ANDROGENS IN MEN DURING ILLNESS, EXERCISE AND PSYCHOLOGICAL STRESS

A Thesis submitted

by

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DECLARATION

Hospital ethical committee permission was granted for all projects in this thesis and informed consent obtained from patients prior to investigation. All publications referred to have been read by and are familiar to myself. I have relied heavily on the expertise of laboratory based colleagues and while I am not familiar with the intricacies of all the techniques utilised I have a full understanding of the principles involved. With the exception of the LH pulse profiles of normal men described in Chapter 14 I personally performed all procedures at patient level, arranged for appropriate biochemical analyses to be performed and statistically analysed resulting data. All associated publications were written by myself as was the entire thesis. Although much of this work has been presented at scientific meetings and has either been published or submitted for publication in medical journals, the contents of this thesis have not been submitted previously for any degree or diploma.

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SUMMARY

My early clinical experience had demonstrated that abnormalities of endocrine testing particularly with regards to thyroid function are common in ill patients.

Interest in endocrine effects of illness was further stimulated by my study of hormone levels of uraemic patients undergoing differing forms of dialysis therapy. Chronic renal failure resulted in abnormal hypothalamic-pituitary-testicular and hypothalamic-pituitary-thyroid function irrespective of the nature of dialysis. Evidence for low levels of adrenal androgen in serum of uraemic patients was also found. As it was well known that abnormalities of thyroid function occurred in a variety of illnesses so it appeared possible that abnormalities of adrenal androgen status and hypothalamic-pituitary-testicular function might also be non-specific consequences of illness.

To investigate this hypothesis these endocrine parameters were measured in several groups of patients:- those ill in a general medical ward, in diabetic men with ketoacidosis, during recovery from burns injury and before and after surgery.

Serum levels of the most abundant adrenal androgen dehydroepiandrosterone sulphate (DHAS) were low in patients with a variety of non-endocrine medical illnesses only if they had been unwell for more than two weeks while serum androstenedione concentrations were higher than in controls irrespective of the duration of illness. Testosterone levels were also low but this was not related to the duration or severity of illness nor could it be accounted for by changes in binding proteins, hyperprolactinaemia nor on the basis of classical primary or secondary testicular failure.

A study of men during an episode of diabetic ketoacidosis revealed that this complication of diabetes mellitus is also accompanied by

depression of serum testosterone concentrations. However serum testosterone concentrations of non-ketotic diabetic men at routine review were not found to be related to diabetic control as assessed by glycosylated haemoglobin estimation. There was some limited evidence in the literature to suggest that adrenal androgen levels in diabetic men might be low and that this might have a deleterious effect on islet cell function but no abnormality of these hormones could be demonstrated in this group of patients.

19 burned men were followed sequentially for several weeks. Profound depression of serum testosterone concentrations for several weeks was found with levels falling into the range found in healthy females in the majority of patients studied. This could not be explained on the basis of binding protein changes nor by alterations in the concentrations of prolactin or gonadotrophins. DHAS levels although normal within 24 hours of admission fell to subnormal levels and remained low for several weeks. Serum androstenedione levels were high following admission and tended to remain so for several weeks. These changes in adrenal androgen concentrations were reminiscent of my observations during medical illness.

Serum gonadotrophin levels of post-menopausal females were studied following cholecystectomy. Unlike the situation in medically ill men this operation led to a marked reduction in gonadotrophin levels sometimes down to the pre-menopausal range. Serum DHAS levels fell to below control values four days after surgery while serum androstenedione concentrations rose transiently post-operatively. The situation was however complicated by the administration of several drugs in particular parenteral opioids which might have affected gonadotrophin levels.

As such profound endocrine changes had been observed with illness, it was of interest to discover whether these were specific to illness or whether other forms of stress such as the physical stress associated

with exercise or the psychological stress accompanying academic examinations might result in similar changes.

Following marathon running there was a small fall in serum testosterone concentrations. The situation differed from that following illness in that there was a massive elevation of serum cortisol as well as a fall in luteinising hormone (LH) levels. On the other hand veteran athletes who had run at least 25 miles a week for many years and who are therefore exposed to recurring and chronic physical stress had normal serum testosterone levels. Neither veteran athletes nor marathon runners showed evidence of a reduction in serum concentrations of adrenal androgens. The stress of physical exercise has a short-lived adverse effect on testicular function but unlike illness there are no changes in adrenal androgen levels.

Review of the literature suggested that psychological stress can adversely affect gonadal function. As illness in general can be psychologically stressful it seemed possible that the endocrine changes found could be a consequence of associated psychological stress. To further elicit the role of psychological stress endocrine function was examined in students several months before and immediately after a series of Degree examinations having excluded other factors which might have affected hormone levels. Increased psychological stress, as assessed by two anxiety rating scales, was reflected in increased excretion of urinary metadrenalines. However, there were no accompanying changes in testicular or adrenocortical hormone levels. The hormonal changes occurring with illness therefore are unlikely to be caused by the associated psychological stress.

Did the low levels of total testosterone found in ill men reflect a reduced availability of this hormone at the cellular level? Free testosterone levels, often considered the physiologically active component of total testosterone, were depressed in burned men to an extent similar

to total testosterone levels suggesting that there is a diminished supply of this hormone to cells. Our burned patients had been found to have low sex hormone binding globulin (SHBG) capacity within 24 hours of admission. This assay depends on the ability of the patients' serum to bind dihydrotestosterone and thus a fall in SHBG capacity might be due to reduced affinity of this binding protein for testosterone. The fall in SHBG capacity was closely mirrored by the change in SHBG measured by an immunoradiometric assay, suggesting that burns injury is followed by a fall in the absolute concentration of SHBG rather than by a reduction in its affinity for testosterone.

Normal hypothalamic-pituitary-testicular function is associated with intermittent rather than constant release of LH. Uraemic men showed impaired LH pulsatility when compared with healthy controls. This might be due to a hypothalamic-pituitary abnormality but altered metabolism of LH occurs in renal failure and might account for this deficit. Nevertheless absence of LH pulsatility might contribute to the low testosterone levels found in uraemic men and encouraged me to search for a similar disturbance in burned men.

Burned men seemed suitable for further investigations of the mechanisms leading to low testosterone levels as these patients have normal renal and liver function and have a prolonged and profound depression of levels of this hormone. Most burned men showed a normal testicular response to stimulation with human chorionic gonadotrophin suggesting that the major defect was at the level of the hypothalamus or pituitary. LH but not follicle stimulating hormone (FSH) levels responded normally to stimulation with luteinising hormone releasing hormone (LHRH) in most men. Multiple blood sampling of burned men revealed evidence of reduced LH pulsatility but the results were very heterogeneous with some individuals having absent pulsatility whilst others pulsed normally. The

biological activity of LH when testosterone levels were suppressed was low and increased following recovery.

The final study described in this thesis examined LH pulse frequency of burned men using a shorter sampling interval and a more refined method of pulse analysis. In addition LH was measured by radioimmunoassay and bioassay through the whole pulse profile for each patient. In this study there was no significant difference in frequency of LH pulses between the burned patients and the control group although the former once again showed a heterogeneous response with most patients pulsing normally but one patient having no detectable pulses. In normal subjects an LH peak was followed by an increase in serum testosterone concentrations but this was not the case following most LH peaks in burned patients. LH pulses in burned men also differed from those in controls in that the biological activity of LH during a peak was less than that during a nadir whilst in normal men the biological activity of LH within a peak was greater than that during a nadir. Thus during illness LH may be secreted in a less pulsatile manner than normal and may be qualitatively abnormal thus being unable to promote testosterone secretion.

Possible mechanisms leading to these changes in serum androgen levels and their consequences to the ill patient are discussed.

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
A'DIONE	Androstenedione
B:I ratio	Ratio of Biological activity to radioImmunoassay activity of luteinising hormone
CAPD	Continuous ambulatory peritoneal dialysis
CBG	Corticosteroid binding globulin
CV	Coefficient of Variation
DHA	Dehydroepiandrosterone
DHAS	Dehydroepiandrosterone sulphate
HCG	Human chorionic gonadotrophin
HD	Haemodialysis
^{125}I	Isotope of iodine
FSH	Follicle stimulating hormone
LH	Luteinising hormone
LHRH	Luteinising hormone releasing hormone
PBI	Protein-bound iodine
PTH	Parathyroid hormone
RIA	Radioimmunoassay
SE	Standard error of the mean
SHBG	Sex hormone binding globulin
SHBG-IA	Sex hormone binding globulin measured by immunoradiometric assay
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine binding globulin
TRH	Thyrotrophin releasing hormone
TSH	Thyroid stimulating hormone
UD	Undetectable
UFC	Urinary free cortisol

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INTRODUCTION

Following appointment to the staff of the Endocrine Unit at Glasgow Royal Infirmary in 1980, I encountered a fascinating case which drew my attention to the effect of serious illness on endocrine function.

Mistaken diagnosis of secondary hypothyroidism

A 76 year old man was admitted with effort dyspnoea and physical signs of right heart failure. Biochemical screening revealed impaired renal function with creatinine clearance only 13 ml/minute as well as thyroid function tests which when repeated over the ensuing few weeks were as follows:-

	T4	T3	TSH
Admission	4	0.7	UD
Week 1	20	0.8	UD
Week 2	34	1.0	1.8 following) 5.0 TRH) 3.6
Week 12	80	1.4	1.6 following) 4.8 TRH) 3.6
Normal range	55-144 nmol/l	1.4-2.8 nmol/l	<0.8-5.0 mU/l

Gonadotrophins were in the high normal range and responded normally to injection of LHRH. Insulin induced hypoglycaemia testing during week 2 showed a basal serum cortisol of 300 nmol/l (normal range 200-700 nmol/l) failing to increase during the test. There was a normal TSH response to TRH (increment >2mU/l with 60 minute value <20 minute value).

Because of low thyroid hormone levels and the poor cortisol response to hypoglycaemia he was diagnosed as having hypothyroidism secondary to partial hypopituitarism and was treated with thyroxine and glucocorticoid replacement therapy. While on this treatment his cardiac failure worsened.

Following specialist referral further scrutiny of the biochemical data showed that his blood glucose during the insulin stress test had not reached hypoglycaemic levels and when this test was repeated he showed a normal cortisol response to adequate hypoglycaemia. Abnormal thyroid function tests were then attributed to systemic disease. Endocrine replacement therapy was subsequently withdrawn and his condition remained stable. Cardiac failure was controlled and he progressed well. Four weeks after stopping replacement therapy thyroid function tests were repeated (Week 12) and were normal.

Subsequent reading of the literature led to the realisation that hormonal changes occur in quite a number of illnesses, different authors having made observations relating to their own area of interest. Thus it was widely held that endocrine changes in chronic renal failure were caused by accumulation of urea and other waste products, in respiratory failure they were blamed on hypoxaemia while as a general endocrinologist I wondered whether they were non-specific markers of ill health.

Most of the literature relating to the field of endocrine adaptation to illness followed the development of specific radioimmunoassays for hormones in serum. However, the principle of endocrine adaptation had been suggested previously by Hans Selye (Selye, 1936) who proposed the term "the general adaptation syndrome" to describe the body's responses to insults as diverse as hypothermia, surgery, exercise and noxious drugs. With access only to rather crude hormone assay methods and relying primarily on animal experimentation, Selye (Selye, 1946) considered the

anterior pituitary gland to be of central importance to "the general adaptation syndrome". He deduced that following exposure to stress the anterior pituitary gland secretes a corticotrophic hormone activating the adrenal cortex to produce essential glucocorticoid; at the same time output of less crucial pituitary hormones, such as reproductive hormones, growth hormone and prolactin appeared to decrease. As assay methods have progressed allowing detailed endocrine investigation of the stressed human being, it has become increasingly clear that a variety of hormonal changes do occur with illness although the advantages to survival are not always clear.

My earliest project focussed on the hormonal changes accompanying chronic renal failure. This subject already commanded an extensive literature. The establishment of a new therapeutic technique, continuous ambulatory peritoneal dialysis, provided new scope to investigate this group of patients. Whereas abnormalities of thyroid function were recognised in a variety of illnesses other than chronic renal failure, there were much less data available on androgen concentrations. I set out to examine the hypothesis that other illnesses would have a similar adverse effect on androgen levels as chronic renal failure. My initial studies had concentrated on abnormalities of hypothalamic-pituitary-testicular function and this system has continued to be of great interest throughout the course of my work. As a chance finding in uraemic patients I had noted unusually low levels of the most abundant adrenal androgen dehydroepiandrosterone sulphate (DHAS) so in subsequent studies adrenal androgen status was monitored to discover whether this finding was specific to the uraemic state or merely a marker of ill health.

Although most of the subsequent thesis is concerned with the effects of illness on endocrine function it was considered important to study other forms of stress. The ill patient may experience significant psychological stress and indeed physical stress unrelated to illness such

as might occur during endoscopic or other invasive procedures. Stresses during university examinations and following marathon running were selected as readily available models for these two differing forms of stress.

To the female reader I must apologise for apparent bias as most of my work has been performed on males. This was a deliberate manoeuvre as studies of endocrine parameters in females are complicated by the cyclical nature of their sex hormones. Additional difficulties arise when collecting control data from female colleagues as exact details of their menstrual cycle as well as oral contraceptive use have to be obtained.

The review of the literature which follows includes some areas which although not studied in detail in this thesis are thought to provide the reader with a better overall grasp of the field.

CHAPTER 1

REVIEW OF LITERATURE

This review aims to give the reader a factual summary of the literature pertinent to this thesis. Some papers are discussed in more depth in the appropriate chapters. Emphasis is placed on those endocrine systems which I have investigated in detail, in particular the hypothalamic-pituitary-testicular and the hypothalamic-pituitary-adrenocortical axes. Changes in hypothalamic-pituitary-thyroid function with illness are also discussed as they have been extensively reported in the literature. An understanding of how this axis is affected by illness may shed light on the mechanisms leading to altered testicular and adrenocortical function.

CONTROL OF ANDROGEN SECRETION

Before reviewing the literature relating to hormonal changes with illness it is appropriate to describe the mechanisms thought to be important in the control of secretion of androgens be they of testicular or adrenocortical origin.

PHYSIOLOGY OF HYPOTHALAMIC-PITUITARY-TESTICULAR FUNCTION

Testosterone secretion from the Leydig cells of the testes depends on secretion of luteinising hormone (LH) by the anterior pituitary. The classic work of Harris (1955) demonstrated that gonadotrophin secretion by the pituitary gland and hence normal gonadal function is dependent on hypothalamic factors and on an intact pituitary portal blood supply connecting the median eminence to the anterior pituitary. A single hypothalamic decapeptide stimulates secretion of both follicle stimulating

hormone (FSH) and LH (Schally et al., 1971). LH secretion is under negative feedback control by testosterone mainly at the level of the hypothalamus (Plant & Dubey, 1984) while FSH release may be suppressed by inhibin or related proteins produced by the Sertoli cells of the testes (Lincoln, McNeilly & Sharpe, 1989).

The LH pulse generator

Krey, Butler, & Knobil (1975) demonstrated that surgical disconnection of the medial basal hypothalamus from the rest of the central nervous system of the rhesus monkey did not affect control of LH secretion, confirming the pivotal role of the hypothalamus rather than higher cerebral centres. LH secretion in man is not constant but intermittent as can be demonstrated by frequent blood sampling (Nankin & Troen, 1971; Naftolin, Yen & Tsai, 1972). Direct sampling of pituitary stalk blood in rhesus monkeys has demonstrated that LHRH secretion is pulsatile (Carmel, Araki & Ferin, 1976) and simultaneous sampling of blood from the jugular veins and pituitary portal system of sheep showed that LHRH and LH pulses are synchronous (Clarke & Cummins, 1982). Measurement of LHRH in the perifusate of the hypothalamus of unanaesthetised sheep also demonstrated LHRH pulses which were synchronous with LH pulses (Levine et al., 1982). Thus intermittent release of LH is under the control of LHRH secreted by the hypothalamus in pulses.

Electrical destruction of the arcuate nucleus in the medial basal hypothalamus of the rhesus monkey blocked LH secretion and the negative feedback effect of sex steroid (Plant et al., 1978). Stereotactic positioning of multiple electrodes into this area demonstrated electrical discharges preceding detection of LH release by a few minutes (Wilson et al., 1984). Thus the activation of a hypothalamic pulse generator seems to be essential for LHRH and hence LH release. Factors such as alpha

receptor blockade and barbiturate anaesthesia which reduced these electrical discharges also decreased the frequency of LH pulses (Wilson et al., 1984; Kaufman et al., 1985).

The intermittent nature of LHRH release is essential for normal pulsatile release of LH; LH secretion in hypogonadotrophic monkeys was restored by pulsatile but not by constant infusion of LHRH (Belchetz et al., 1978). Replacement of the normal pulsatile release of LH by a steady state situation as occurs with chronic use of an LHRH agonist in man results in very low testosterone levels even though absolute levels of LH as measured by radioimmunoassay are less affected (Faure et al., 1982). The negative feedback effect of testosterone on LH secretion is mediated by a suppressive effect on the hypothalamic pulse generator (Plant & Dubey, 1984) although an inhibitory effect at the level of the pituitary may also play a part (Caminos, Ma & Snyder, 1977).

LH biological activity

A given immunoassay value for LH does not equate with biological activity which can be measured in a bioassay such as that based on the ability of mouse interstitial cells to secrete testosterone in vitro (Van Damme, Robertson & Diczfalusy, 1974). When the biological activity of LH is measured following the chronic use of an LHRH agonist it is very low even though LH by radioimmunoassay may be normal (St Arnaud et al., 1986). Biological activity of LH varies in health. For example it reduces with age in men (Warner, Dufau & Santen, 1985). LH secreted in a pulse has a higher biological activity than that secreted between peaks (Dufau et al., 1983; Veldhuis et al., 1983). Oestradiol infusion into normal men reduces LH biological activity disproportionately to its effect on LH measured by radioimmunoassay (Veldhuis & Dufau, 1987).

Immunoassay of a polypeptide hormone such as LH measures its absolute concentration by assessing the ability of the antibody to bind

to the antigenic determinants of the polypeptide structure. This does not necessarily reflect biologically activity (Robertson, Lambert & Loveridge, 1987) which depends largely upon the configuration of that part of the molecular structure involved in binding to the receptor on the effector cell. The antibody used in the immunoassay may be directed against a site discrete from this binding area. The antibody may therefore recognise a variety of polypeptides identical at the antibody binding region yet differing with respect to this receptor binding site and hence biological activity. Moreover most available LH immunoassays employ a polyclonal antiserum which may cross-react with a multiplicity of molecular forms of LH or indeed with its constituent sub-units.

Heterogeneity of LH

LH in man is known to be heterogeneous with several distinct peaks when subjected to electrofocussing on a sucrose density gradient (Strollo et al., 1981; Reader, Robertson & Diczfalusy, 1983). The biological activity of the LH in each peak (when obtained from human pituitary tissue or when secreted from human pituitary cells in culture) has been shown to vary with acidic forms having lower biological activity than more basic forms (Lichtenberg et al., 1984; Snyder et al., 1989).

LH is a dimer composed of an α subunit and a β subunit. The former is common to LH, FSH, HCG and TSH with the β subunit conferring the unique nature of the polypeptide. The heterogeneous nature of LH is not caused by changes in the amino acid sequence of the α or β chains but to the diverse nature of the type of sugars that bind to these polypeptides (Green & Baenziger, 1988). The presence of these sugars may be important for activity of polypeptide hormones. Van Hall et al. (1971) showed that removal of sialic acid from HCG by enzyme digestion did not alter HCG levels measured by radioimmunoassay but reduced biological activity. There remained the anxiety that the amino acid configuration of HCG may

have been damaged by the enzyme digestion and that the altered biological activity was caused by this rather than desialylation. Matzuk, Keene & Boime (1989) avoided this complicating factor by using mutated cell lines secreting HCG dimers which lack oligosaccharides at individual sites on the α chain, the β chain or both. Mutant strains of HCG without oligosaccharides on the α subunit while binding apparantly normally to the HCG receptor had a reduced biological activity. These mutant strains of HCG reduced the ability of native HCG to produce cyclic AMP in vitro thus acting as a competitive inhibitor.

There is some evidence that this type of competitive inhibition between different molecular forms of the same polypeptide hormone occurs in vivo. Administration of an LHRH antagonist to hypogonadal women caused a 30% reduction in FSH by radioimmunoassay but 60% by bioassay (Dahl, Bicsak & Hsueh, 1988). Chromatography demonstrated four peaks of different biological activity, the least active increasing after LHRH antagonist administration. This relatively inactive isohormone inhibited the ability of native FSH to stimulate oestrogen secretion by ovarian granulosa cells. Thus the pituitary does seem capable of producing isomers of polypeptide hormones with differing biological activities and this may be relevant when the abnormalities of hypothalamic-pituitary-testicular function with illness are described later in this thesis.

CONTROL OF ADRENAL ANDROGEN SECRETION

Adrenal androgen is secreted primarily by the zona reticularis of the adrenal cortex whilst cortisol is secreted primarily by the zona fasciculata. The mechanisms controlling adrenal androgen secretion are not fully understood. Adrenal androgens are under the influence of ACTH; short term ACTH administration certainly leads to increased output of adrenal androgen and intramuscular administration of long-acting ACTH over two days results in a sustained increase in DHAS ((Vaitukaitis, Dale

& Melby, 1969; Yamaji & Ibayashi, 1969). Patients with hypopituitarism and secondary adrenocortical insufficiency have low adrenal androgen levels which are returned to normal by ACTH administration (Pang et al., 1987; Yamaji et al., 1987).

Adrenocortical activation does not necessarily affect cortisol and adrenal androgen levels in the same way. For example serum levels of the adrenal androgens, DHA and DHAS increase several years before puberty, reach a peak in early adult life and gradually decline to levels around 20% of maximal in old age; all this with little change in the secretory rate of cortisol (Parker & Odell, 1980). Adrenal androgen status in patients with pituitary dependent Cushing's syndrome is of interest as DHAS levels are normal or only modestly elevated in comparison to the degree of cortisol excess (Yamaji & Ibayashi, 1969).

Observations such as those in the preceding paragraph have led to a debate about factors additional to ACTH which might influence adrenal androgen secretion. Two hypotheses exist: one postulates that adrenal androgen secretion is under control of a specific adrenal androgen stimulating hormone (Parker & Odell, 1980); and the other that secretion is affected by morphological and functional changes within the adrenal cortex induced locally by changing concentrations of steroid (Anderson, 1980). In vitro studies using human adrenal tissue have shown that intra-adrenal enzyme systems are affected in differing ways by build up of substrate (Couch, Muller & Winter, 1986). These authors found that 17,20 desmolase, the enzyme crucial to adrenal androgen synthesis, is inhibited in a more sensitive fashion than other adrenocortical enzyme systems by the accumulation of endogenous steroids. Indeed the intraadrenal steroid concentrations found in man are sufficient to exert an inhibitory effect on 17,20 desmolase (Dickerman et al., 1984). Such mechanisms may be involved in the divergence of cortisol and adrenal androgen levels mentioned above and described later in this thesis.

ILLNESS AND THYROID FUNCTION

Of all the endocrine changes which occur with illness, those affecting thyroid function are most widely recognised. This has arisen because thyroid disease is common and has adverse effects on many other systems. In addition certain clinical syndromes may mimic thyroid disease; the facies in chronic renal failure or hypothermia may resemble that of hypothyroidism whilst symptoms and signs of hyperthyroidism may simulate heart disease. Such diagnostic confusion is not unusual so an ability to interpret thyroid function tests in non-thyroidal illness has assumed increasing importance in contemporary clinical practice.

Low T3 concentrations and non-thyroidal illness

Subnormal levels of triiodothyronine (T3) are found in many clinical situations, including surgery (Burr et al., 1975), myocardial infarction (Wiersinga, Lie & Tauber, 1981), diabetic ketoacidosis (Naeije et al., 1978) and febrile illness (Talwar, Sawhney & Rastogi, 1977). The low T3 state is not exclusive to illness. Subjects who are fasted show a fall in serum T3 levels even if given thyroxine (T4) in sufficient quantities to suppress endogenous thyroid hormone secretion (Vagenakis et al., 1975). This finding suggested that low levels of T3 are due to altered thyroid hormone metabolism rather than reduced secretion of T3.

Serum T3 is largely derived from peripheral conversion of T4. Studies using ^{125}I-labelled T4 administered to patients with severe chronic illness have demonstrated reduced conversion of T4 to T3 (Carter et al., 1976). Low T3 states are also characterised by a reciprocal increase in the metabolically inactive reverse T3 (Vagenakis et al., 1975; Chopra et al., 1975). The production rate of reverse T3 in the low T3 state is not increased, the accumulation of reverse T3 occurring because

of decreased catabolism (Chopra, 1976; Kaptein et al., 1982a). In vitro evidence suggests that the conversion of T4 to T3 and that of reverse T3 to its diiodothyronine derivative may be regulated by the same 5'-deiodinase (Chopra et al., 1978). The inhibition of this enzyme system might account for most of the changes in the low T3 state.

Low T4 concentrations and non-thyroidal illness

In more seriously ill patients both T4 and T3 are low (Carter et al., 1974), a situation commonly referred to as the low T4 syndrome or the "sick euthyroid syndrome". T4 levels correlate inversely with severity of illness, the patients with the lowest levels having the highest mortality (Slag et al., 1981; Kaptein et al., 1982b).

The mechanisms leading to low T4 levels are not fully understood. Thyroid hormones are protein bound, primarily to thyroxine binding globulin (TBG) but also to albumin and in the case of T4 to thyroxine binding prealbumin (Hagen & Elliott, 1973) and thus alterations in binding influence T4 and T3 concentrations. The dialyzable fraction of T4 which is the free, non-protein bound fraction of T4 is increased in non-thyroidal illness despite normal or only slightly reduced levels of T4 binding proteins (Chopra et al., 1979a). The realisation that absolute changes in the concentration of T4 binding proteins in non-thyroidal illness are insufficient to account for the increase in the free fraction has led to the search for factors in serum which would displace thyroid hormones from their binding proteins.

An early study which provided evidence for such an agent demonstrated an increase in the free fraction of T4 when serum of patients with non-thyroidal illness was added to normal serum (Chopra et al., 1979b). Evidence also exists for inhibition of T4 binding to solid matrices as well as to serum proteins (Oppenheimer et al., 1982). When estimations of free T4 are calculated from the resin uptake of T3 and the

total T4 concentration (the free T4 index), as opposed to the more reliable equilibrium dialysis method, values are often in the hypothyroid range (Chopra et al., 1979a). This discrepancy may thus be explained by the presence of an inhibitor in serum which reduces binding not only to protein but to resin. Further work from Chopra's group revealed a substance in extrathyroidal tissues that reduces binding affinity to serum proteins (Chopra et al., 1982). So in ill patients it was postulated that such a substance might be released into the circulation perhaps as a consequence of tissue damage.

It has been suggested that the T4 binding inhibitor may be a free fatty acid such as oleic acid; they displace T4 from binding proteins especially when levels of albumin and thyroxine-binding prealbumin are low as they often are in non-thyroidal illness (Chopra et al., 1986). Lim et al. (1988) confirmed that free fatty acids displace T4 from binding proteins but only at concentrations three times those found in man. Further doubt concerning the role of oleic acid as the binding inhibitor was cast by Haynes et al. (1989) who found that T4 concentrations in serum of ill patients bore no relation to those of oleic acid or other free fatty acids.

Free thyroid hormone concentrations

The estimation of free thyroid hormones in serum is fraught with difficulty. Equilibrium dialysis methods are commonly considered the gold standard but are time consuming and not available for routine use. However, by this more reliable method free T4 is usually found to be normal or raised and free T3 normal or low in patients with the low T4 syndrome (Chopra et al., 1979a; Woeber & Maddux, 1981). Several recent methods of measuring free T4 permit its routine estimation but most have proved disappointing in the evaluation of patients with non-thyroidal

illness as free T4 levels are often found to be spuriously low (Chopra et al., 1980; Melmed et al., 1982).

Recent doubts have been cast on the reliability of equilibrium dialysis as a method of detecting free thyroid hormone levels as this method usually involves dilution of serum which may alter the effect of binding inhibitors (Faber et al., 1987; Surks et al., 1988). Both these groups used an ultrafiltration method which employs undiluted serum and described normal free T4 levels in ill patients with either normal (Faber et al., 1987) or low free T3 levels (Surks et al., 1988).

TSH concentrations and non-thyroidal illness

Unlike the situation in primary hypothyroidism when thyroid stimulating hormone (TSH) levels are high, low T4 levels in non-thyroidal illness are accompanied by normal TSH levels. TSH responses to thyrotrophin releasing hormone (TRH) are often within the normal range in individuals but may be diminished in a series of ill patients when compared with a healthy control group (Kaptein et al., 1981). The contribution, if any, of altered TSH secretion to the low T4 syndrome is uncertain. TSH levels of fasting mildly hypothyroid patients sometimes fall to within the normal range (Borst et al., 1983). Ill patients given iodide which further reduces thyroid hormone levels failed to show the expected increase in TSH levels (Maturlo et al., 1980) in contrast to fasting but otherwise healthy subjects similarly treated who showed an augmented TSH response to TRH (Gardner et al., 1979).

Ill patients with low circulating T4 have a reduced T4 secretion rate despite an increased metabolic clearance rate (Kaptein et al., 1982a). As the main influence on T4 secretion is the circulating TSH concentration, it is possible that low TSH levels contribute to the low T4 state of critical illness. Indeed Bacci, Schussler & Kaplan (1982) have demonstrated a rise in TSH as T3 levels rose with recovery from severe

illness. Using more sensitive TSH assays Wehman et al. (1985) found a low TSH concentration in association with the low T4 syndrome while both Hamblin et al. (1986) and Wehman et al. (1985) found increasing TSH levels during recovery. Although these authors suggested that reduced TSH secretion may contribute to the low T4 syndrome, other groups have found normal TSH levels in ill patients (Faber et al., 1987; Surks et al., 1988). This raised the possibility that the changes in TSH concentrations in the earlier studies may be a short-lived and secondary phenomenon; thus displacement of T4 from its binding proteins at the onset of the low T4 syndrome would lead to an increase in free T4 and consequent suppression of TSH with the reverse occurring during recovery. Romijn & Wiersinga (1990) found that the nocturnal surge of TSH found in healthy subjects was reduced in ill patients. Thus evidence exists to associate hypothalamic-pituitary dysfunction with the low T4 syndrome but what contribution this makes to the reduction in thyroid hormone levels is uncertain.

High T4 concentrations and non-thyroidal illness

Concentrations of serum T4 may occasionally be high in particular groups of patients with non-thyroidal illness. Damaged hepatocytes in certain liver diseases, in particular primary biliary cirrhosis and hepatitis both acute and chronic, release increased amounts of TBG resulting in levels of T4 and T3 which are either normal or high (Schussler et al., 1978; Gardner, Carithers & Utiger, 1982). TSH levels may be raised in some patients with chronic active hepatitis possibly due to an associated autoimmune thyroiditis (Schussler et al., 1978). A high incidence of hyperthyroxinaemia in acute psychiatric admissions has been reported and appears to resolve spontaneously within two weeks (Cohen & Swigar, 1979). This association has to be borne in mind if an inappropriate diagnosis of thyrotoxicosis is to be avoided.

Low T4 syndrome - adaptive phenomenon or tissue hypothyroidism?

It is not known whether low T4 and T3 states are a desirable adaptation to illness. Conventional wisdom is that only the free fraction of thyroid hormones is available for transport into tissues although it has been suggested that a proportion of the protein bound fraction may also be available (Pardridge et al., 1981). If circulating levels of free T3 are low in ill patients, its availability to tissues would be expected to be reduced. Why is there not clear evidence of tissue hypothyroidism? Physiological correlates of thyroid function such as systolic time intervals or ankle jerk relaxation time remain normal (Chopra et al., 1974; Spector et al., 1975).

More than 50% of T3 within nuclei (where it exerts its main action) may be derived from intracellular conversion from T4 (Crantz, Silver & Larsen, 1982). When intracellular T3 levels are high, conversion of T4 to T3 is inhibited and the converse may also apply (Koenig et al., 1984). Such a mechanism would prevent large fluctuations in intracellular T3 levels when circulating levels are low. The major tissue effects of T3 are mediated via T3 nuclear receptor proteins (Samuels et al., 1988). Williams et al. (1989) found increased levels of T3 receptor messenger RNA in cells of patients with the low T4 syndrome. They suggested that this was a compensatory mechanisms; low free T3 levels in serum and then intracellularly would promote nuclear receptor messenger RNA synthesis and hence increased supply of T3 receptor protein which would compensate for the low free T3 levels and maintain the euthyroid state.

ILLNESS AND TESTICULAR FUNCTION

Gonadal function which would seem to be of little immediate importance to the management of the ill patient has not been studied in such detail as thyroid function. The gonads have two main roles; to produce sufficient numbers of spermatozoa and ova for fertility and to

secrete sex hormones thus maintaining an appropriate endocrine milieu for sexual function. There is increasing recognition that both these aspects may be abnormal in a variety of illnesses.

Hypothalamic-pituitary-testicular function and chronic renal failure

Interest in the gonadal status of patients with chronic renal failure arose because loss of libido, impotence and infertility are common clinical problems (Elstein, Smith & Curtis, 1969; Abram et al., 1974). The importance of this subject has increased as advances in the management of end stage renal failure have led to longer life expectancy. Oligospermia and azoospermia are commonly seen in uraemic men (Lim & Fang, 1975). Serum testosterone concentrations are low and are associated with raised LH levels. The testes secrete low quantities of testosterone which are cleared normally from the circulation (Stewart-Bentley, Gans & Horton, 1974). LH levels peak normally after the administration of LHRH although they remain elevated longer than usual (Schalch et al., 1975), a finding attributable to the reduced metabolic clearance of LH (Holdsworth, Atkins & De Kretser, 1977). FSH levels are often raised, the patients with azoospermia having the highest values (Lim & Fang, 1975). Normality may be restored by renal transplantation but haemodialysis does not improve the situation (Lim & Fang, 1975).

The findings are consistent with a primary testicular defect. An additional deficit at the hypothalamic-pituitary level may exist as patients with the lowest testosterone levels do not necessarily have the highest LH levels (Lim, Kathpalia & Henriquez, 1978). Moreover an exaggerated LH response to LHRH is seen in other examples of primary testicular failure such as Klinefelter's syndrome (De Kretser, Burger & Dumpys, 1975) but does not occur in chronic renal failure (Holdsworth et al., 1977).

Hypothalamic-pituitary-testicular function and liver disease

The gynaecomastia and testicular atrophy of patients with cirrhosis have stimulated an interest in the gonadal effects of liver disease. Serum testosterone concentrations are low or normal but because of a high sex hormone binding globulin (SHBG) capacity, which is surprising in view of its hepatic synthesis, free (non-protein bound) hormone levels are frankly low (Van Thiel, Lester & Sherins, 1974; Baker et al., 1976). Oestradiol levels have been found to be normal or low (Van Thiel et al., 1974; Baker et al., 1976) whereas oestrogens of lesser potency, oestrone and oestriol, are raised the highest levels being seen in patients with gynaecomastia (Green et al., 1976). These high levels are thought to be due to increased hepatic conversion from adrenal androgens (Van Thiel & Loriaux, 1979). Gonadotrophin levels are normal or raised but the presence of normal LH levels in some individuals with low testosterone values has been cited as evidence for a hypothalamic-pituitary defect in addition to a primary testicular abnormality (Van Thiel et al., 1974).

Hypothalamic-pituitary-testicular function and other illnesses

Hypogonadism is a feature of several conditions in which the disease process seems to involve the hypothalamic-pituitary-testicular axis; for example, haemochromatosis, in which it is probably caused by iron deposition in the hypothalamus or pituitary (Bezwoda et al., 1977). Patients with lepromatous leprosy frequently have testicular atrophy due to direct invasion of the testes by acid fast bacilli (Morley et al., 1977). Hypogonadism due to primary testicular failure is a characteristic feature of myotonic dystrophy (Febres et al., 1975).

Low serum testosterone concentrations have been recognised in other clinical situations in which the primary pathology does not relate directly to the hypothalamic-pituitary-testicular axis - for example, following surgery (Matsumoto et al., 1970), myocardial infarction (Wang et

al., 1978a), burns (Dolecek et al., 1979a), head injury (Rudman et al., 1977), malignant disease (Blackman et al., 1988), acute rheumatoid arthritis (Gordon et al., 1986) and respiratory failure (Semple et al., 1981). Investigators have usually sought an explanation specific to the condition studied; thus in malignancy the associated malnutrition or altered sex steroid metabolism by tumour were proposed as possible mechanisms (Blackman et al., 1988). In the case of respiratory failure it was suggested that hypoxaemia may have had an adverse effect on hypothalamic-pituitary-testicular function. I collaborated with the author of this paper (Semple et al., 1981) on a study of men with cyanotic congenital heart disease who were otherwise fit and found normal hypothalamic-pituitary-testicular function (Semple et al., 1985). This finding as well as the disparate nature of the conditions with which low testosterone concentrations had been associated suggested to me that they may be a non-specific consequence of illness and encouraged me to embark on further studies.

Spermatogenesis is less easy to investigate in ill patients. Attempts to store semen from men with lymphoreticular malignancy prior to chemotherapy are often thwarted by finding unexplained oligospermia (Hendry et al., 1983). Abnormal spermatogenesis is often seen in patients with chronic renal failure or chronic liver disease (Lim & Fang, 1975; Mowat et al., 1976) while patients dying from severe burns not directly affecting the gonads have abnormal germinal epithelium on testicular histology (Dolecek et al., 1983).

Possible mechanisms leading to hypogonadism following illness

Several mediators of this anti-gonadal effect have been postulated. Cortisol which is often elevated during acute illness (Melby & Spink, 1958) may adversely affect the gonads directly (Cumming, Quigley & Yen, 1983) as well as cause hypothalamic-pituitary suppression (Luton et al.,

1977). An intravenous infusion of adrenaline leads to a small decrease in serum testosterone concentration (Levin et al., 1967) but there is no evidence that endogenous catecholamines might have such an effect.

The only situation in which low LH levels have been unequivocally implicated in the low testosterone state is following traumatic brain injury when LH levels are low but respond normally or in an exaggerated fashion to LHRH (Rudman et al., 1977; Clark, Raggatt & Edwards, 1988). This suggests a hypothalamic abnormality in the presence of normal pituitary synthesis and storage of LH. Acute rheumatoid arthritis also differs from other conditions in which low serum testosterone levels are found. LH levels are raised suggesting a defect at the testicular level possibly due to rheumatoid vasculitis involving the testes (Gordon et al., 1986).

Gonadotrophin status in other conditions is complex. Following surgery there is a rise in LH concentrations within 60 minutes of the first incision falling to below preoperative levels on the second postoperative day and increasing above baseline again on the sixth day (Aono et al., 1972). The early rise in LH has since been attributed to the effects of general anaesthesia (Nakashima et al., 1975). Although some authors have confirmed the fall in LH levels within 48 hours of operation (Charters, Odell & Thompson, 1969; Hagen, Brandt & Kehlet, 1980), others have found no change (Carstensen et al., 1973) or an increase (Wang, Chan & Yeung, 1978b). These discrepancies may be explained by differences in general anaesthesia or surgical procedure. Low testosterone levels were associated with low or normal LH levels in hypoxic men (Semple et al., 1981) but with raised levels following myocardial infarction (Wang et al., 1978a). In burned patients with very low testosterone levels Dolecek et al. (1979) found no consistent change.

Normal testosterone secretion is accompanied by the presence of regular secretory pulses of LH (Nankin & Troen, 1971) which occur

secondary to the pulsatile release of LHRH by the hypothalamus (Carmel et al., 1976). Any factor which interferes with the hypothalamic pulse generator might lead to abnormalities in LH pulsatility and reduced testosterone secretion. For example the opioid antagonist, naloxone, increases the rate and amplitude of LH pulses in normal men (Grossman et al., 1981) suggesting that excessive opioid tone might depress LH pulsatility. Endogenous opioid peptides are increased concurrently with adrenocorticotrophic hormone (ACTH) and thus are likely to be increased in ill patients (Guillemin et al., 1977). Evidence of lack of LH pulsatility was found in two severely ill post-menopausal women during multiple sampling (Warren et al., 1977). An abnormal hypothalamic signal in ill patients resulting in a failure to generate normal LH pulses might contribute to the low testosterone state. This hypothesis has been investigated in more detail in my own studies.

ILLNESS AND ADRENOCORTICAL FUNCTION

The adrenal cortex secretes three different types of steroid; glucocorticoid, mineralocorticoid and adrenal androgens.

Cortisol levels and illness

Urinary excretion of glucocorticoid is increased after surgery and other trauma, the duration and extent of the elevation increasing with severity of the insult (Moore et al., 1955; Espiner, 1966). Serum levels may be high (Melby & Spink, 1958) but are sometimes within the normal range (Carey, Cloutier & Lowery, 1971; Bane et al., 1974).

About 7 percent of cortisol is free or unbound, the rest bound to corticosteroid binding globulin (CBG) and to a lesser extent albumin (Daughaday & Mariz, 1961). CBG is saturated by levels of serum cortisol around 550 nmol/l above which the percentage of cortisol which is free increases rapidly (Daughaday & Mariz, 1961). Studies on ill subjects which

have included measurement of the free cortisol fraction in the circulation have found that this increases more than total cortisol (Murray, 1967; Barton & Passingham, 1981). Those authors who have found high normal total cortisol levels in ill subjects (Carey et al., 1971; Bane et al., 1974) might have found unequivocally raised free cortisol levels had these been measured. Plasma ACTH concentrations although rarely measured, correlate poorly with cortisol levels (Vaughan et al., 1982) possibly reflecting the insensitivity of earlier ACTH assays to changes within the physiological range.

Aldosterone levels and illness

Secretion of the major mineralocorticoid aldosterone is influenced by ACTH although the main controlling factor is the renin-angiotensin system. The rise in aldosterone values seen following surgery and burns injury is accompanied by activation of the renin-angiotensin system and may persist for several weeks in burned patients (Bane et al., 1974; Griffiths et al., 1983). This probably protects by counteracting the volume depletion of trauma or other acute illness. In contrast to burned patients, Zipser et al. (1981) found inappropriately low aldosterone levels with raised cortisol and renin values in persistently hypotensive patients in an intensive care unit. Luger et al. (1984) compared ten patients on admission to an intensive care unit with ten who had been there for one week; the more chronically ill had lower aldosterone levels than the recent arrivals despite higher angiotensin II levels, a phenomenon they attributed to concurrent drug administration. Findling, Waters & Raff (1987) found that 21 per cent of seriously ill patients had a low serum aldosterone level despite a high plasma renin activity. Wade et al. (1988) found raised aldosterone levels in men within 24 hours of admission to an intensive care unit, the extent of the elevation depending

upon severity of illness. It appears therefore that only the more chronically ill may have unexpectedly low aldosterone levels.

Adrenal androgen levels and illness

Because adrenal androgens are influenced by ACTH (Vaitukaitis et al., 1969), one might expect adrenocortical activation following illness to lead to increased adrenal androgen levels.

Early investigators found little or no change following surgery in the excretion of urinary 17-ketosteroids in contrast to the pronounced rise in urinary excretion of glucocorticoids (Forbes et al., 1947; Moore et al., 1955). Urinary 17-ketosteroids represent the excretory products of a number of androgenic steroids and contain a variable gonadal component. As previously discussed, serum testosterone concentrations may fall after surgery and other illnesses. Failure of urinary 17-ketosteroid excretion to increase with illness could be due to a fall in serum gonadal steroid levels masking an increase in adrenal androgen secretion or alternatively adrenal androgen concentrations may not increase despite adrenocortical activation.

