nocturnal total testosterone levels in 12 depressed patients before and after recovery. Recovery was defined as a HDRS

score below 5 points for at least 4 weeks and at least 2 weeks withdrawal from antidepressant medication. These authors found statistically significant increase (approx. 23 %) in Total T on remission.

However, other studies have failed to support the present finding: Cooper et al. (1989) studied hormone levels pre- and post ECT in 14 patients during depression and on remission. Their results showed no correlation between depression and testosterone levels. There are no clear explanation of the inconsistency in the results between the present study and Cooper's study. Clinical features of depression, for example: severity and duration of depression were comparable in both studies. However, the patients in Cooper et al.'s (1989) study were on average older than the patients in the present study (52.4 yrs and 42.4 yrs respectively). Given the large body of evidence of an age-related decline of testosterone, it is likely that the discrepancies between the present results and Cooper et al.'s (1989) might be reflective of the age difference between the two study groups.

More significantly, the study design employed by Cooper might have precluded the authors of observing any difference in testosterone, as these authors sampled testosterone 15 minutes after each ECT session. There is quite a consistent body of literature which suggests that testosterone is adversely affected by physical (Grant et al. 1997), as well as psychological stress (Christiansen and Hars, 1995). Therefore, the lack of difference in testosterone that may have accompanied the change in clinical status might have been due

to the stress-related decrease in testosterone. In fact, these authors did report a trend for lower testosterone levels following each session of ECT.

Another study, by Sachar, et al (1973), also failed to report a difference in testosterone levels during depression compared to remission. Compared to the patients in our study, these patients were also considerably older (mean age 61.5 yrs and 42.4 yrs respectively). It is a widely accepted observation that testosterone levels decrease with age. Therefore, while there may have been a trend for an increased testosterone following remission, the difference may not have been large enough to be detected by statistical methods.

It is unfortunate that, as far as we are aware, none of the above studies, nor other reports, have examined free T, as well as SHBG, in addition to total T, in relation to clinical outcome of depression, therefore, we have no basal studies to which the present results could be compared. In the present study, the percentage change in free T on remission was only minimally larger than the change in Total T.

7.4 Possible explanations of the results

While the present study aimed to investigate the relationship between HPG measures and depression, we did not set out to determine the causes or the direction of the relationship, thus, the mechanisms underlying the causes for the relationship between testosterone and depression can not be elucidated by the design of the present study. Nevertheless, identification of a causal factor (or factors) would be a very difficult task, owing to the

strong possibility that the relationship is most likely to be bidirectional and influenced by a multitude of biological and social factors.

Review of previous results, as well as results from the present study generates two hypotheses for a relationship between depression and testosterone: firstly, there is the possibility that low testosterone initially causes depressive symptoms that may or may not progress into a depressive illness, and secondly, the presence of depressive symptoms may lead to blunting of the HPG axis function.

It is physiologically possible that low testosterone levels to start with may directly precipitate physical symptoms such as muscle wasting, anorexia, fatigue, disrupted sleep and decreased libido. These alterations might in turn have a degrading effect on mood and precipitate depression in vulnerable individuals. There are several lines of evidence which suggest that low testosterone is related to these above physical symptoms. For example, as outlined above, several clinical trials investigating the effects of testosterone administration on mood have concluded that testosterone's beneficial effect on mood is attributed to its primary effect on somatic features, such as improvement in sleep, weight, energy levels and libido (Grinspoon et al. 2000; Rabkin et al. 2000). The improvement in these features then acts to improve mood. In addition, other studies have outlined the correlation between testosterone and somatic features in normal subjects (Schiavi et al., 1992) as well as patients with HIV (Grinspoon, et al. 2000) and depression (Baumgatner et al. 1988). Furthermore, findings that pharmacologically induced hypogonadism by the administration of GnRH antagonists induces depressive symptoms are consistent with the

hypothesis that low levels of testosterone may initiate depressive symptoms in some individuals (Rosenblatt and Mellow, 1995; Schmidt et al., 2004). Finally, this hypothesis is strengthened by observations that patients with hypogonadism show a greater increase in the risk of incident depression compared to normal patients (Shores, et al 2004).

The observation that the somatic features factor, over and above the other depression factors, was the only factor which significantly contributed to the variability in free and total testosterone in the present study, supports the above hypothesis that the relationship between depression and testosterone can be attributed to somatic features. Another observation which suggest that testosterone is related to somatic features is provided by the observation in the current study that the patients with melancholic features exhibited lower testosterone levels compared to patients without melancholic features.

Melancholia, as defined by the DSM-IV, is characterised by the presence of somatic symptoms, such as decreased appetite and sleep and psychomotor disturbance (APA, 1994).