Until recent years the data on adrenal androgens in serum of ill men have been scanty. Low levels of DHAS had been found in uraemic patients (Zumoff et al., 1980), a finding refuted by Winer et al. (1982) who found normal values. The earlier paper used sera from normal subjects as a control whereas the latter used sera from chronically ill patients. An alternative explanation for the findings of Winer and colleagues (1982) might be that all chronically patients whether uraemic or not have low levels of DHAS. This hypothesis is investigated further in this thesis.

ILLNESS AND OTHER HORMONES

Hormones and energy supply

During illness energy requirements alter and there is an increased demand for supply of energy substrate. Activation of the sympathetic nervous system in ill patients leads to catecholamine release, usually short-lived but sometimes prolonged in very ill patients in whom there may be a rapid fall before death (Benedict & Grahame-Smith, 1978). Catecholamines promote hepatic glycogenolysis and gluconeogenesis as well as stimulating lipolysis with subsequent release of free fatty acids and glycerol. They also stimulate glucagon release and inhibit insulin secretion (Gerich, Karam & Forsham, 1973).

Glucagon is therefore raised in illness, usually in proportion to its severity; insulin levels are inappropriately low in relation to the elevated blood glucose values (Rocha et al., 1973). This alteration in the balance between insulin and glucagon secretion despite hyperglycaemia allows persistence of glucagon-mediated events in the liver, in particular glycogenolysis and gluconeogenesis using the products of enhanced lipolysis and protein breakdown.

Plasma growth hormone also increases after injury despite levels of plasma glucose which in the normal person would be expected to inhibit its secretion (Carey et al., 1971). Levels usually fall rapidly to normal although raised levels may be sustained for weeks in severely burned patients (Wilmore et al., 1975).

The elevated cortisol levels previously described have multiple effects on glucose metabolism. In most tissues glucocorticoid inhibits glucose uptake; in the liver glycogen synthesis and gluconeogenesis is stimulated an effect enhanced by its catabolic effect on protein leading to increased supply of amino acids as a substrate for gluconeogenesis. (Baxter & Forsham, 1972).

These endocrine changes explain the hyperglycaemia which is a common accompaniment of illness. It may be distinguished from diabetes mellitus by normal glucose tolerance following recovery.

Hormones and calcium metabolism

Calcium is essential for a wide variety of metabolic processes and in health serum levels are kept within a narrow range by endocrine homeostatic mechanisms. A number of factors may alter total serum calcium including changes in serum albumin caused by reduced protein synthesis in ill health, venostasis or changes in posture which alter total serum calcium but not ionised calcium.

Low total serum calcium levels are common in severely ill patients. Chernow et al. (1982) found 64% of intensive care unit patients to be thus affected. A reduction in both total and ionised calcium has been found in a number of conditions including burns (Szyfelbein, Drop & Martyn, 1981), sepsis (Taylor et al., 1978) and acute pancreatitis (Robertson et al., 1976). Changes are usually short-lived although in severely burned patients hypocalcaemia may persist for a number of weeks (Szyfelbein et al., 1981).

The hormones primarily involved in calcium homeostasis are parathyroid hormone (PTH), vitamin D and to a lesser extent calcitonin. Hypocalcaemia, which is the main physiological stimulus to PTH secretion, does not cause the expected hormone rise in ill patients and therefore suppression of PTH has been suggested as the main cause of hypocalcaemia (Robertson et al., 1976; Taylor et al., 1978). Paradoxically, a rise in both PTH and calcitonin has been found in hypocalcaemic burned patients (Lovén, Nordström & Lennquist, 1984). However, problems with PTH assay methodology render changes within the normal range difficult to interpret. Much of the data on ionised calcium may be suspect as the electrodes employed can give misleading results in the presence of low

albumin levels which are so often a feature of ill patients (Payne, 1982). Thus the mechanism of these ionic changes is unclear and may not be attributable solely to endocrine changes.

ENDOCRINE RESPONSES TO EXERCISE

The endocrine system is intimately involved in the physiological response to exercise. Resulting hormonal changes are reviewed with particular emphasis on the systems later investigated in my own studies.

Exercise and thyroid function

Serum T4 levels have been found to be increased (Refsum & Strömme, 1979; Schürmeyer et al., 1984) or unchanged (Galbo et al., 1977) after single bouts of prolonged exercise. A five day combat course led to a fall in both T4 and T3 levels (Aakvaag et al., 1978a) while in other situations T3 values have been found to be increased (Refsum & Strömme, 1979) or unchanged after exercise (Galbo et al., 1977). Literature on TSH levels is similarly contradictory with values after exercise described as increased (Refsum & Strömme, 1979), unchanged (Terjung & Tipton, 1971) or decreased (Grossman et al., 1984). Doubt also exists whether thyroid hormone levels are significantly altered by training. A six week training programme resulted in 11 percent reduction in T4 with no change in T3 (Balsam & Leppo, 1975). However, running more than 30 miles per week for at least two weeks led to 9 percent reduction in T3 with no change in T4 or TSH values, although the latter showed an exaggerated response to TRH (Boyden et al., 1982).

The field is complicated by the variety of exercise programmes investigated. Resulting fluid shifts might be expected to cause changes in serum concentration of hormones. Failure to find any major change in thyroid hormones with exercise does not necessarily imply constant

secretion as metabolism of thyroid hormone may change with exercise. Irvine (1968) reported increased degradation of T4 with unchanged serum hormone levels after one week of a training programme and therefore suggested an increased thyroidal secretion but Balsam and Leppo (1975) found decreased degradation rate in a similar situation.

Changes in the hypothalamic-pituitary-thyroid axis with exercise are so minor and contradictory that it would be surprising if this system played an important role in physiological response to exercise or training.

Exercise and testicular function

After short term exertion, serum testosterone concentrations increase (Sutton et al., 1973; Galbo et al., 1977; Kindermann et al., 1982; Grossman et al., 1984). Levels of LH have been found unchanged (Sutton et al., 1973) or increased (Grossman et al., 1984). No change in SHBG capacity accompanies the increase in serum testosterone concentrations (Sutton et al., 1973) which has been attributed to factors other than increased secretion such as plasma volume changes (Kindermann et al., 1982) or a decreased metabolic clearance rate due to reduced hepatic blood flow (Sutton, Coleman & Casey, 1978; Keizer, Poortmann & Bunnik, 1980).

The increase in serum testosterone concentration wanes after more prolonged exercise (Galbo et al., 1977) and may fall below baseline values in association with unchanged or reduced LH levels (Dessypris, Kuoppasalmi & Adlercreutz, 1976; Aakvaag et al., 1978b; Kuusi et al., 1984; Schürmeyer, Jung & Nieschlag, 1984). The low serum testosterone concentration in one study was attributed to low SHBG capacity (Kuusi et al., 1984) but was accompanied by increased SHBG capacity in another (Aakvaag et al., 1978b).

Trained female athletes often have secondary amenorrhoea with consequent reduction in oestradiol levels (Baker et al., 1981). In male athletes the situation is less clear. Seven fit athletes had higher serum testosterone concentrations than an unfit age matched but strikingly overweight control group (Young et al., 1976). 39 army recruits showed a significant increase in concentrations of testosterone, androstenedione and LH after a six month physical training programme (Remes, Kuoppasalmi & Adlercreutz, 1979). In contrast to these results, 31 endurance athletes had lower serum testosterone concentrations but similar SHBG capacity and gonadotrophin levels when compared to an age matched control group (Wheeler et al., 1984). These studies are difficult to compare because of differences in the type of training programme, in the timing of blood samples with respect to the most recent exercise and in the choice of control subjects.

Prolonged exercise like illness seems to affect hypothalamic-pituitary-gonadal function. This stimulated my own studies which attempted to compare and contrast the hormonal status of ill patients with the hormonal changes occuring with exercise and training.

Exercise and adrenocortical function

Mild to moderate exercise leads to a reduced or unchanged serum cortisol concentration (Cornil et al., 1965; Raymond, Sode & Tucci, 1972; Davies & Few, 1973). Serum cortisol levels seem only to increase with higher work loads, in particular when oxygen uptake exceeds 60 percent of the individuals' maximum (Davies & Few, 1973). Disappearance of cortisol from blood accelerates progressively with severity of exercise, possibly due to increased uptake by skeletal muscle and other tissues (Few, 1974). Any increased production of cortisol with modest effort is masked by increased disposal. At higher exercise intensity a substantial release of glucocorticoid will overwhelm any increased elimination and serum levels

will rise. It is logical that glucocorticoid levels should increase only with strenuous effort as it is in this situation that there is a greater requirement for glucocorticoid activation of gluconeogenesis and mobilisation of amino acid and fat stores.

Aldosterone levels also increase with exercise in relation to its intensity and this is largely due to activation of the renin-angiotensin system although ACTH stimulation may also contribute (Grossman et al., 1984). This allows for retention of sodium and water by the kidney in a situation where there are increased losses in sweat and breath, thus promoting conservation of the intravascular volume.

Adrenal androgens have not been studied frequently in this situation but both androstenedione and dehydroepiandrosterone sulphate (DHAS) levels appear to increase following long distance running (Dessypris et al., 1976; Baker et al., 1982).

Exercise and other hormones

Activation of the sympathetic nervous system is an essential component of the cardiovascular response to exercise. Adrenaline and noradrenaline levels rise in proportion to severity of exercise with noradrenaline increasing at a lower work intensity than adrenaline (Galbo, Holst & Christensen, 1975).

Catecholamines have important effects on the endocrine pancreas and, as in acute illness, glucagon levels rise and insulin levels fall although to a lesser extent in trained than untrained individuals (Gyntelberg et al., 1977). Exercise results in greater insulin sensitivity (Leblanc et al., 1981) with trained individuals showing evidence of increased membrane binding of the insulin molecule (Leblanc et al., 1979). Unlike acutely ill patients, exercising individuals often have low plasma glucose concentrations which may partly account for the release of catecholamines (Felig et al., 1982). Growth hormone also increases with

exercise (Schalch, 1967), a finding commonly exploited as a test of growth hormone deficiency. As in acute illness counterregulatory hormones predominate leading to increased catabolism and substrate supply.

Calcium regulatory hormones do not appear to play a significant part in the physiological response to exercise (Ljunghall et al., 1984).

ENDOCRINE RESPONSES TO PSYCHOLOGICAL STRESS

Psychological stress and thyroid function

An increase in serum protein bound iodine (PBI), measured as a marker of thyroid hormone levels, was found in the venous effluent of exteriorized thyroid glands of sheep following exposure to fireworks or barking dogs (Falconer & Hetzel, 1964). An increase in circulating PBI was also found in monkeys following a 72-hour avoidance session (Mason et al., 1968a). An eight hour period of sensory deprivation in humans led to a small increase in TSH levels without any change in PBI levels (Zuckermann et al., 1966). A small increase in TSH without any change in serum T4 was observed in eight subjects in anticipation of a novel and exhausting exercise session (Mason et al., 1973). As previously discussed, some patients with acute psychiatric disturbance have a raised serum T4 level (Cohen & Swigar, 1979). Whereas psychiatric illness may affect thyroid hormone concentrations, psychological stress in humans appears not to do so to any significant extent.

Psychological stress and testicular function

Although it is recognised that the female menstrual cycle, and thus hypothalamic-pituitary-ovarian function, may be affected by psychological stress (Lachelin & Yen, 1978), it is uncertain whether the hypothalamic-pituitary-testicular axis may be similarly affected.

Monkeys subjected to continuous psychological stress for 72 hours had low urinary excretion of testosterone (Mason et al., 1968b). Rats exposed to chronic immobilisation stress showed reduced serum testosterone and LH levels even if previously adrenalectomized, suggesting that any changes in the hypothalamic-pituitary-testicular axis had occurred independently of adrenocortical activation (Tache et al., 1980).

Early work in humans had shown unchanged excretion of urinary androgen metabolites following academic examination stress (Connell, Cooper & Redfearn, 1958) but reduced excretion in soldiers awaiting battle (Rose et al., 1969). Low serum testosterone concentrations with unchanged LH levels were found in eight army cadets during a psychologically stressful combat course (Aakvaag et al., 1978b). A similar study showed lower serum testosterone concentrations during the early stressful part of an officer examination course of six months duration compared with the less stressful later part (Kreuz, Rose & Jennings, 1972). The effects of exercise which was an integral part of both these military courses were not taken into consideration in either of these studies.

Thus evidence exists to suggest that hypothalamic-pituitary-testicular function may be altered by psychological stress. However, human studies have been complicated by failure to examine this in isolation from physical effort.

Psychological stress and adrenocortical function

The human adrenal cortex is able to respond to psychological stress, as it does to illness and exercise, but in a less consistent fashion. Students were found to have a twofold elevation of serum cortisol following an examination (Hodges, Jones & Stockham, 1962). Patients in the 24 hours preceding cardiac surgery were found to have similar serum cortisol concentrations to controls except for a surge whilst undergoing

preoperative skin preparation (Czeisler et al., 1976). Urinary excretion of glucocorticoids was normal in soldiers awaiting attack in Vietnam but had been previously raised during combat training with its accompanying heavy exercise programme (Rose et al., 1969). Heterogeneous responses to cardiac catheterisation were seen with calm patients having unchanged but anxious patients raised serum cortisol concentrations (Greene et al., 1970). A varied response was also seen in air crews following aircraft carrier landings: pilots showed much greater increases in serum and urinary cortisol levels than navigators despite similar levels of anxiety as judged by psychological testing (Millar et al., 1970).

There is considerable variation between subjects in the glucocorticoid changes with psychological stress, individuals who are able to cope with stress better having a lesser adrenocortical response (Rose, Poe & Mason, 1968; Miyabo et al., 1976). In general acute stresses seem to have a greater effect than chronic stresses such as awaiting combat or surgery. The response varies with the magnitude of the stress.

The data on adrenal androgen responses to psychological stress is sparse. Stressed monkeys had decreased excretion of androgen metabolites with increased urinary excretion of glucocorticoid (Mason et al., 1968c). Soldiers awaiting attack had reduced excretion of urinary androgen metabolites (Rose et al., 1969). The data from these papers do not permit differentiation between gonadal and adrenal origin of these changes.

Psychological stress and other hormones

Other hormones are well known to be affected by psychological stress. Goodall & Berman (1960) showed that urinary catecholamine excretion increased in human volunteers in anticipation of centrifugation of the person which involved rapid rotation to a 12G forward acceleration. Naval aircrew likewise showed increased excretion following aircraft carrier landings (Rubin et al., 1970). Growth hormone and

prolactin levels rose during a demanding flight in novice but not experienced pilots (Pinter et al., 1979). Similarly neurotic subjects showed increased growth hormone levels during psychological stress but normal subjects did not (Miyabo et al., 1976).

Thus several endocrine systems may be affected by psychological stress. The field has proved a difficult one to research because this type of stress is so difficult to quantify. A situation may be stressful to one individual but not to another. This contrasts with exercise where comparable subjects can be stressed accurately to a specified percentage of their predicted maximal capacity. In addition, psychological stress is often associated with other factors such as exertion or illness which may alter endocrine function independently.

The observation that psychological stress might affect the hypothalamic-pituitary-testicular axis raised the possibility that some of the hormonal changes occurring with illness might be related to associated psychological stress. It was clear that many studies in this field had been complicated by other factors which might alter endocrine systems and so I resolved to search for a suitable model to investigate the effects of psychological stress free from contamination by illness, exercise or other relevant parameters.

In Chapter 2 which follows the methodology involved in the various projects is detailed. In Chapters 3 to 7 the endocrine changes in various clinical situations are described. The effects of exercise and psychological stress are documented in Chapters 8 to 10. The mechanisms involved in the changes occurring with illness are investigated in Chapters 11 to 14.

CHAPTER 2

METHODS

Assay Methodology

Estimations of blood glucose, serum creatinine, haemoglobin, albumin and arterial pH were performed using standard laboratory techniques.

Testosterone was measured in ether extracts of serum using a double antibody radioimmunoassay based on a rabbit anti-testosterone-3-0-carboxymethyl oxime-BSA serum and a ^{125}I radioligand prepared from the histamine derivative of testosterone-3-0-carboxymethyl oxime. The sex hormone binding globulin (SHBG) capacity of serum was assessed by saturation with [^{3}H]-dihydrotestosterone using a modification of the method of Rosner (1972). Derived free testosterone concentrations were obtained by multiplying the total serum testosterone concentration by the percentage free testosterone concentration as calculated from the formula:-

$$\text{percentage free testosterone} = 2.28 - 1.38 \times \log_{10} \text{SHBG}$$

(Anderson et al., 1975).

Serum cortisol was estimated in a direct, solid-phase radio-immunoassay system (McConway & Chapman, 1986). Urinary free cortisol (UFC) was measured without prior extraction in the same assay system and was expressed as the cortisol:creatinine ratio.

Androstenedione analysis involved ether extraction prior to radio-immunoassay which utilises a sheep antiserum raised against androstenedione - carboxyethylthioether ovalbumin (Guildhay Reference No. Hp/S/673-1A), [1,2,6,7-^{3}H] androstenedione (Amersham TRK 454), and double antibody separation (reagents from the Scottish Antibody Production Unit, Law Hospital, Lanarkshire, Scotland). The assay for DHAS was an unextracted radioimmunoassay using a rabbit antiserum (Dr B T Rudd,

Birmingham), [7-^{3}H] DHAS (New England Nuclear) and double antibody separation.

Serum FSH, LH and prolactin were measured using conventional double antibody radioimmunoassays (Beastall et al., 1987). Gonadotrophin assays were standardised using the Second International Reference Preparation MRC 78/549 for FSH and the First International Reference Preparation MRC 68/40 for LH. The prolactin assay was calibrated against MRC 75/504. The LH antibody employed was raised in the rabbit against human pituitary LH (code 87/2, a gift from Professor W.R. Butt, University of Birmingham). Iodinated LH tracer was produced using material obtained from the Chelsea Hospital for women, London. The assay performance based on external quality controls from the National External Quality Assessment Scheme for gonadotrophins was within the accepted limits of bias and variability (<9%).

Serum thyroxine (T4) and triiodothyronine (T3) were measured using a modification to previously published radioimmunoassays in which sheep antibodies were coupled to microfine cellulose particles (Challand, Ratcliffe & Ratcliffe, 1975). Serum TSH was estimated using an immunoradiometric assay employing an ^{125}I-labelled monoclonal antibody covalently linked to Sepharose (McConway, Biggart & Chapman, 1987). Following a series of saline washes sensitivity of at least 0.8 mU/l may be achieved.

Urine metadrenalines were measured by an adaptation of the method of Pisano (1960) and were expressed as the metadrenaline:creatinine ratio in order to correct for possible incomplete urine collection.

Serum for assay was separated and stored at -20°C. To exclude the effects of inter-assay variation all samples from the same individual were assayed together.

Reference ranges and Coefficients of Variation are shown in Table 1.

TABLE 1 Coefficients of variation and reference ranges (male) of assays used in this thesis.

	Reference range	Coefficient of Variation (%) Intra-assay	Inter-assay
T4	55-144 nmol/l	4.5	9.0
T3	1.4-2.8 nmol/l	5.5	9.0
TSH	<0.8-5.0 mU/l	3.5	10.0
Testosterone	11-36 nmol/l	7.0	9.0
SHBG capacity	5-45 nmol/l	7.0	12.0
FSH*	UD-6.4 U/l (24-60 years)	5.5	10.0
LH*	UD-8.6 U/l (24-60 years)	3.1	8.0
Prolactin	UD-360 mU/l	5.0	10.0
Cortisol	280-720 nmol/l	7.0	9.0
Androstenedione	2-11 nmol/l	7.5	15.0
DHAS*	UD-9 µmol/l	7.5	15.0
Urinary free cortisol	<25 µmol/mol creatinine	7.5	15.0
Urinary metadrenaline	<300 µmol/mol creatinine	6.0	9.0

UD = undetectable

* Reference range of these hormones varies with age.

The intra-assay CV was calculated from the results obtained using 20 replicates of pooled serum run within the same assay. The inter-assay CV was the mean CV of three serum pools containing different analyte concentrations run in 20 consecutive assays.

Dynamic testing

The ability of the pituitary gland to secrete TSH and/or gonadotrophins was tested by measuring TSH, FSH and LH at standard time intervals (0, 20 or 30, and 60 minutes) after the intravenous injection of TRH (200 μg) and/or LHRH (100 μg).

The ability of the testes to secrete testosterone was tested by measuring the serum testosterone concentration on day 5 after the intramuscular injection of 2000 units of human chorionic gonadotrophin (HCG) on days 1 and 3. A normal capacity of the testes to secrete testosterone is usually accepted when levels double and reach the normal range by day 5 (Anderson et al., 1972).

LH bioassay

The biological activity of LH was measured using a modification of the isolated interstitial cell bioassay (Van Damme et al., 1974). Two male mice were killed by cervical dislocation. Their testes were removed, decapsulated and placed in Dulbecco's Modified Eagle's Minimum Medium (DMEM) without bicarbonate and with HEPES (20 mM) containing 4% donor calf serum. The testes were cut into small pieces using fine scissors and were dispersed by mechanical agitation for 10 minutes at 37°C in air. The resulting cell suspension was filtered through nylon (100 μm mesh), collected by centrifugation (150g, 5 minutes at 4°C), resuspended in DMEM with serum and allowed to incubate for one hour at 37°C with gentle shaking. Cells were collected again by centrifugation, resuspended in medium at a concentration of 0.66×10^6 viable cells per ml. Routine cell viability is in excess of 90% as assessed by the trypan blue exclusion method. Three quality controls, graded doses of LH (First International Reference Preparation MRC 68/40) or dilutions of unknown plasma samples each at 50 μl were dispensed into a 96 well tissue culture plate and treated with 50 μl of cell suspension.

After a four hour incubation culture plates were frozen until testosterone levels could be estimated in duplicate samples by standard radioimmunoassay. All samples from the same individual were assayed within one assay to eliminate inter-assay variation. Each plasma sample was assayed at two dilutions thus allowing calculation of the intra-assay coefficient of variation for each assay run. This figure was consistently less than 6%. Human FSH, TSH, ACTH , LHRH, growth hormone, prolactin, oxytocin and antidiuretic hormone did not influence the bioassay method at levels likely to be found in biological samples. Parallelism in the dose response curve was observed between the plasma samples and the reference preparation. The sensitivity of this assay (defined as the minimum concentration of LH that was significantly different from control) was 1.9 U/l.

The biological activity of LH is usually expressed as the ratio of LH as measured by bioassay to that measured by radioimmunoassay (B:I ratio) (Dufau et al., 1983; Warner et al., 1985).

LH Pulsatility Studies

These are described in Chapters 12 and 14 because different sampling procedures and methods of pulse analysis were employed in the various studies described.

Presentation of data

Error bars in illustrations represent the standard error of the mean of the data (SE). Values given in text and tables refer to mean ± SE.

Because much of the data in this thesis were not normally distributed statistical analyses were performed using non-parametric methods. Wilcoxon Rank tests for paired and unpaired data were used to test for significance between groups and Spearman Rank tests were used to obtain correlation coefficients.

Ethics

Hospital ethical committee permission was granted for all projects in this thesis and informed consent obtained from patients prior to investigation.

CHAPTER 3

ENDOCRINE FUNCTION IN CHRONIC RENAL FAILURE

Comparison of Haemodialysis (HD) and Continuous Ambulatory Peritoneal Dialysis (CAPD)

Theoretical reasons had existed for suspecting that CAPD might alter the hormonal milieu of uraemic patients but this study found pituitary-thyroid and pituitary-testicular function disturbed to a similar extent in patients treated by HD and by CAPD. The incidental and unexpected finding of low levels of DHAS in uraemic patients raised the possibility that adrenal as well as testicular androgen levels might be affected by chronic illness.

Introduction

My earliest researches into the endocrine consequences of illness were on patients with chronic renal failure. The advent of a new form of renal replacement therapy, Continuous Ambulatory Peritoneal Dialyis (CAPD), stimulated study of thyroid and testicular function in patients undergoing this form of treatment.

Abnormalities of thyroid and testicular function are common in chronic renal failure (Spector et al., 1975; Lim & Fang, 1975). Compared to Haemodialysis (HD), CAPD is more efficient at removing molecules of 500-5000 daltons (Moncrief et al., 1978). These "middle molecules" may be responsible for some of the manifestations of chronic renal failure (Nolph, 1977) and their more efficient clearance might account for the improved biochemical control and higher haemoglobin levels found in CAPD patients (Gokal et al., 1980). If these "middle molecules" were to affect the hormonal milieu adversely, then CAPD might be expected to lead to improved thyroid and testicular function. On the other hand, peritoneal dialysis has been shown to remove thyroid hormones from thyrotoxic rats (Herrmann, Schmidt & Krüskemper, 1973) and has been used therapeutically

in patients with thyroid crisis (Herrman et al., 1971). Thus by clearing hormones from the circulation, CAPD might have an unfavourable effect on the endocrine status of uraemic patients.

This study was designed to compare the pituitary-thyroid and pituitary-testicular axes of uraemic patients on CAPD with those of patients on HD and with healthy control subjects.

Methods

Nine male patients (age range 43-60, mean 52 years) who had been on CAPD for at least six months were compared with nine men on HD (age range 34-60, mean 48 years) and nine healthy men (age range 40-65, mean 50 years). Because of the novelty of CAPD at the time of study it was not possible to match for duration of dialysis which was longer in the HD group (range 6-40, mean 24 months) than the CAPD group (range 6-12, mean 9 months). There was no significant difference in the mean serum creatinine concentrations between the two uraemic groups (CAPD v HD, 1000 v 1050 μmol/l) at the time of sampling.

In CAPD patients two litres of dialysis fluid remain within the peritoneal cavity for at least four hours and overnight during the last cycle of the day. After filling the peritoneal cavity the empty bag was attached to the belt until the next exchange when it was used to drain fluid. Each patient had five exchanges per day. Haemodialysis patients were in an intermittent thrice weekly hospital based dialysis programme.

All studies were performed between 0900 and 1200 hours before the second exchange of the day in CAPD patients and before dialysis in the HD group. Basal blood samples were withdrawn from an indwelling venous cannula and thereafter stimulation testing with LHRH and TRH was performed.

Comparisons were made using the Wilcoxon Rank Sum test for unpaired data.

Results

Basal endocrine results are shown in Table 2. Serum T4 and T3 concentrations were lower than controls in both dialysis groups with T3 being lower in the CAPD than the HD group. Basal TSH concentrations were similar in the three groups but TSH responses to TRH (Figure 1) were blunted in both dialysis groups to a similar extent.

Mean serum testosterone concentrations were below the control mean in both dialysis groups but the reduction was only statistically significant in the HD group. Because the sex hormone binding globulin (SHBG) capacity tended to be high in the uraemic patients, the derived free testosterone levels in both the CAPD and HD patients were significantly depressed as compared with the control group. Although serum FSH levels tended to be higher in the uraemic groups, the differences were not significant as there was a wide scatter of results with most patients having normal results but several having high values in the range compatible with damage to the germinal epithelium. LH concentrations were elevated to a similar extent in both dialysis groups. FSH and LH responses to LHRH were similar in all three groups (Figure 1). Hyperprolactinaemia was found to a similar degree in both patient groups.

Concentrations of the major adrenal androgen DHAS were measured and were found to be low when all uraemic patients were compared to the control group ($p<0.001$). Both dialysis groups had lower concentrations than the control group but this only reached statistical significance with respect to the HD group ($p<0.01$).

Discussion

Hormone results were similar in both dialysis groups although higher haemoglobin concentrations in the CAPD group as compared with the HD group (mean, 8.3 g/dl v 7.2 g/dl) implied a more efficient clearance of "middle molecules".

TABLE 2 Hormone levels in nine male patients on continuous ambulatory peritoneal dialysis (CAPD) or haemodialysis (HD) compared with nine normal controls (mean ± SE).

		CAPD	*HD*	*CONTROLS*
T4	(nmol/l)	78.1 ± 7.5*	68.7 ± 7.6**	103 ± 7.0
T3	(nmol/l)	1.0 ± 0.1***,α	1.4 ± 0.1**	2.2 ± 0.1
TSH	(mU/l)	2.0 ± 0.5	2.7 ± 0.6	2.1 ± 0.4
Testosterone	(nmol/l)	12.0 ± 1.4	9.7 ± 0.7**	15.1 ± 1.2
SHBG	(nmol/l)	52.4 ± 4.5**	42.6 ± 3.6	34.1 ± 2.1
Derived free Testosterone	(pmol/l)	153 ± 15**	138 ± 10***	238 ± 17
FSH	(U/l)	6.6 ± 1.8	15.0 ± 6.1	4.3 ± 1.3
LH	(U/l)	19.8 ± 4.5**	18.9 ± 6.0*	6.5 ± 0.5
Prolactin	(mU/l)	2575 ± 1308***	2778 ± 1828***	189 ± 27
DHAS	(μmol/l)	3.5 ± 1.3	2.0 ± 0.9**	6.3 ± 0.5

CAPD or HD vs controls : * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

CAPD vs HD : α $p<0.05$.

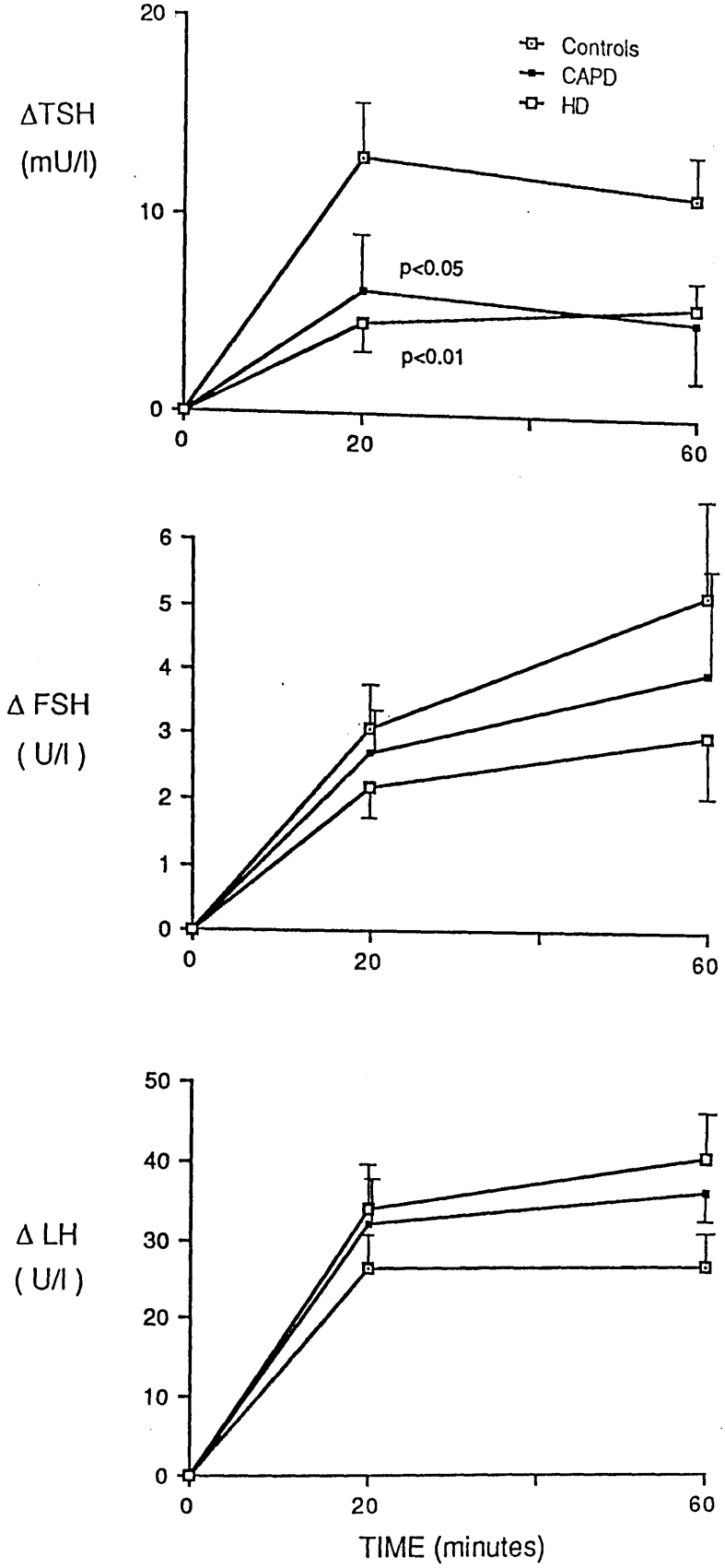


Figure 1 Serum TSH, FSH and LH responses (mean ± SE) of CAPD patients, HD patients and an age and sex matched control group to the intravenous injection of TRH + LHRH expressed as an increment (Δ) over basal values.

The groups differed significantly only with respect to T3 concentrations which were lower in the CAPD patients. The duration of dialysis was longer in the HD than in the CAPD patients yet thyroid function is not thought to be improved by increasing duration of dialysis (Silverberg et al., 1973). T3 might be lost in the dialysate so this was examined in five patients. Using conventional assays for T4 and T3, dialysate concentrations were below the detection limit of the assays (10 nmol/l and 0.5 nmol/l respectively). The dialysate concentration was also below the detection limit of a free T4 assay (2 pmol/l) that is based on a two step principle and so is unaffected by variations in protein content. As 10 litres of dialysate are exchanged every day significant quantities of thyroid hormones might be lost even if concentrations in the dialysate were below the sensitivity of the assays. More recent work using a sophisticated concentrating technique has revealed only minute amounts of T3 but up to 10% of total thyroxine output in the peritoneal dialysate (Kerr et al., 1986). Four CAPD patients were receiving β-adrenergic blocking drugs in contrast to only one HD patient. This provides a likely explanation for the lower T3 levels in the CAPD patients as these drugs inhibit conversion of T4 to T3 (Lotti et al., 1977).

The sex hormone status of uraemic patients is more profoundly suppressed than reflected by routine serum testosterone estimations. Their high SHBG capacity leads to increased protein binding leaving a smaller free fraction as represented by the low derived free testosterone levels found in both groups. There was no important loss of testosterone in the peritoneal dialysate as pooled dialysate from eight patients was examined and only 4 nmol of this steroid was measured over a 24 hour period as compared with an estimated production rate in uraemic patients of 9-25 μmol/24 hours (Stewart-Bentley et al., 1974). The tendency towards higher SHBG capacity in uraemic patients probably only reflects

their hypogonadal state as low testosterone concentrations are associated with high SHBG capacity (Vermeulen et al., 1969). Raised prolactin concentrations were found in both HD and CAPD patients. There was a large scatter of results as expressed in the high standard error (range 260-10900 mU/l, CAPD: 250-17300 mU/l, HD) but there was no significant correlation between the degree of hyperprolactinaemia and the serum testosterone concentration ($r=-0.02$, CAPD; $r=0.38$, HD). These findings were similar to those of Hagen et al. (1976) and Cowden et al. (1978).

This study was the first to show that levels of thyroid and testicular hormones of CAPD patients are not favourably affected by the improved removal of "middle molecules" and uraemic toxins by this process (Semple et al., 1982a; Semple et al., 1982b). There has been no similar work examining pituitary-testicular function in the two different dialysis modalities although other investigators have investigated the pituitary-thyroid axis in later years.

Selgas et al. (1983) found reduced levels of T4 and T3 in serum of 16 CAPD patients with no detectable T4 or free T4 in the dialysate; results were compared with a normal control group but not with HD patients. Thysen et al. (1983) compared thyroid hormone levels of nine CAPD patients with those of eight HD patients and a normal control group; T4 and T3 concentrations were reduced in both dialysis groups, the T4 levels being lower in the HD than the CAPD group. Reverse T3 levels were also measured in this study and were reported to be high in the CAPD patients and low in the HD patients. It is of interest that the same authors were less sure of this finding in a different rendering of the same data (Charytan et al., 1983); they stated that "serum rT3 concentrations were within normal limits in both treatment populations, (though a markedly elevated value in one CAPD patient caused a significant upwards displacement of the mean)". Ross et al. (1985) studied thyroid function in larger groups of CAPD and HD patients with similar

findings. Kerr et al (1986) compared eight CAPD patients with eight HD patients and a normal control group; patient groups were matched for duration of dialysis, an advantage not available to earlier workers because of the novelty of CAPD in the early 1980's. T4 and T3 were depressed to a similar extent in both patient groups as were free T4, free T3 and reverse T3 although no mention was made of the difficulties in measuring free hormone levels in the serum of ill patients.

The incidental finding of low DHAS levels in uraemic patients was unexpected and was not included in the original description of this research (Semple et al., 1982b). The significance of this observation only arose at the time of planning the studies described in the following chapters. Review of the literature then revealed controversy about adrenal androgen status in chronic renal failure. Zumoff and colleagues (1980) found low levels of DHAS in the sera of uraemic patients but Winer et al. (1982) were unable to confirm this association. The discrepancy between these two papers is explained by the choice of control populations, the earlier paper using sera from healthy subjects in contrast to the later paper in which sera from hospitalised patients with stable chronic illnesses were used. This divergence of results could thus be explained if chronic illness of any kind leads to depressed adrenal androgen levels. The hypothesis that serum concentrations of androgens of either testicular or adrenocortical origin are depressed in ill men is investigated in subsequent chapters.

The study which followed set out to examine whether the abnormal androgen levels which were found in uraemic patients were also found in patients with a variety of medical illnesses.

CHAPTER 4

ANDROGEN LEVELS IN MEDICALLY ILL MEN

To investigate whether androgen levels in serum are depressed as a non-specific consequence of illness 30 medically ill men were compared with 30 healthy men. Serum testosterone concentrations were reduced in acute and chronic illness while serum DHAS concentrations were depressed only in those ill for more than two weeks. The reduction in serum testosterone levels could not be explained by altered concentrations of binding proteins, gonadotrophins or prolactin.

Introduction

As a low serum testosterone concentration had been discovered in a number of unrelated conditions, it appeared possible that this change was a general response to illness as opposed to being a specific consequence of certain disorders.

The finding of unusually low levels of the adrenal androgen DHAS in serum of uraemic patients led to the suspicion that this might be a further general marker of illness. Ill patients often have elevated serum and urinary cortisol levels suggesting adrenocortical activation (Melby & Spink, 1958; Espiner, 1966). Urinary excretion of 17-ketosteroids is modestly raised in the early days following trauma but may be reduced in the chronically ill (Forbes et al., 1947). 17-ketosteroids are the metabolites of androgens of both testicular and adrenocortical origin. Alterations in the excretion of 17-ketosteroids may reflect changes in secretion, metabolism or renal handling of androgen and give little clue about serum levels of testicular or adrenal androgen.

I embarked on a study of medically ill men in an effort to discover whether serum levels of testosterone and adrenal androgens are depressed as a general response to illness.

Methods

Blood samples were withdrawn between 0800 and 1000 hours from 30 ill men (mean age 45, range 18-65 years) who were in-patients with a broad spectrum of medical illness (Table 3). Samples were also obtained at the same time of day from a healthy age and sex matched control group (mean age 44, range 20-66 years). No patients or controls were on drugs known to influence adrenocortical or gonadal function. No patient had significant renal or hepatic impairment as judged by standard laboratory tests. Patients who had recently undergone surgery or who were suffering from any condition associated with low serum testosterone concentrations were excluded.

Samples were assayed for T4, T3, testosterone, SHBG capacity, FSH, LH, prolactin, androstenedione and DHAS. Statistical analyses were performed using the Wilcoxon Rank test for unpaired data. Correlation coefficients were calculated using the Spearman Rank test.

Results (Table 4)

Serum testosterone levels were decreased in the ill patients with 50 percent having levels lower than the normal controls range (Figure 2). The mean value of serum SHBG capacity was somewhat higher in patients than controls though not significantly so. The difference could be attributed largely to high values in two patients with bronchial carcinoma (85,156 c.f. control SHBG range 14-64 nmol/l). Derived free testosterone and total testosterone concentrations were depressed to a similar extent. There was no significant difference in levels of FSH, LH, and prolactin between the groups. Serum concentrations of androstenedione (Figure 3) were increased whereas those of serum DHAS (Figure 4) were reduced in the ill group as compared to the control group.

To investigate these findings further the ill patients were subdivided; 14 who had been ill for two weeks or less and 16 who had

TABLE 3 Clinical details of 30 medically ill men.

Patient	*Age (years)*	*Diagnosis*	*Duration of Illness (weeks)*
1	50	pneumonia	<1
2	54	subarachnoid haemorrhage	<1
3	64	pneumonia	<1
4	48	gastrointestinal bleed	<1
5	63	bronchial carcinoma	12
6	65	acute myeloid leukaemia	12
7	64	acute myeloid leukaemia	8
8	64	pneumonia	1
9	35	acute lymphatic leukaemia	4
10	58	bronchial carcinoma	8
11	21	meningitis	1
12	26	infective endocarditis	4
13	28	gastrointestinal haemorrhage	<1
14	49	pneumonia	1
15	37	subarachnoid haemorrhage	8
16	56	septic arthritis	6
17	63	bronchial carcinoma	12
18	42	Crohn's disease	2
19	62	gastric carcinoma	4
20	46	Crohn's disease	12
21	40	empyema	4
22	29	hypertensive encephalopathy	1
23	56	bronchial carcinoma	12
24	47	necrotizing fasciitis	4
25	18	Stevens-Johnson syndrome	3
26	19	diabetic ketoacidosis	<1
27	30	diabetic ketoacidosis	<1
28	50	diabetic ketoacidosis	<1
29	28	diabetic ketoacidosis	<1
30	50	diabetic ketoacidosis	3

TABLE 4 Hormone levels of 30 medically ill men and 30 age matched healthy men (mean ± SE).

		ILL PATIENTS	*CONTROLS*
Testosterone	(nmol/l)	8.9 ± 1.1 $p<0.001$	18.2 ± 1.4
SHBG	(nmol/l)	39.3 ± 4.9	33.6 ± 2.3
Derived free Testosterone	(pmol/l)	131 ± 15 $p<0.001$	288 ± 22
FSH	(U/L)	5.6 ± 1.1	3.7 ± 0.5
LH	(U/l)	8.3 ± 1.2	5.4 ± 0.4
Prolactin	(mU/l)	208 ± 42	199 ± 29
Androstenedione	(nmol/l)	6.6 ± 0.7 $p<0.001$	4.0 ± 0.3
DHAS	(µmol/l)	3.4 ± 0.6 $p<0.001$	6.0 ± 0.7
T4	(nmol/l)	84 ± 5 $p<0.05$	100 ± 4
T3	(nmol/l)	1.3 ± 0.1 $p<0.001$	2.1 ± 0.1

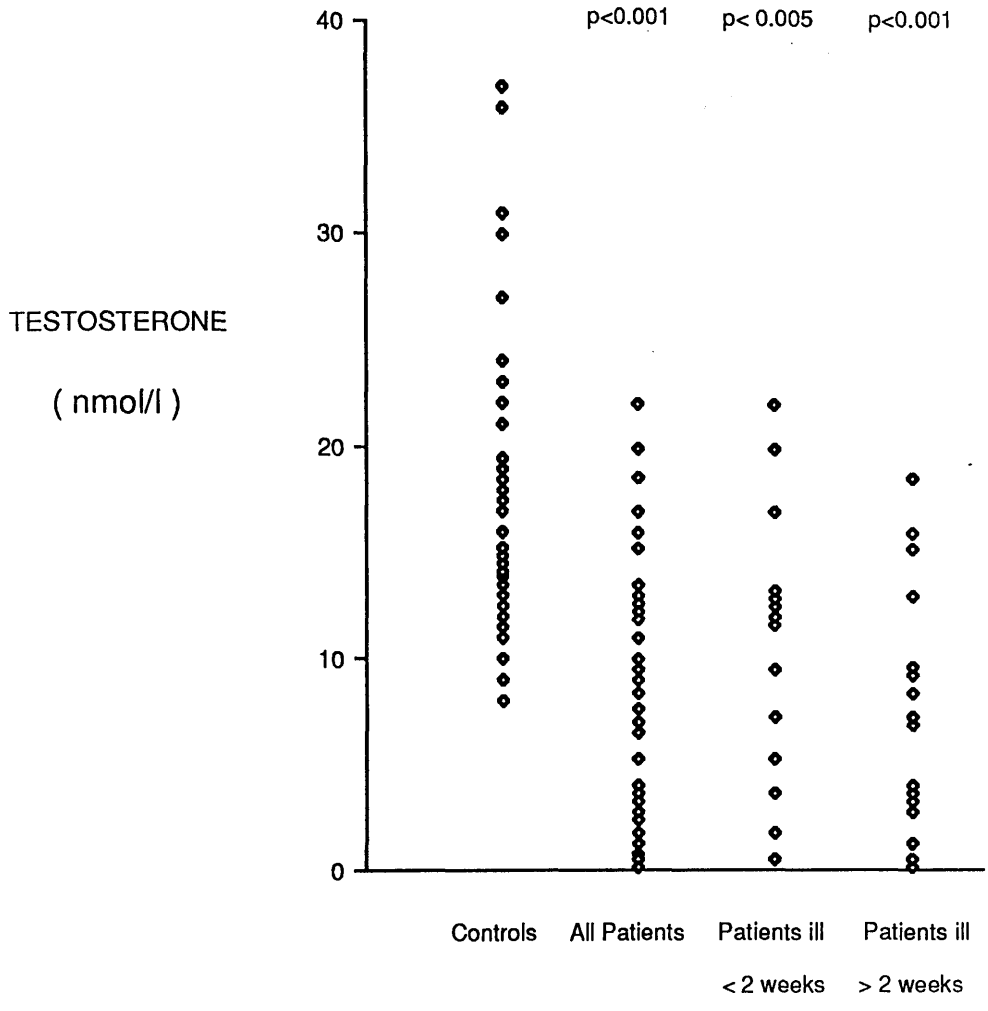


Figure 2 Serum testosterone concentrations of 30 healthy men and 30 medically ill men further subdivided into those ill for for less than two weeks (14) and those ill for more than two weeks (16). P values are for comparison with the control group.

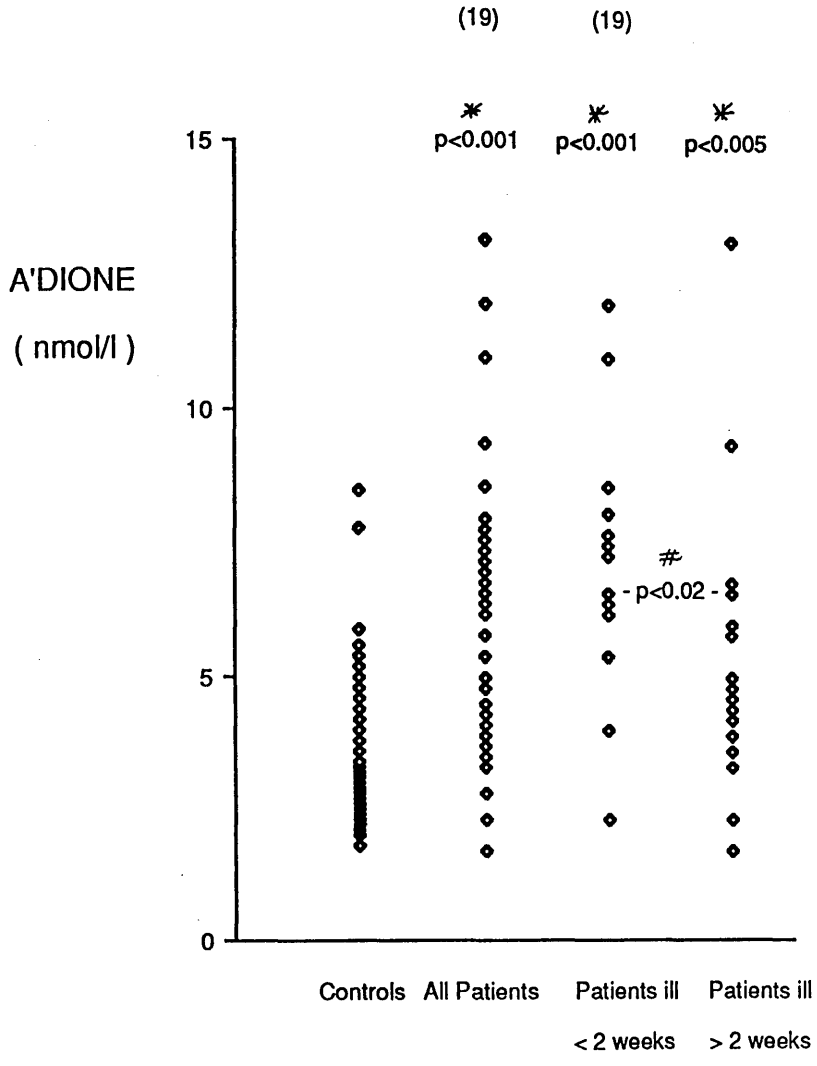


Figure 3 Serum androstenedione concentrations of 30 healthy men and 30 medically ill men further subdivided into those ill for less than two weeks (14) and those ill for more than two weeks (16). The number in brackets at the top of the figure refers to a patient with high levels off the end of the scale. P values * are for comparison with the control group and # for comparison between groups.

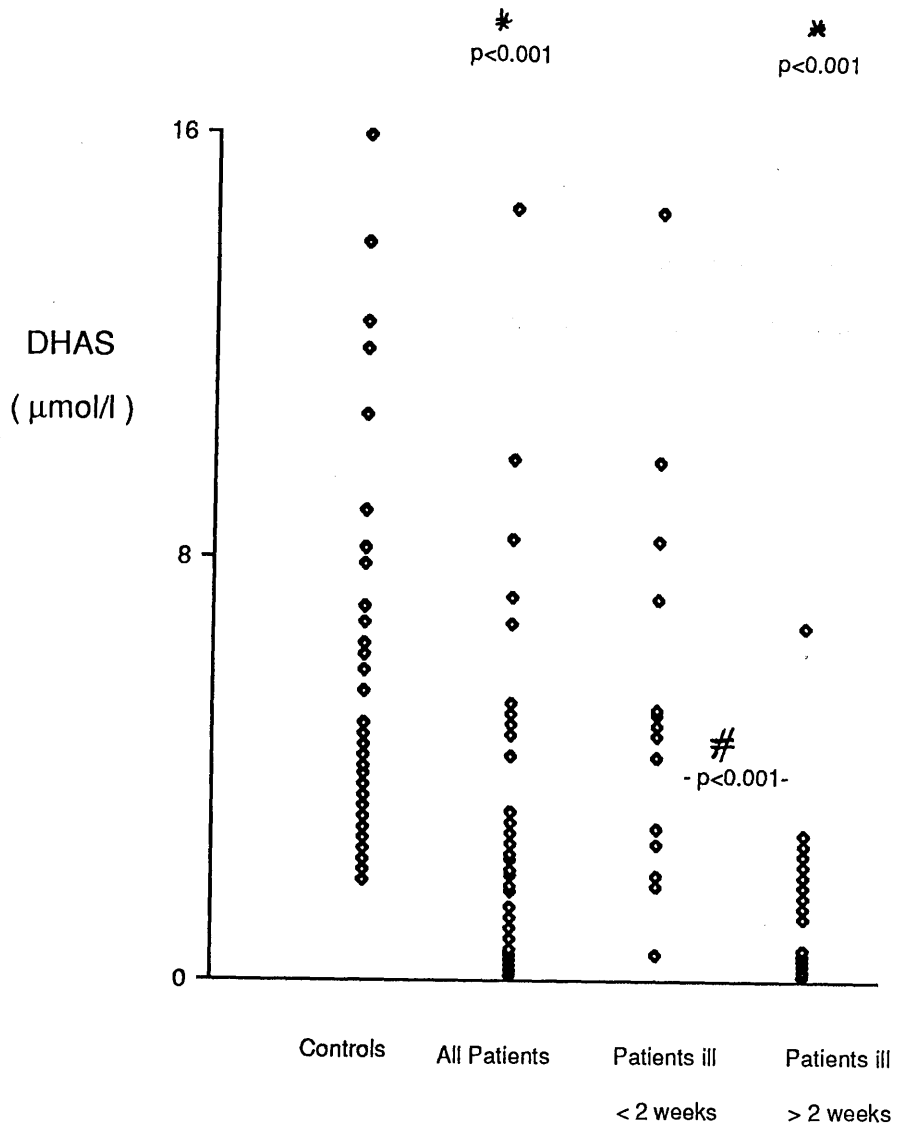


Figure 4 Serum DHAS concentrations of 30 healthy men and 30 medically ill men further subdivided into those ill for less than two weeks (14) and those ill for more than two weeks (16). P values * are for comparison with the control group and # for comparison between adjacent groups.

been ill for more than two weeks. Serum testosterone concentrations (Figure 2) were depressed to a similar degree in the acutely ill (10.6 ± 1.7 nmol/l, $p<0.005$ c.f. controls) as in the more chronically ill (7.4 ± 1.4 nmol/l, $p<0.001$ c.f. controls). Levels of serum androstenedione (Figure 3) were higher in the acutely ill (8.0 ± 1.1 nmol/l) than in the chronically ill (5.4 ± 0.7 nmol/l, $p<0.02$) whereas those of serum DHAS (Figure 4) was lower in the chronically ill (1.7 ± 0.4 μmol/l) than in the acutely ill (5.3 ± 1.0 μmol/l, $p<0.001$).

Serum testosterone concentrations of the ill patients showed statistically significant correlations with serum T3 ($r=0.423$, $p<0.05$) but not with serum T4 ($r=0.339$), SHBG capacity ($r=0.340$), FSH ($r=0.135$), LH ($r=0.059$), or prolactin ($r=-0.299$) concentrations. Serum DHAS concentrations failed to correlate significantly with serum levels of T4 ($r=0.119$), T3 ($r=0.285$), testosterone ($r=0.328$) or any other hormone measured.

Discussion

This study (Semple, Gray & Beastall, 1987a) demonstrated that reduced serum testosterone concentrations occur commonly in a variety of illnesses both acute and chronic and are not merely a consequence of certain illnesses such as chronic renal failure or respiratory failure as the literature had previously suggested. This study (Semple, Gray & Beastall, 1987b) also confirmed and extended the findings of Parker and colleagues (Parker, Levin & Lifrak, 1985) who had found low levels of DHAS in the critically ill and who published their paper during the final stages of my own study.

Low serum testosterone concentrations were found in patients with a variety of illnesses. Few patients were extremely ill as patients were studied in a general medical ward rather than in an intensive care unit where drugs such as steroids, dopamine and opiates are often

administered. Two patients (one with necrotising fasciitis, one with infectious endocarditis) died within two weeks of sampling and had very low levels of testosterone (<2 nmol/l). Three others (one with diabetic ketoacidosis, one with empyema and one with bronchopneumonia) had similarly low levels but survived. Like low T3 states, low testosterone levels do not seem to confer a particularly poor prognosis. Parallels between serum T3 and testosterone concentrations are not confined to illness and it is interesting to note that levels of both hormones fall in healthy men who are fasted (Vagenakis et al., 1975; Klibanski et al., 1981).

Although there was a significant correlation between levels of testosterone and T3 and although low testosterone levels may be found in patients with low T4 and low T3 states, there is no evidence to suggest that a common mechanism is involved in their evolution. Low T4 states are primarily due to changes in protein binding of T4 whilst reduced peripheral conversion of T4 to T3 is the main mechanism leading to low T3 states. Testosterone is secreted in the male directly from the testes and in the circulation is bound by SHBG and albumin. No significant change in the binding capacity of SHBG was seen in medically ill patients in contrast to uraemic patients. Changes in serum albumin concentration, which occur commonly with illness, may alter protein binding of testosterone. However due to the low affinity low saturation nature of albumin binding, alterations in albumin concentrations are unlikely to lead to substantial reductions in serum total testosterone concentrations (Dunn, Nisula & Rodbard, 1981). The correlation between testosterone and T3 in this study may just represent parallel changes in these hormones with illness rather than imply a biologically significant association.

Metabolism of testosterone is decreased in ill patients (Zumoff et al., 1971) so altered metabolism will not account for low serum concentrations. Increased clearance is a possible mechanism but normal

17-ketosteroid excretion during illness makes this unlikely (Forbes et al., 1947). Reduced testicular secretion seems likely to be a major factor leading to the low testosterone state associated with illness.

LH levels of men with normal hypothalamic-pituitary function increase in response to low serum testosterone concentrations indicating primary testicular failure as in Klinefelter's syndrome. On the other hand, if hypothalamic-pituitary suppression with subsequent secondary testicular failure was the only factor leading to low testosterone concentrations, one would expect to find low LH levels. The lack of any correlation between the LH and testosterone concentrations of ill patients suggests that factors other than hypothalamic-pituitary suppression are implicated in the genesis of the low testosterone state and that it cannot be explained on the basis of classical primary or secondary testicular failure. Nor in view of the lack of correlation between serum testosterone and serum prolactin and SHBG capacity can low serum testosterone levels be attributed to changes in SHBG capacity or hyperprolactinaemia.

There are other possible explanations for this state of relative hypogonadism. Normal LH levels using a single radioimmunoassay determination do not necessarily imply physiological secretion which is normally episodic with pulses every 60-120 minutes (Nankin & Troen, 1971). Moreover LH can be secreted in biologically active or inactive forms possibly due to secretion of LH fragments which are biologically inactive but cross-react with LH antiserum (Dufau et al., 1983). Thus alterations in biological potency or pulsatility of LH might lead to reduced testosterone secretion without changing absolute LH concentrations.

DHAS levels were low in patients ill for more than two weeks. This might be due to reduced adrenocortical secretion, reduced sulphation of dehydroepiandrosterone (DHA) or increased metabolism. Reduced sulphation

is unlikely to be a factor as low levels of both DHAS and the unsulphated DHA in the critically ill have recently been described (Parker et al., 1985). The metabolism of DHA has been shown to be reduced in ill patients (Zumoff et al. 1971) so it is unlikely that low DHAS concentrations in this context are due to accelerated metabolic clearance. Reduced adrenocortical secretion seems probable.

Serum androstenedione concentrations did not follow the same pattern as DHAS in medically ill patients. Rather than fall levels of androstenedione rose in the acute situation only to fall to control values after two weeks. The physiological role of adrenal androgens is at present uncertain and likewise the reason for the blood levels of androstenedione and DHAS behaving differently is unclear.

Thus androgens of both testicular and adrenocortical origin join T4 and T3 in a list of hormones which show a non-specific adaptation to illness. In the following three chapters these endocrine changes are studied in patients with diabetes mellitus, following burns injury and following surgery.

CHAPTER 5

ANDROGENS AND DIABETES MELLITUS

Patients in varying degrees of diabetic ketoacidosis had low serum testosterone levels which rose following recovery but glycaemic control of non-ketotic diabetic men was unrelated to testosterone levels. Although previous authors had suggested that adrenal androgens might be abnormal in diabetic men, their levels were found to be similar to those of healthy non-diabetic men.

Introduction

Interest in the sex steroid profiles of men with diabetes arose because impotence is such a common feature of this condition and may occur in up to 50 percent of patients (Schöffling et al., 1963). Early endocrine studies of such impotent patients reported conflicting findings, urinary excretion of 17-ketosteroids being low (Miller & Mason, 1945), normal (Faerman et al., 1972) or high (Schöffling et al., 1963).

Most authors measuring serum hormone concentrations rather than urinary metabolite levels have found normal serum testosterone concentrations in diabetic men whether impotent or not (Ellenberg, 1971; Faerman et al., 1972; Kolodny et al., 1974) and it is now commonly accepted that the impotence of diabetes is not caused by androgen deficiency. However, several papers have reported serum testosterone concentrations of diabetic men to be lower than those of control populations. Shahwan et al. (1978) found such concentrations in sulphonylurea-treated diabetic men to be lower than in either insulin requiring patients, patients on diet alone or normal control subjects. A study of 41 diabetic men (age range 23-55, mean 37.5 years), only five of whom were receiving insulin, showed serum testosterone levels less than control values but usually within the normal reference range (Andò, Rubens & Rottiers, 1984) while levels of newly diagnosed and mildly

ketotic insulin dependent diabetic men have been reported to rise following insulin therapy (Gluud et al., 1982).

The low serum testosterone concentrations found in the studies of Shahwan et al. (1978) and of Andò et al. (1984) might have been due to general ill health consequent to poor diabetic control. The latter study employed a relatively young group of diabetic men the majority of whom one would have expected to be on insulin therapy; the fact that only five of 41 were so treated makes it unlikely that they were well controlled. Sulphonylurea therapy was implicated as a possible cause of the low testosterone levels in the former study but again from the data supplied it is not possible to exclude an effect of poor glycaemic control.

In view of my own interest in depression of serum testosterone concentration as a consequence of ill health it was logical to investigate whether ill diabetic men with ketoacidosis are similarly affected and also whether blood glucose control in patients without ketoacidosis influences testicular function.

Scanty reports of alterations in serum or urine levels of adrenal androgens or their metabolites in diabetic patients have appeared in the literature but seemed of little clinical relevance. For example Schöffling et al. (1963) found that diabetic men had increased urinary excretion of DHA but not of other androgens; on the contrary Alasandro et al. (1982) found reduced excretion of DHA as well as other androgens. Szpunar, Blair & McCann (1977) found raised androstenedione and DHA levels in plasma of diabetic females but adolescent diabetic boys had low DHAS levels when matched for bone age with boys who had presented with constitutional delayed puberty (Cohen et al., 1984).

Recent research has suggested that adrenal androgen levels of diabetic patients may be of more than academic significance. Feeding DHA or DHAS to genetically obese diabetic mice prevented hyperglycaemia and the progressive islet cell atrophy usually found in this animal model

(Coleman, Schwizer & Leiter, 1984). Low serum levels of adrenal androgens if found in adult men as reported in adolescent diabetic boys might then have a deleterious effect on islet cell activity. Moreover recent work has suggested that a low level of DHAS may be an independent risk factor for coronary artery disease and premature death both worrying problems to diabetic patients and their physicians (Barrett-Connor, Khaw & Yen, 1986). The time seemed ripe to investigate the adrenal androgen status of adult males with diabetes mellitus.

Methods

To investigate the effect of diabetic ketoacidosis on testicular function eight male patients affected to a varying extent by this complication (Table 5) were venesected within 24 hours of hospital admission and following recovery at the same time of day four weeks later. The diagnosis of diabetic ketoacidosis was made on the basis of acidaemia (i.e. pH<7.3) or heavy ketonuria by ketostix testing (i.e. > ++ ketonuria). Paired samples were assayed for testosterone and SHBG capacity.

The androgen status of men with diabetes mellitus was studied by comparing 15 patients with insulin dependent diabetes (age range 19-61, mean 41 years) as well as 15 with non-insulin dependent diabetes (age range 37-59, mean 52 years) with appropriately age matched healthy non-diabetic men. Patients were selected from the diabetic out-patient clinic at random apart from the following exclusions:- ketonuria, patients on drugs known to affect hypothalamic-pituitary-testicular function, the presence of other illnesses as far as could be elicited from history taking and inspection of the case record, and age over 60 years because of difficulties in finding healthy controls in this age group.

Blood sampling was performed between 0900 and 1100 hours in patients and controls, the samples being assayed for glycosylated

TABLE 5 Clinical details and serum testosterone levels during acute episode and following recovery from diabetic ketoacidosis.

Age of Patient (years)	*Clinical Problem*	*Testosterone level (nmol/l)* *Early*	*Recovery*
42	longstanding diabetes; pH - 7.0; glucose - 70 mmol/l	1.2	10.1
55	sulphonylurea failure; 12 Kg weight loss; ketonuria +++; glucose 25 mmol/l	8.7	9.8
37	sulphonylurea failure; 10 Kg weight loss; ketonuria ++++; glucose 22 mmol/l	4.6	11.2
25	newly diagnosed diabetes; ketonuria ++++; glucose 17 mmol/l	10.2	12.1
50	newly diagnosed diabetes; pH - 7.2; glucose 32 mmol/l	5.2	19.8
19	newly diagnosed diabetes; pH - 6.95; glucose 101 mmol/l	3.6	22.0
28	longstanding diabetes; pH - 7.1, glucose 40 mmol/l	9.5	15.0
35	longstanding diabetes; pH - 7.1; glucose 35 mmol/l	0.5	9.8

haemoglobin to give a measure of glycaemic control as well as for testosterone, SHBG capacity, androstenedione and DHAS. Glycosylated haemoglobin was measured by agar gel electrophoresis using a commercial kit (Corning Medical and Scientific Ltd) which has a normal range of 6-9 percent.

Statistical analyses were performed using the Wilcoxon Rank test for unpaired data and the Spearman Rank test to calculate correlation coefficients.

Results

The mean serum testosterone concentration of the patients with ketoacidosis was 5.4 ± 1.3 nmol/l increasing to 13.7 ± 1.7 nmol/l four weeks later ($p<0.02$). Individual values are shown in Table 5. There was no significant difference in mean SHBG capacity at these two times (28.6 ± 3.6 v 36.2 ± 6.0 nmol/l). Derived free testosterone increased from 95 ± 26 nmol/l to 203 ± 25 nmol/l ($p<0.02$).

There was no significant difference in serum levels of testosterone, androstenedione and DHAS between either insulin dependent or non-insulin dependent diabetic men as compared with the appropriate control groups (Table 6). Glycaemic control was variable with glycosylated haemoglobin values ranging from 7.1 percent to 15.6 percent (mean 11.5 percent) in patients receiving insulin and 7.2 percent to 16.6 percent (mean 10.9 percent) in the non-insulin dependent group. To assess the effect, if any, of glycaemic control on testicular function both diabetic groups were considered together; there was no significant correlation between glycosylated haemoglobin and serum testosterone concentration ($r=0.17$).

Discussion

This study was the first to demonstrate that diabetic ketoacidosis is associated with a reduction in serum testosterone concentrations and

TABLE 6 Androgen status of diabetic men (mean ± SE).

Group under study (*age, years*)	*Testosterone* (*nmol/l*)	*Androstenedione* (*nmol/l*)	*DHAS* (*μmol/l*)
Insulin dependent diabetes (40.6 ± 4.0)	19.7 ± 1.5	5.3 ± 0.5	7.4 ± 1.0
Control group (40.7 ± 3.8)	20.0 ± 2.4	4.6 ± 0.6	7.2 ± 0.8
Non-insulin dependent diabetes (52.1 ± 6.0)	14.2 ± 1.2	5.9 ± 0.5	4.0 ± 0.7
Control group (52.6 ± 5.3)	14.9 ± 1.2	5.4 ± 0.7	4.5 ± 0.3

that glycaemic control in stable non-ketotic patients does not correlate with serum testosterone concentrations (Semple, Gray & Beastall, 1988). This suggests that hyperglycaemia does not adversely affect testosterone production in the absence of ketacidosis.

The observation that DHAS therapy was beneficial to diabetic rats (Coleman et al., 1984) raises the possibility that DHAS deficiency might be deleterious to islet cell function. The finding of normal adrenal androgen status in adult diabetic men was the first such observation (Semple et al., 1988) and suggests that the abnormal islet cell function found in insulin dependent and non-insulin dependent diabetic patients is unlikely to be related to alteration in adrenal androgen levels. Moreover the increased susceptibility of diabetic patients to atheroma formation and early mortality is unlikely to be related to low serum DHAS concentrations which have recently been shown to be an independent risk factor for cardiovascular disease (Barrett-Connor et al., 1986).

Thus early studies had suggested that different forms of illness might lower both testicular and adrenal androgen levels. Further studies were performed on burned patients as outlined in the following chapter in an attempt to discover the time of onset and duration of these abnormalities.

CHAPTER 6

ENDOCRINE CONSEQUENCES OF BURNS INJURY

19 men were investigated for four weeks following admission for treatment of burns. Profound depression of serum testosterone concentrations was found. This often persisted for several weeks and could not be accounted for by alterations in protein binding, serum prolactin or LH levels. Serum DHAS concentrations were also depressed one week after injury and remained low over the period of study.

Introduction

Burns injury represents one of the greatest stresses to which the human body can be exposed. Clinically significant burns are accompanied by marked metabolic changes many of which are controlled by endocrine systems. These patients are particularly suitable for investigation as they are usually previously healthy and thus are not already in receipt of medications known to alter endocrine function. Opioid analgesics may be given following admission but their use in the West of Scotland Burns Unit is usually short term as it is felt that chronic use may prejudice long term recovery by depressing appetite and promoting chest infections. Indeed many patients do not require potent analgesia because full thickness burns are painless due to damage to peripheral nerve endings. Inpatient treatment tends to be protracted and thus serial measurements are relatively straightforward to perform.

Previous studies have shown substantial reductions in serum testosterone concentrations (Dolecek et al., 1979a; Vogel, Peake & Rada, 1985). In the latter study mean levels were depressed to 19 percent of control values; this compares with a reduction to 48 percent following surgery (Wang et al., 1978b), 65 percent in respiratory failure (Semple et al., 1981) and only 83 percent following myocardial infarction (Wang et al., 1978a). Only patients in deep coma following head injury have shown

a greater suppression (to 5 percent of control values) and here the picture was complicated by high dose dexamethasone treatment. Head injury also differs from these other clinical situations in that definite hypothalamic-pituitary suppression has been observed with gonadotrophin levels below the normal reference ranges (Rudman et al., 1977).

Dolecek et al. (1979a) found LH levels to be raised within 48 hours of burns injury, returning to normal thereafter; FSH levels were subnormal within 48 hours and remained low for several weeks. Serum testosterone concentrations were low within 48 hours of injury, reached a nadir during the second week, and often remained low for several weeks. Gonadotrophin responses to LHRH appeared depressed during the second or third weeks after injury although no control data were available for comparison (Dolecek et al., 1983). Serum testosterone concentrations increased following stimulation with HCG but failed to reach the normal range in patients who had very low basal levels (Dolecek et al, 1979a). Vogel et al. (1985) found low LH levels within the first week of injury but levels were similar to control values in the ensuing weeks despite low serum testosterone concentrations. FSH levels were not measured in this study. SHBG capacity was lower than that of a control group in the first post-burn week only.

Burns trauma is similar to other pathological states with regards to changes in thyroid hormone levels. Low serum T3 concentrations are found and may persist for several weeks (Smeds et al., 1981). Low T4 levels are also seen especially in those with more extensive burns (Dolecek et al., 1979b; Vaughan et al., 1985).

Adrenocortical activation as indicated by increased urinary excretion of cortisol metabolites is found in more severely burned patients (Wilson, Lovelace & Hardy, 1955). Serum cortisol, a less sensitive measure of adrenocortical activation, has been found to be normal or increased in this situation (Balogh et al., 1984; Dolecek, 1984).

Little information is available on adrenal androgen responses. Urinary excretion of 17-ketosteroids show a short-lived increase before falling to subnormal levels (Wilson et al., 1955). Dolecek (1984) apparently found normal serum androstenedione and low levels of serum DHAS but failed to provide supporting data.

As most previous authors had concentrated on each system in isolation, it was decided to study testicular, thyroid and adrenocortical function following burns trauma in a coordinated fashion in the same patient group.

Methods

19 men (age range 20-69, mean 37 years) admitted to a regional burns unit were studied. Burns injury varied in intensity from 4 to 70 percent of total surface area (mean 20 percent). Blood samples were obtained between 0800 and 1000 hours within 24 hours of admission and thereafter at weekly intervals for four weeks. Not all patients were able to be studied at each time interval due to death (2 patients), early discharge, inadequate venous access or an insufficient serum sample. The number of patients on whom data are based is given in parenthesis in the appropriate figure. Samples were obtained at the same time of day from 19 healthy men (age range 20-66, mean 38 years). Samples were assayed for serum concentrations of testosterone, SHBG capacity, FSH, LH, prolactin, T4, T3, cortisol, androstenedione and DHAS.

It was not possible to exclude entirely the effects of drugs in this study as six patients received parenteral opiates within 24 hours of admission. None received this treatment for more than 48 hours. Analgesia was provided where necessary with a paracetamol mixture containing only 16 mg codeine per dose.

Statistical analyses were performed using the Wilcoxon Rank test for paired data. Correlations were performed using Spearman's Rank test.

Results

Serum testosterone concentrations (Figure 5) were low following admission (week 1) (6.8 ± 1.8 v 18.9 ± 1.4 nmol/l, $p<0.001$) and remained low during the study, reaching a nadir of 4.1 ± 1.4 nmol/l in week 3. SHBG capacity (Figure 5) was low on admission (21.2 ± 3.2 v 29.9 ± 1.8 nmol/l, $p<0.02$) but increased to levels higher than control values by week 4 (38.2 ± 4.1 nmol/l, $p<0.02$). Derived free testosterone levels (Figure 5) showed a trend similar to total testosterone concentrations. FSH concentrations (Figure 6) were lower than in the control group on admission though not significantly (2.1 ± 0.4 v 3.2 ± 0.5 U/l) but became so by week 2 (1.7 ± 0.4 U/l, $p<0.05$). LH levels (Figure 6) were unchanged and similar to control values throughout the study. Prolactin levels on admission (Figure 6) were not significantly different from controls (247 ± 58 v 204 ± 18 mU/l) showing only a slight and insignificant tendency to increase.

Both T4 and T3 levels (Figure 7) were low on admission (50 ± 4.1 v 100 ± 4.2 nmol/l, $p<0.001$; 1.0 ± 0.1 v 2.2 ± 0.1 nmol/l, $p<0.001$) and remained lower than controls for the duration of the study.

There was evidence of adrenocortical activation following injury. Serum cortisol concentrations (Figure 8) were high following admission (661 ± 91 v 359 ± 30 nmol/l, $p<0.005$) and remained high over the study period. Serum androstenedione concentrations (Figure 8) were also high following admission (7.5 ± 1.0 v 3.9 ± 0.3 nmol/l, $p<0.02$) and remained higher than the control group thereafter although there was a downward drift. Serum DHAS concentrations (Figure 8) were similar to control values in week 1 (6.8 ± 1.2 v 5.2 ± 0.7 μmol/l), fell to levels significantly lower than controls reaching a nadir in week 3 and remained low for the duration of the study.

Figure 9 illustrates the serum testosterone and DHAS concentrations of three patients who had further estimations performed up to 24 weeks

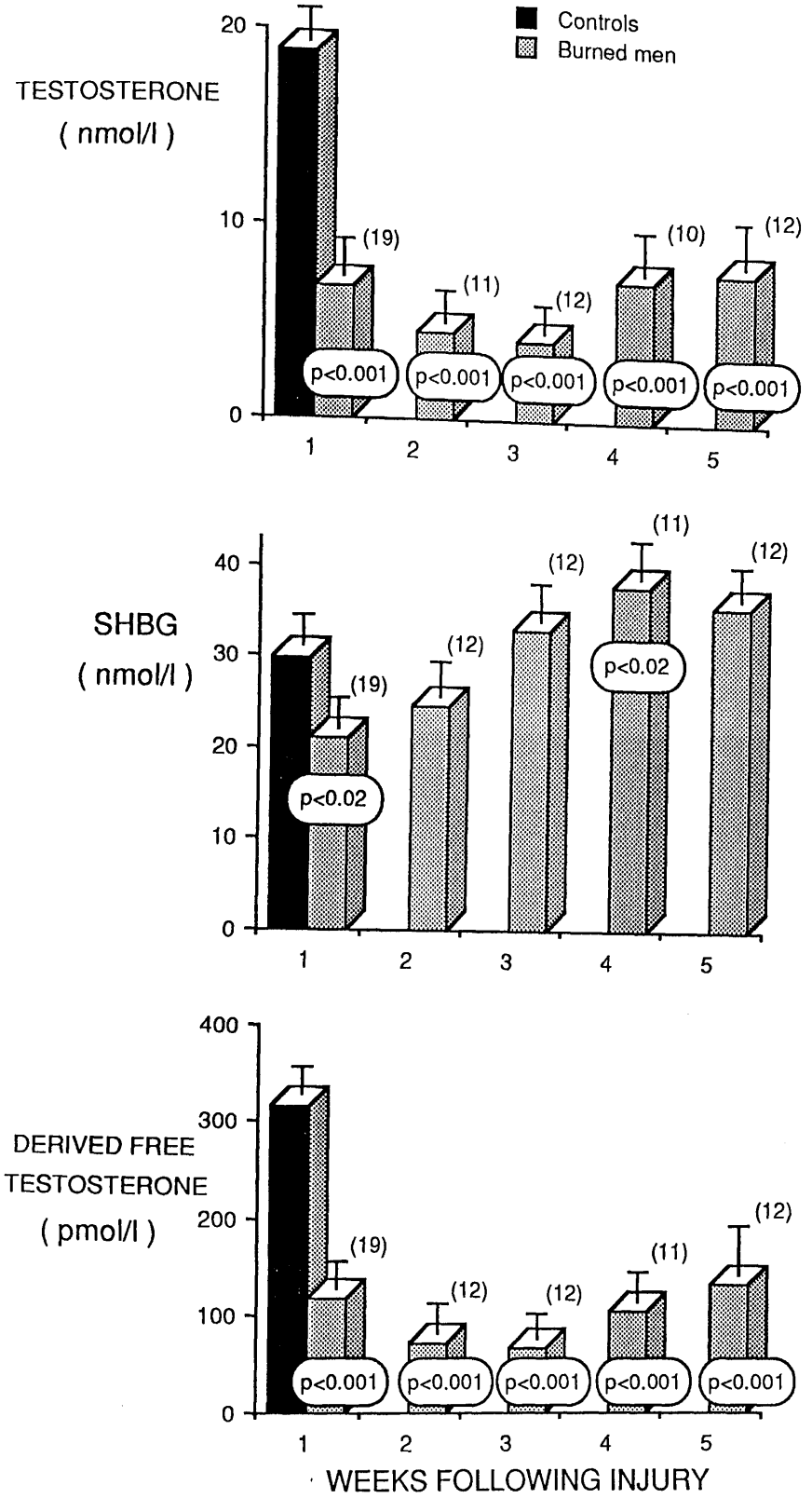


Figure 5 Serum testosterone, SHBG capacity and derived free testosterone concentrations of burned men following admission (week 1) and for four weeks thereafter are compared with results from a control group. Figures in brackets by error bars indicate the number of patients sampled at each time interval. P values compare patients' results with those of the normal control group.

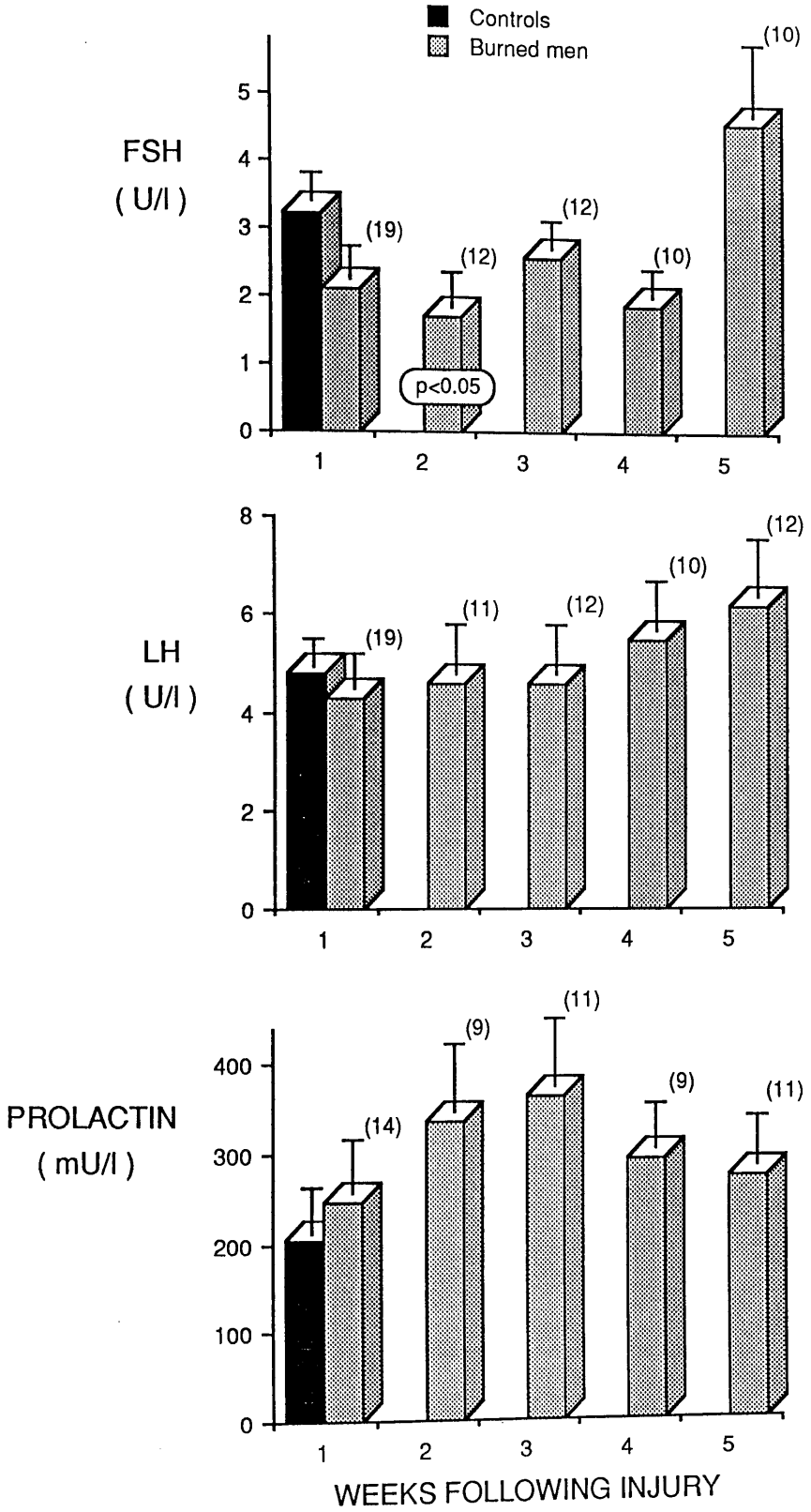


Figure 6 Serum FSH, LH and prolactin concentrations of burned men following admission (week 1) and for four weeks thereafter are compared with results from a control group. Figures in brackets by error bars indicate the number of patients sampled at each time interval. P values compare patients' results with those of the normal control group.

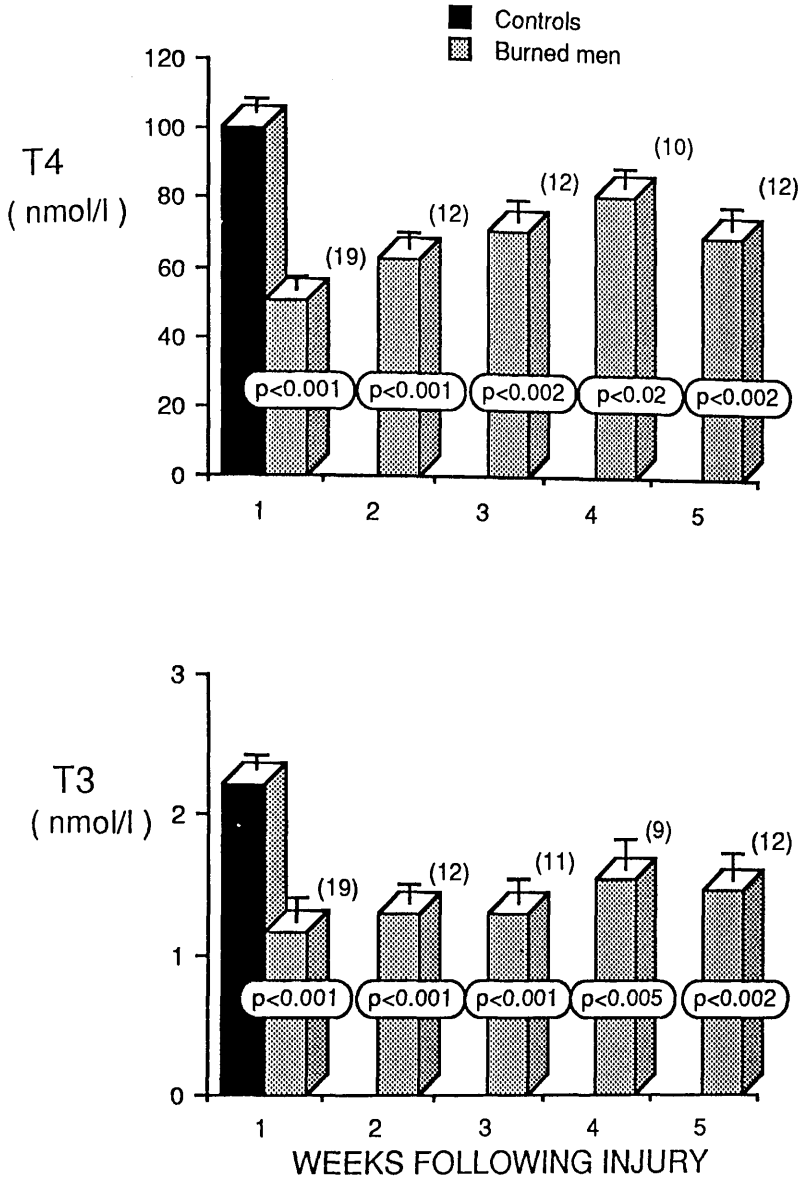


Figure 7 Serum T4 and T3 concentrations of burned men following admission (week 1) and for four weeks thereafter are compared with results from a control group. Figures in brackets by error bars indicate the number of patients sampled at each time interval. P values compare patients' results with those of the normal control group.

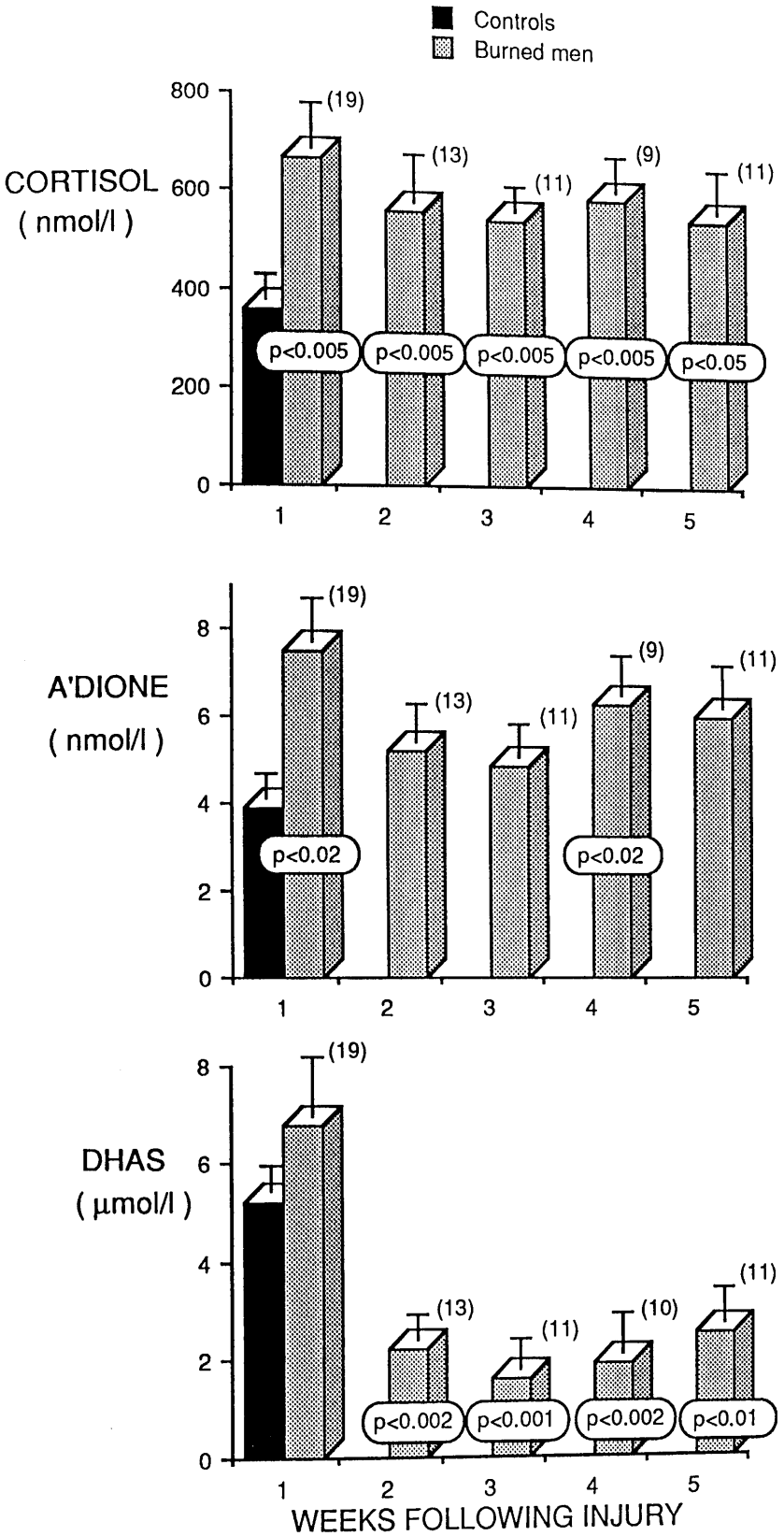


Figure 8 Serum cortisol, androstenedione and DHAS concentrations of burned men following admission (week 1) and for four weeks thereafter are compared with results from a control group. Figures in brackets by error bars indicate the number of patients sampled at each time interval. P values compare patients' results with those of the normal control group.