The second hypothesis evoked in the explanation of the relationship between testosterone and depression concerns the possibility that depressive illness leads to blunting of the HPG axis. This hypothesis has been mostly derived from observations of the effects of psychological aspects of depression on testosterone. Namely, the hypothesis is proposed that testosterone levels may be mediated by a psychological construct akin to a victory-failure axis. There exists a large body of both, primate and human studies, which provide evidence that failure (submissiveness, losing a competition, social defeat) reduces

testosterone levels. Animal models show lowered testosterone levels with losing a high position in the social hierarchy following defeat by the dominant male (Rose, et al. 1975). In men, there seems to exist a similar pattern of testosterone response as a function of defeat or success. A number of studies show that experiences of winning a competition, feeling dominant, or a rise in the social hierarchy increase testosterone levels (Mazur and Lamb, 1980; Ehrenkranz & Bliss et al, 1974). In contrast, experiences of being dominated, social defeat and losing a competition decreases testosterone levels (Booth, et al 1989; Krauss, Heistermann and Kappeler 1999). The symptoms of sense of failure, hopelessness, submission, loss of control and apathy are some of the central characteristics of depression (APA, 1994). Thus, it is likely that the prolonged presence of feelings associated with defeat, failure and hopelessness leads to a subsequent decrease in testosterone levels. Indeed, the observation of a strong negative relationship between testosterone and duration of depressive episode support the notion of a progressive decrease of testosterone with depression.

7.5 Implications of the current results

The current findings have clinical, as well as research implications. Firstly, the present findings may increase clinicians' awareness of the associations between depression and testosterone who might be prompted to consider screening patients for testosterone levels, particularly patients with somatic complaints.

Secondly, as depression has reached a status of a major health burden and is a high risk factor for suicide, particularly in men, identifying conditions (such as low testosterone

levels) that could potentially increase the risk of depressive illness could provide crucial opportunities for early intervention and treatment. By the same token, education and counseling initiatives could be implemented which educate men about depression and how to recognise and acknowledge its symptoms and seek help as early as possible. It is likely that prolonged delay in treatment of depression would not only lead to severe depression, but it can also precipitate problems associated with low testosterone, such as muscle wasting, decrease in bone density (Schweiger, et al. 1994) and may increase the risk for the development of diabetes and myocardial infraction (Tibblin, Adlerberth, Lindstedt et al., 1996).

7.6 Methodological considerations:

Results from the present investigation must be considered in light of several methodological and conceptual limitations:

Firstly, the emotional stress of the experimental situation itself may affect hormone levels, this potentially being a stronger factor for patients with depression. Psychological and laboratory literature indicates that testosterone levels decrease in stressful conditions (Christiansen and Hars, 1995). This is a potentially significant factor which must be considered in future studies.

Another feature that could possibly be confounding the present conclusion is that the results are based on a single blood sample. This could potentially have effects on the observed results due to the circadian nature of testosterone as well as the intraindividual

variation of hormone. While there is no total consensus in the literature as to the circadian rhythms of testosterone, most studies indicate that in normal men, levels are highest in the early morning, and then gradually decrease throughout the day (Winters, 2001). The potential significance of this observation for the present results is compounded by the lack of strong evidence of the pattern and magnitude of circadian rhythms during depressive illness. However, the present results are in accordance with authors using multiple (Mason, 1989), as well as 24-hour sampling of testosterone (Schweiger, 1999). In addition, we have standardized our sampling time for all of the patients to between the hours of 8.30 am and 10.30 am, thereby reducing any variability in testosterone levels attributable to circadian rhythms.

Regarding intraindividual variation, testosterone, however, has been shown to have a high intraindividual correlation, which indicates that a single assay characterises an individual reliably (Cauley, Gutai, Kuller et al 1991). In addition, there is evidence that a single total T sample is a reliable measure of testosterone status: In a study that assessed eight testosterone measurements over one year in 169 middle-aged and elderly men, the first sample was highly correlated ($r = 0.90$) with the annual mean testosterone level from all samples (Vermeulen & Verdonck, 1992). However, the use of a single hormone measurement might have produced misguided results for LH levels due to this gonadotropin's pulsatile nature.

Another potentially confounding effect in the present study is the use of various medications by the patients for the duration of the study. Psychotropic medications may

affect the HPG axis at many levels and may also influence levels of gonadal hormones by interfering with all aspects of hormone production: secretion, transportation, protein binding and metabolism. Furthermore, some patients were taking antipsychotics or mood-stabilisers in addition to antidepressants. Although there exist inconsistencies in the literature (Brambilla, 1975; Hunter, Christie, Whalley et al., 1989), there are reports which indicate that antipsychotics and mood stabilisers may affect HPG functioning (Brown, 1981; Whalley, 1987), the effect of an administration of these in combination with antidepressants on the HPG hormones is not known. The present methodological design allowing the use of psychiatric medication was due to ethical and practical reasons.