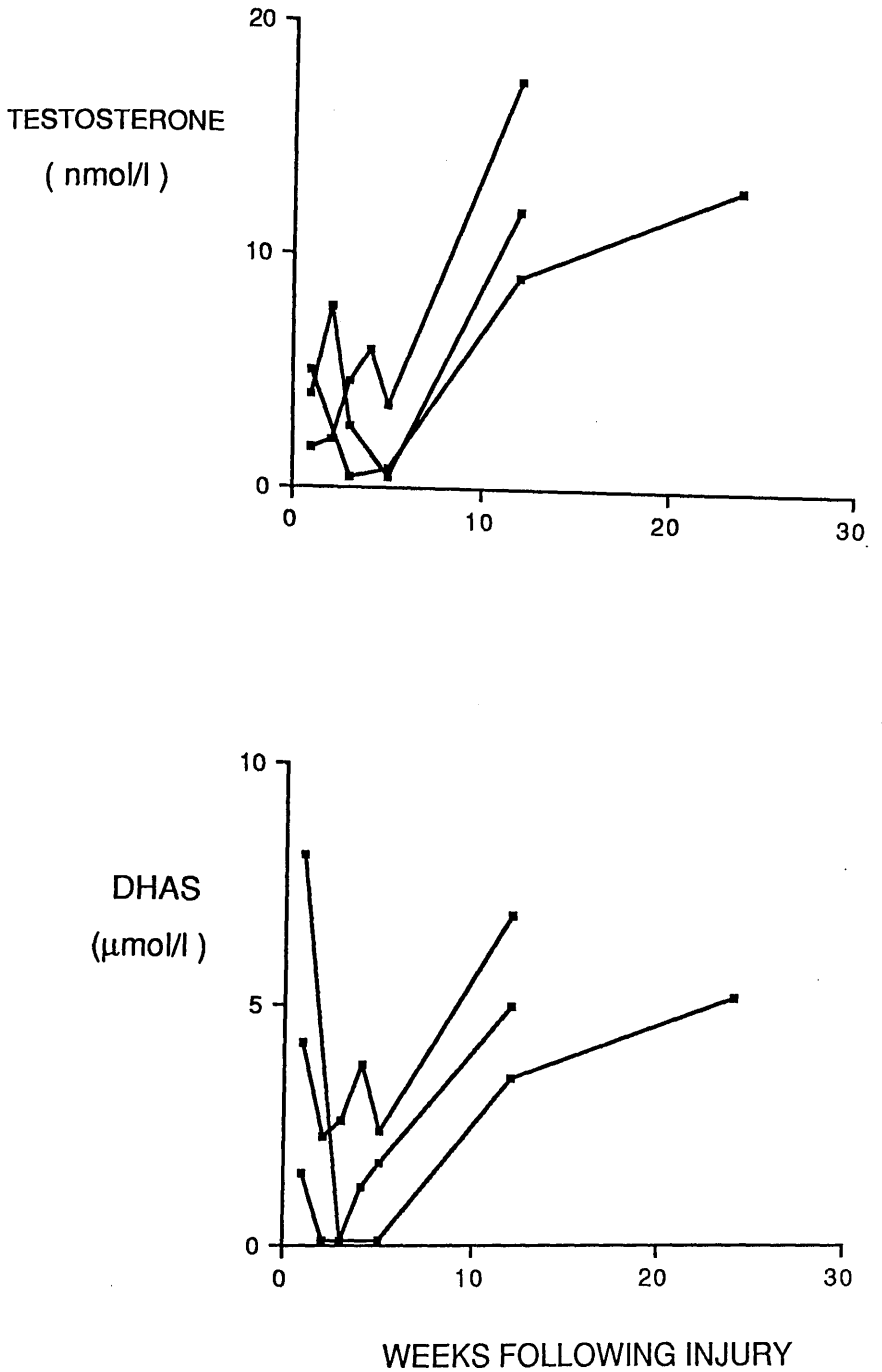


Figure 9 Serum testosterone and DHAS concentrations of three burned patients who were studied for up to 24 weeks following injury.

after injury. Concentrations of both steroids returned to within the normal range.

The size of the burn showed a significant correlation with serum T4 concentrations on admission ($r=-0.742$, $p<0.002$) but not with any other variable. Serum testosterone concentrations on admission did not correlate with any other hormone level measured.

Discussion

This study confirmed the finding of low serum testosterone concentrations following burns injury. A low SHBG capacity was also found but this was shortlived and levels increased to greater than control values three weeks after admission. It is possible that the fall in SHBG capacity is due to changes in testosterone binding similar to those occuring between T4 and its binding proteins in ill patients. Indeed free fatty acids which have been proposed as the thyroid hormone binding inhibitor in the low T4 syndrome (Chopra et al., 1986) may reduce the binding of testosterone to SHBG and albumin (Mooradian, et al., 1988). Alternatively the increased protein breakdown occurring following burns injury may allow increased catabolism of SHBG. This question is addressed further in Chapter 11. In any case the changes in SHBG did not correlate with alterations in serum testosterone concentrations and were not of significant magnitude to account for these changes. One might expect increased catabolism of circulating albumin in this situation; this might lead to small changes in serum testosterone concentrations but not of the magnitude of those seen in many of our patients (Dunn et al., 1981).

Like the situation in medically ill patients LH levels were unchanged despite low testosterone levels. FSH levels were significantly depressed. The failure of LH to increase in response to a reduction in serum testosterone concentrations does imply hypothalamic-pituitary dysfunction although one would expect to find levels of LH below control

values if this were the main mechanism leading to low serum testosterone concentrations. Prolactin concentrations showed a tendency to increase but, because of the wide scatter of results, this did not achieve statistical significance. Hyperprolactinaemia can cause low testosterone levels but levels required are usually many times higher than those found in our patients (Carter et al., 1978).

The LH in serum of burned and ill patients does not seem effective in stimulating testosterone secretion. Perhaps this is because the LH secreted in these patients is structurally abnormal and therefore biologically inactive but sufficiently similar to LH in healthy men to be measured in standard LH radioimmunoassays. Possibly the regular secretory pulses of LH found in healthy men (Nankin & Troen, 1971) are disrupted by illness and the consequent non-pulsatile LH secretion is less active in promoting testicular steroidogenesis. A relative insensitivity to the action of LH at the level of the testes might also occur in association with either or both of these putative abnormalities of hypothalamic-pituitary function. These possibilities are investigated in more detail in Chapters 13 and 14.

Prolonged depression of serum T4 and T3 concentrations were seen in burned patients. The mean nadirs were 52 and 55 percent of mean control values as compared with 22 percent in the case of serum testosterone concentrations which seem more sensitive to this type of stress. For example one patient, a 60 year old man with a relatively minor burn of only four percent involving his cheek, had a serum testosterone concentration of only 2.6 nmol/l two weeks after injury subsequently recovering to 12.0 nmol/l; T4 and T3 levels reached a nadir of 58 and 1.2 nmol/l compared with levels of 77 and 2.3 nmol/l after recovery. Thus not only men very severely burned are affected in this way. As many as 12 of our 19 patients had serum testosterone concentrations within the female range (<2 nmol/l) on at least one occasion. Indeed serum testosterone

concentrations are so sensitive to burns injury that they give no indication of severity; hence the lack of correlation between surface area affected and serum testosterone concentration.

It is only fair to state that the observations made with regard to serum testosterone concentrations in this study (Semple et al., 1987c) in part duplicated the findings of previous workers (Dolecek et al., 1979a; Vogel et al., 1985) although my own study was in progress at the time of the latter publication. The extent and duration of the depression of serum testosterone concentrations as confirmed in this study as well as the comparatively sparing use of drugs confirmed that burned patients would be particularly suitable for investigation of the underlying mechanisms leading to low serum testosterone levels in ill men; these further studies are detailed in Chapters 13 and 14.

There was definite evidence of sustained adrenocortical activation following burns injury. Despite the prolonged increase in serum cortisol and to a lesser extent androstenedione concentrations, serum DHAS levels were normal on admission, fell to low levels one week thereafter and remained low for the remainder of the study period. This divergence between serum concentrations of androstenedione and DHAS is reminiscent of the situation in those patients who had been medically ill for more than two weeks. It is reassuring to note that both serum testosterone and DHAS levels do ultimately return to normal.

While this study was being carried out, there was no previous detailed assessment of adrenal androgen levels in burned patients. However I was beaten to press (Semple et al., 1987b) by Parker & Baxter (1985) who found a progressive reduction in serum DHAS concentrations over four weeks after burns injury in 19 adult men. Further work by this group (Lephart, Baxter & Parker, 1987) revealed that the low DHAS levels in these patients are accompanied by normal or reduced levels of urinary 17-ketosteroids. This suggests that the low serum DHAS levels are not the

result of increased urinary excretion of DHAS or of its 17-ketosteroid metabolites. Moreover serum levels of DHA were low demonstrating that low DHAS levels are not due to impaired sulphation of DHA by DHA sulphakinase. Serum levels of androstenediol and its sulphate were also low; these are metabolites of DHA and DHAS respectively and this finding is in favour of reduced synthesis rather than increased metabolism being the cause of low DHA and DHAS levels in burned men.

In a search for further situations which would be suitable for the serial study of hormone changes with illness, the situation following surgical stress was examined in an investigation outlined in the following chapter.

CHAPTER 7

HORMONAL CHANGES FOLLOWING CHOLECYSTECTOMY

Gonadotrophin levels of post-menopausal females were reduced following surgery. Increased serum and urine cortisol levels were accompanied by a transient elevation of androstenedione concentrations although DHAS levels fell below baseline one week after surgery.

Introduction

Having shown that both medically ill and burned men had altered hormonal status with respect to adrenocortical and testicular steroidogenesis I decided to continue my observations in patients following surgery.

The finding of normal LH levels in medically ill and burned patients with low serum testosterone concentrations was intriguing. Although low serum testosterone concentrations following surgery have been described by several authors there is no consensus as to whether gonadotrophin levels of men in this situation are unchanged (Carstensen et al., 1973), high (Wang et al., 1978b) or low (Charters et al., 1969; Hagen et al., 1980).

Depressed gonadotrophin levels had been reported previously in elderly medically ill women (Warren et al., 1977) and in post-menopausal women who also had the low T4 syndrome (Quint & Kaiser, 1985). Post-menopausal women have high gonadotrophin levels due to reduced negative feedback fron declining oestrogen secretion. These high levels may be particularly sensitive to the effects of illness. High gonadotrophin levels are only found in men with primary testicular failure such as in Klinefelter's syndrome. It would be of great interest to study such men following illness or surgery but due to their relative scarcity this is not practical. I considered it of interest to study the gonadotrophin

levels of post-menopausal women following surgery in order to look for evidence of hypothalamic-pituitary suppression. Surgery also provided a model for the further study of adrenal androgen secretion. Would adrenocortical activation following surgery lead to increased DHAS levels or would the postoperative period be associated with low levels of DHAS as in chronically ill and burned men?

Methods

Nine female post-menopausal patients (mean age 57, range 49-64 years) undergoing elective cholecystectomy agreed to take part in this study. No patients were taking any drugs known to affect endocrine function. An aliquot of urine and a blood sample were collected between 0800 and 1000 hours on the day prior to surgery (day -1) and on days +1, +4, +8 and +28 post-operatively.

Patients received an intramuscular opiate in combination with atropine as pre-medication. Induction of anaesthesia was obtained with thiopentone sodium supplemented in some patients by opiate analgesia. Maintenance of anaesthesia was achieved with a nitrous oxide/oxygen mixture supplemented by either halothane or enflurane and/or opiate analgesia. All patients received either suxamethonium or vecuronium as a muscle relaxant as well as parenteral opiate analgesia for between 48 and 72 hours following surgery.

Urine samples were assayed for urinary free cortisol (UFC) and expressed as the cortisol:creatinine ratio. Serum samples were assayed for FSH, LH, prolactin, cortisol, androstenedione and DHAS.

Statistical analyses were performed using the Wilcoxon Rank test for paired data.

Results

Serum cortisol and UFC (Figure 10) showed the expected rise in the first post-operative day (day +1) before gradually returning to baseline values. Serum androstenedione (Figure 11) increased from 4.3 ± 0.5 to 6.3 ± 0.7 nmol/l ($p<0.05$) on day +1 but returned to normal thereafter. Serum DHAS (Figure 11) was unchanged on day +1, fell to a nadir on day +8 (1.7 ± 0.6 v 0.5 ± 0.2 μmol/l, $p<0.02$) but had returned to baseline levels by day +28.

Both FSH (22.7 ± 2.9 v 48.3 ± 7.2 U/l, $p<0.02$) and LH (18.4 ± 5.4 v 50.5 ± 13.5 U/l, $p<0.02$) were significantly decreased on day +1 (Figure 12) but rapidly returned to normal. All patients had pre-operative gonadotrophin levels within the range consistent with the post-menopausal state (>30 U/l). Four patients had LH levels on day +1 less than 10 U/l. Serum prolactin concentrations (Figure 12) were raised post-operatively but to a relatively modest extent with day +1 levels ranging from 65 to 1260 mU/l (pre-operative range 20-530 mU/l).

Discussion

Adrenocortical activation following surgery was demonstrated in the early years of scientific endocrinology by investigators who found increased urinary excretion of 17-hydroxycorticoids in the absence of any appreciable change in 17-ketosteroids (Forbes et al. 1947; Moore et al. 1955). UFC gives a measure of the rate of clearance of cortisol from the circulation and relects the free, non-protein bound and presumed physiologically active moiety of serum cortisol. A comparison of the changes in UFC and serum cortisol in the first post-operative day is of interest; the former increased by a factor of 14 while the latter increased only by a factor of two (Figure 10). This highlights the importance of the interaction between cortisol binding globulin (CBG) and cortisol when the adrenal cortex is activated. CBG capacity readily

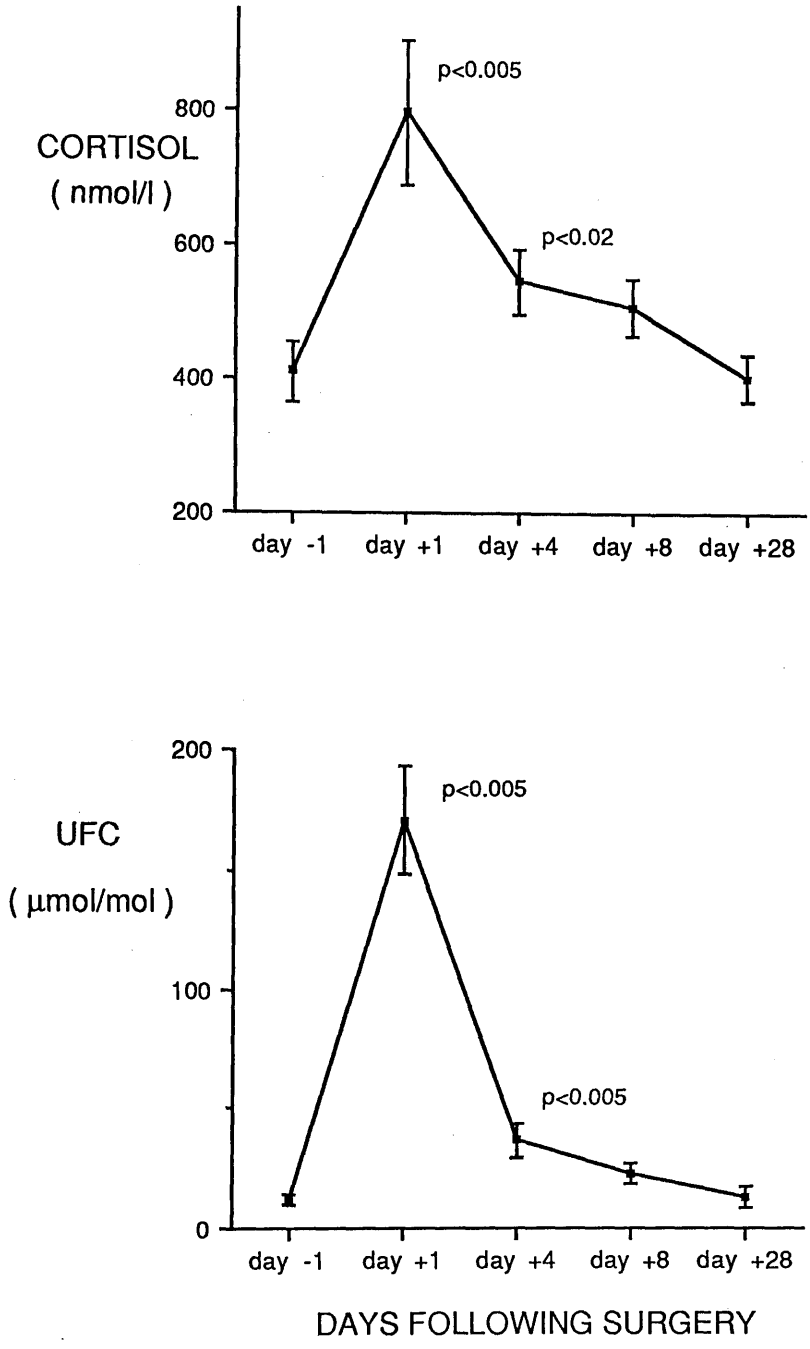


Figure 10 Serum and urinary free cortisol (UFC) concentrations (mean ± SE) before (day -1) and after cholecystectomy (days +1, +4, +8, +28) in 9 post-menopausal women. P values are for comparison with the pre-operative day.

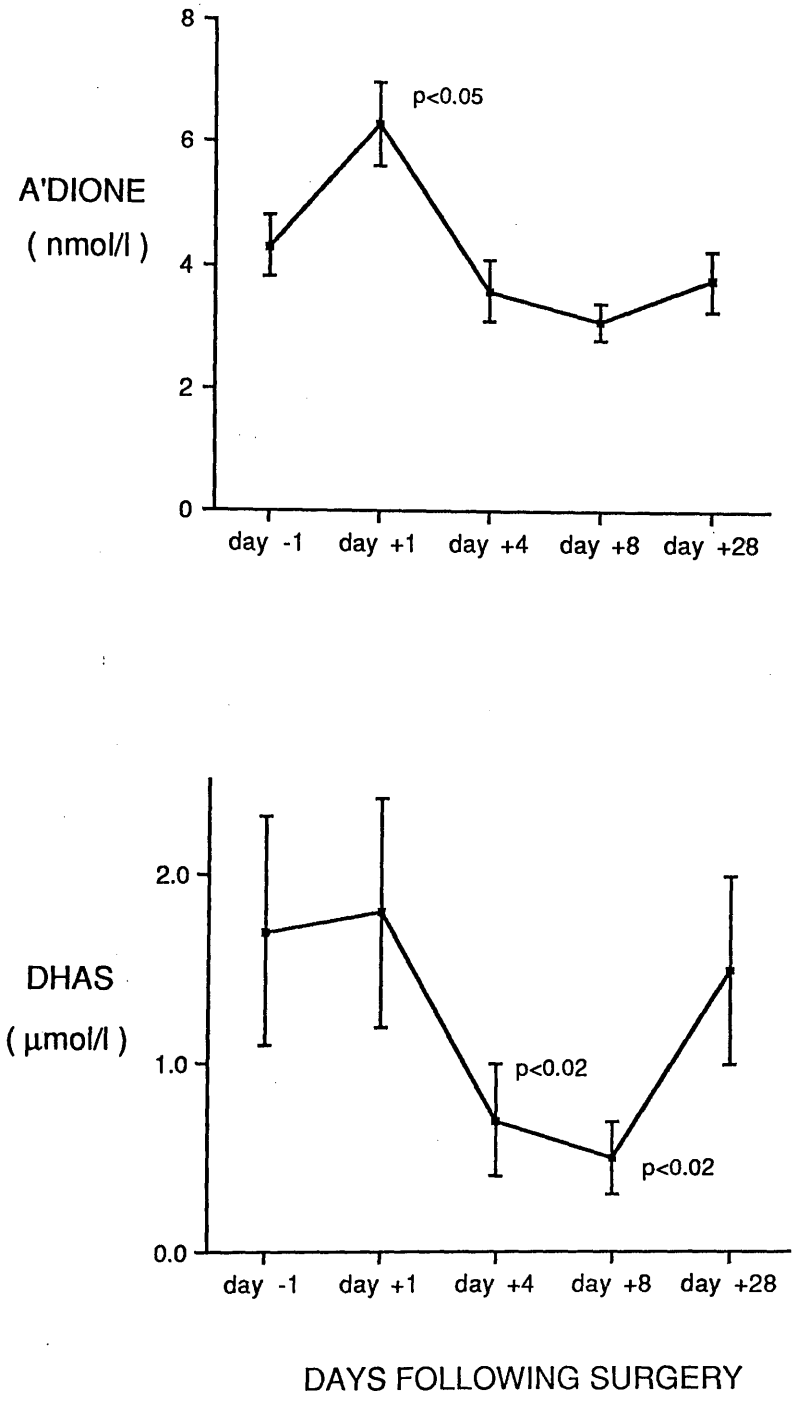


Figure 11 Serum androstenedione and DHAS concentrations (mean ± SE) before (day -1) and after cholecystectomy (days +1, +4, +8, +28) in 9 post-menopausal women. P values are for comparison with the pre-operative day.

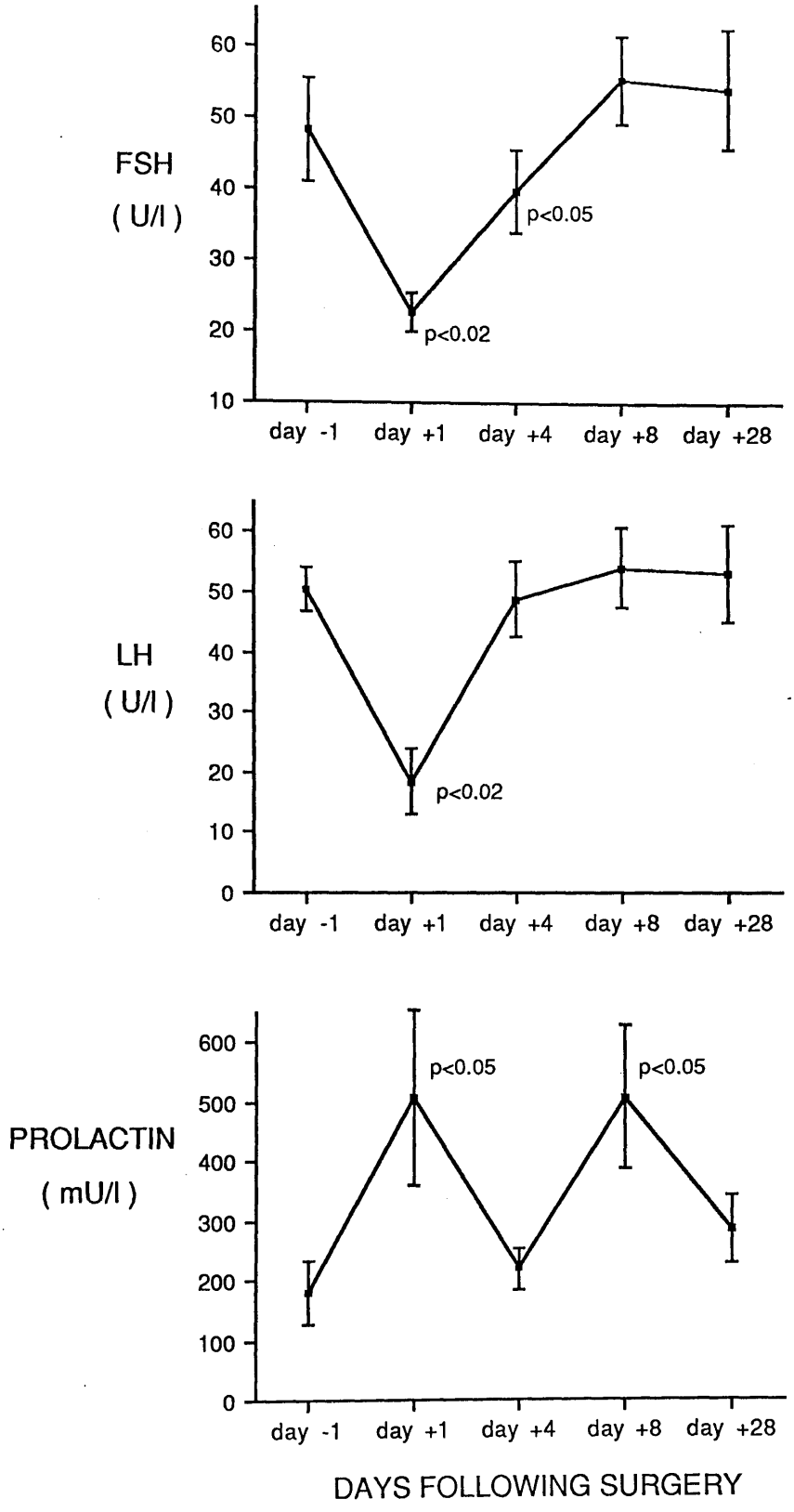


Figure 12 Serum FSH, LH and prolactin concentrations (mean ± SE) before (day -1) and after cholecystectomy (days +1, +4, +8, +28) in 9 post-menopausal women. P values are for comparison with the pre-operative day.

becomes saturated leading to much greater increases in free as opposed to total cortisol (Daughaday & Mariz, 1961).

Our description of adrenal androgen changes in serum of post-operative patients was the first in the literature (Semple et al., 1987b). Adrenocortical activation was not reflected in any substantial change in adrenal androgens. Androstenedione levels showed a small increase on day +1 only while DHAS showed no early change but fell to below basal levels on days +4 and +8 (Figure 11). There are similarities here with the results in our medically ill and burned patients. Acute medical or surgical stress may lead to a short-lived increase in androstenedione levels but more prolonged stresses seem to lead to a fall in DHAS levels.

Surgical stress was accompanied by modest hyperprolactinaemia and a reduction in gonadotrophin concentrations to pre-menopausal levels in several patients suggesting a transient hypogonadotrophic state (Figure 12). This change cannot simply be attributed to surgical stress as these patients received a variety of drugs both intra-operatively and post-operatively. All our subjects received parenteral opiates for 48 hours post-operatively and it would obviously not be ethical to study patients after major surgery without such potent analgesia. Previous studies in men have suggested that the drugs used in general anaesthesia may increase rather than decrease gonadotrophin concentrations (Nakashima et al., 1975). However prolonged usage of opioid drugs as in the case of drug addicts has an adverse effect on hypothalamic-pituitary-testicular function (Mendelson & Mello, 1975) and it is possible that short-term but intensive administration of opiates post-operatively may contribute to the fall in gonadotrophin levels.

Stress following surgery like that associated with medical illness and burns is accompanied by profound changes in the hormonal milieu. Changes in adrenal androgen levels were found which closely mirrored those found in burned patients except that the latter group seemed to be

affected in a more profound and longlasting way. The hope that the data on gonadotrophin levels of these postoperative patients might shed light on the mechanisms leading to a reduction in serum testosterone levels in ill men proved simplistic. Interpretation of the early changes in gonadotrophin levels in these patients was complicated by multiple drug administration. In particular the liberal use of parenteral opiates in the post-operative period rendered this data of little value.

Thus far several clinical situations had been studied and abnormalities of androgen levels in serum had been found to be common. Hormonal activation is found in other forms of stress such as following exercise or psychological stress. Were the abnormalities found in the situations already described specific to illness or would they be found in other stressful circumstances? The search for the answer to this question is addressed in the following three chapters.

CHAPTER 8

ENDOCRINE RESPONSES TO MARATHON RUNNING

10 men were studied one week before and on completion of a marathon. A massive rise in serum cortisol and a small increase in androstenedione and DHAS concentrations were found. Serum testosterone and LH concentrations fell. Extreme physical effort lowers testosterone levels but to a lesser degree than occurs during illness and unaccompanied by low levels of DHAS.

Introduction

The finding of abnormal gonadal function in ill men stimulated interest in endocrine function during other stressful situations. Ever since a soldier from the defending Greek army ran to Athens from the battlefield at Marathon, announced victory and died, it has been widely held that marathon running is the supreme test of physical fitness and provides a physiological stress which is difficult to surpass. Indeed the above soldier was not to be the last individual to die at or near the finishing line. In the years preceding this study (pre-1984) marathon running had increased greatly in popularity providing the opportunity for study of participants who would not be classified as top class athletes.

Exercise is known to lead to increased blood levels of several hormones in particular adrenocortical and adrenomedullary hormones, growth hormone and prolactin. Several studies had suggested that short term physical effort could increase serum testosterone concentrations (Sutton et al., 1973; Galbo et al., 1977; Kindermann et al., 1982; Grossman et al., 1984) but other investigators had found that levels had fallen with more intense exercise (Dessypris et al., 1976; Aakvaag et al., 1978a). When my interest arose in this subject only one previous study had described endocrine responses to marathon running, reporting a fall in mean serum testosterone concentrations in 13 runners from 23.9 before to

15.6 nmol/l after a marathon (Dessypris et al., 1976). However the ambient temperature was 30°C during the race which might have resulted in major fluid shifts and indeed might have been an additional stressor in its own right. No attempt was made to estimate the extent of dehydration in these athletes.

An investigation of endocrine function following marathon running was therefore performed in an effort to discover whether the stress of illness and extreme effort have any common effects on the hormonal milieu.

Methods

10 healthy adult males (age range 23-49, mean 33 years) agreed to take part in this study. All had been in training for at least three months for the Glasgow marathon but none were competitive runners. Pre-marathon samples were withdrawn during the week before the race between 1230 and 1330 hours, considered to be the likely finishing time. Fluids were freely available en route and weather conditions were cool with the average temperature 12°C. Post-marathon sampling was performed within 30 minutes of the finish (range 6-30, mean 20 minutes).

Samples were assayed for testosterone, SHBG capacity, FSH, LH, prolactin, cortisol, androstenedione and DHAS. To assess hydration status each individual's microhaematocrit was measured before and after the race using the Hawksley method after centrifugating anticoagulated blood samples at 13000 G for five minutes.

Statistical analyses were performed using the Wilcoxon Rank Test for paired data.

Results (Table 7)

All 10 runners completed the race in reasonable physical condition (mean duration 232, range 182-258 minutes). There was no significant

TABLE 7 Hormone levels in 10 men before and after marathon running (mean ± SE).

		PRE-MARATHON	*POST-MARATHON*	
Cortisol	(nmol/l)	338 ± 46	1640 ± 280	$p<0.01$
Androstenedione	(nmol/l)	3.6 ± 0.3	7.1 ± 0.4	$p<0.01$
DHAS	(µmol/l)	6.7 ± 1.0	9.8 ± 1.7	$p<0.01$
Testosterone	(nmol/l)	18.5 ± 1.6	14.9 ± 1.1	$p<0.01$
Derived free Testosterone	(pmol/l)	312 ± 32	245 ± 30	$p<0.01$
SHBG	(nmol/l)	30.5 ± 2.8	30.0 ± 2.3	
FSH	(U/l)	3.7 ± 0.5	3.4 ± 0.3	
LH	(U/l)	4.1 ± 0.6	2.5 ± 0.4	$p<0.05$
Prolactin	(mU/l)	140 ± 32	334 ± 90	$p<0.01$

difference between the microhaematocrit before and after the race (47 ± 0.8 v 46 ± 0.4 percent).

Adrenocortical activation was reflected in a massive increase in serum cortisol concentrations with a smaller but statistically significant rise in the adrenal androgens, androstenedione and DHAS. A fall in serum testosterone concentrations was accompanied by a drop in LH levels, an increased prolactin level but unchanged SHBG levels. Changes in levels of cortisol, testosterone and LH are shown in Figure 13.

Discussion

A small but statistically significant reduction in serum testosterone concentrations occurred as a consequence of marathon running (Semple, Thomson & Beastall, 1985) confirming the earlier work of Dessypris et al. (1976) and the more recent study of Kuusi et al. (1984). In the latter study the fall in testosterone levels could be accounted for by a similar fall in SHBG capacity leading to an unchanged derived free testosterone concentration. In the study described in this chapter SHBG capacity was unchanged and derived free testosterone closely mirrored the changes in total testosterone. The finding of a fall in LH levels contrasted with unchanged levels found by Dessypris et al. (1976) but agreed with the findings of Kuusi et al. (1984) in men and Hale et al. (1983) in women.

The finding that the microhaematocrit of athletes was unchanged by the race shows that they were not signficantly dehydrated so any alteration in hormone levels could not be attributed to fluid shifts. This finding is perhaps a little surprising in view of the duration and intensity of exercise but weather conditions were cool and participants were supplied with plentiful fluids at regular intervals.

The suppression of testosterone levels seen with effort differs in several ways from that following illness. Firstly it is less pronounced;

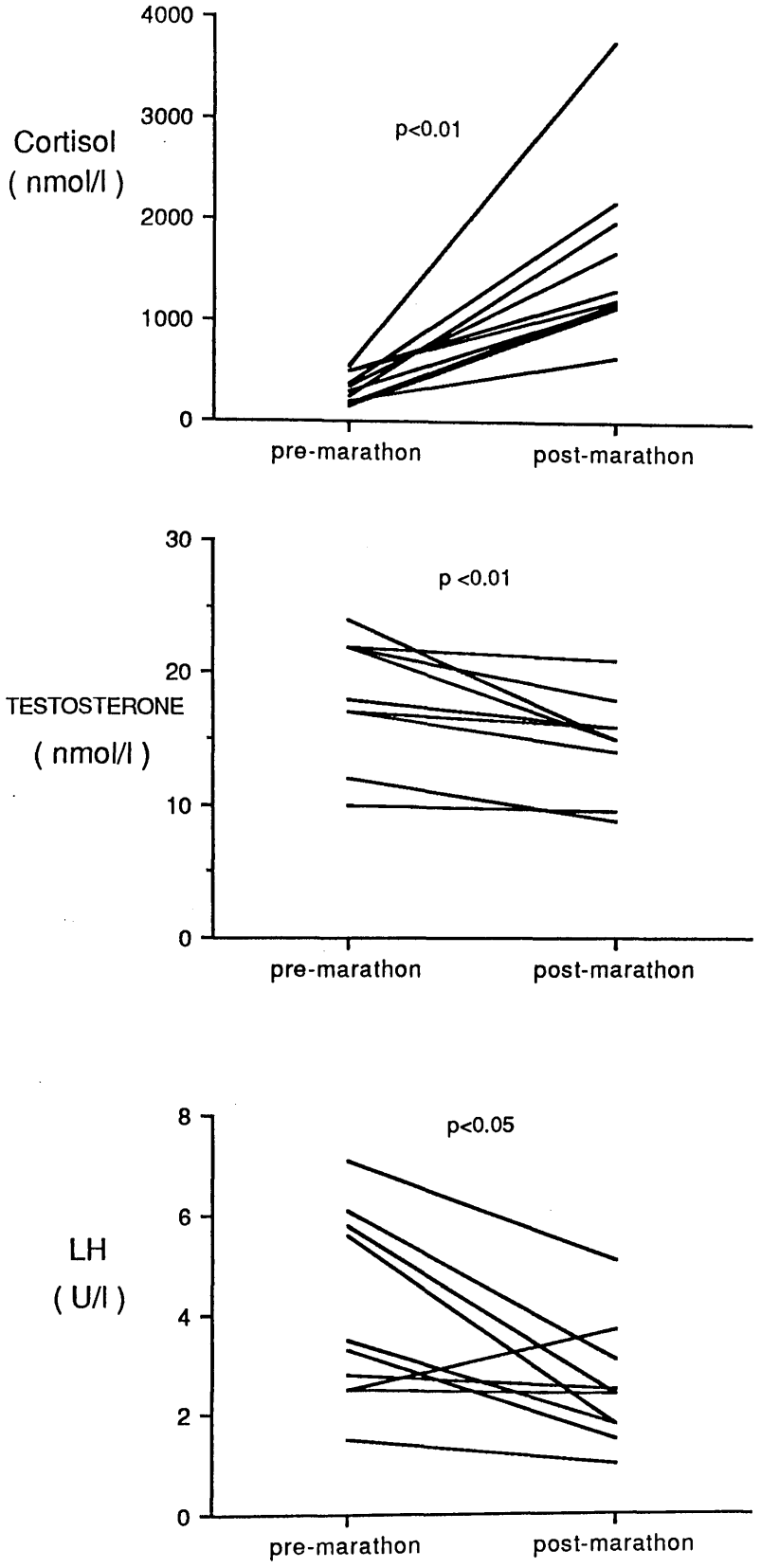


Figure 13 Serum cortisol, testosterone and LH of 10 men during the week prior to and immediately after a marathon.

for example the burned patients described in Chapter 6 had very low serum levels of testosterone whereas all the marathon runners had levels within the normal male range. While in athletes there is a massive outpouring of glucocorticoid the reduction in serum testosterone concentration with illness may occur in the absence of demonstrable adrenocortical activation. Thus extreme physical effort may lead to a reduction in testosterone levels but of lesser degree than that associated with illness and unaccompanied by low levels of the adrenal androgen DHAS.

Endogenous opioid peptides have an inhibitory effect on hypothalamic-pituitary-testicular function (Grossman et al., 1981) and it had been widely suspected that the "anti-gonadal" effect of exercise in men and women might be mediated by an increase in these substances with exercise. Early work using assays of low specificity had suggested that β-endorphin was increased by exercise (Colt, Wardlaw & Frantz, 1981; Farrell et al., 1982) but Elias et al. (1986) using more specific assays observed a β-lipotropin increase but no β-endorphin response to acute exercise. As mentioned above the fall in LH levels with exercise is not consistent. Indeed when pulsatile LH release was examined immediately following exercise no change in LH pulse frequency was found as compared with pre-exercise values (McColl et al., 1989). Hyperprolactinaemia may exert an "antigonadal" effect but at much higher levels than found in this study and this effect is mediated centrally. There remains then the difficulty in explaining a reduction in serum testosterone levels in the absence of any obvious change in central control. An alteration in the biological activity of LH with exercise is a possiblity but has not been investigated in the literature.

Increased glucocorticoid levels in this situation were expected but the extent of the elevation was surprising. In a previous publication (Semple et al., 1982c) the mean serum cortisol concentration after

injection of pharmacological doses of synthetic ACTH (Synacthen) into 10 heathy men was 865 nmol/l as compared with 1640 nmol/l after marathon running. Metabolism of cortisol increases with exercise (Few, 1974) and thus the increased serum levels found in this situation are likely to be due to increased secretion rather than reduced elimination. This intense adrenocortical activation was also reflected by an increase in the adrenal androgens, androstenedione and DHAS. This contrasts with patients following surgery, burns injury and medical illness in whom androstenedione but not DHAS levels increased . This may be related to the duration of the stresses involved. In the marathon they were relatively short-lived and of great intensity.

In the chapter which follows I studied androgen levels of men who undertook regular levels of physical exertion over many years, the so called veteran athletes. Would the chronic nature of this stimulus and presumed recurrent adrenocortical activation lead to any lasting changes in androgen levels?

CHAPTER 9

GONADAL FUNCTION IN VETERAN ATHLETES

13 veteran athletes were compared with 13 sedentary controls. Extreme and longstanding physical fitness had no discernible effect on pituitary-testicular function or on adrenal androgen levels.

Introduction

Menstrual abnormalities such as amenorrhoea or oligomenorrhoea are common in female athletes (Baker et al., 1981). Amenorrhoeic runners have low serum oestradiol levels (Baker et al., 1981). Evidence suggesting hypothalamic-pituitary-testicular suppression had been found following prolonged exertion and this raised the possibility that exercise of this nature, repeated and prolonged over many years, might have a more permanent "anti-gonadal" effect. If the low DHAS levels found in ill men are caused by adrenocortical adaptation to chronic stimulation then might the same effect be present in veteran athletes whose repeated and sustained exertions might be expected to lead to repeated and sustained adrenocortical activation.

The opportunity arose to study members of the Scottish Veteran Harriers Club which is comprised of members over 40 years old most of whom had been distance training for many years.

Methods

13 men, age range 44-60 years agreed to take part in this study. All had been running more than 25 miles per week (range 25-110 miles) for at least 20 years (range 20-39 years). To avoid the acute effects of exertion subjects were asked not to train during the 24 hours prior to sampling. Blood samples were obtained between 0800 and 1000 hours from these subjects and from age matched normal controls who did not take

regular exercise. Samples were assayed for serum concentrations of testosterone, SHBG capacity, FSH, LH, androstenedione and DHAS.

Statistical analyses were performed using the Wilcoxon Rank test for unpaired data.

Results (Table 8)

There was no significant difference between the athletes and the controls with regard to any of the parameters measured.

Discussion

Several previous papers have addressed this question and their conclusions are summarised below:-

Authors	*Subjects*	*Testosterone*
Young et al., 1976	7 "high fit" v 7 "low fit"	↑
Remes et al., 1979	39 recruits- 6 month training	↑
Wheeler et al., 1984	31 athletes v 18 controls	↓
Kuoppasalmi & Adlercreuz, 1985	16 athletes v 7 controls	no difference
MacConnie et al., 1986	6 athletes v 6 controls	no difference
Wheeler et al., 1986	49 athletes v 18 controls	↓
Hackney, Sinning & Bruot (1988)	11 trained v 11 untrained	↓

When low serum testosterone concentration were found following marathon running it seemed unlikely that athletes in the resting state would have increased testosterone levels as the literature available at that time suggested. The results of Wheeler et al. (1984) then spurred me on to perform my own study the results of which are similar to

TABLE 8 Hormone levels of 13 veteran athletes and 13 age matched controls (mean ± SE).

	VETERAN ATHLETES	*CONTROLS*
Age (years)	53.6 ± 1.5	52.7 ± 1.8
Years in training	25 ± 5	--
Miles per week	51 ± 7	--
Testosterone (nmol/l)	15.8 ± 1.2	16.6 ± 1.8
SHBG capacity (nmol/l)	41.2 ± 3.3	36.6 ± 2.8
Derived free Testosterone (pmol/l)	225 ± 11	248 ± 22
Androstenedione (nmol/l)	4.9 ± 0.7	4.5 ± 0.7
DHAS (µmol/l)	4.3 ± 0.4	4.2 ± 0.7
FSH (U/l)	5.3 ± 0.8	4.6 ± 0.8
LH (U/l)	5.4 ± 0.6	6.0 ± 0.5

Kuoppasalmi & Adlercreuz (1985) and MacConnie et al. (1986). What explanation might there be for these differing results?

The first study cited was very small; moreover the control group seemed strikingly overweight (mean weight 73 Kg v 94 Kg), a factor which can have an adverse effect on gonadal function (Glass et al., 1977). Veteran athletes in the study described in this chapter were leaner than control subjects (mean body mass index 22.0, range 19.5-24.5 v 24.1, 22.0-26.5) but none of the latter were obese (obesity = body mass index > 30). The second study employed recruits before and after a six month training course. It is possible serum testosterone concentrations might rise over this relatively short term yet return to normal when, as in the case of the veterans, training is sustained over many years. Wheeler et al. (1984) and Hackney et al (1988) studied athletes similar to the Scottish veterans. Differences in training schedules, age ranges and timing of sampling in relation to exercise might account for the different results. That this might be the case is suggested by the later work of Wheeler et al. (1986) in an extension to their earlier study (Wheeler et al. 1984). 49 athletes were examined using different training schedules; only those running more than 40 miles per week showed a statistically significant fall in serum testosterone concentrations whereas those running between 25 and 40 miles per week did not.

A recent review of this subject has come down in favour of a mild lowering of testosterone levels with heavy training (Cumming, Wheeler & McColl, 1988). However, there may be a bias towards positive results reaching publication; reearchers finding no difference between trained athletes and controls may (like myself) not have submitted their work for publication or if they have done so it may not have been of sufficient interest to merit acceptance.

Evidence for abnormalities of LH secretion of trained athletes is equally conflicting. Female athletes have evidence of an abnormality of LH

pulsatility whether menstruating normally or amenorrhoeic (Cumming et al., 1985; Veldhuis et al., 1985). MacConnie et al (1986) found decreased frequency and amplitude of LH pulses in male athletes despite normal levels of serum testosterone and suggested that increased levels of testosterone occurring after oft-repeated short term exertion (Sutton et al., 1973; Galbo et al., 1977; Kindermann et al., 1982; Grossman et al., 1984) might lead to a degree of hypothalamic-pituitary suppression. However Rogol and colleagues (1984) found no evidence of abnormal LH pulsatility in endurance trained athletes although their sampling period commenced just after the completion of a 10-15 mile run. Hackney et al. (1988) found normal LH pulsatility in their group of endurance trained athletes who had had no exercise for 36 hours and who had lower testosterone levels than their control group.

In my study I was unable to detect any evidence of low testosterone levels with long term endurance training. If any "anti-gonadal" effect does occur it must be of a minor degree; it is unlikely to have adverse physiological sequelae; and it bares no similarity to the profound and prolonged reduction in serum testosterone concentrations found in ill men.

Veteran athletes had adrenal androgen levels similar to controls. Thus regular exercise with presumed oft repeated adrenocortical activation has no discernable effect on adrenal androgen levels in serum.

Illness is usually accompanied by a considerable degree of psychological stress. Might the endocrine changes in ill patients described in earlier chapters be related to this psychological stress rather than to the illness itself? In the project which follows I studied the endocrine response to examination stress to discover whether there there were any similarities with the results found in ill men.

CHAPTER 10

ENDOCRINE EFFECTS OF EXAMINATION STRESS

To determine the effects of pure psychological stress on endocrine function, nine men were studied 16 weeks before and immediately after Final Degree examinations. Increased psychological stress was accompanied by increased urinary excretion of metadrenalines but there were no significant changes in gonadal or adrenocortical hormones.

Introduction

Having demonstrated that the stress associated with exercise and illness may alter levels of hormones not usually considered "stress hormones", I decided to investigate endocrine function associated with psychological stress which is often a consequence of illness. Previous studies in this field had proved difficult to interpret because of the presence of stressors such as illness or exercise while certain factors which influence endocrine function such as medication or alcohol ingestion had been ignored.

Psychological stress may lead to increased secretion of glucocorticoids and catecholamines (Hodges et al., 1962; Rubin et al., 1970) but responses vary considerably between individuals and depend on the type of exposure. For example pilots show a much greater adrenocortical response to the stress of aircraft carrier landings than do navigators despite having lower levels of anxiety and fear (Miller et al., 1970) and individuals who cope well with stress have a lesser response than those who cope badly (Miyabo et al., 1970).

Interest in testicular function associated with psychological stress was stimulated by the finding of reduced urinary excretion of androgen metabolites in soldiers awaiting attack (Rose et al., 1969) although unchanged excretion had been demonstrated previously during examination stress (Connell et al., 1958). Low serum testosterone concentrations were

found in eight army cadets during a five day combat course; although this was undoubtedly a psychologically stressful experience, the study was complicated by the stress of intense physical training (Aakvaag et al., 1978b). Similarly, during a 23 week officer training course low serum testosterone levels were found during the third as compared with the penultimate week (Kreuz et al., 1972); while the authors related this observation to a reduction in psychological stress, it is possible that the physical training component of the course led to increased fitness with a reduction in the physical stress of exercise in the later weeks of the course.

The aim of the current study was to examine hormone changes associated with university examination stress whilst taking into account other factors known to affect endocrine function.

Methods

Nine final year physiology students (age range 20-22, mean 21 years) agreed to take part in the study, permission having been obtained from University authorities. Following a four week vacation and 16 weeks prior to Final examinations initial blood sampling was carried out between 1200 and 1300 hours. Repeat venepuncture was performed at the same time of day immediately following a three hour written examination in the middle of a series of Final Degree examinations. Twenty four hour urine collections were obtained terminating at 0900 hours on the days of each venepuncture as well as 10 ml aliquots of urine from the first sample of the day for estimation of urinary free cortisol. All subjects had endured six hours of written examinations during the 24 hours preceding the second venepuncture.

Serum samples were assayed for cortisol, androstenedione, testosterone, SHBG capacity, DHAS, FSH, LH, and prolactin. Aliquots of early morning urine were measured for urinary free cortisol and 24 hour

collections were assayed for urinary metadrenaline excretion as expressed as μmol/mol creatinine in order to correct for possible incomplete urine collection.

Subjects were asked to fill in a questionnaire regarding exercise, cigarette smoking (Deslypere & Vermeulen, 1984) and alcohol ingestion (Gordon et al., 1976) all factors which may alter gonadal endocrine function. No subject was taking any drugs or medication.

In order to assess the extent of psychological stress, subjects were asked to complete two self-rating anxiety assessments during the 24 hours preceding each venepuncture. The Zung self-rating anxiety scale (Zung, 1971) consists of 20 statements which encompass many physiological consequences of anxiety; for example - "I feel more nervous and anxious than usual" and "I have to empty my bladder more often". Subjects were asked to rate each of the 20 items as to how it applied to him during the past week in the following terms:- none or a little of the time, some of the time, part of the time, most or all of the time. Each reply was scored a value of 1, 2, 3, or 4 in such a way that less anxious subjects have a low score on the scale which ranges from a minimum of 25 to a maximum of 100 when expressed as a percentage. As the Zung scale had been designed to assess anxiety associated with psychiatric disorders, further assessment was made using a visual analogue scale (Figure 14) employing the subjects' responses to the following words:- tense, excited, fearful, relaxed, 'wound up', apprehensive, confident, worrying, calm, and tremulous. Subjects were asked to place a cross on a 10 cm line indicating the extent to which each word applied to them at the present time or during the preceding week. Results were expressed on a scale of zero to 100, the higher the score the greater the anxiety.

Statistical analyses were performed using the Wilcoxon Rank Sum test for paired data. The chances of statistical significance being fortuitously obtained increases with the number of parameters measured.

ANALOGUE RATING SCALE

Below you will see a list of words which describe how a person feels or acts. You have to consider each description carefully and decide how true it could be of you during the past week / or at the present time. Place a cross anywhere along the line opposite each description to indicate how true it could be. Obviously the closer you put the cross to the "extremely" end the more true you feel the description is and so on.

TENSE not at all_____extremely

EXCITED _____

FEARFUL _____

RELAXED _____

WOUND UP _____

APPREHENSIVE _____

CONFIDENT _____

WORRYING _____

CALM _____

SHAKY or TREMULOUS _____

Figure 14 This visual analogue rating scale was completed in the 24 hours prior to both pre-exam and post-exam blood sampling. Each response was scored out of 10 with the aid of a ruler in such a way that higher scores indicated greater anxiety. All 10 scores were added up to give a total score out of 100.

When this study was submitted for publication to Clinical Science it was suggested that the Bonferroni correction ((Wallenstein, Zucker & Fleiss, 1980) be invoked. To reduce the chance of a false positive statistical result to less than the 5% level in this study of 13 variables significance was only claimed when P was less than 0.004 (i.e. 0.05 ÷ 13).

Results (Table 9)

Both anxiety rating scales showed significant increases during the post-examination assessment period. The mean pre-examination value for the Zung scale was 36.1 and was similar to that found by Zung (Zung, 1971) in 100 normal controls (33.8) supporting the assumption that students were not unduly stressed during the pre-examination sampling period. There was no significant change in exercise taken or alcohol or cigarettes consumed between the two study periods (Table 10). 24 hour urinary metadrenalines reflected increased psychological stress with all subjects showing increased excretion at the time of examinations. No other hormone levels showed any significant change.

Discussion

This study (Semple et al., 1988) demonstrating an unchanged hypothalamic-pituitary-testicular axis with psychological stress was unusual in that confounding factors such as exercise and alcohol were taken into consideration and in that the presence of definite psychological stress was confirmed by psychological testing. In a similar study Johansson et al. (1988) found serum testosterone levels of medical students unchanged immediately prior to a three hour examination but LH levels were reduced. Whilst it may be premature on the strength of these findings to exclude any effect of psychological stress on serum testosterone concentrations, previous literature has often failed to take into consideration other important factors such as the effect of exercise.

TABLE 9 Psychological assessment and hormone levels before and after examinations in 9 male university students.

	PRE-EXAM	*POST-EXAM*	
Zung anxiety rating	36.1 ± 1.7	46.5 ± 2.8	$p<0.004$
Visual analogue scale	32.2 ± 4.4	60.9 ± 3.8	$p<0.001$
Urinary metadrenalines (µmol/mol creatinine)	108.7 ± 12.7	190.9 ± 12.7	$p<0.001$
Urinary free cortisol (µmol/mol creatinine)	10.4 ± 1.2	13.3 ± 3.0	
Cortisol (nmol/l)	409 ± 40	318 ± 38	
Androstenedione (nmol/l)	6.3 ± 0.5	6.1 ± 0.5	
DHAS (µmol/l)	9.3 ± 0.5	9.1 ± 0.8	
Testosterone (nmol/l)	24.3 ± 1.8	21.0 ± 1.9	
SHBG (nmol/l)	33.6 ± 4.3	32.7 ± 4.3	
Derived free Testosterone (pmol/l)	390 ± 37	343 ± 30	
FSH (U/l)	3.2 ± 0.5	2.8 ± 0.5	
LH (U/l)	5.3 ± 0.6	6.2 ± 0.9	
Prolactin (mU/l)	155 ± 38	71 ± 21	

TABLE 10 Three other variables which have previously been described as influencing endocrine function in 9 male university students.

SUBJECT NO.	*EXERCISE (miles/week)*		*CIGARETTES (cigs/day)*		*ALCOHOL (Units/week)*	
	PRE	POST	PRE	POST	PRE	POST
1	30	17	0	0	20	0
2	0	0	0	0	1	1
3	0	0	0	0	1	2
4	3	3	5	5	50	20
5	0	0	0	0	40	30
6	0	0	0	0	0	0
7	0	0	0	0	8	6
8	6	0	10	10	40	50
9	0	0	0	0	16	8

The subject is of some clinical relevance as it has been suggested that altered testosterone secretion with stress may contribute to the increased risk of coronary artery disease in subjects with Type A personalities (Henry, 1986). Until further studies have refuted these findings it should not be assumed that pure psychological stress depresses serum testosterone concentrations.

The timing of sample collection posed particular problems during this study. It was not considered practical to collect urine during the course of examinations themselves. Thus blood and urine samples were not obtained at the same time of day. It is possible that urine was collected prior to examinations when psychological stress may have been greater than following examinations when venesection was performed and when anxiety may have been waning.

The stress of examinations was reflected in an increase in urinary catecholamine excretion but serum and urinary cortisol concentrations were unaffected. Adaptation occurs more rapidly with cortisol; once the stressful situation has lost its novelty, cortisol secretion may not increase while catecholamine secretion remains responsive to stress (Rubin et al., 1970; Rose et al., 1969; Czeisler et al., 1976).

In clinical practice it is widely assumed that a raised prolactin level may be explained by psychological stress such as attendance at an out-patient clinic. When factors such as drugs, trauma, illness and exercise are carefully excluded, the association between psychological state and prolactin levels is less clear (Brooks et al., 1986). The students studied in this chapter had a reduced prolactin level following examinations ($p<0.05$, not significant following Bonferroni correction). There are several possible explanations for this failure to find prolactin concentrations increasing with psychological stress. Firstly, prolactin secretion may not increase with pure psychological stress; secondly, blood sampling may have been performed after the time of maximum anxiety

and a prolactin peak may have been missed; thirdly, adaptation may occur, as in the case of cortisol secretion, so that only novel or acute stresses rather than familiar or chronic ones increase prolactin levels; finally, continued stimulation of prolactin secretion with prolonged psychological stress may have resulted in depletion of a readily releasable pool. The observation that prolactin levels of medical students were raised immediately prior to examinations but fell to below control levels immediately afterwards suggests that adaptation may occur (Johansson et al., 1983). Allen et al. (1985) found that both cortisol and prolactin levels were increased after a 15 minute oral examination but that there was no significant trend in prolactin levels with increasing psychological stress prior to examinations. However, novel stresses such as preparing for a first parachute jump have failed to elevate prolactin levels (Noel et al., 1976). Thus considerable doubt still remains as to whether prolactin secretion is affected by psychological stress.

The endocrine responses to stress associated with examinations seem to have little in common with the changes seen following marathon running or during illness. There is no evidence of adrenocortical activation or suppression of adrenal androgen secretion; pituitary-testicular function is not affected.

The studies described so far in this thesis have mainly involved observation of endocrine changes with illness and other stressful situations. The chapters which follow describe studies which were designed to study the mechanisms involved in these changes particularly with respect to pituitary-testicular function.

CHAPTER 11

THE NATURE OF PROTEIN BINDING OF TESTOSTERONE IN BURNED PATIENTS

Changes in SHBG capacity following burns injury were closely mirrored by changes in SHBG concentration measured by immunoradiometric assay suggesting that low SHBG capacity is due to a fall in absolute concentration rather than an alteration in binding affinity. Burned men with low serum total testosterone concentrations also had low levels of free testosterone measured by equilibrium dialysis.

Introduction

The main abnormality leading to low T4 levels in ill patients is an alteration in the binding of T4 to its binding proteins so free T4 levels measured by equilibrium dialysis are not usually low. A similar abnormality might contribute to the low serum testosterone concentrations found in ill men. The SHBG capacity of burned men following admission was low; this might be due to changes in testosterone binding similar to those occuring between T4 and its binding proteins in ill patients. Indeed free fatty acids which have been proposed as the thyroid hormone binding inhibitor in the low T4 syndrome (Chopra et al., 1986) may reduce the binding of testosterone to SHBG and albumin (Mooradian, et al., 1988). Alternatively the increased protein breakdown occuring following burns injury may allow increased catabolism of SHBG and lead to fall in the absolute concentration of SHBG. To investigate whether the changes in serum SHBG capacity following burns injury were due to altered binding kinetics or a change in its absolute concentration, SHBG was measured by an immunoradiometric assay (SHBG-IA) as well as by the more usual binding capacity method.

The free (non-protein bound) component of total testosterone is usually considered the physiologically active moiety as it is able to

pass intracellularly although Pardridge & Mietus (1979) found that albumin binding did not inhibit transport of testosterone into rat brain cells and thus albumin bound testosterone may also be important. It is thus of interest to investigate whether free testosterone measured directly (rather than derived as in previous chapters in this thesis) is reduced to a similar extent to total testosterone. Accordingly free testosterone levels were measured by equilibrium dialysis and compared with total testosterone and derived free testosterone levels.

Methods - Assays

The absolute concentration of SHBG (SHBG-IA) was measured using a non-competitive immunoradiometric assay (Farmos Diagnostica, Finland) in which a mouse ^{125}I-labelled anti-SHBG monoclonal antibody and rabbit anti-SHBG antiserum are added to the serum sample. Following incubation solid phase donkey anti-rabbit IgG antiserum is added. The mixture is then centrifuged with the resulting pellet containing radioactivity in proportion to the concentration of SHBG. The SHBG in the unknown sample is extrapolated after reference to a standard curve. The intra-assay and inter-assay coefficients of variation were 3.2 and 11.2 percent respectively. SHBG capacity was measured as described in Chapter 2.

Equilibrium dialysis was performed with small aliquots of serum using the Dianorm dialysis machine (Weber, Schildknecht & Kesselring, 1971). 200 µl of serum is injected into one half of a Teflon cell and is separated by a cellulose dialysis membrane (Spectropore 2 - Pierce, UK, Ltd) from H.E.P.E.S. buffer (N-2-Hydroxyethylpiperazine-N'-2-Ethane-sulphonic acid) pH 7.4 in the other side of the cell. The cells rotate in the machine for 12 hours at 37°C allowing equilibration of the unbound testosterone with the buffer solution. The concentration of testosterone in the buffer side of the cell should be the same as the free testosterone concentration in the serum sample at equilibrium.

Testosterone in the buffer solution is measured after ether extraction by radioimmunoassay with a second antibody separation system. The key to this procedure is the use of a high specificity, high sensitivity testosterone antiserum which was raised in sheep against testosterone-3-carboxymethyloxime linked to bovine serum albumin and was used with a testosterone-3-carboxymethyloxime-iodohistamine label (Webb et al., 1985). This assay will measure free testosterone concentrations >10 pmol/l and shows only significant cross-reactivity (12%) with 5α-dihydro-testosterone. Results produced by this procedure were shown to be independent of the degree of sample dilution.

Methods - Patients studied

SHBG studies were performed on 17 burned men for whom sera was available from their first venesection after admission. 14 of these patients were also studied using sera from the venesection performed either in the third or fourth week after admission. Data were compared with results from 15 age-matched healthy men.

Serum testosterone, free testosterone by equilibrium dialysis and the derived free testosterone concentrations were measured in 12 burned men with low serum testosterone concentrations and in 13 age matched healthy men.

Results were compared using the Wilcoxon Rank Test for unpaired data while correlation coefficients were calculated using the Spearman Rank Test.

Results

The SHBG capacity of burned men on admission was 20.4 ± 2.9 nmol/l increasing to 36.8 ± 4.2 nmol/l with recovery as compared with 33.5 ± 3.1 nmol/l in the control group. The SHBG-IA concentrations of burned men on admission were 21.1 ± 2.6 nmol/l increasing to 33.4 ± 3.4 nmol/l with

recovery as compared with 30.5 ± 3.0 nmol/l in the control group. There was no significant difference between the SHBG capacity and the SHBG-IA results in any of these groups. Correlation coefficients between the two methods were:- r=0.90, p<0.001 for all three groups combined, r=0.80, p<0.002 for the burned patients following admission and r=0.93, p<0.001 for the control group. The relationship between SHBG capacity and SHBG-IA is shown graphically in Figure 15.

In burned men with low serum testosterone levels (0.3-10.1, mean 4.0 nmol/l) the mean free testosterone concentration was not significantly different from the derived free testosterone (free testosterone 76.9 ± 21.7 v derived free testosterone 73.4 ± 15.6 pmol/l). Free testosterone correlated significantly with total testosterone (r=0.78, p<0.01) and derived free testosterone (r=0.69, p<0.05). In normal men the mean free testosterone concentration was not significantly different from the derived free testosterone (273 ± 20 v 270 ± 26 pmol/l). Free testosterone correlated significantly with total testosterone (r=0.71, p<0.02) and derived free testosterone (r=0.65, p<0.05). The relationships between free testosterone and total testosterone and free testosterone and derived free testosterone are shown in Figure 16.

Discussion

SHBG when measured by immunoradiometric assay closely correlated with that measured by the binding capacity method. This study has demonstrated for the first time that the low SHBG level following burns injury is due to a genuine fall in the concentration of this binding protein rather than to a change in its affinity for testosterone. This contrasts with the situation in the low T4 syndrome where there is a fall in the affinity of binding proteins for T4 with little or no change in their absolute concentration. A reduction in the hepatic synthesis of SHBG would not be expected to result in such an early fall in its

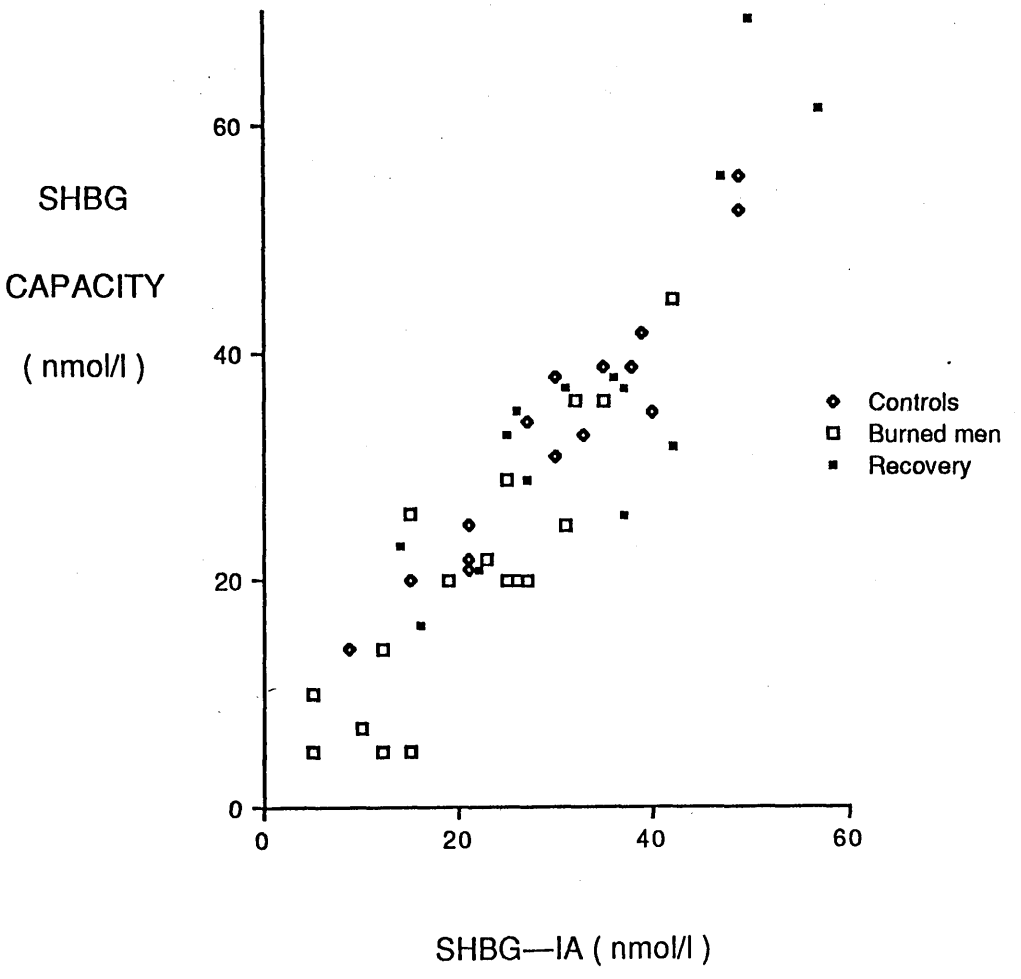
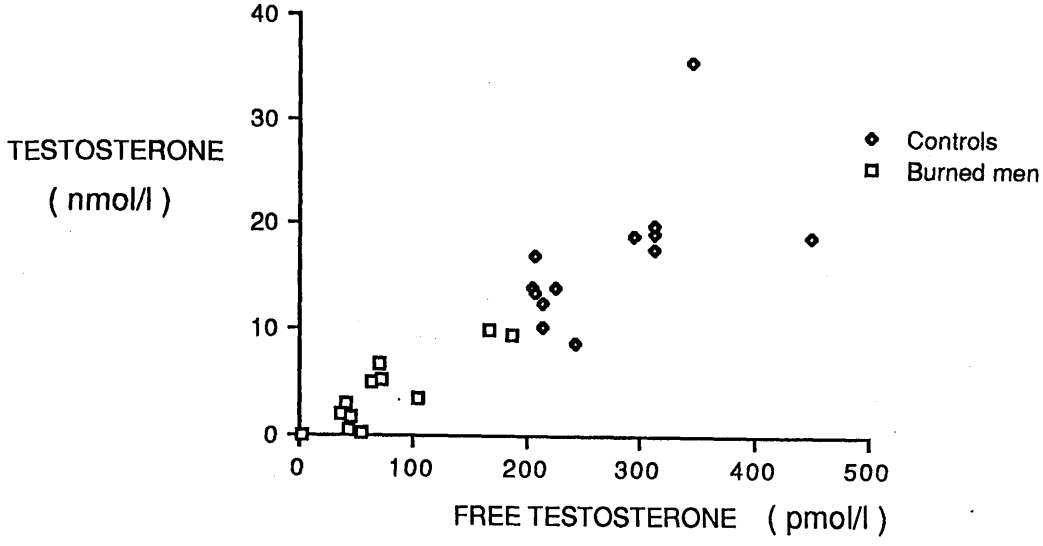


Figure 15 Relationship between SHBG measured by immunoradiometric assay (SHBG-IA) and SHBG binding capacity in healthy men, burned men with low SHBG capacity and burned men following recovery.



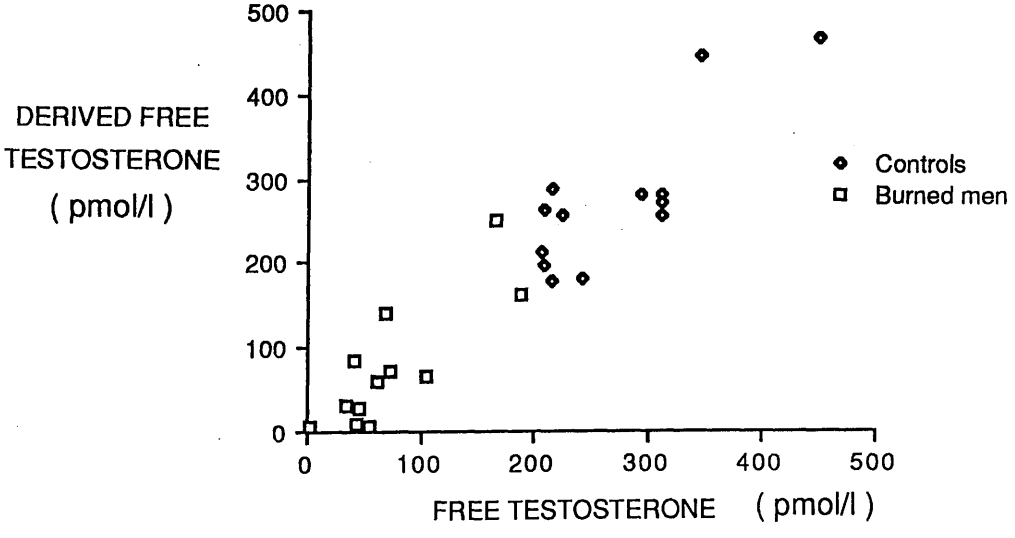


Figure 16 The relationship between free testosterone measured by equilibrium dialysis and total testosterone and derived free testosterone.

concentration. Burns injury is followed by an intense catabolic state and it is possible that this early fall after burns injury is due to increased metabolism of this protein. Alternatively the fall may be due to extravasation at the site of injury.

The finding that burned patients with low serum testosterone concentrations had low levels of free testosterone is also novel. The relationships between free testosterone and derived free testosterone were similar in ill patients and normal controls. This suggests that the normal relationship between testosterone and its binding proteins holds true in this context despite a fall in serum albumin concentrations (mean 37.5 ± 1.7 v 45.0 ± 1.3 g/l in control group). Moll & Rosenfield (1979) found that changes in albumin concentrations within the normal range had little effect on testosterone binding and Dunn et al. (1981) calculated that it would require a reduction in serum albumin concentrations of around 65% for the free testosterone fraction to double.

The fall in total testosterone concentrations found following burns injury and probably in other pathological situations is likely to lead to a reduction in testosterone available to cells. The physiological consequences of this reduced tissue exposure to testosterone are uncertain.

CHAPTER 12

LH PULSATILITY STUDIES IN MEN WITH CHRONIC RENAL FAILURE

The pulsatile nature of LH secretion was studied in six healthy and six uraemic men. Only two of the latter but all six healthy men had evidence of LH pulses. This difference may contribute to the low testosterone levels found in uraemia and raised the possibility that disordered LH pulsatility might account for the low levels found in other clinical situations.

Introduction

In chapter 3 it was shown that a low serum testosterone concentration is a consistent feature in uraemic men irrespective of the nature of renal dialysis therapy but the mechanism leading to this is uncertain. Stimulation with human chorionic gonadotrophin (HCG) leads to an increase in serum testosterone concentrations in such men (Chen et al., 1970) but probably not to the same extent as in the healthy (Holdsworth et al., 1977). The usually elevated LH levels would point to a primary testicular defect although these levels are often less high than one would expect if this were the sole mechanism. So there may be an additional abnormality of the hypothalamic-pituitary system in the uraemic state.

LH secretion in normal man has an intermittent quality (Nankin & Troen, 1971; Naftolin et al., 1972) probably due to the pulsatile release of LHRH by the hypothalamus (Carmel et al., 1976). If this varying pattern of LH secretion is abolished by the administration of LHRH agonists, serum testosterone concentrations fall to the range seen in castrated men even though absolute LH concentrations may remain within the normal range (Faure et al., 1982; St Arnaud et al., 1986). It seemed possible that uraemic men might lack normal LH pulsatility leading to less effective stimulation of testosterone secretion.

It was decided therefore to investigate the mode of LH secretion in uraemic men to discover whether levels are constant or pulsatile in nature.

Methods

Six uraemic men (age range 20-49 mean 34 years) who had been on dialysis (HD - 5, CAPD - 1) for 1-10 years and six age-matched healthy men were studied. In addition a 20-year old man known to have Klinefelter's syndrome but otherwise healthy was investigated to obtain an example of the LH pulse profile associated with a pure primary testicular defect.

Statistical analyses were performed using the Wilcoxon Rank test for unpaired data.

Methods - Pulsatility Studies

At 0900 hours on the day of study an indwelling venous cannula was inserted in the antecubital fossa and kept patent with 0.5 ml heparin solution (10 U/ml). 5 mls of blood were withdrawn every 15 minutes for six hours. Serum was stored at -20°C prior to assay and all samples from the same individual were assayed in the same assay batch.

Pulse analysis was performed by a modification of the method of Santen and Bardin (1973). These authors defined a significant pulse as an increment from nadir to peak greater than 20 percent. Their sampling interval was 20 minutes but it has since been demonstrated that more pulses are detected if this is shortened (Crowley et al., 1985; Veldhuis et al., 1986). An interval of 15 minutes was chosen in order not to subject patients to the additional blood loss of more frequent sampling. To minimise the possibility of random changes in LH concentrations being labelled as a secretory pulse a significant peak had to include two points each with a nadir-to-peak increment of at least 20 percent.

Results from each subject are expressed as the mean of all LH levels obtained, the number of pulses during each six hour study period, the inter-pulse interval denoting the time in minutes between pulses and the mean amplitude of each subject's pulses. The amplitude was calculated by subtracting values from the preceding nadir from the mean of the two values constituting a peak, expressed both as a percentage and an absolute increment.

Results (Table 11)

The serum testosterone concentrations of the uraemic patients were less than those of the controls (13.1 ± 2.1 v 24.4 ± 4.4 nmol/l) although with the small numbers involved this did not reach statistical significance.

The mean LH level of all samples from each individual was greater in the uraemic subjects than controls ($p<0.005$). The patient with Klinefelter's syndrome had similar raised values. LH pulsatility was demonstrated in all normal subjects but in only two of the six uraemic patients ($p<0.02$). The patient with Klinefelter's syndrome had LH pulses of greater amplitude than those of either groups.

The LH pulse profile of a normal man, a uraemic patient and the patient with Klinefelter's syndrome are represented in Figure 17.

Discussion

The normal controls had LH levels which were not constant but variable with peaks every 90-120 minutes. The patient with Klinefelter's syndrome also had LH pulses which were of greater amplitude than those of either normal or uraemic subjects, a finding consistent with the results of Winters and Troen (1983).

Uraemic patients appear to have less LH pulsatility than normal and certainly do not have the exaggerated pulsatility seen in patients with

TABLE 11 LH pulsatility in uraemic and normal men and a patient with Klinefelter's syndrome. Values for LH, inter-pulse interval and amplitude are the mean for each pulse series. The testosterone value refers to a single estimation from the first sample taken during the pulsatility study.

	TESTOSTERONE (nmol/l)	*MEAN LH (U/l)*	*PULSES per 6 hrs*	*INTER-PULSE INTERVAL (mins)*	*AMPLITUDE (%)*	*AMPLITUDE (U/l)*
Control subjects						
	11.6	4.1	3	95	51	1.8
	14.6	3.7	3	140	42	1.3
	37.8	4.0	3	85	72	2.7
	30.0	6.1	4	55	51	2.6
	33.0	3.4	3	95	85	2.0
	19.2	4.2	3	125	50	1.6
mean	24.4	4.3	3.2	99	58	2.0
Uraemic subjects						
	12.6	17.3	3	110	39	5.7
	17.8	6.4	2	165	29	2.0
	4.9	13.5	0	--	--	--
	19.4	13.6	0	--	--	--
	11.8	9.6	0	--	--	--
	12.3	18.4	0	--	--	--
mean	13.1	13.1 ($p<0.005$)	0.8 ($p<0.02$)	142	34	3.8
Klinefelter's						
	10.2	17.3	2	120	110	13.0

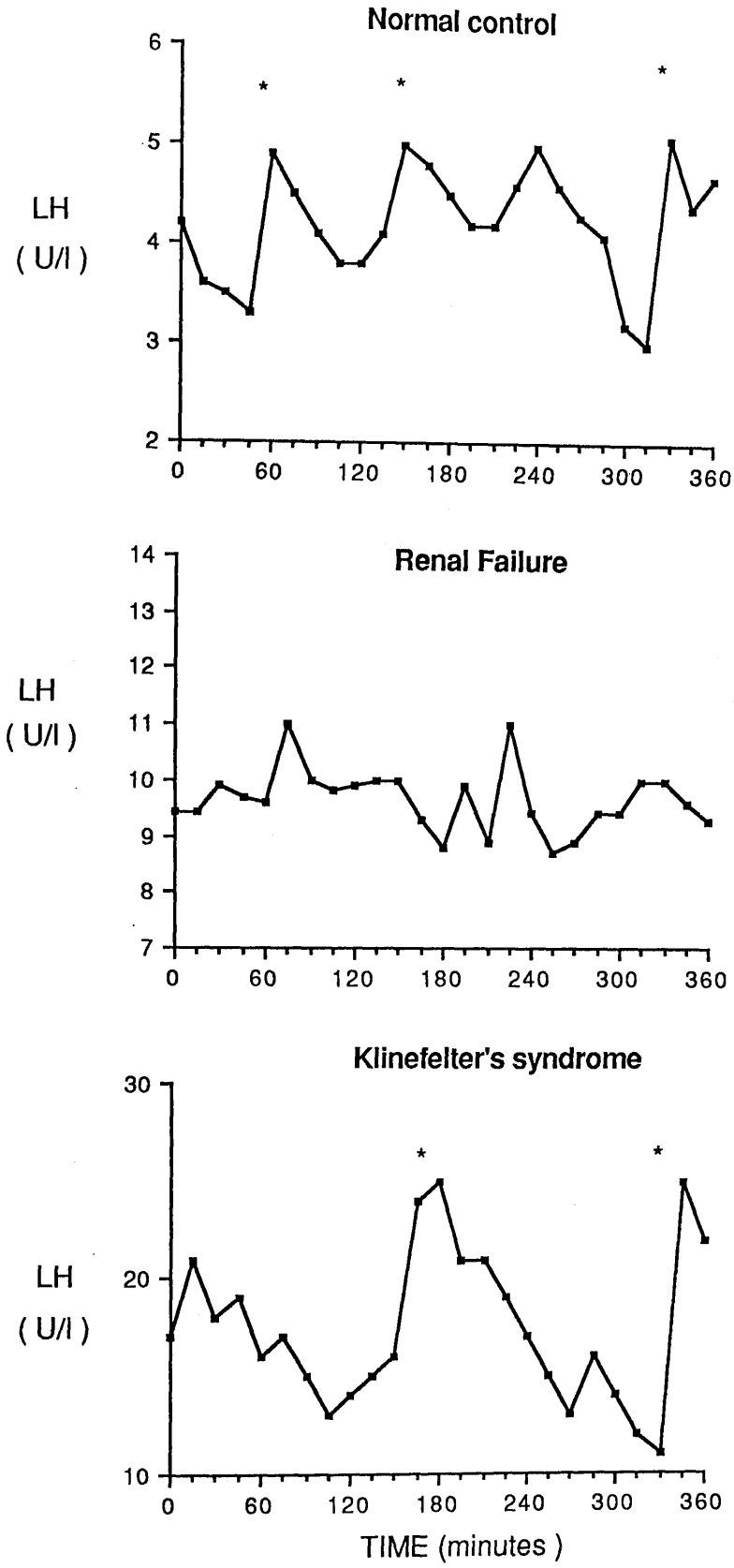


Figure 17 LH pulse profiles of three subjects:- a normal man, a patient with chronic renal failure and a patient with Klinefelter's syndrome. Asterisks denote LH pulses.

pure primary testicular failure. These results suggest the presence of a hypothalamic-pituitary abnormality. Other groups have investigated this subject. Rodger et al. (1985) published their study whilst my own was in its final stages. Their findings were essentially very similar. They compared six uraemic and six normal men finding 3.0 pulses per six hours in the former and 0.25 pulses per six hours in the latter with only one of the six patients having detectable pulses. They also demonstrated normal LH pulsatility in renal transplant recipients whose gonadal function is known to be superior to that of uraemic men (Lim & Fang, 1975). Wheatley et al. (1987) found that seven of ten of their uraemic patients had pulses but with a mean frequency of only 1.0 in seven hours as compared with 3.3 in normal men.

It is appropriate at this stage to insert a note of caution in the interpretation of these findings. The reduced LH pulsatility of uraemic patients may not necessarily be due to impaired hypothalamic release of LHRH. LH metabolism is known to be inhibited in chronic renal failure (Holdsworth et al., 1977) and this might lead to elevated LH levels swamping LH secretory surges of normal amplitude. Whatever the mechanism the reduction of LH pulsatility may lead to loss of effective stimulation of testosterone secretion. Although intermittent release of LHRH seems to be critical for normal hypothalamic-pituitary-testicular function, it is less certain that LH secretion requires to be pulsatile in order to stimulate testosterone secretion. Twice weekly intramuscular administration of LH in the form of HCG while effectively stimulating this secretion is likely to lead to constant rather than pulsatile LH concentrations. Thus although the constancy of LH concentrations of uraemic men may be a marker of a hypothalamic abnormality it is not necessarily the sole explanation for the hypogonadism seen in these patients.

The finding of altered LH pulsatility in these patients raised the possibility that hypothalamic-pituitary dysfunction might contribute to the low testosterone levels associated with other types of illness.

The following chapter describes my more detailed observations on hypothalamic-pituitary-testicular function of burned men including an assessment of LH pulsatility similar to that detailed in this chapter.

CHAPTER 13

AN INVESTIGATION OF THE MECHANISMS LEADING TO LOW SERUM TESTOSTERONE CONCENTRATIONS IN BURNED MEN

Five of six burned men showed a normal response of serum testosterone to HCG stimulation. Five had a normal LH response to LHRH with one having an exaggerated response. Three showed an impaired FSH response to LHRH. LH pulsatility was variable with two patients having no pulses. LH biological activity of burned men with low serum testosterone concentrations was less than that of healthy men and of the same patients when testosterone levels had returned to normal. Changes in LH pulsatility and biological activity may be factors leading to low testosterone concentrations.

Introduction

A low serum testosterone concentration seems to be an inevitable consequence of a clinically significant burn (Chapter 6). This could not be explained on the basis of an alteration in the levels of LH, changes in SHBG capacity nor by hyperprolactinaemia. Burns injury provides a suitable model for study of the mechanisms leading to the low testosterone state associated with illness because the abnormality is so profound and persistent. In addition because those patients with relatively minor burns are thus affected it is possible to study patients who are ambulant, otherwise healthy and who have good venous access.

The finding of reduced LH pulsatility in uraemic subjects (Chapter 12) raised the possibility that altered hypothalamic-pituitary function might contribute to the low serum testosterone levels found in patients with chronic renal failure and possibly other illnesses. Lack of pulsatility of LH levels in uraemic patients might be related to altered metabolism of LH in this condition (Holdsworth et al., 1977) rather than to hypothalamic-pituitary dysfunction. I therefore decided to investigate

in detail the hypothalamic-pituitary-testicular axis of burned patients with normal renal function.

Methods

During the second week following burns injury seven men underwent studies of LH pulsatility. Prior to pulsatility testing all had been found to have a subnormal serum testosterone level. To assess pituitary responsiveness to appropriate stimulation LHRH testing (as decribed in Chapter 2) was performed on completion of the pulsatility study. Thereafter an HCG stimulation test was carried out (as decribed in Chapter 2). One patient declined to undergo the LHRH and HCG tests. Although all patients studied had a low serum testosterone level at some stage after burns injury, one patient had a level within the normal range at the time of study. The age, surface area of burn, the minimum serum testosterone and the serum testosterone (testo) concentrations on the day of the pulsatility study are illustrated as follows:-

AGE (years)	SURFACE AREA	TESTO (day after burn) (nmol/l)	TESTO (day of study) (nmol/l)
20	20%	4.5 (day 3)	12.6 (day 14)
51	19%	0.5 (day 1)	2.3 (day 7)
60	4%	2.6 (day 14)	2.6 (day 14)
25	10%	5.1 (day 2)	8.2 (day 10)
30	20%	1.7 (day 7)	1.7 (day 7)
36	14%	1.0 (day 7)	1.0 (day 7)
33	15%	2.0 (day 3)	4.5 (day 10)

To analyse the biological potency of LH eight burned men were studied and compared with eight age matched healthy men. Sera were

available at a time when serum testosterone levels were low and later when these had recovered towards the normal range.

Results

The data from the HCG stimulation tests are shown graphically in Figure 18. A normal capacity of the testes to secrete testosterone in men with low serum testosterone concentrations is usually accepted when levels double and reach the normal range by day 5 (Anderson et al., 1972). Five patients showed a normal response while one showed no response whatsoever.

LHRH stimulation tests are shown in Figure 19. The shaded area demonstrates the the range of responses found in ten healthy men (Semple et al., 1982d). Five patients showed a normal LH response to LHRH with one showing an exaggerated response. This patient had a serum testosterone concentration of 1.0 nmol/l and a very low mean LH of 1.0 U/l through the pulsatility study. Three patients had a reduced FSH response.

An analysis of LH pulsatility is shown in Table 12. Six of the seven burned patients had very low serum testosterone concentrations at this stage of the investigation and these are considered together. The remaining patient had a level within the normal range at the time of study although a previously measured serum testosterone concentration had been unequivocally low (4.5 nmol/l). As this patient seemed to have entered a recovery phase his results were considered separately. The six with low serum testosterone levels had significantly fewer pulses than normal controls ($p<0.02$) although they were similar with regard to mean LH levels. Pulses when present were similar in character to those of the controls. The patient whose serum testosterone level had already recovered had a normal pulse frequency but increased amplitude. The LH pulse profiles of a normal man, a burned patient with a low serum

TABLE 12 LH pulsatility in burned and normal men. Values for LH, inter-pulse interval and amplitude are the mean for each pulse series. The testosterone value refers to a single estimation from the first sample taken during the pulsatility study.