Similarly, we were only able to measure the total level of LH in this study. While absolute values of LH are of primal importance and relevance to the examination of the HPG axis functioning, pulse frequency is also a very important parameter measure which ought to be examined to assess whether this is the same as in normal men or its affected by depression.

Another consideration for the implication of the present results is that the “gold standard” definition of remission, a cutoff score of 7 on the 17-item Hamilton depression scale, has been questioned by some investigators who have suggested that this cutoff score is too high (Judd, 2000; Nierenberg, Keefe, Leslie et al., 1999). It is argued that a cutoff of 7 on the Hamilton depression scale allows for the presence of residual depressive symptoms, and it has even been suggested that some patients may even qualify for a depressive disorder diagnosis at this level of symptomatology (Nierenberg et al., 1999). Indeed, in a

longitudinal study following patients for up to 15 months, Paykel (1995) found residual symptoms on remission to be a common phenomenon in depression, occurring in 32% of patients who remitted.

Definition of remission of depression has been the subject for much debate, especially given the chronicity, and complexity of depressive symptoms. It is possible that some, or even most of our patients who were classified as remitters at followed-up did have residual depressive symptoms. The cut-off score of 7 or less on the HDRS in the present study was chosen as it reflects the vast majority of clinical research practice.

A further potential methodological consideration of the present study is that exercise was not assessed. Given the current evidence that exercise is a potential factor which might affect HPG functioning, and that levels of energy are a common symptom in depression, examination of exercise levels, or some other indicator of energy levels would have yielded more information in the present study. The decision not to include an exercise questionnaire was twofold: firstly, as this study was performed in an inpatient population, there is a relatively good reason to assume that patients would be undertaking similar activities during their admission; secondly, given that the present population of patients was suffering from a relatively severe depressive episode, where fatigue levels are quite high, energy expenditure was not measured due to an attempt to minimise the number of questionnaires and therefore the time required for assessment of each patient, and thirdly, and most importantly, as far as we are aware, there does not exist a validated, reliable

energy and exercise assessment tool which standardised for use in population of inpatients with depression.

Additionally, as the current study involves a longitudinal aspect, the possibility of seasonal variations of testosterone can not be entirely excluded as having an effect on the results. However, the evidence for seasonal variations is very weak and this variation seems to be prominent in countries which experience extreme seasonal variation in sunlight and temperature, such as Scandinavian countries. In addition, circannual variation of testosterone is most prominent in the animal community where seasonal breeding is crucial for survival.

Finally, as this study focused on men admitted to a private tertiary psychiatric hospital for the treatment of depression, the findings may not be applicable to other clinical populations or patients in primary health care. Future studies are needed whereby the existence of this relationship in different population groups will be examined.

7.7 Final conclusions

While the notion of a relationship between depression and testosterone has been conceptualised many years ago, there has been an ongoing debate and speculation as to the nature and the magnitude of the relationship. The present explorative study has yielded restricted, but valuable information regarding the relationship between testosterone and depression. Addressing some of the methodological issues pertaining to previous studies in this field (such as the number and heterogeneity of subject size, design

of study, measure of depression severity), we were able to demonstrate previously reported decrease in free and total testosterone as a function of age, although, in the present study, both free and total testosterone exhibited similar rate of decline with age. In addition, the present study reflects previous reports of an inverse relationship between severity of depression and levels of testosterone. By applying multiple regression analyses using factor structures obtained from the present, as well as previous HDRS scores, we were able to demonstrate that the relationship between severity of depression and testosterone is mostly attributable to somatic features of depression. Finally, the current results expand the available body of literature of an association between endocrine systems and depression, which has included major hypothalamic-pituitary systems such as the HPA, HPT and HPGH.

7.8 Future Directions

This research has been exploratory in nature and the observed relationship between testosterone and depression requires further investigation. Future research studies should analyse whether there is a difference between somatic features and other features of depression and confirm these findings with larger number of patients. In addition, further studies on the effects of antidepressant medication on testosterone are needed to investigate the possibility that relationships observed between testosterone and depression are due to the effects of antidepressants.

Furthermore, we observed a high component of somatic symptoms reported, which supports earlier findings that men tend to report somatic symptoms more than women.

The reason for this gender bias in reporting symptoms might be that it is socially more acceptable for men to report somatic symptoms, as they might be more salient and easier to describe, report and recall. Studies specifically designed to disentangle these factors are needed. In addition, the reinforcement of developing and trialing diagnostic tools or questionnaires specifically designed for men, which reflect this possibility is in order.

Finally, in light of observations that testosterone increases with improvement of depression, as well as the relative low risk of side-effects of testosterone supplementation, future research attempts need to be made that assess the efficacy of testosterone supplementation for the treatment of depression.

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