	TESTOSTERONE (nmol/l)	*LH (U/l)*	*PULSES per 6 hours*	*INTER-PULSE INTERVAL (mins)*	*AMPLITUDE (%)*	*(U/l)*
Control subjects						
	11.6	4.1	3	95	51	1.8
	14.6	3.7	3	140	42	1.3
	37.8	4.0	3	85	72	2.7
	30.0	6.1	4	55	51	2.6
	33.0	3.4	3	95	85	2.0
	19.2	4.2	3	125	50	1.6
mean	24.4	4.3	3.2	99	58	2.0
Burned subjects						
	2.6	4.1	0	--	--	--
	2.3	5.8	3	95	39	1.9
	1.7	5.1	2	100	72	2.7
	8.2	5.5	1	--	58	2.9
	4.5	3.8	1	--	250	4.0
	1.0	1.0	0	--	--	--
mean	3.4 ($p<0.005$)	4.2	1.2 ($p<0.02$)	98	105	2.9
Burned subject recovery phase						
	12.6	12.1	3	90	82	7.6

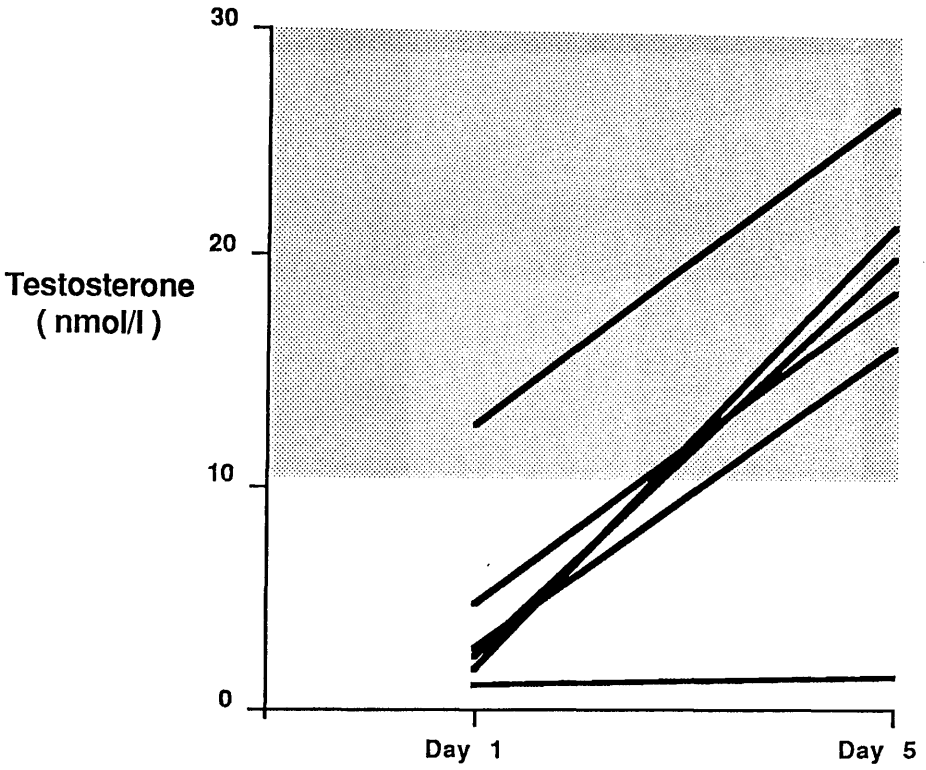


Figure 18 Serum testosterone responses of six burned men to HCG stimulation. The shaded area represents the range of serum testosterone concentrations of 19 healthy men.

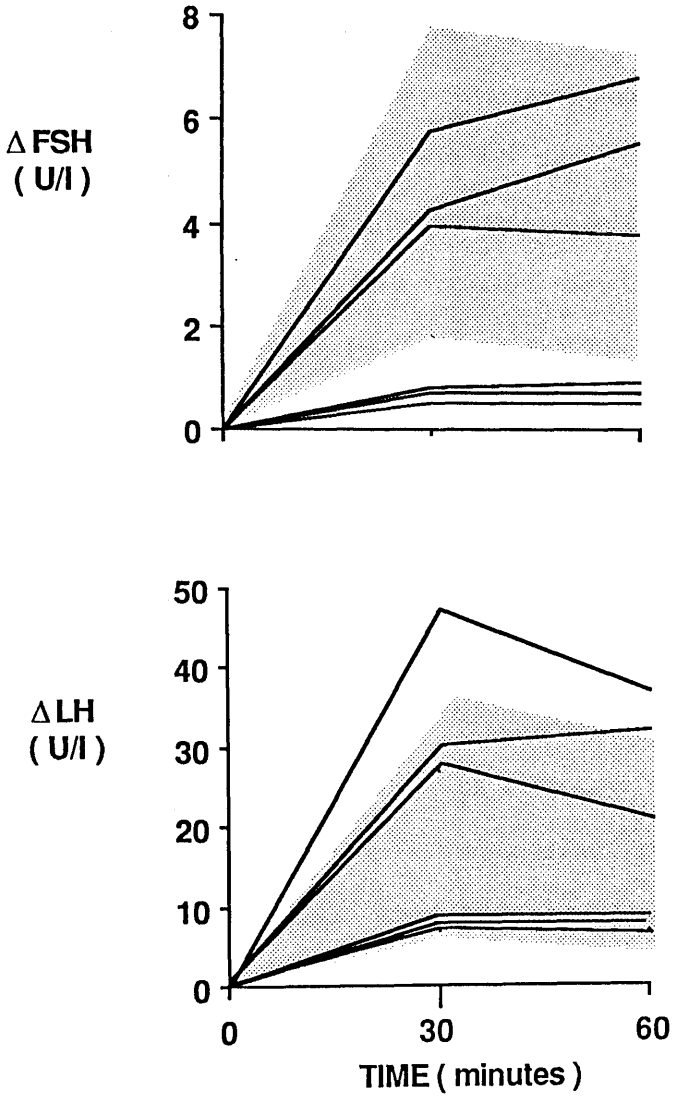


Figure 19 Serum FSH and LH responses of six burned men to the intravenous injection of LHRH expressed as an increment over basal values (Δ). The shaded area represents the range of responses found in 10 healthy men.

testosterone level and the burned patient who had entered a recovery phase are shown in Figure 20.

LH bioactivity data are shown in Figure 21. The serum testosterone concentrations of these eight patients increased from 2.4 ± 1.0 nmol/l to 15.6 ± 2.1 nmol/l. LH measured by radioimmunoassay (LH-RIA) showed no significant change (6.2 ± 1.3 v 6.4 ± 1.3 U/l). LH measured by bioassay (LH-BIO) increased but not in a statistically significant manner (17.3 ± 2.7 v 23.1 ± 3.3 U/l). It is widely accepted that LH biological activity is more appropriately expressed in terms of the B:I ratio (LH-BIO ÷ LH-RIA) (Warner et al., 1985; St-Arnaud et al., 1986). The B:I ratio was significantly lower in the burned patients with low serum testosterone concentrations (3.2 ± 0.6) than in the control group (4.4 ± 0.5, $p<0.005$) or indeed in the same patients following recovery (4.2 ± 1.0, $p<0.02$).

Discussion

Five of six patients showed brisk responses to HCG suggesting that the testes in this situation are able to react normally to appropriate stimuli. Dolecek et al. (1979a) found that some patients with particularly severe burns failed to respond to HCG. The complete absence of a response in one subject is difficult to explain as this patient was not exceptional with regard to the severity of his burn (14%).

There was a normal LH response to LHRH in most patients suggesting a satisfactory secretory reserve of LH. It is interesting that the single patient with the exaggerated response had a very low level of LH before LHRH stimulation. This raises the possibility that LH may be synthesised and stored but not released in some burned patients reminiscent of the situation in men following head injury (Clark et al., 1988). Basal FSH levels had been shown to be depressed in Chapter 6. Three patients showed subnormal FSH responses to LHRH. This may be of relevance to the disruption of the germinal epithelium described by Dolecek et al. (1983)

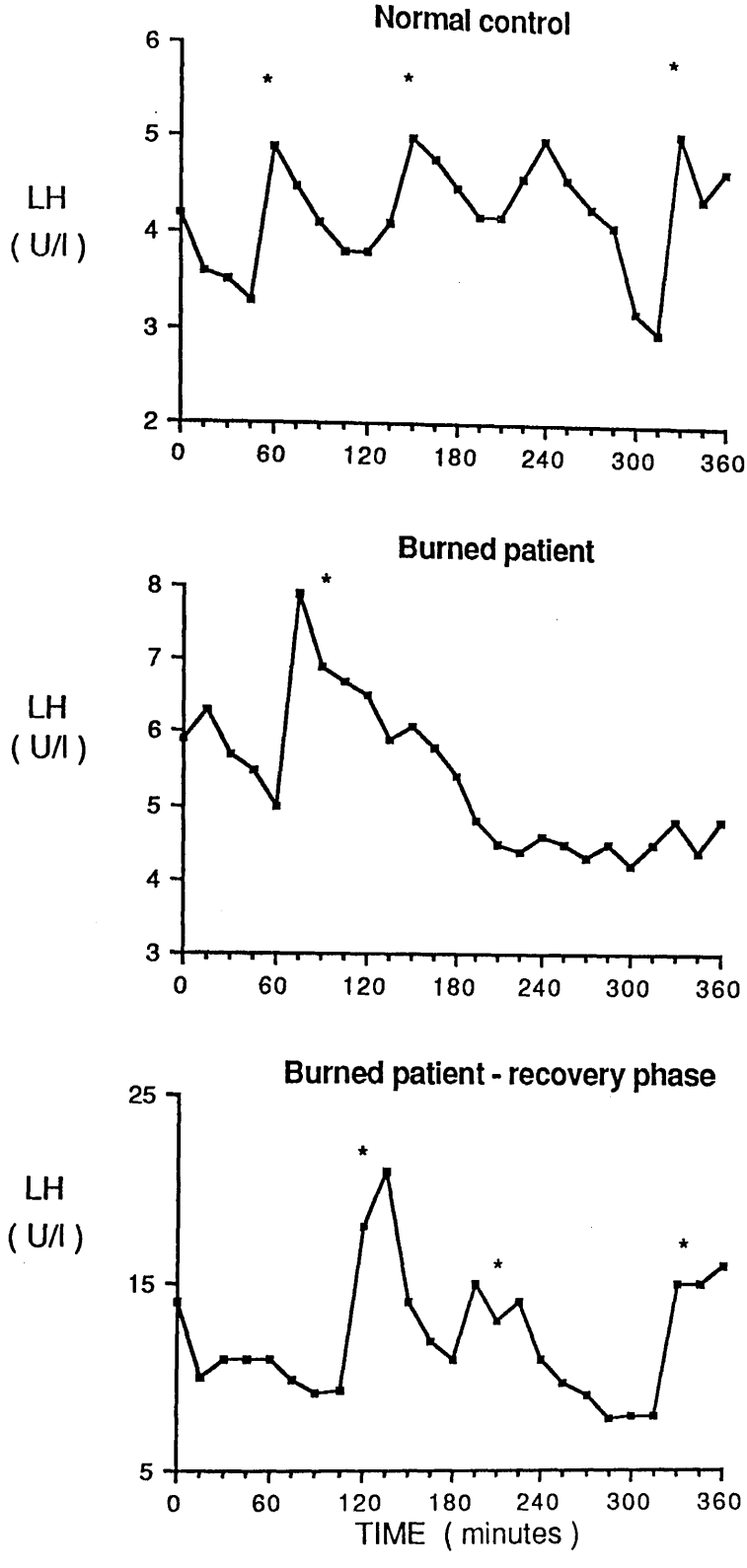


Figure 20 LH pulse profiles of three subjects:- a normal man, a burned patient with a low serum testosterone concentration (2 nmol/l) and a burned man whose serum testosterone concentration had returned to within the normal range. Asterisks denote LH pulses.

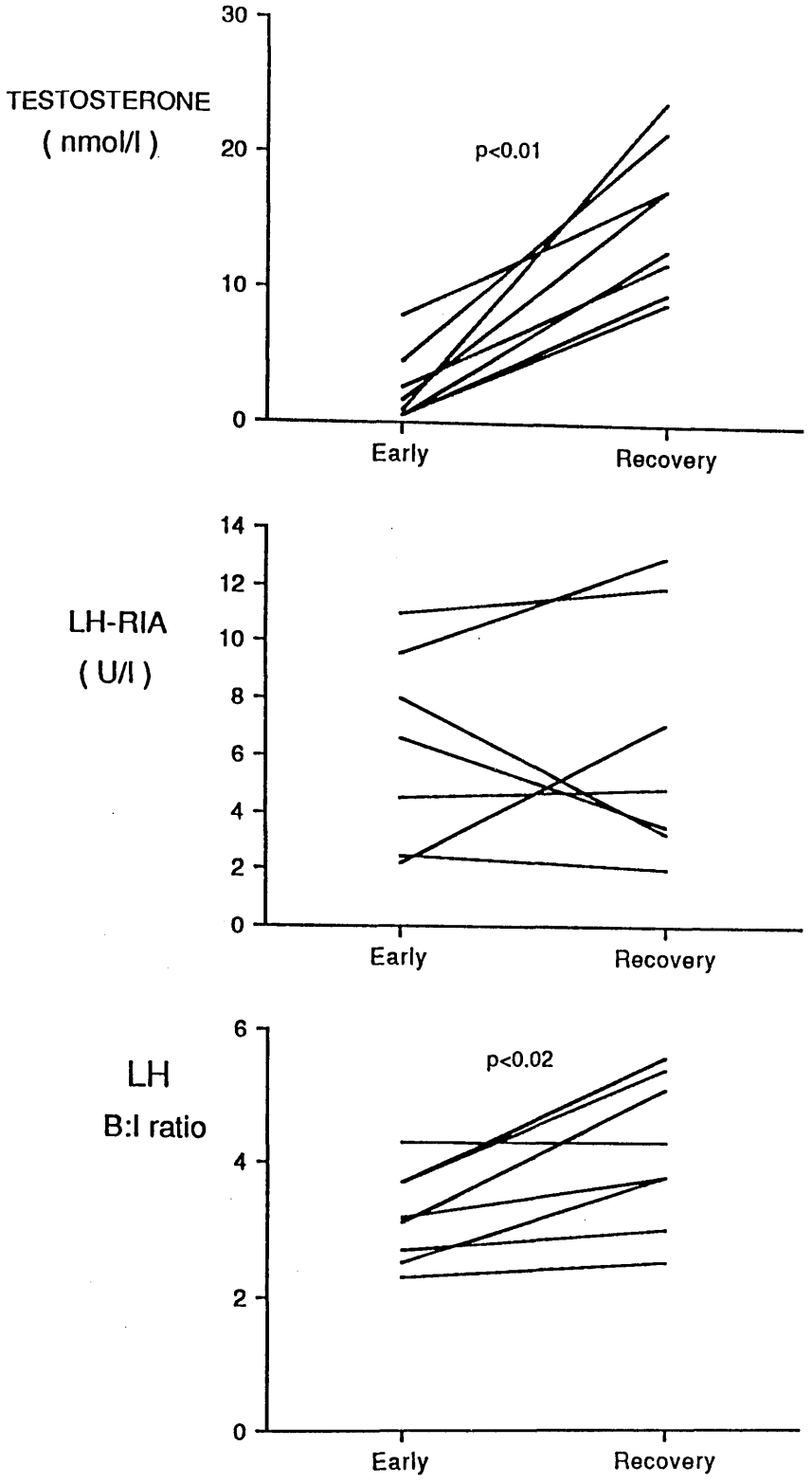


Figure 21 Serum testosterone concentrations, LH concentrations by radioimmunoassay (LH-RIA) and LH biological activity (B:I ratio) of eight men during the early and late weeks following burns injury.

in post-mortem specimens of severely burned men. In vitro work using cultured porcine Leydig cells has suggested that FSH may stimulate the Sertoli cells of the testis to secrete proteins which enhance the action of LH (Benahmed et al., 1985). The low FSH levels found in burned men could also contribute to the inhibition of testosterone secretion.

The literature on LH pulses in illness is scanty. Warren et al., 1977) failed to observe any variation in LH concentrations during serial LH sampling of two ill post-menopausal women. Reduced LH pulsatility has been described in conditions such as hyperprolactinaemia (Winters & Troen, 1984) and amenorrhoea related to weight loss (Reame et al., 1985) but not in non-endocrine disorders until recent work demonstrating reduced pulse frequency in men with chronic renal failure (Rodger et al., 1985; Wheatley et al., 1987) and liver disease (Bannister et al., 1986). Neither of these latter conditions are ideal models for the study of gonadal function in non-endocrine disorders because both result in altered sex hormone metabolism (Holdsworth et al., 1977; Gordon et al., 1975).

LH levels of burned patients with low serum testosterone concentrations were less pulsatile than LH levels of controls. When these data were published (Semple et al., 1987c), they were the first description of LH pulsatility in non-endocrine disease other than renal and kidney failure. Some readers objected to my interpretation of the data because of the exclusion of the patient whose serum testosterone concentration had risen to within the normal range at the time of the pulsatility study. If all seven patients were compared with the control group the difference in LH pulsatility would have failed to reach statistical significance. I considered that my analysis of the data was justified because the initial aim of the study was to examine LH pulsatility in burned men with low serum testosterone levels and I therefore considered it acceptable to exclude from analysis the patient

with the normal serum testosterone concentration. What is certainly true is that some burned patients have absent LH pulsatility although some may pulse normally. It may be that soon after burns injury these patients all pass through a stage of reduced LH pulsatility which after a variable time interval gradually recovers. The heterogeneous nature of these results may merely reflect the stage at which sampling occurs.

The realisation that the detection of LH in serum by radio-immunoassay is not synonymous with biological activity is relatively recent (Dufau et al., 1983). B:I ratios are higher during an LH peak than a nadir (Dufau et al., 1983; Veldhuis et al., 1983). Warner, Dufau & Santen (1985) found a reduced B:I ratio in 20 ill men who had LH and testosterone levels similar to a control group. Although there was a reduced LH B:I ratio in burned men in my own study, this change was small in comparison with the fall in serum testosterone concentrations. It is not clear what a modest reduction in LH bioactivity, as measured using mice Leydig cells in vitro, means in the human subject. Thus while it is premature to suggest that low biological potency of LH is a major factor leading to low serum testosterone levels, it is tempting to assume that it may at least contribute.

Burns injury provides a suitable model for the study of endocrine changes following non-endocrine illness. The available evidence points to an abnormality at the level of the hypothalamus and/or pituitary leading to secretion of LH of reduced biological potency and with reduced pulsatility.

The study which follows describes a more detailed investigation of LH biological activity and pulsatility and their relationship to testosterone levels in burned men.

CHAPTER 14

A FURTHER INVESTIGATION OF LH PULSATILITY IN BURNED MEN

Further pulsatility studies using a 10 minute sampling interval were performed in six burned patients and compared with data from 11 normal men. All samples were assayed for LH-BIO, LH-RIA and testosterone. There was no significant difference in frequency of bioactive or immunoreactive pulses although one burned patient had no pulses. In normal men the B:I ratios were higher at a pulse peak than at the nadir and an increase in serum testosterone levels usually followed an LH peak. In burned men B:I ratios were lower at a pulse peak than a nadir and an increase in the serum testosterone concentration did not usually follow an LH peak.

Introduction

When my paper entitled "Mechanisms leading to hypogonadism in burned men" was published in 1987 (Semple et al., 1987c), it was the first such study in burned men and indeed was the first paper to include an assessment of both LH pulsatility and biological activity in ill men. B:I ratios are higher during an LH peak than during an inter-pulse interval in normal subjects (Dufau et al., 1983; Veldhuis et al., 1983) and the low B:I ratio described in burned patients with low serum testosterone concentrations in Chapter 13 might merely reflect reduced LH pulsatility. I therefore decided to investigate LH levels by both radioimmunoassay (LH-RIA) and bioassay (LH-BIO) through the whole sampling period.

Lincoln (1977) demonstrated a temporal association between LH and testosterone peaks. More recently Veldhuis et al. (1987) demonstrated testosterone pulses in men at a mean frequency of 3.3/6 hours with a mean amplitude of 8.4 nmol/l. Testosterone pulses correlated with LH pulses with a time lag of between 50 and 70 minutes. Testosterone levels were therefore measured through pulse profiles to investigate whether

testosterone peaks were present and whether they had any temporal relationship to LH peaks.

Assessment of the increasing literature on methods of pulse detection led me to question some of my earlier methodology. Veldhuis et al. (1983) in their earlier studies had used a sampling interval of 20 minutes but in their later work demonstrated that pulses were increasingly likely to be missed as the sampling interval increased beyond five minutes (Veldhuis et al., 1986). The sampling interval was therefore decreased to 10 minutes as a compromise between the ideal study design and patients' comfort and health. Over the last few years a perplexing array of methods for pulse analysis have been developed (Crowley et al., 1985). Manual methods compare favourably with complex computerised systems and have the advantage that the presence or absence of a pulse can be checked by visual inspection (Reame et al., 1984). With the increasing trend in the literature to more precise methods of pulse analysis, a further modification (Tsatsoulis et al., 1989) of the method of Santen & Bardin (1973) was employed and is described in the Methods section.

Methods - Subjects

The characteristics of the burned men are described as follows:-

Age (years)	Surface area	mean serum testosterone during sampling period (nmol/l)	day after burn
31	10%	7.4	day 19
25	15%	12.9	day 14
29	23%	4.3	day 10
24	60%	1.5	day 25
21	18%	7.4	day 23
21	20%	6.6	day 25

11 normal men (age range, 20-40 years, mean age 27 years) were studied. As mentioned in the Declaration, studies on normal men were not carried out by the author in person. Values were derived from Talbot et al. (in press). All samples were, however, treated in the same manner and all assays were carried out in the same laboratory.

Methods - Blood Sampling and Assays

Pulsatility studies were carried out on six burned men and 11 normal controls using a six hour sampling period between 1400 and 2200 hours. When this study was being performed there was some doubt about the stability of LH as measured by bioassay although it has since been demonstrated to be unaffected by temperature changes or by being left at room temperature for several hours (Lambert et al., 1986). Samples were collected as described in Chapter 12 except that blood was withdrawn every 10 minutes and transferred into Lithium Heparin tubes on ice and centrifuged with plasma being snap frozen and stored at -20°C until hormone assay.

All samples were assayed in duplicate for LH-RIA, LH-BIO and testosterone so that a mean coefficient of variation could be calculated for each set of samples from each patient. All samples from a subject were assayed in the same assay.

The LH bioassay is described in Chapter 2. Although the radio-immunoassays of testosterone and LH were carried out in a different laboratory, they were essentially the same as those described in Chapter 2. (The LH tracer was obtained from the Chelsea Hospital for Women. The tracer used in the testosterone assay was testosterone-3-O-(carboxymethyl)-histamine-^{125}I [Amersham]). The intra-assay and inter-assay coefficients of variation for LH were 5.3% and 6.1% respectively. The intra-assay and inter-assay coefficients of variation for the testosterone assay were 3.8% and 7.1% respectively.

Methods - Pulse Definition

A pulse was detected if there were at least two points greater than the preceding nadir by at least three times the mean intra-assay coefficient of variation for the samples in that pulse profile. Each peak also had to have a descending limb (Tsatsoulis et al., 1989). A testosterone peak was defined in a similar manner with the additional criterion of a minimum increment from nadir to peak of ≥2nmol/l.

Results

Serum testosterone concentrations of the burned patients were lower than controls (6.7 ± 1.5 v 19.6 ± 2.4 nmol/l, $p<0.01$). Mean LH-BIO levels were 12.5 ± 1.7 U/l in burned patients as compared with 11.9 ± 1.3 U/l in the controls. Mean LH-RIA levels were 6.3 ± 0.9 U/l compared with 4.7 ± 0.5 U/l (NS). LH-RIA levels were lower than LH-BIO in all samples.

There was no significant difference in LH pulsatility between the two groups. Examples of pulse profiles of normal men are shown in Figures 22 and 23 and of burned men in Figures 24, 25 and 26. LH secretion was pulsatile in all normal subjects with 23 bioactive (mean 2.1/6 hours) and 19 immunoreactive pulses (mean 1.7/6 hours) detected. In burned men there were 13 bioactive (mean 2.2/6 hours) and 12 immunoreactive pulses (mean 2.0/6 hours). One patient showed no pulsatility. All immunoreactive pulses were synchronous with bioactive pulses. Frequency, amplitude, fractional amplitude and interpulse intervals of bioactive and immunoreactive pulses were similar in both groups and are shown in Tables 13 and 14.

The mean B:I ratio was greater ($p<0.01$) in normal subjects (2.6 ± 0.2) than in burned patients (1.9 ± 0.1). In normal subjects the B:I ratios were higher ($p<0.01$) at pulse peaks than at the nadirs (2.9 ± 0.2 v 2.2 ± 0.1). In contrast B:I ratios of burned men were lower ($p<0.01$) at pulse peaks than at the nadirs (1.8 ± 0.1 v 2.0 ± 0.1). The changes in

TABLE 13 Characteristics of bioactive LH (LH-BIO) pulses. Values for testosterone, LH-BIO, inter-pulse interval and amplitude are the mean for each pulse series.

	TESTOSTERONE (nmol/l)	*LH-BIO (U/l)*	*PULSES per 6 hrs*	*INTER-PULSE INTERVAL (mins)*	*AMPLITUDE (%)*	*(U/l)*
Control subjects						
	16.3	8.9	1	--	142	8.6
	16.1	21.3	3	60	88	14.4
	10.9	11.1	3	105	71	5.6
	11.5	13.5	3	135	86	8.2
	10.9	7.1	2	170	123	11.8
	14.3	6.8	1	--	116	5.0
	23.9	9.7	2	190	45	3.6
	25.9	11.0	1	--	48	4.2
	35.5	19.3	2	140	67	10.0
	23.9	12.9	2	160	95	9.6
	25.9	15.3	3	120	68	8.4
mean	19.6	12.5	2.1	135	86	8.1
Burned subjects						
	4.3	10.4	2	200	33	3.1
	12.9	16.6	1	--	62	9.2
	1.5	11.4	4	80	35	3.4
	7.4	18.0	4	90	84	10.6
	6.7	8.3	2	80	101	6.3
	7.4	6.5	0	--	--	---
mean	6.7 ($p<0.01$)	11.9	2.2	112	63	6.5

TABLE 14 Characteristics of immunoreactive LH (LH-RIA) pulses. Values for testosterone, LH-RIA, inter-pulse interval and amplitude are the mean for each pulse series.

	TESTOSTERONE (*nmol/l*)	*LH-RIA* (*U/l*)	*PULSES* *per 6 hrs*	*INTER-PULSE INTERVAL* (mins)	*AMPLITUDE* (%)	(U/l)
Control subjects						
	16.3	3.2	1	--	135	3.7
	16.1	4.9	2	150	78	5.5
	10.9	3.4	2	100	153	3.5
	11.5	5.5	2	200	119	6.3
	10.9	4.9	2	190	96	3.3
	14.3	2.8	1	--	98	2.0
	23.9	4.7	1	--	73	2.5
	25.9	4.6	1	--	38	1.5
	35.5	5.7	2	140	63	2.9
	23.9	5.6	2	160	81	3.5
	25.9	5.9	3	115	55	2.8
mean	19.6	4.7	1.7	150	90	3.4
Burned subjects						
	4.3	5.2	2	190	60	2.7
	12.9	9.3	1	--	78	7.1
	1.5	6.7	4	80	66	3.4
	7.4	8.2	4	100	95	5.1
	6.7	4.6	1	--	185	6.0
	7.4	3.5	0	--	--	---
mean	6.7 ($p<0.01$)	6.3	2.0	120	97	4.9

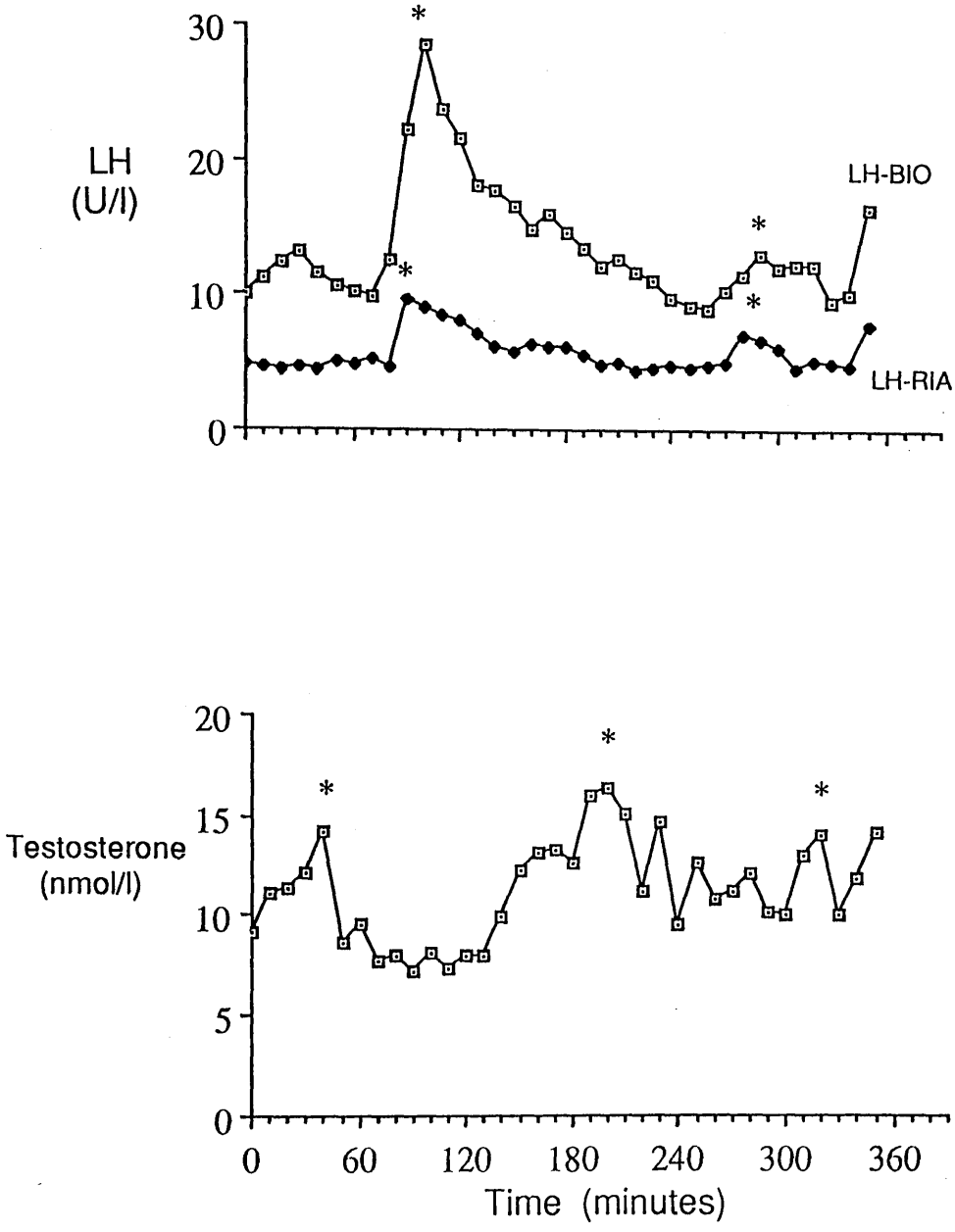


Figure 22 LH and testosterone pulse profiles of a normal man. LH-BIO denotes LH measured by bioassay and LH-RIA denotes LH measured by radioimmunoassay. Asterisks indicate pulses.

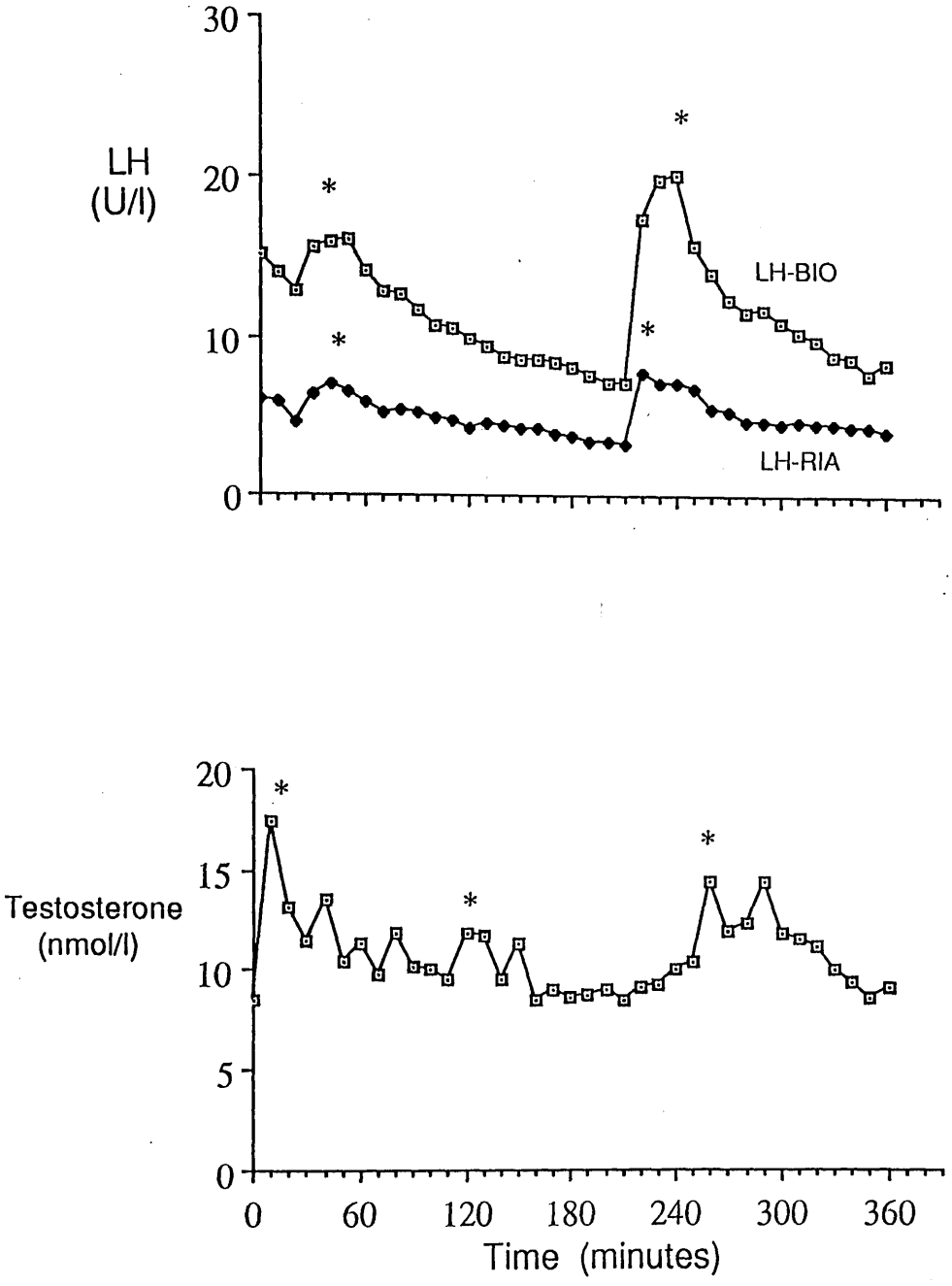


Figure 23 LH and testosterone pulse profiles of a normal man. LH-BIO denotes LH measured by bioassay and LH-RIA denotes LH measured by radioimmunoassay. Asterisks indicate pulses.

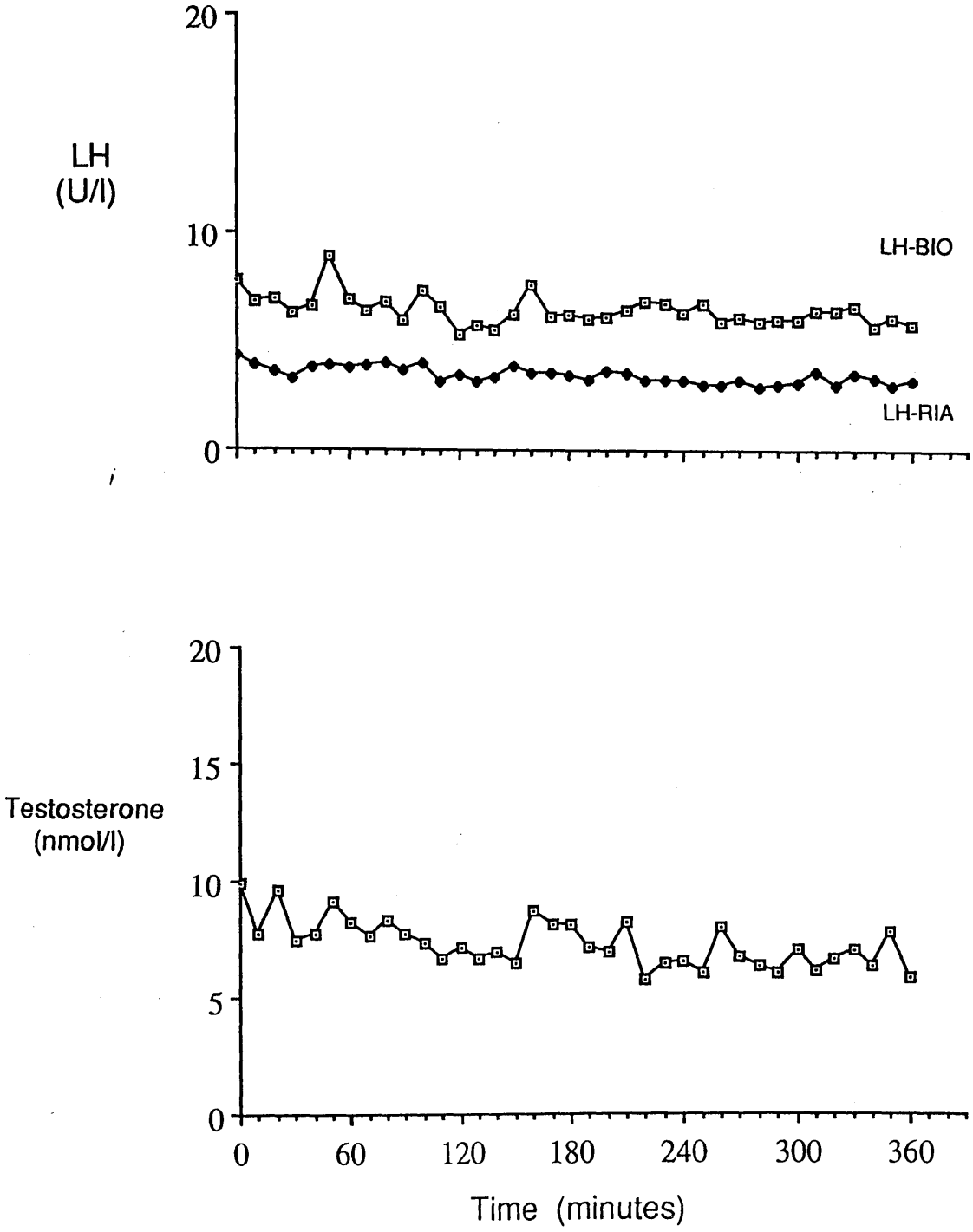


Figure 24 LH and testosterone pulse profiles of a burned man. LH-BIO denotes LH measured by bioassay and LH-RIA denotes LH measured by radioimmunoassay. Asterisks indicate pulses.

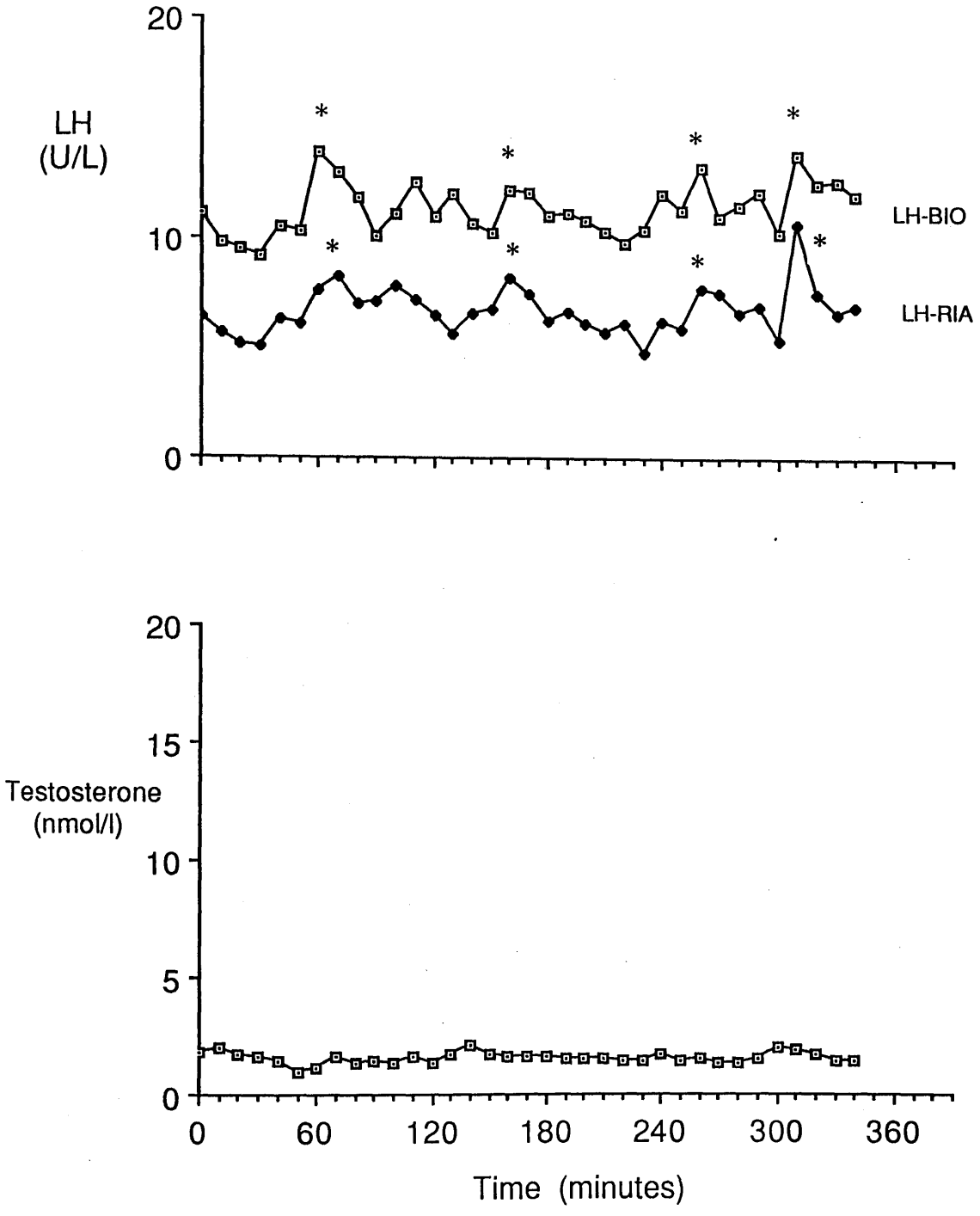


Figure 25 LH and testosterone pulse profiles of a burned man. LH-BIO denotes LH measured by bioassay and LH-RIA denotes LH measured by radioimmunoassay. Asterisks indicate pulses.

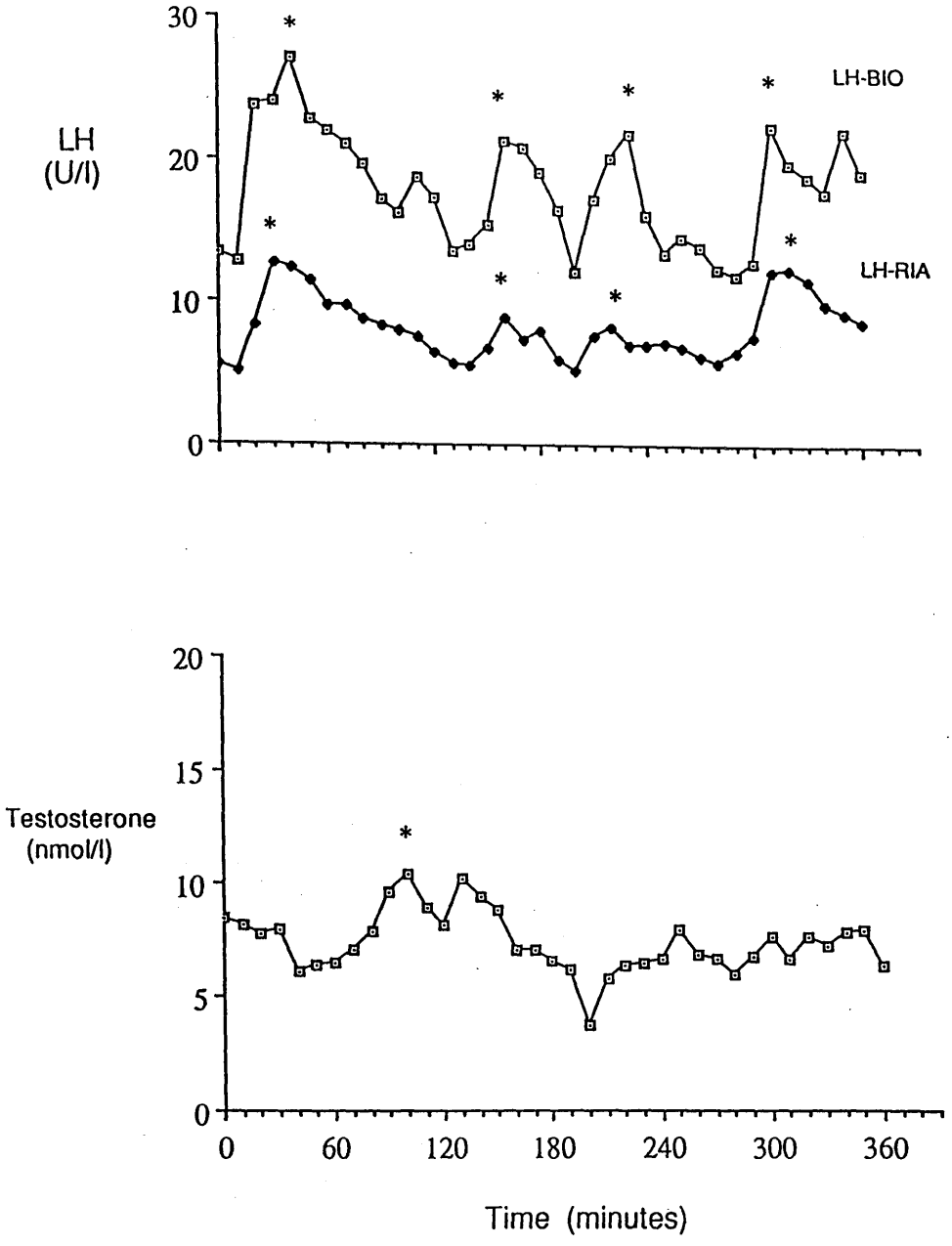


Figure 26 LH and testosterone pulse profiles of a burned man. LH-BIO denotes LH measured by bioassay and LH-RIA denotes LH measured by radioimmunoassay. Asterisks indicate pulses.

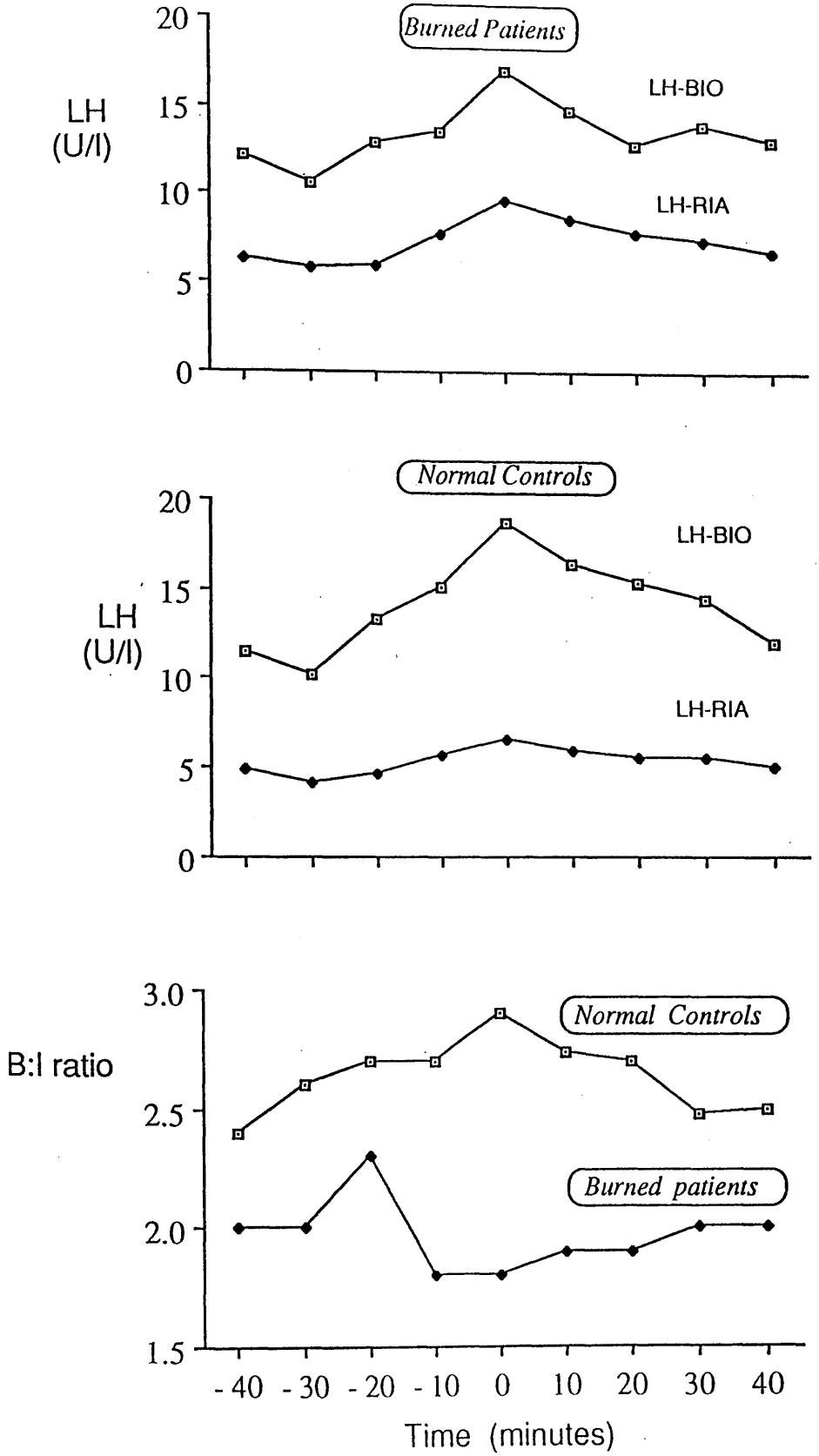


Figure 27 Mean bioactive LH (LH-BIO), immunoreactive LH (LH-RIA) and LH B:I ratios of burned patients and normal controls synchronised with time point zero given to the pulse peak.

mean LH-BIO, LH-RIA and B:I ratios through the pulses were synchronised with time point zero given to the pulse peak and are shown graphically in Figure 27. In normal men LH-BIO increased from 11.5 ± 2.3 U/l at -40 minutes to 18.7 ± 1.6 U/l at zero minutes; LH-RIA increased from 4.9 ± 1.0 U/l to 6.6 ± 0.5 U/l; and B:I ratios increased from 2.4 ± 0.1 to 2.9 ±0.2 . In burned men LH-BIO increased from 12.1 ± 1.1 U/l to 17.0 ± 1.8 U/l; LH-RIA increased from 6.3 ± 0.8 U/l to 9.7 ± 0.9 U/l; and B:I ratios decreased from 2.0 ± 0.1. to 1.8 ± 0.1

Testosterone pulses were seen in all normal subjects studied (range 2-3/6 hours). All but three were associated with an LH-BIO peak (mean time lag 40 minutes). The mean incremental amplitude was 6.1 nmol/l (range 3.1-9.3 nmol). Only two testosterone peaks were detected in the six burned men (incremental amplitude 4.3, 4.9 nmol/l) both being associated with an LH peak (time lag 40, 70 minutes). The relationship between LH and testosterone in normal men and in burned men is illustrated in Figures 22-26.

Discussion

This study is unprecedented in the literature as it has examined both LH-BIO and LH-RIA through pulse series in burned men. Previous authors have only studied LH-RIA in patients with either chronic renal failure (Rodger et al., 1985; Wheatley et al., 1987) or liver disease (Bannister et al., 1986). The low B:I ratio described in burned men in the previous chapter has been confirmed but has been shown not to be due to a reduction in LH pulse frequency.

The method for pulse analysis used in this study improves on that used in Chapters 12 and 13 in which an increment of 20% between the nadir and peak was required before assigning a pulse. This took no account of the precision of the assay used in each sampling series. The intra-assay coefficient of variation mentioned in Chapter 2 is given as

3.1% but this takes no account of changes in precision on a day to day basis. Measuring all samples in duplicate allowed an intra-assay coefficient of variation to be calculated for each sampling series. Three times the intra-assay coefficient of variation was used to signify a peak; this is a widely used parameter and should mean that the chances of detecting a true peak do not vary from day to day and assay to assay.

It was felt necessary to require an increment of ≥2 nmol/l in serum testosterone concentration in addition to the standard definition of three times the intra-assay CV before assigning a peak. This is rather arbitrary but was considered necessary because of the low serum testosterone levels in burned men. The mean CV of serum testosterone concentrations for all pulse series was 4.9% (range 3.6%-7.3%). Using the standard pulse definition without the ≥2 nmol/l stipulation would have resulted in an increase of serum testosterone concentration of ≤0.3 nmol/l being labelled a peak in one patient. The testosterone peaks detected in normal men all had an increment ≥2nmol/l (range 3.1-9.3 nmol/l) using the standard pulse definition.

In contrast to the study in Chapter 13 there was no significant difference in LH pulsatility between burned patients and normals. In both studies there was a heterogeneous pattern in burned patients with one or two patients in each group having absent pulsatility a finding not found in normal subjects. The study described in this chapter was carried out rather later (mean 19, range 10-25 days) after burn injury than the study in Chapter 13 (mean 10, range 7-14 days). The timing of LH pulsatility investigations had not appeared critical as the initial studies on burned patients in Chapter 6 had demonstrated suppression of serum testosterone concentrations for at least five weeks after burns injury.

Different patterns of LH and testosterone pulsatility were found in burned patients. A single patient showed no peaks of LH or testosterone (Figure 24). Three patients showed LH but not testosterone peaks (eg

Figure 25). Two patients showed LH peaks with a single testosterone peak (eg Figure 26). It is possible that there is a more consistent depression of LH pulsatility in the early days after injury with recovery occurring in a variable manner. This could only be clarified by sequential pulsatility studies which would not be compatible with patient care. More consistent findings might have been obtained if patients had been sampled within one week of injury but again considerations of patient care often require these studies to be delayed.

LH-BIO levels were consistently higher than LH-RIA levels in all subjects a finding in common with other authors (Dufau et al., 1983; Warner et al., 1985). The value for the LH B:I ratio in plasma has been found to vary from 0.7 to 7.0 depending on which reference preparation is employed as standard in the bioassay (Lichtenberg et al., 1983). The absolute value of LH B:I ratio is by itself of little significance but a change gives useful information if the same standard is employed throughout a study (Robertson et al., 1987) as was the case in this study and in those described in Chapters 12 and 13.

Pulse profiles of burned men differed from normal in two ways. The biological activity as expressed as the B:I ratio was lower at the pulse peak than at the nadir. The reverse was true in normals, in agreement with Dufau et al. (1983) and Veldhuis et al. (1983). LH pulses were usually followed by surges of testosterone in normal men but this only happened twice in burned men. These differences suggest that either the testes of burned men are refractory to stimulation by an LH secretory episode or that the LH secreted at the pulse peak is qualitatively abnormal and is unable to stimulate testosterone secretion. The fact that most burned men seem to respond normally to HCG might suggest that the testes are not refractory to stimulation. However this test uses pharmacological doses of HCG and and may not reflect normal physiology.

This study has confirmed the low B:I ratio of LH following burns injury. This is not due to reduced LH pulse frequency. LH secreted in a pulse in burned men has a lower B:I ratio than that secreted during an inter-pulse interval, a finding that contrasts with normal men. The LH thus secreted seems to be unable to promote testosterone secretion.

CHAPTER 15

CONCLUSIONS

This thesis has demonstrated that low serum levels of androgens of both testicular and adrenocortical origin are commonly found during illness. These changes seem to be specific to illness and cannot be attributed to associated psychological disturbance. Indeed the androgenic milieu associated with exercise and psychological stress showed few similarities to that associated with illness. It may be that tissue injury is a prerequisite for the profound changes in serum androgen levels described in various clinical situations in this thesis. Although Hans Selye (1946) considered the anterior pituitary gland to be central to "the general adaptation syndrome", many endocrine changes with illness are not readily explained by altered secretion of known pituitary hormones.

Hypothalamic-pituitary-testicular axis

While investigating this endocrine system abnormalities of hypothalamic-pituitary function were identified as possible important factors leading to low testosterone levels. What factors specific to illness might result in hypothalamic-pituitary dysfunction?

Hyperprolactinaemia may abolish the pulsatility of LH secretion (Winters & Troen, 1984) and cause male hypogonadism but the prolactin levels required are much higher (Carter et al., 1978) than those found in either ill patients or athletes.

Cortisol excess is known to have an adverse effect on this axis. Patients with Cushing's syndrome often have low testosterone concentrations but in association with low gonadotrophin levels (Luton et al., 1977). Acute administration of exogenous corticosteroid leads to a fall in serum testosterone levels without any change in serum LH levels

(Cumming et al., 1983). However long term administration of corticosteroid only depresses testosterone levels by around 40 percent (Reid et al., 1985). An adverse effect of raised corticosteroid activity on this axis is unlikely to account for the often prolonged and profound depression of serum testosterone concentrations in ill patients but may be responsible for the small reduction found in association with very high serum cortisol levels at the end of a marathon run.

Administration of an opiate antagonist such as naloxone to normal men results in enhanced LH pulsatility (Grossman et al., 1981) implying that endogenous opioid tone in normal man suppresses LH pulsatility. Increased levels of endogenous opioid peptides which have been reported with exercise (Colt et al., 1981; Farrell et al., 1982) might lead to reduced LH pulsatility thus contributing to the low serum testosterone levels found following prolonged exertion. However the available evidence suggests that LH pulsatility is normal in this situation (McColl et al., 1989) and indeed there is some doubt whether endogenous opioids are indeed increased (Elias et al., 1986). Preliminary data suggest that elevated levels of endogenous opiates are found following burns injury only when complicated by septicaemia (Elliot et al., 1985) whereas very low serum testosterone concentrations and disordered LH pulsatility are found in uncomplicated burned patients. The techniques required to measure these peptides are complex and still evolving and it is possible that additional endogenous opiates not measurable by current techniques might be increased in this situation. Moreover normal levels in the circulation do not exclude the possibility of raised levels in the environment of the hypothalamus and pituitary.

Raised plasma catecholamine concentrations following head injury were found to be inversely related to low serum testosterone concentrations (Woolf et al., 1986) but such association does not necessarily imply causality. An adrenaline infusion has been shown to

lower testosterone production rates in man although the mechanism for this was not explored (Levin et al., 1967). The catecholamine dopamine reduces LH levels when infused intravenously into volunteers (Leblanc et al., 1976) but when this experiment was repeated using physiological rather than pharmacological doses of dopamine no effect was found (Connell et al., 1984). It is possible that raised catecholamine levels alter the pulsatile nature of LH secretion and might exert a testosterone lowering effect but there is no evidence in the literature to support this.

Definite evidence of reduced LH pulsatility was found in uraemic men and in some men following burns injury. The heterogeneity of response in burned men suggests that some patients might have been sampled while their LH pulse generator was recovering but before their hypothalamic-pituitary-testicular axis had returned completely to normal.

My finding of low LH B:I ratios in burned men with low serum testosterone concentrations has since been confirmed (Plymate et al., 1987). The study described in Chapter 13 had suggested that this might merely reflect reduced LH pulsatility as biologically rich LH is secreted preferentially in LH peaks (Dufau et al., 1983; Veldhuis et al., 1983). However my later pulsatility study demonstrated that LH pulses are heterogeneous rather than uniformly diminished and it was not possible to attribute the low LH B:I ratios to reduced LH pulsatility. It is possible that the low LH B:I ratios might be a consequence of low serum testosterone levels rather than a cause but men with primary testicular failure seem to have high LH B:I ratios returning to normal with testosterone replacement therapy suggesting that this is unlikely to be the case (Tsatsoulis et al., 1990).

What mechanisms might exist to account for the alteration in biological activity of LH? This glycoprotein in man is synthesised and secreted in several distinct forms each with different biological activity

(Strollo et al., 1981; Reader, et al., 1983; Lichtenberg et al., 1984; Snyder et al., 1989). This heterogeneity is not caused by changes in the amino acid sequence of the α or β chains but to variations in the nature of the carbohydrate moieties of the hormone (Green & Baenziger, 1988). The presence of these sugars may be important for activity of polypeptide hormones. Deglycosylation of α subunits prior to recombination of the α and β subunits resulted in a glycoprotein with increased receptor binding but with no response from the target cell (Sairam & Bhargavi, 1985). Matzuk et al. (1989) using mutant cell lines secreting HCG dimers with different oligosaccharides attached found that dimers lacking oligosaccharides on the α chain had reduced biological activity and indeed some forms of HCG acted as a competitive inhibitor binding to the HCG receptor but blocking production of cyclic AMP by native HCG.

LH of increased biological activity is secreted at a pulse peak in normal men and in men with hypogonadotrophic hypogonadism whose LH pulsatility is restored by LHRH therapy (Dufau et al., 1983; Tsatsoulis et al., 1989). Low but not pharmacological doses of exogenously administered LHRH stimulate release of biologically rich LH (Veldhuis, Johnson & Dufau, 1987). In vitro LHRH stimulates subunit glycosylation in pituitary cells from castrated rats (Krummen & Baldwin, 1988). LHRH increases the glycosylation of LH released from quartered rat anterior pituitary glands; moreover the carbohydrate content of LH released in response to LHRH differs from that released in its absence (Liu & Jackson, 1978). Thus in vivo and in vitro evidence exists to suggest that LHRH may alter glycosylation of LH; release of LH of high biological activity may thus depend on normal LHRH pulse generator activity.

A possible course of events might be as follows:- soon after burns injury the LH pulse generator is switched off; intermittent LHRH release is mandatory for release of biologically active LH and possibly also for its synthesis; the LH secreted whilst capable of detection by radio-

immunoassay may have altered glycosylation and is not able to stimulate testosterone secretion; when the pulse generator begins to recover, release of LH of lower biological activity may continue until biologically richer LH becomes available.

How might burns injury and other illnesses have such an effect on the LH pulse generator? Several possibilities exist. The possible influence of endogenous opioid peptides and catecholamines has been discussed. Oestrogen levels are raised in uraemic, burned and septicaemic patients to an extent related to severity of illness (Rodger et al., 1985; Plymate et al., 1987; Christeff et al., 1988). An oestradiol infusion in healthy men reduces LH pulse frequency and LH biological activity with the oestrogen antagonist tamoxifen having the opposite effect suggesting that oestrogens in health may have an inhibitory effect on the LH pulse generator (Veldhuis & Dufau, 1987).

Endotoxin induced shock in male rats is accompanied by a fall in testosterone and an increase in oestrogen levels, these endocrine changes being prevented by either adrenalectomy or orchidectomy (Christeff et al., 1987). This animal experimentation led Christeff et al. (1988) to propose that the raised oestrogen levels in ill men resulted from testicular conversion of adrenal androgen to oestrogen. There is some evidence to suggest that such an interaction between the adrenal cortex and the testes may occur in rats (Feek, Tuzi & Edwards, 1989) but no such evidence exisis as yet in the human. Oestradiol in man is secreted by the testes (Longcope, Widrich & Sawin, 1972) but is also derived from aromitisation from testosterone (Longcope, Kato & Horton, 1969). No information is available in the literature concerning the source of the increased oestrogen levels in ill men. Whatever the mechanism leading to raised oestrogen levels in ill men, they may have an adverse effect on the LH pulse generator similar to an oestradiol infusion thus adversely affecting hypothalmic-pituitary-testicular function.

Severe illness is accompanied by release of a large number of locally acting inflammatory mediators and in vitro research has shown that some of these may affect endocrine systems. For example fibroblast growth factor and interleukin-1 inhibit LH or HCG stimulated testosterone secretion in cultured rat Leydig cells (Fauser, Baird & Hsueh, 1988; Calkins et al., 1988). Platelet activating factor inhibited LHRH release from the rat median eminence in vitro (Junier et al., 1988). There is some evidence to suggest that such effects may be of relevance in vivo. Rivier & Vale (1989) found that interleukin-1α injected into the lateral ventricle of castrated rats led to a dose dependent decrease of LH secretion whilst having no effect on LHRH stimulated LH release, implying an effect on endogenous LHRH release. There is no evidence in humans that these substances mediate the endocrine consequences of illness but they remain plausible candidates.

Hypothalamic-pituitary-adrenocortical axis

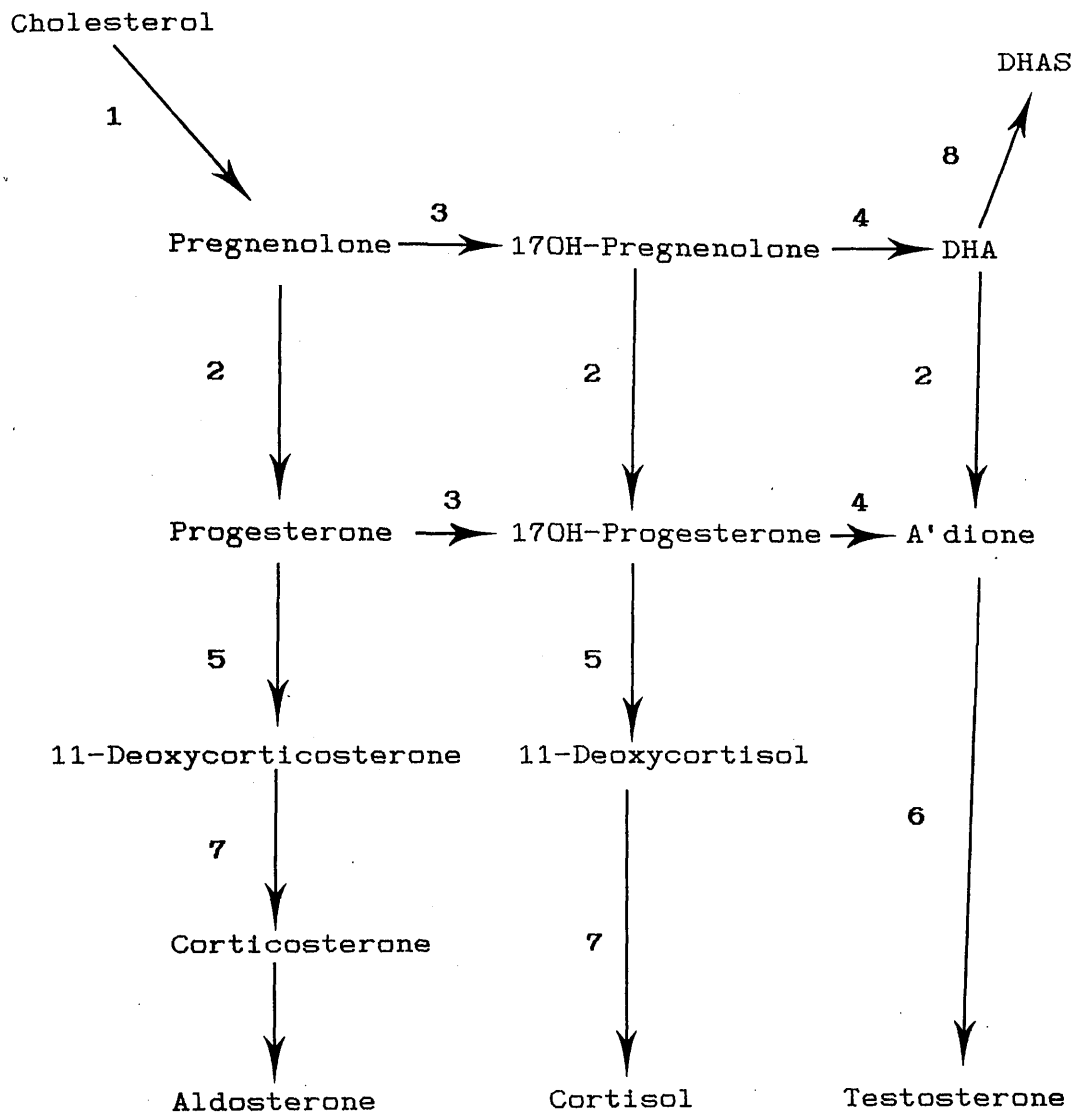
Although this thesis has demonstrated that DHAS levels often fall with longstanding illness I was unable to provide evidence to explain this finding. Several possibilities exist:- There may be decreased adrenocortical production of DHA and DHAS, reduced sulphation of DHA or reduced excretion or altered metabolism of DHAS.

Early investigators found unchanged urinary 17-ketosteroid excretion with increased excretion of glucocorticoid following trauma (Forbes et al., 1947; Moore et al., 1955). Lephart et al. (1987) obtained similar results in burned men with high serum cortisol but depressed serum DHAS concentrations. Urinary DHA excretion was reduced in ill men with low serum DHA and DHAS concentrations (Parker et al., 1985). Zumoff et al. (1971) administered isotopically labelled DHA to ill men and controls and found a smaller amount of the radioactivity in the urine of the ill than in that of the healthy. Increased urinary excretion of DHAS or its

metabolites would appear improbable. Serum DHA concentrations of burned men are depressed to a similar degree to those of DHAS suggesting that reduced sulphation of DHA is not an explanation for the low DHAS levels (Parker et al., 1985; Lephart et al., 1987). In addition Lephart et al. (1987) found reduced levels of androstenediol and androstenediol sulphate, important metabolites of DHA and DHAS respectively. The evidence therefore points to inhibition of adrenocortical secretion of DHAS being the main mechanism leading to low serum concentrations in ill men.

If ACTH were the only factor regulating cortisol and adrenal androgen secretion one might expect levels of cortisol and DHAS to change in parallel in ill men. The divergence between cortisol and DHAS in illness and other situations has led to continuing controversy relating to control of adrenal androgen secretion discussed in Chapter 1. A reduction in the level of the putative pituitary hormone, adrenal androgen stimulating hormone, with illness is one possible explanation but as there is as yet no firm evidence for its existence it is necessary to seek alternative mechanisms.

Recent in vitro studies using human adrenocortical tissue have shown that intra-adrenal enzyme systems are affected in differing ways by build up of substrate (Couch et al., 1986). These authors found that 17,20 desmolase the enzyme crucial to adrenal androgen synthesis (Figure 28) is inhibited in a more sensitive fashion than 17-hydroxylase by the accumulation of endogenous steroids; indeed the known intra-adrenal concentrations in man are sufficient to exert an inhibitory effect on 17,20 desmolase with relative sparing of 17-hydroxylase (Dickerman et al., 1984). Prolonged adrenocortical stimulation, as might be found after several days of illness, may lead to increased adrenal blood flow promoting passage of increased concentrations of intra-adrenal steroids into the inner zone of the gland, the zona reticularis, which is responsible for the majority of adrenal androgen production; these



1) Cholesterol desmolase
2) 3β-Hydroxysteroid dehydrogenase (3β-HSD)
3) 17α-hydroxylase
4) 17,20-desmolase
5) 21-hydroxylase
6) 17β-HSD
7) 11β-hydroxylase
8) DHA sulphokinase

Figure 28 A simplified representation of the major steroid synthetic pathways.

increased steroid concentrations may then inhibit 17,20 desmolase leading to a fall in adrenal androgen production.

A reduction in the activity of 17,20 desmolase with relative sparing of 17-hydroxylase does not explain the divergence between androstenedione and DHAS levels in serum. A further alteration in intra-adrenal enzyme systems might account for this. For example, ACTH stimulation seems to lead to increased activity of the 3ß-HSD enzyme system (Simonian & Gill, 1981) thus promoting corticosteroid synthesis and also stimulating conversion of DHA to androstenedione. Thus although adrenal androgen synthesis in total may be reduced, there may be a shift from DHA and DHAS to androstenedione synthesis. Control of steroid synthesis by local intra-adrenal mechanisms does not readily explain the low DHAS levels in uraemic patients who probably secrete cortisol normally although may have raised circulating levels due to reduced metabolism (Wallace et al., 1980). In addition if chronic adrenocortical stimulation secondary to illness leads to reduced secretion of adrenal androgen, why do patients with Cushing's disease have normal or modestly raised DHAS levels rather than low levels (Yamaji & Ibayashi, 1969)? Many such questions will remain unanswered until a fuller understanding of the mechanisms controlling adrenocortical secretion is reached.

The possibility that factors produced at the site of tissue damage might affect hypothalmic-pituitary-testicular function has been discussed. There is some evidence that thay might also influence adrenocortical function. Interleukin-1 seems to activate the hypothalamic-pituitary-adrenocortical axis of rats (Besedovsky & del Rey, 1987) and epidermal growth factor has a similar effect in rhesus monkeys (Luger et al., 1988) both effects mediated by increased corticotrophin releasing hormone release. Insulin-like growth factor I increases cortisol secretion by cultured bovine cells by increasing the activity of several enzyme systems (Penhoat et al., 1988) whereas Type B transforming growth factor

seems to depress cortisol secretion (Feige, Cochet & Chambaz, 1986). It is likely that many such factors are released from injured or diseased tissues. Whether they influence hypothalamic-pituitary-adrenocortical function in man and are released in sufficient quantities to have such a systemic effect remains to be established.

Implications for survival of changes in androgen levels with illness

The situation with regard to the low T4 syndrome is of interest; there is no evidence to suggest that replacement therapy with thyroid hormone is beneficial. Burned patients with low levels of T4 and T3 showed no change in metabolic rate or mortality when treated with T3 (Becker et al., 1982). Septicaemic rats with low thyroid hormone levels showed increased mortality and decreased time to death when treated with T4 (Little, 1985). Medically ill patients with the low T4 syndrome have not been improved by T4 replacement therapy (Brent & Hershman, 1986). Changes in thyroid hormone levels with illness may have a protective effect possibly by reducing protein catabolism (Gardner et al., 1979).

It is relatively straightforward to find evidence of biochemical hypogonadism in ill men as reflected by low serum testosterone concentrations. It is difficult if not impossible to discover whether patients are biologically hypogonadal as suggested by decreased libido and frequency of sexual activity. No attempt to research this was attempted as the problems involved in attempting to disentangle the effects of low serum testosterone concentrations from the general malaise induced by illness and hospitalisation presented insurmountable difficulties. A low serum hormone level does not necessarily equate with a deficiency state. In the case of thyroid hormone there are several intracellular mechanisms which may compensate for low levels of hormone reaching the cell (Koenig et al., 1984; Williams et al., 1989). Similar

mechanisms may exist in the case of androgens but no evidence for this exists at present.

It is tempting to speculate that depressed serum testosterone levels with illness may be a teleological mechanism promoting decreased libido and fertility when the reserves of the patient would be more suitably expended on recovery from illness rather than on sexual activity and prospective parenthood. A short-term reduction in serum testosterone concentrations is unlikely to be harmful as many patients, such as those with prostatic carcinoma treated by surgical castration, experience low serum testosterone levels in the long-term without ill effects.

Although DHA and DHAS are the most abundant circulating adrenal steroids, they are only weak androgens with little virilising or anabolic properties. There is limited evidence from animal experimentation to suggest that adrenal androgens may have a beneficial effect on the immune system. Loria et al. (1989) showed that DHA but not aetiocholanolone had a protective effective against a potentially lethal viral infection in mice. The hypothesis that low adrenal androgen levels in ill subjects has an immunologically deleterious effect remains to be tested.

It seems more likely that adrenal androgens provide a reservoir of adrenocortical activity which can be "tapped" in times of chronic illness. Thus steroid synthesis in times of chronic illness may be diverted from adrenal androgen to corticosteroid pathways ensuring maintained secretion of cortisol which is essential for the recovery of ill patients. Other factors may also favour corticosteroid production at the expense of other adrenocortical products. Thus aldosterone levels may be inappropriately low in some chronically and severely ill patients (Zipser et al., 1981; Luger et al., 1984; Findling et al., 1987) suggesting that mineralo-corticoid as well as adrenal androgen synthetic pathways may be inhibited in this situation. Not only does the environment of ill patients favour increased cortisol secretion but it also leads to reduced

elimination (Sandberg et al., 1956). The metabolism of cortisol to cortisone accounts for a major proportion of the elimination of cortisol (Fukushima et al., 1960) and this process may be inhibited in the severely ill (Gold et al., 1958). Thus reduced adrenal androgen secretion in the ill may in combination with other mechanisms promote a sustained and pronounced increase in cortisol levels which are crucial for recovery.

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The work described in this thesis has in great measure occupied the author over the last few years always helped by various willing colleagues. Profound changes in the hormonal milieu of ill patients have been documented and some pathophysiological mechanisms have been explored. The abnormalities found have been difficult to explain in terms of classical endocrine control systems. Further research is warranted to to investigate the mechanisms leading to the observed changes.

For example a more detailed study of the structure of LH secreted in a pulse peak as compared to that present during a nadir with comparisons between normal and burned patients would allow confirmation of the hypothesis that variation in glycosylation is the cause of altered LH biological activity. The effect of giving ill patients replacement therapy with adrenal androgen would be of interest but not ethically justifiable at present. A similar experiment using an animal model might suggest whether such treatment was likely to be of value and would be necessary before embarking on a trial in humans. Studies using radiolabelled steroids administered to ill men could be performed in order to investigate the hypothesis that 17,20 desmolase is inhibited with relative sparing 17-hydroxylase and might also be able to shed light on the source of the increased oestrogen levels. It may be possible to determine whether the increased oestrogens in the circulation of ill men are implicated in the abnormality of hypothalamic-pituitary-testicular

function by studying the biological activity of LH through a pulse series in burned men treated with either the oestrogen antagonist tamoxifen or placebo.

Further investigation of these situations may lead to a better understanding of the mechanisms controlling hypothalmic-pituitary-testicular and hypothalamic-pituitary-adrenocortical function in health and disease.

REFERENCES

Aakvaag, A., Sand, T., Opstad, P.K. & Fonnum, F. (1978a) Hormonal changes in serum in young men during prolonged physical strain. European Journal of Applied Physiology, 39, 283-291.

Aakvaag, A., Bentdal, O., Quigstad, K., Walstad, P., Ronningen, H. & Fonnum, F. (1978b) Testosterone and testosterone binding globulin (TeBG) in young men during prolonged stress. International Journal of Andrology, 1, 22-31.

Abram, H.S., Hester, L.R., Epstein, G.M. & Sheridan, W.F. (1974) Sexual activity and renal failure. In Proceedings of the 5th International Congress of Nephrology, Vol. 3, 207-210. Basel: Karger.

Alasandro, M., Wiesler, D., Rhodes, G. & Novotny, M. (1982) Quantitative alterations of steroid urinary profiles associated with diabetes mellitus. Clinica Chimica Acta, 126, 243-255.

Allen, P.I.M., Batty, K.A., Dodd, C.A.S., Herbert, J., Hugh, C.J., Moore, G.F., Seymour, M.J., Shiers, H.M., Stacey, P.M. & Young, S.K. (1985) Dissociation between emotional and endocrine responses preceding an academic examination in male medical students. Journal of Endocrinology, 107, 163-170.

Anderson, D.C. (1980) The adrenal androgen-stimulating hormone does not exist. Lancet, 2, 454-456.

Anderson, D.C., Marshall, J.C., Young, J.L. & Fraser, T.R. (1972) Stimulation tests of pituitary-leydig cell function in normal male subjects and hypogonadal men. Clinical Endocrinology, 1, 127-140.

Anderson, D.C., Thorner, M.O., Fisher, R.A., Woodham, J.P., Goble, H.L. & Besser, G.M. (1975) Effects of hormonal treatment on plasma unbound androgen levels in hirsute women. Acta Endocrinologica (Copenh), 199 (Suppl), 224.

Andò, S., Rubens, R. & Rottiers, R. (1984) Androgen plasma levels in male diabetics. Journal of Endocrinological Investigation, 7, 21-24.

Aono, T., Kurachi, K., Mizutani, S., Hamanaka, Y., Uozumi, T., Nakasima, A., Koshiyama, K. & Matsumoto, K. (1972) Influence of major surgical stress on plasma levels of testosterone, luteinizing hormone and follicle stimulating hormone in male patients. Journal of Clinical Endocrinology and Metabolism, 35, 535-542.

Bacci, B., Schussler, G.C. & Kaplan, T.B. (1982) The relationship between serum triiodothyronine and thyrotropin during systemic illness. Journal of Clinical Endocrinology and Metabolism, 54, 1229-1235.

Baker, E.R., Mathur, R.S., Kirk, R.F., Landgrebe, S.C., Moody, L.O. & Williamson, H.O. (1982) Plasma gonadotrophins, prolactin, and steroid hormone concentrations in female runners immediately after a long-distance run. Fertility and Sterility, 38, 38-41.

Baker, H.W.G., Paulsen, C.A., Burger, H.G., De Kretser, D.M., Dulmanis, A., Hudson, B., O'Connor, S., Purcell, N., Rennie, G.C., Seah, C.S., Taft, H.P. & Wang, C. (1976) A study of the endocrine manifestations of hepatic cirhosis. Quarterly Journal of Medicine, **45**, 145-178.

Balogh, D., Moncayo, R. & Bauer, M. (1984) Hormonal dysregulations in severe burns. Burns, **10**, 257-263.

Balsam, A. & Leppo, L.E. (1975) Effect of physical training on the metabolism of thyroid hormones in man. Journal of Applied Physiology, **38**, 212-215.

Bane, J.W., McCaa, R.E., McCaa, C.S., Read, V.H., Turney, W.H. & Turner, M.D. (1974) The pattern of aldosterone and cortisone blood levels in thermal burn patients. Journal of Trauma, **14**, 605-611.

Bannister, P., Handley, T., Chapman, C. & Losowsky, M.S. (1986) Hypogonadism in chronic liver disease: impaired release of luteinising hormone. British Medical Journal, **293**, 1191-1193.

Barrett-Connor, E., Khaw, K-T. & Yen, S.S.C. (1986) A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. New England Journal of Medicine, **315**, 1519-1524.

Barton, R.N. & Passingham, B.J. (1981) Effect of binding to plasma proteins on the interpretation of plasma cortisol concentrations after accidental injury. Clinical Science, **61**, 399-405.

Baxter, J.D. & Forsham, P.H. (1972) Tissue effects of glucocorticoids. American Journal of Medicine, **53**, 573-589.

Beastall, G.H., Ferguson, K.M., O'Reilly, D.S., Seth, J. & Sheridan, B. (1987) Assays for follicle stimulating hormone and luteinising hormone: guidelines for the provision of a clinical biochemistry service. Annals of Clinical Biochemistry, **24**, 246-262.

Belchetz, P.E., Plant, T.M., Nakai, Y., Keogh, E.J. & Knobil, E. (1978) Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science, **202**, 631-633.

Benahmed, M., Grenot, C., Tabone, E., Sanchez, P. & Morera, A.M. (1985) FSH regulates cultured Leydig cell function via Sertoli cell proteins: an in vitro study. Biochemical and Biophysical Research Communications, **132**, 729-734.

Benedict, C.R. & Grahame-Smith, D.G. (1978) Plasma noradrenaline and adrenaline concentrations and dopamine-β-hydroxylase activity in patients with shock due to septicaemia, trauma and haemorrhage. Quarterly Journal of Medicine, **47**, 1-20.

Besodovsky, H. & del Rey, A. (1987) Neuroendocrine and metabolic responses induced by interleukin-1. Journal of Neuroscience Research, **18**, 172-178.

Bezwoda, W.R., Bothwell, T.H., van der Walt, L.A., Kronheim, S. & Pimstone, B.L. (1977) An investigation into gonadal dysfunction in patients with idiopathic haemochromatosis. Clinical Endocrinology, **6**, 377-385.

Blackman, M.R., Weintraub, B.D., Rosen, S.W. & Harman S.M. (1988) Comparison of the effects of lung cancer, benign lung disease, and normal aging on pituitary-gonadal function in men. Journal of Clinical Endocrinology and Metabolism, **66**, 88-95.

Borst, G.C., Osburne, R.C., O'Brian, J.T., Georges, L.P. & Burman, K.D. (1983) Fasting decreases thyrotropin responsiveness to thyrotropin-releasing hormone: a potential cause of misinterpretation of thyroid function tests in the critically ill. Journal of Clinical Endocrinology and Metabolism, **57**, 380-383.

Boyden, T.W., Pamenter, R.W., Stanforth, P., Rutkis, T. & Wilmore, J.H. (1982) Evidence for mild thyroidal impairment in women undergoing endurance training. Journal of Clinical Endocrinology and Metabolism, **53**, 53-56.

Brent, G.A. & Hershman, J.M. (1986) Thyroxine therapy in patients with severe nonthyroidal illness and low serum thyroxine concentration. Journal of Clinical Endocrinology and Metabolism, **63**, 1-8.

Brooks, J.E., Herbert, M., Walder, C.P., Selby, C. & Jeffcoate, W.J. (1986) Prolactin and stress: some endocrine correlates of pre-operative anxiety. Clinical Endocrinology, **24**, 653-656.

Burr, W.A., Griffiths, R.S., Black, E.G., Hoffenberg, R., Meinhold, H. & Wenzel, K.W. (1975) Serum triiodothyronine and reverse triiodothyronine concentrations after surgical operation. Lancet, **2**, 1277-1279.

Calkins, J.H., Sigel, M.M., Nankin, H.R. & Lin, T. (1988) Interleukin-1 inhibits leydig cell steroidogenesis in primary cultures. Endocrinology, **123**, 1605-1610.

Caminos-Torres, R., Ma, L. & Snyder, P.J. (1977) Testosterone-induced inhibition of the LH and FSH responses to gonadotrophin-releasing hormone occurs slowly. Journal of Clinical Endocrinology and Metabolism, **44**, 1142-1153.

Carey, L.C., Cloutier, C.T. & Lowery, B.D. (1971) Growth hormone and adrenal cortical response to shock and trauma in the human. Annals of Surgery, **174**, 451-460.

Carmel, P.W., Araki, S. & Ferin, M. (1976) Pituitary stalk portal blood collection in rhesus monkeys: evidence for pulsatile release of gonadotrophin-releasing hormone (GnRH). Endocrinology, **99**, 243-248.

Carstensen, H., Amér, B., Amér, I. & Wide, L. (1973) The postoperative decrease of plasma testosterone in man, after major surgery, in relation to plasma FSH and LH. Journal of Steroid Biochemistry, **4**, 45-55.

Carter, J.N., Eastman, C.J., Corcoran, J.M. & Lazarus, L. (1974) Effect of severe, chronic illness on thyroid function. Lancet, **2**, 971-974.

Carter, J.N., Tyson, J.E., Tolis, G., Van Vliet, S., Faiman, C. & Friesen, H.G. (1978) Prolactin-secreting tumors and hypogonadism in 22 men. New England Journal of Medicine, **299**, 847-852.

Challand, G.S., Ratcliffe, W.A. & Ratcliffe, J.G. (1975) Semi-automated radioimmunoassays for total serum thyroxine and triiodothyronine. Clinica Chemica Acta, **60**, 25-32.

Charters, A.C., Odell, W.D. & Thompson, J.C. (1969) Anterior pituitary function during surgical stress and convalescence. Radioimmunoassay measurement of blood TSH, LH, FSH and growth hormone. Journal of Clinical Endocrinology, 29, 63-71.

Charytan, C., Thysen, B., Gatz, M., Freeman, R. & Alpert, B.E. (1983) Thyroid function tests in uremic patients on maintenance dialysis: a comparison of CAPD and hemodialysis. Peritoneal Dialysis Bulletin, 3, S27-S29.

Chen, J.C., Vidt, D.G., Zorn, E.M., Hallberg, M.C. & Wieland, R.G. (1970) Pituitary-Leydig cell function in uremic males. Journal of Clinical Endocrinology, 31, 14-17.

Chernow, B., Zaloga, G., McFadden, E., Clapper, M., Kotler, M., Barton, M. & Rainey, T.G. (1982) Critical Care Medicine, 10, 848-851.

Chopra, I.J. (1976) An assessment of daily production and significance of thyroidal secretion of 3,3',5'-triiodothyronine (reverse T3) in man. Journal of Clinical Investigation, 58, 32-40.

Chopra, I.J., Chopra, U., Smith, S.R., Reza, M. & Solomon, D.H. (1975) Reciprocal changes in serum concentrations of 3,3',5'-triiodothyronine (reverse T3) and 3,3',5-triiodothyronine (T3) in systemic illness. Journal of Clinical Endocrinology and Metabolism, 41, 1043-1049.

Chopra, I.J., Chua Teco, G.N., Nguyen, A.H. & Solomon, D.H. (1979b) In search of an inhibitor of thyroid hormone binding to serum proteins in nonthyroidal illnesses. Journal of Clinical Endocrinology and Metabolism, 49, 63-69.

Chopra, I.J., Huang, T.S., Solomon, D.H., Chaudhuri, G. & Chua Teco, G.N. (1986) The role of thyroxine (T4)-binding serum proteins in oleic acid-induced increase in free T4 in nonthyroidal illnesses. Journal of Clinical Endocrinology and Metabolism, 63, 776-779.

Chopra, I.J., Solomon, D.H., Chopra, U., Young, R.T. & Chua Teco, G.N. (1974) Alterations in circulating thyroid hormones and thyrotropin in hepatic cirrhosis: evidence for euthyroidism despite subnormal serum triiodothyronine. Journal of Clinical Endocrinology and Metabolism, 39, 501-511.

Chopra, I.J., Solomon, D.H., Chua Teco, G.N. & Eisenberg, J.B. (1982) An inhibitor of the binding of thyroid hormones to serum proteins is present in extrathyroidal tissues. Science, 215, 407-409.

Chopra, I.J., Solomon, D.H., Hepner, G.W. & Morgenstein, A.A. (1979a) Misleadingly low free thyroxine index and usefulness of reverse triiodothyronine measurement in nonthyroidal illnesses. Annals of Internal Medicine, 90, 905-912.

Chopra, I.J., Van Herle, A.J., Chua Teco, G.N. & Nguyen, A.H. (1980) Serum free thyroxine in thyroidal and nonthyroidal illnesses: a comparison of measurements by radioimmunoassay, equilibrium dialysis, and free thyroxine index. Journal of Clinical Endocrinology and Metabolism, 51, 135-143.

Chopra, I.J., Wu, S-Y., Nakamura, Y. & Solomon, D.H. (1978) Monodeiodination of 3,5,3'-triiodothyronine and 3,3',5'-triiodothyronine to 3,3', diiodothyronine in vitro. Endocrinology, 102, 1099-1106.

Christeff, N., Auclair, M-C., Benassayag, C., Carli, A. & Nunez, E.A. (1987) Endotoxin-induced changes in sex steroid hormone levels in male rats. Journal of Steroid Biochemistry, **29**, 435-440.

Christeff, N., Benassayag, C., Carli-Vielle, C., Carli, A. & Nunez, E.A. (1988) Elevated oestrogen and reduced testosterone levels in the serum of male septic shock patients. Journal of Steroid Biochemistry, **29**, 435-440.

Clark, J.D.A., Raggatt, P.R. & Edwards, O.M. (1988) Hypothalamic hypogonadism following major head injury. Clinical Endocrinology (Oxf), **29**, 153-165.

Clarke, I.J. & Cummins, J.T. (1982) The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology, **111**, 1737-1739.

Cohen, H.N., Paterson, K.R., Wallace, A.M., Beastall, G.H., Manderson, W.G. & MacCuish, A.C. (1984) Dissociation of adrenarche to gonadarche in diabetes mellitus. Clinical Endocrinology, **20**, 717-724.

Cohen, K.L. & Swigar, M.E. (1979) Thyroid function screening in psychiatric patients. Journal of the American Medical Association, **242**, 254-257.

Coleman, D.L., Schwizer, R.W. & Leiter, E.H. (1984) Effect of genetic background on the therapeutic effects of dehydroepiandrosterone (DHEA) in diabetes-obesity mutants and in aged normal mice. Diabetes, **33**, 26-32.

Colt, E.W.D., Wardlaw, S.L. & Frantz, A.G. (1981) The effect of running on plasma ß-endorphin. Life Science, **28**, 1637-1640.

Connell, A.M., Cooper, J. & Redfearn, J.W. (1958) The contrasting effects of emotional tension and physical exercise on the excretion of 17-ketogenic steroids and 17-ketosteroids. Acta Endocrinologica (Copenh), **27**, 179-194.

Connell, J.M.C., Ball, S.G., Inglis, G.C., Beastall, G.H. & Davies, D.L. (1984) The effect of low-dose dopamine infusion on anterior pituitary hormone secretion in normal female subjects. Clinical Science, **67**, 219-223.

Cornil, A., De Coster, A., Copinschi, G. & Franckson, J.R.M. (1965) Effect of muscular exercise on the plasma level of cortisol in man. Acta Endocrinologica (Copenh), **48**, 163-168.

Couch, R.M., Muller, J. & Winter, J.S.D. (1986) Regulation of the activities of 17-hydroxylase and 17,20 desmolase in the human adrenal cortex: kinetic analysis and inhibition by endogenous steroids. Journal of Clinical Endocrinology and Metabolism, **63**, 613-618.

Cowden, E.A., Ratcliffe, W.A., Ratcliffe, J.G., Dobbie, J.W. & Kennedy, A.C. (1978) Hyperprolactinaemia in renal disease. Clinical Endocrinology, **9**, 241-248.

Crantz, F.R., Silva, J.E. & Larsen, P.R. (1982) An analysis of the sources and quantity of 3,5,3'-triiodothyronine specifically bound to nuclear receptors in rat cerebral cortex and cerebellum. Endocrinology, **110**, 367-375.

Crowley, W.F., Filicori, M., Spratt, D.I. & Santoro, N.F. (1985) The physiology of gonadotrophin-releasing hormone (GnRH) secretion in men and women. Recent Progress in Hormone Research, **41**, 473-526.

Cumming, D.C., Quigley, M.E. & Yen, S.S.C. (1983) Acute suppression of circulating testosterone levels by cortisol in men . Journal of Clinical Endocrinology and Metabolism, **57**, 671-673.

Cumming, D.C., Vickovic, M.M., Wall, S.R. & Fluker, M.R. (1985) Defects in pulsatile LH release in normally menstruating runners. Journal of Clinical Endocrinology and Metabolism, **60**, 810-812.

Cumming, D.C., Wheeler, G.D. & McColl, E.M. (1989) The effects of exercise on reproductive function in man. Sports Medicine, **7**, 1-17.

Czeisler, C.A., Ede, M.C.M., Regestein, Q.R., Kisch, E.S., Fang, V.S. & Ehrlich, E.N. (1976) Episodic 24 hour cortisol secretory patterns in patients awaiting elective cardiac surgery. Journal of Clinical Endocrinology and Metabolism, **42**, 273-283.

Dahl, K.D., Bicsak, T.A. & Hsueh, A.J.W. (1988) Naturally occurring antihormones: secretion of FSH antagonists by women treated with a GnRH analog. Science, **239**, 72-74.

Daughaday, W.H. & Mariz, I.K. (1961) Corticosteroid-binding globulin: its properties and quantitation. Metabolism, **10**, 936-950.

Davies, C.T.M. & Few, J.D. (1973) Effects of exercise on adrenocortical function. Journal of Applied Physiology, **35**, 887-891.

De Kretser, D.M., Burger, H.G. & Dumpys, R. (1975) Serum LH and FSH response in four-hour infusions of luteinizing hormone-releasing hormone in normal men, Sertoli cell only syndrome, and Klinefelter's syndrome. Journal of Clinical Endocrinology and Metabolism, **41**, 876-886.

Deslypere, J.P. & Vermeulen, A. (1984) Leydig cell function in normal men: effect of age, life-style, residence, diet and activity. Journal of Clinical Endocrinology and Metabolism, **59**, 955-962.

Dessypris, A., Kuoppasalmi, K. & Adlercreutz, H. (1976) Plasma cortisol, testosterone, androstenedione and luteinizing hormone (LH) in a non-competitive marathon run. Journal of Steroid Biochemistry, **7**, 33-37.

Dickerman, Z., Grant, D.R., Faiman, C. & Winter, J.S.D. (1984) Intraadrenal steroid concentrations in man: zonal differences and developmental changes. Journal of Clinical Endocrinology and Metabolism, **59**, 1031-1036.

Dolecek, R. (1984) Burn stress and its endocrine consequences. Acta Chirurgiae Plasticae (Prague), **26**, 107-127.

Dolecek, R., Adámková, M., Sotorníková, T., Slosarcík, K. & Závada, M. (1979a) Syndrome of afterburn peripheral gland involvement - very low plasma testosterone levels in burned male patients. Acta Chirurgiae Plasticae (Prague), **21**, 114-119.

Dolecek, R., Adámková, M., Sotorníková, T., Závada, M. & Kracmer, P. (1979b) Endocrine response after burn. Scandinavian Journal of Plastic and Reconstructive Surgery, **13**, 9-16.

Dolecek, R., Dvorácek, C., Jczek, M, Sajnar, J. & Závada, M. (1983) Very low serum testosterone levels and severe impairment of spermatogenesis in burned male patients. Correlations with basal levels and levels of FSH, LH, and PRL after LHRH + TRH. Endocrinologia Experimentalis, **17**, 33-45.

Dufau, M.L., Veldhuis, J.D., Fraioli, F., Johnson, M.L. & Beitins, I.Z. (1983) Mode of secretion of bioactive luteinizing hormone in man. Journal of Clinical Endocrinology and Metabolism, 57, 993-1000.

Dunn, J.F., Nisula, B.C. & Rodbard, D. (1981) Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. Journal of Clinical Endocrinology and Metabolism, 53, 58-68.

Elias, A.N., Iyer, K., Pandian, M.R., Weathersbee, P., Stone, S. & Tobis, J. (1986) β-endorphin/β-lipotropin release and gonadotropin secretion after acute exercise in normal males. Journal of Applied Physiology, 61, 2045-2049.

Ellenberg, M. (1971) Impotence in diabetes: the neurologic factor. Annals of Internal Medicine, 75, 213-219.

Elliot, D., Everitt, A.S., Gault, D., Quaba, A.A., Hackett, M.E.J., Howlett, T.A., Tomlin, S.J. & Rees, L.H. (1985) The role of endorphins in septicaemic shock: a pilot study in burned patients. Burns, 11, 387-392.

Elstein, M., Smith, E.K.M. & Curtis, J.R. (1969) Reproductive potential of patients treated by maintenance haemodialysis. British Medical Journal, 2, 734-736.

Espiner, E.A. (1966) Urinary cortisol excretion in stress situations and in patients with Cushing's syndrome. Journal of Endocrinology, 35, 29-44.

Faber, J., Kirkegaard, C., Rasmussen, B., Westh, H., Busch-Srensen, M. & Jensen, I.W. (1987) Pituitary-thyroid axis in critical illness. Journal of Clinical Endocrinology and Metabolism, 65, 315-320.

Faerman, I., Vilar, O., Rivarola, M.A., Rosner, J.M., Jadzinsky, M.N., Fox, D., Perez Lloret, A., Bernstein-Hahn, L. & Saraceni, D. (1972) Impotence and diabetes. Studies of androgenic function in diabetic impotent males. Diabetes, 21, 23-30.

Falconer, I.R. & Hetzel, B.S. (1964) Effect of emotional stress and TSH on thyroid vein hormone level in sheep with exteriorized thyroids. Endocrinology, 75, 42-48.

Farrell, P.A., Gates, W.K., Maksud, M.G. & Morgan, W.P. (1982) Increases in plasma β-endorphin/β-lipotropin immunoreactivity after treadmill running in humans. Journal of Applied Physiology, 52, 1245-1249.

Faure, N., Labrie, F., Lemay, A., Bélanger, A, Gourdeau, Y., Laroche, B. & Robert, G. (1982) Inhibition of serum androgen levels by chronic intranasal and subcutaneous administration of a potent luteinizing hormone-releasing hormone (LHRH) agonist in adult males. Fertility and Sterility, 37, 416-424.

Fauser, B.C.J.M., Baird, A & Hsueh, A.J.W. (1988) Fibroblast growth factor inhibits luteinizing hormone-stimulated androgen production by cultured rat testicular cells. Endocrinology, 123, 2935-2941.

Febres, F., Scaglia, H., Lisker, R., Espinosa, J., Morals, T., Shkurovich,M. & Perez-Palacios, P. (1975) Hypothalamic-pituitary-gonadal function in patients with myotonic dystrophy. Journal of Clinical Endocrinology and Metabolism, 41, 833-840.

Feek, C.M., Tuzi, N.L. & Edwards, C.R.W. (1989) The adrenal gland and progesterone stimulates testicular steroidogenesis in the rat in vitro. Journal of Steroid Biochemistry, 32, 573-579.

Feige, J.J., Cochet, C. & Chambaz, E.M. (1986) Type B transforming growth factor is a potent modulator of differentiated adrenocortical cell functions. Biochemical Biophysical Research Communications, 139, 693-700.

Felig, P., Cherif, A., Minagawa, A. & Wahren, J. (1982) Hypoglycaemia during prolonged exercise in normal men. New England Journal of Medicine, 306, 895-900.

Few, J.D. (1974) Effect of exercise on the secretion and metabolism of cortisol in man. Journal of Endocrinology, 62, 341-353.

Findling, J.W., Waters, V.O. & Raff, H. (1987) The dissociation of aldosterone and renin in critical illness. Journal of Clinical Endocrinology and Metabolism, 64, 592-595.

Forbes, A.P., Donaldson, E.C., Reifenstein, E.C. & Albright, F. (1947) The effect of trauma and disease on the urinary 17-ketosteroid excretion in man. Journal of Clinical Endocrinology, 7, 264-288.

Fukushima, D.K., Bradlow, H.L., Zumoff, B. & Gallagher, T.F. (1960) Metabolic transformation of hydrocortisone-4-C^{14} in normal men. Journal of Biological Chemistry, 235, 2246-2252.

Galbo, H., Holst, J.J. & Christensen, N.J. (1975) Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. Journal of Applied Physiology, 38, 70-76.

Galbo, H., Hummer, L., Petersen, I.B., Christensen, N.J. & Bie, N. (1977) Thyroid and testicular hormone responses to graded and prolonged exercise in man. European Journal of Applied Physiology, 36, 101-106.

Gardner, D.F., Carithers, R.L. & Utiger, R.D. (1982) Thyroid function tests in patients with acute and resolved hepatitis B virus infections. Annals of Internal Medicine, 96, 450-452.

Gardner, D.F., Kaplan, M.M., Stanley, C.A. & Utiger, R.D. (1979) Effect of tri-iodothyronine replacement on the metabolic and pituitary responses to starvation. (1979) New England Journal of Medicine, 300, 579-584.

Gerich, J.E., Karam, J.H. & Forsham, P.H. (1973) Stimulation of glucagon secretion by epinephrine in man. Journal of Clinical Endocrinology and Metabolism, 37, 479-481.

Glass, A.R., Swerdloff, R.S., Bray, G.A., Dahms, W.T. & Atkinson, R.L. (1977) Low serum testosterone and sex-hormone-binding-globulin in massively obese men. Journal of Clinical Endocrinology and Metabolism, 45, 1211-1219.

Gluud, C., Madsbad, S., Krarup, T. & Bennett, P. (1982) Plasma testosterone and androstenedione in insulin dependent patients at time of diagnosis and during the first year of insulin treatment. Acta Endocrinologica (Copenh), 100, 406-409.

Gokal, R., McHugh, M., Fryer, R., Ward, M.K. & Kerr, D.N.S. (1980) Continuous ambulatory peritoneal dialysis therapy: one year's experience in a UK dialysis unit. British Medical Journal, 281, 474-477.

Gold, N.I., Singleton, E., MacFarlane, D.A. & Moore F.D. (1958) Quantitative determination of the urinary cortisol metabolites, "tetrahydro F", "allo-tetrahydro F" and "tetrahydro E": effects of adrenocorticotrophin and complex trauma in the human. Journal of Clinical Investigation, 37, 818-823.

Goodall, M. & Berman, M.L. (1960) Urinary output of adrenaline, noradrenaline, and 3-methoxy-4-hydroxymandelic acid following centrifugation and anticipation of centrifugation. Journal of Clinical Investigation, 39, 1533-1538.

Gordon, D., Beastall, G.H., Thomson, J.A. & Sturrock, R.D. (1986) Androgenic status and sexual function in males with rheumatoid arthritis and ankylosing spondylitis. Quarterly Journal of Medicine, 60, 671-679.

Gordon, G.G., Altman, K., Southren, A.L., Rubin, E. & Lieber, C.S. (1976) Effect of alcohol (ethanol) administration on sex-hormone metabolism in normal men. New England Journal of Medicine, 295, 793-797.

Gordon, G.G., Olivo, J., Rafii, F. & Southren, A.L. (1975) Conversion of androgens to estrogens in cirrhosis of the liver. Journal of Clinical Endocrinology and Metabolism, 40, 1018-1026.

Green, E.D. & Baenziger, J.U. (1988) Asparagine-linked oligosaccharides on lutropin, follitropin & thyrotropin. I. Structural elucidation of the sulfated and sialylated oligosaccharides on bovine , ovine and human pituitary glycoprotein hormones. Journal of Biological Chemistry, 263, 25-35.

Green, J.R.B., Mowat, N.A.G., Fisher, R.A. & Anderson, D.C. (1976) Plasma oestrogens in men with chronic liver disease. Gut, 17, 426-430.

Greene, W.A., Conron, G., Schalch, D.S. & Schreiner, B.F. (1970) Psychologic correlates of growth hormone and adrenal secretory responses of patients undergoing cardiac catheterisation. Psychosomatic Medicine, 32, 599-614.

Griffiths, R.W., Millar, J.G.B., Albano, J. & Shakespeare, P.G. (1983) Observations on the activity of the renin-angiotensin-aldosterone (RAA) system after low volume colloid resuscitation for burns injury. Annals of the Royal College of Surgeons of England, 65, 212-215.

Grossman, A., Bouloux, P., Price, P., Drury, P.L., Lam, K.S.L., Turner, T., Thomas, J., Besser, G.M. & Sutton, J. (1984) The role of opioid peptides in the hormonal responses to acute exercise in man. Clinical Science, 67, 483-491.

Grossman, A., Moult, P.J.A., Gaillard, R.C., Delitalia, G., Toff, W.D., Rees,L.H. & Besser, G.M. (1981) The opioid control of LH and FSH release: effects of met-enkephalin analogue and naloxone. Clinical Endocrinology, 14, 41-47.

Guillemin, R., Vargo, T., Rossier, J., Minick, S., Ling, N., Rivier, C., Vale, W. & Bloom, F. (1977) Beta-endorphin and adrenocorticotrophin are secreted concomitantly by the pituitary gland. Science, 197, 1367-1369.

Gyntelberg, F., Rennie, M.J., Hickson, R.C. & Holloszy, J.O. (1977) Effect of training on the response of plasma glucose to exercise. Journal of Applied Physiology, 43, 302-305.

Hacknay, A.C., Sinning, W.E. & Bruot, B.C, (1988) Reproductive profile of endurance-trained and untrained males. Medicine and Science in Sports and Exercise, 20, 60-65.

Hagen, C., Brandt, M.R. & Kehlet, H. (1980) Prolactin, LH, FSH, GH and cortisol response to surgery and the effect of epidural analgesia. Acta Endocrinologica (Copenh), 94, 151-154.

Hagen, C., Olgaard, K., McNeilly, A.S. & Fisher, R. (1976) Prolactin and the pituitary-gonadal axis in male uraemic patients on regular dialysis. Acta Endocrinologica (Copenh), 82, 29-38.

Hagen, G.A. & Elliott, W.J. (1973) Transport of thyroid hormones in serum and cerebrospinal fluid. Journal of Clinical Endocrinology and Metabolism, 37, 415-422.

Hale, R.W., Kosasa, T., Krieger, J. & Pepper, S. (1983) A marathon: the immediate effect on female runners' luteinizing hormone, follicle-stimulating hormone, prolactin, testosterone and cortisol levels. American Journal of Obstetrics and Gynaecology, 146, 550-556.

Hamblin, P.S., Dyer, S.A., Mohr, V.S., Le Grand, B.A., Lim, C-F., Tuxen, D.V., Topliss, D.J. & Stockigt, J.R. (1986) Relationship between thyrotropin and thyroxine changes during recovery from severe hypothyroxinemia of critical illness. Journal of Clinical Endocrinology and Metabolism, 62, 717-722.

Harris, G.W. (1955) In Neural control of the pituitary gland, Arnold: London.

Haynes, I.G., Lockett, S.J., Farmer, M.J., Fitch, N.J., Bradwell, A.R., Sheppard, M.C. & Ramsden, D.B. (1989) Is oleic acid the thyroxine binding inhibitor in the serum of ill patients. Clinical Endocrinology, 31, 25-30.

Hendry, W.F., Stedronska, J., Jones, C.R., Blackmore, C.A., Barrett, A. & Peckham, M.J. (1983) Semen analysis in testicular cancer and Hodgkin's disease: pre- and post-treatment findings and implications for cryopreservation. British Journal of Urology, 55, 769-773.

Henry, J.P. (1986) Mechanisms by which stress can lead to coronary heart disease. Postgraduate Medical Journal, 62, 687-693.

Herrmann, J., Krüskemper, H.L., Grosser, K.D., Hübner, W. & Böhm, W. (1971) Peritonealdialyse in der Behandlung der thyreotoxischen Krise. Deutsche Medizinische Wochenschrift, 96, 742-745.

Herrmann, J., Schmidt, H.J. & Krüskemper, H.L. (1973) Thyroxine elimination by peritoneal dialysis in experimental thyrotoxicosis. Hormone and Metabolic Research, 5, 180-183.

Hodges, J.R., Jones, M.T. & Stockham, M.A. (1982) Effect of emotion on blood corticotrophin and cortisol concentrations in man. Nature, 193, 1187-1188.

Holdsworth, S., Atkins, R.C. & De Kretser, D.M. (1977) The pituitary-testicular axis in men with chronic renal failure. New England Journal of Medicine, 296, 1245-1249.

Irvine, C.H.G. (1968) Effect of exercise on thyroxine degradation in athletes and non-athletes. Journal of Clinical Endocrinology, 28, 942-948.

Johansson, G.G., Karonen, S-L. & Laakso, M-L. (1983) Reversal of an elevated level of prolactin during prolonged psychological stress. Acta Physiologica Scandinavica, **119**, 463-464.

Johansson, G.G., Laakso, M-L., Peder, M. & Karonen, S-L. (1988) Examination stress decreases plasma levels of luteinizing hormone in male students. Psychsomatic Medicine, **50**, 286-294.

Junier, M.P., Tiberghien, C., Rougeot, C., Fafeur, Y. & Dray, F. (1988) Inhibitory effect of platelet-activating factor (PAF) on luteinizing hormone-releasing hormone and somatostatin release from rat median eminence in vitro correlated with the characterisation of specific PAF receptor sites in rat hypothalamus. Endocrinology, **123**, 72-80.

Kaptein, E.M., Grieb, D.A., Spencer, C.A., Wheeler, W.S. & Nicoloff, J.T. (1981) Thyroxine metabolism in the low thyroxine state of critical nonthyroidal illnesses. Journal of Clinical Endocrinology and Metabolism, **53**, 764-771.

Kaptein, E.M., Robinson, W.J., Grieb, D.A. & Nicoloff, J.T. (1982a) Peripheral serum thyroxine, triiodothyronine and reverse triiodothyronine kinetics in the low thyroxine state of acute nonthyroidal illness. Journal of Clinical Investigation, **69**, 526-535.

Kaptein, E.M., Weiner, J.M., Robinson, W.J., Wheeler, W.S. & Nicoloff, J.T. (1982b) Relationship of altered thyroid hormone indices to survival in nonthyroidal illnesses. Clinical Endocrinology, **16**, 565-574.

Kaufman, J-M., Kesner, J.S., Wilson, R.C. & Knobil, E. (1985) Electrophysiological manifestation of luteinizing hormone-releasing hormone pulse generator activity in the rhesus monkey: influence of α-adrenergic and dopaminergic blocking agents. Endocrinology, **116**, 1327-1333.

Keizer, H.A., Poortman, J. & Bunnik, G.S.J. (1980) Influence of physical exercise on sex-hormone metabolism. Journal of Applied Physiology, **48**, 765-769.

Kerr, D.J., Singh, V.K., McConnell, K.N., Junor, B.J.R. & Alexander, W.D. (1986). Serum and peritoneal dialysate thyroid hormone levels in patients on continuous ambulatory peritoneal dialysis. Nephron, **43**, 164-168.

Kindermann, W., Schnabel, A., Schmitt, W.M., Biro, G., Cassens, J. & Weber, F. (1982) Catecholamines, growth hormone, cortisol, insulin and sex hormones in anaerobic and aerobic exercise. European Journal of Applied Physiology, **49**, 389-399.

Klibanski, A., Beitins, I.Z., Badger, T., Little, R. & McArthur, J.W. (1981) Reproductive function during fasting in men. Journal of Clinical Endocrinology and Metabolism, **53**, 258-263.

Koenig, R.J., Leonard, J.L., Senator, D., Rappaport, N., Watson, A.Y. & Larsen, P.R. (1984) Regulation of thyroxine 5'-deiodinase activity by 3,5,3'-triiodothyronine in cultured rat anterior pituitary cells. Endocrinology, **115**, 324-329.

Kolodny, R.C., Kahn, C.B., Goldstein, H.H. & Barnett, D.M. (1973) Sexual function in diabetic men. Diabetes, **23**, 306-309.

Kreuz, L.E., Rose, R.M. & Jennings, J.R. (1972) Suppression of plasma testosterone levels and psychological stress. Archives of General Psychiatry, **26**, 479-482.

Krey, L.C., Butler, W.R. & Knobil, E. (1975) Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. I. Gonadotropin secretion. Endocrinology, **96**, 1073-1087.

Krummen, L.A. & Baldwin, D.M. (1988) Regulation of luteinizing hormone subunit biosynthesis in cultured male anterior pituitary cells: effects of gonadotropin-releasing hormone and testosterone. Endocrinology, **123**, 1868-1878.

Kuoppasalmi, K. & Adlercreuz, H. (1985) Interaction between catabolic and anabolic steroid hormones in muscular exercise. In Exercise Endocrinology, eds. Fotherby, K. & Pal, S.B., pp 65-68. de Gruyter: Berlin, New York.

Kuusi, T., Kostiainen, E., Vartiainen, E., Pitkänen, L., Ehnholm, C., Korhonen, H.J., Nissinen, A. & Puska, P. (1984) Acute effects of marathon running on levels of serum lipoproteins and androgenic hormones in healthy males. Metabolism, 33, 527-531.

Lachelin, G.C.L. & Yen, S.S.C. (1978) Hypothalamic chronic anovulation. American Journal of Obstetrics and Gynecology, **130**, 825-831.

Lambert, A., Tsatsoulis, A., Shalet, S., Frost, J. & Robertson W.R. (1986) Bioactive and immunoreactive luteinising hormone, stability in vitro in human blood and plasma. Journal of Endocrinology, **111 (Suppl)**, Abstract 59.

Leblanc, H., Lachelin, G.C.L., Abu-Fadil, S. & Yen, S.S.C. (1976) Effects of dopamine infusion on pituitary hormone secretion in humans. Journal of Clinical Endocrinology and Metabolism, **43**, 668-674.

Leblanc, J., Nadeau, A., Boulay, M. & Rousseau-Migneron, S. (1979) Effects of physical training and adiposity on glucose metabolism and ^{125}I-insulin binding. Journal of Applied Physiology, **46**, 235-239.

Leblanc, J., Nadeau, A., Richard, D. & Tremblay, A. (1981) Studies on the sparing effect of exercise on insulin requirements in human subjects. Metabolism, **30**, 1119-1124.

Lephart, E.D., Baxter, C.R. & Parker, C.R. (1987) Effect of burn trauma on adrenal and testicular steroid hormone production. Journal of Clinical Endocrinology and Metabolism, **64**, 842-848.

Levin, J., Lloyd, C.W., Lobotsky, J. & Friedrich, E.H. (1967) The effect of epinephrine on testosterone production. Acta Endocrinologica (Copenh), **55**, 184-192.

Levine, J.E., Pau, K-Y.F., Ramirez, V.D. & Jackson G.L. (1982) Simultaneous measurement of luteinizing hormone-releasing hormone and luteinizing hormone release in unaesthetized ovariectomized sheep. Endocrinology, **111**, 1449-1155.

Lichtenberg, V., Pahnke, V.G., Graesslin, D. & Bettendorf, G. (1983) Biological and immunological potencies of lutropin (LH) in human serum: comparative studies using different reference preparations. Hormone and Metabolic Research, **14**, 39-45.

Lichtenberg, V., Weise, H.C., Graesslin, D. & Bettendorf, G. (1984) Polymorphism of human pituitary lutrotropin (LH) . Effect of the seven isohormones on mouse Leydig cell function. FEBS Letters, **169**, 21-24.

Lim, C-F., Bai, Y., Topliss, D.J., Barlow, J.W. & Stockigt, J.R. (1988) Drug and fatty acid effect on thyroid hormone binding. Journal of Clinical Endocrinology and Metabolism, **67**, 682-688.

Lim, V.S. & Fang, V.S. (1975) Gonadal dysfunction in uremic men. A study of the hypothalamic-pituitary-testicular axis before and after renal transplantation. American Journal of Medicine, **58**, 655-662.

Lim, V.S., Kathpalia, S.C. & Henriquez, C. (1978) Endocrine abnormalities associated with chronic renal failure. Medical Clinics of North America, **62**, 1341-1361.

Lincoln, D.W., McNeilly, A.S. & Sharpe, R.M. (1989) Reproductive physiology of inhibin and related peptides. In Recent Advances in Endocrinology and Metabolism, eds. Edwards, C.R.W. & Lincoln, D.W., pp 77-107. Churchill Livingstone: Edinburgh, London.

Lincoln, G.A. (1977) Luteinising hormone and testosterone in man. Nature, **252**, 232-233.

Little, J.S. (1985) Effect of thyroid hormone supplementation on survival after bacterial infection. Endocrinology, **117**, 1431-1435.

Liu, T-C. & Jackson, G.L. (1978) Modifications of luteinizing hormone biosynthesis and release by gonadotropin-releasing hormone, cycloheximide and actinomycin D. Endocrinology, **103**, 1253-1263.

Ljunghall, S., Joborn, H., Benson, L., Fellström, R., Wide, L. & Yen, S.S.C. (1984) Effects of physical exercise on serum calcium and parathyroid hormone. European Journal of Clinical Investigation, **14**, 469-473.

Longcope, C., Kato, T. & Horton, R. (1969) Conversion of blood androgens to estrogens in normal adult men and women. Journal of Clinical Investigation, **48**, 2191-2201.

Longcope, C., Widrich, W. & Sawin, C.T. (1972) The secretion of estrone and estradiol-17β by human testis. Steroids, **20**, 439-448.

Loria, R.M., Inge, T.H., Cook, S.S., Szakal, A.K. & Regelson, W. (1988) Protection against acute lethal viral infections with the native steroid Dehydrepiandrosterone (DHEA). Journal of Medical Virology, **26**, 301-314.

Lotti, G., Delitalia, G., Devilla, L., Alagna, S. & Masala, A. (1977) Reduction of plasma triiodothyronine (T3) induced by propranolol. Clinical Endocrinology, **6**, 405-410.

Lovén, L., Nordström, H. & Lennquist, S. (1984) Changes in calcium and phosphate and their regulating hormones in patients with severe burn injuries. Scandinavian Journal of Plastic and Reconstructive Surgery, **18**, 49-53.

Luger, A., Graf, H., Prager, R. & Stummvoll, H.K. (1984) Alterations in the renin-angiotensin-aldosterone system in the critically ill. Hormone and Metabolic Research, **16**, 213-214.

Luton, J-P., Thieblot, P., Valcke, J-C., Mahoudeau, J.A. & Bricaire, H. (1977) Reversible gonadotrophin deficiency in male Cushing's disease. Journal of Clinical Endocrinology and Metabolism, **45**, 488-495.

McColl, E.M., Wheeler, G.D., Gomes, P., Bhambhani Y. & Cummings D.C. (1989) The effects of acute exercise on pulsatile LH release in high-mileage male runners. Clinical Endocrinology, **31**, 617-621.

MacConnie, S.E., Barkan, A., Lampman, R.M., Schork, M.A. & Beitins, I.Z. (1986) Decreased hypothalamic gonadotrophin-releasing hormone secretion in male marathon runners. New England Journal of Medicine, **315**, 411-417.

McConway, M.G., Biggart, E.M. & Chapman, R.S. (1987) Performance of the two-site immunoradiometric assay for serum thyroid-stimulating hormone. Effects of changes in solid-phase matrix and antibody coupling. Journal of Immunological Methods, **104**, 87-92.

McConway, M.G. & Chapman, R.S. (1986) Development and evaluation of a simple, direct solid-phase radioimmunoassay of serum cortisol from readily available reagents. Clinica Chimica Acta, **158**, 59-70.

Mason, J.W., Hartley, L.H., Kotchen, T.A., Wherry, F.E., Pennington, L.L. & Jones, L.G. (1973) Plasma thyroid-stimulating hormone response in anticipation of muscular exercise in the human. Journal of Clinical Endocrinology and Metabolism, **37**, 403-406.

Mason, J.W., Kenion, C.C., Collins, D.R., Mougey, E.H., Jones, J.A., Driver, G.C., Brady, J.V. & Beer, B. (1968b) Urinary testosterone response to 72-hr avoidance sessions in the monkey. Psychosomatic Medicine, **30**, 721-732.

Mason, J.W., Mougay, E.H., Brady, J.V. & Tolliver, G.A. (1968a) Thyroid (plasma butanol-extractable iodine) responses to 72-hr avoidance sessions in the monkey. Psychosomatic Medicine, **30**, 682-695.

Mason, J.W., Tolson, W.W., Robinson, J.A., Brady, J.V., Tolliver, G.A. & Johnson, T.A. (1968c) Urinary androsterone, etiocholanolone, and dehydroepiandrosterone responses to 72-hr avoidance sessions in the monkey. Psychosomatic Medicine, **30**, 710-720.

Matsumoto, K., Takeyasu, K., Mizutani, S., Hamanaka, Y. & Uozumi, T. (1970) Plasma testosterone levels following surgical stress in male patients. Acta Endocrinologica (Copenh), **65**, 11-17.

Maturlo, S.J., Rosenbaum, R.L., Pan, C. & Surks, M.I. (1980) Variable thyrotropin response to thyrotropin-releasing hormone after small decreases in plasma free thyroid hormone concentrations in patients with nonthyroidal diseases. Journal of Clinical Investigation, **66**, 451-456.

Matzuk, M.M., Keene, J.L. & Boime, I. (1989) Site specificity of the chorionic gonadotropin N-linked oligosaccharides in signal transduction. Journal of Biological Chemistry, **264**, 2409-2414.

Melby, J.C. & Spink, W.W. (1958) Comparative studies on adrenal cortical function and cortisol metabolism in healthy adults and in patients with shock due to infection. Journal of Clinical Investigation, **37**, 1791-1798.

Melmed, S., Geola, F.L., Reed, A.W., Pekary, E., Park, J. & Hershman, J.M. (1982) A comparison of methods for assessing thyroid function in nonthyroidal illness. Journal of Clinical Endocrinology and Metabolism, **54**, 300-306.

Mendelson, J.H. & Mello, N.K. (1975) Plasma testosterone levels during chronic heroin use and protracted abstinence. A study of Hong Kong addicts. Clinical Pharmacology and Therapeutics, **17**, 529-533.

Miller, R.G., Rubin, R.T., Clark, B.R., Crawford, W.R. & Arthur, R.J. (1970) The stress of aircraft carrier landings. 1. Corticosteroid responses in naval aviators. Psychosomatic Medicine, **32**, 581-588.

Miller, S. & Mason, H.L. (1945) The excretion of 17-ketosteroids by diabetics. Journal of Clinical Endocrinology, **5**, 220-225.

Miyabo, S., Hisada, T., Asato, T., Mizushima, N. & Ueno, K. (1976) Growth hormone and cortisol responses to psychological stress: comparison of normal and neurotic subjects. Journal of Clinical Endocrinology and Metabolism, **42**, 1158-1162.

Moll, G.W. & Rosenfield, R.L. (1979) Testosterone binding and free plasma androgen concentrations under physiological conditions: characterization by flow dialysis techniques. Journal of Clinical Endocrinology and Metabolism, **49**, 730-736.

Moncrief, J.W., Nolph, K.D., Rubin, J. & Popovich, R.P. (1978) Additional experience with continuous ambulatory peritoneal dialysis (CAPD). Transactions of the American Society for Artificial Internal Organs, **24**, 476-483.

Moore, F.D., Steenberg, R.W., Ball, M.R., Wilson, G.M. & Myrden, J.A. (1955) Studies in surgical endocrinology. 1. The urinary excretion of 17-hydroxycorticoids, and associated metabolic changes, in cases of soft tissue trauma of varying severity and in bone trauma. Annals of Surgery, **141**, 145-174.

Mooradian, A.D., Pamplona, D.M., Viosca, S.P. & Korenman, S.G. (1988) Effect of free fatty acids on the bioavailability of plasma testosterone and dihydrotestosterone. Journal of Steroid Biochemistry, **29**, 369-370.

Morley, J.E., Distiller, L.A., Sagel, J., Kok, S.H., Kay, G., Carr, P. & Katz, M. (1977) Hormonal changes associated with testicular atrophy and gynaecomastia in patients with leprosy. Clinical Endocrinology, **6**, 299-303.

Mowat, N.A.G., Edwards, C.R.W., Fisher, R., McNeilly, A.S., Green, J.R.B. & Dawson, A.M. (1976) Hypothalamic-pituitary-gonadal function in men with cirrhosis of the liver. Gut, **17**, 345-350.

Murray, D. (1967) Cortisol binding to plasma proteins in man in health, stress and at death. Journal of Endocrinology, **39**, 571-591.

Naftolin, F., Yen, S.S.C. & Tsai, C.C. (1972) Rapid cycling of plasma gonadotrophins in normal men as demonstrated by frequent sampling. Nature [New Biology], 236, 92-93.

Nakashima, A., Koshiyama, K., Uozumi, T., Monden, Y., Hamanaka, Y., Kurachi, K., Aono, T., Mizutani, S. & Matsumoto, K. (1975) Effects of general anaesthesia and severity of surgical stress on serum LH and testosterone in males. Acta Endocrinologica (Copenh), 78, 258-269.

Nankin, H.R. & Troen, P. (1971) Repetitive luteinizing hormone elevations in serum of normal men Journal of Clinical Endocrinology and Metabolism, 33, 558-560.

Noel, G.L., Dimond, R.L., Earll, J.M. & Frantz, A.G. (1976) Prolactin, thyrotropin and growth hormone release during stress associated with parachute jumping. Aviation Space and Environmental Medicine, 47, 543-547.

Nolph, K.D. (1977) Short dialysis, middle molecules, and uremia. Annals of Internal Medicine, 86, 93-97.

Oppenheimer, J.H., Schwartz, H.L., Mariash, C.N. & Kaiser, F.E. (1982) Evidence for a factor in the sera of patients with nonthyroidal disease which inhibits iodothyronine binding by solid matrices, serum proteins, and rat hepatocytes. Journal of Clinical Endocrinology and Metabolism, 54, 757-766.

Pang, S., Legido, A., Levine, L.S., Temeck, J.W. & New, M.I. (1987) Adrenal androgen response to metyrapone, adrenocorticotropin and corticotropin-releasing hormone stimulation in children with hypopituitarism. Journal of Clinical Endocrinology and Metabolism, 65, 282-289.

Pardridge, W.M. & Mietus, L.J. (1979) Transport of steroid hormones through the rat blood-brain barrier. Journal of Clinical Investigation, 64, 145-154.

Pardridge, W.M., Slag, M.F., Morley, J.E., Elson, M.K., Shafer, R.B. & Mietus, L.J. (1981) Hepatic bioavailability of serum thyroid hormones in nonthyroidal illness. Journal of Clinical Endocrinology and Metabolism, 53, 913-916.

Parker, C.R. & Baxter, C.R. (1985) Divergence in adrenal steroid secretory pattern after thermal injury in adult patients. Journal of Trauma, 25, 508-510.

Parker, L.N., Levin, E.R. & Lifrak, E.T. (1985) Evidence for adrenocortical adaptation to severe illness. Journal of Clinical Endocrinology and Metabolism, 60, 947-952.

Parker, L.N. & Odell, W.D. (1980) Control of adrenal androgen secretion. Endocrine Reviews, 1, 392-410.

Payne, R.B. (1982) Clinically significant effect of protein concentration on ion-selective electrode measurements of ionised calcium. Annals of Clinical Biochemistry, 19, 233-237.

Pinter, E.J., Tolis, G., Guyda, H. & Katsarkas, A. (1979) Hormonal and free fatty acid changes during strenuous flight in novices and trained personnel. Psychoneuroendocrinology, 4, 79-82.

Pisano, J.J. (1960) A simple analysis for normetanephrine and metanephrine in urine. Clinica Chimica Acta, 5, 406-414.

Plant, T.M. & Dubey, A.K. (1984) Evidence from the rhesus monkey (Macaca mulatta) for the view that negative feedback control of luteinizing hormone secretion by the testis is mediated by a deceleration of hypothalamic gonadotropin-releasing hormone pulse frequency. Endocrinology, **115**, 2145-2153.

Plant, T.M., Krey, L.C., Moossy, J., McCormack, J.T., Hess, D.L. & Knobil, E. (1978) The arcuate nucleus and the control of gonadotropin and prolactin secretion in the female rhesus monkey (Macaca mulatta). Endocrinology, **102**, 52-62.

Plymate, S.R., Vaughan, G.M., Mason, A.D. & Pruitt, B.A. (1987) Central hypogonadism in burned men. Hormone Research, **27**, 152-158.

Quint, A.R. & Kaiser, F.E. (1985) Gonadotropin determinations and thyrotropin-releasing hormone and luteinising hormone-releasing hormone testing in critically ill postmenopausal women with hypothyroxinemia. Journal of Clinical Endocrinology and Metabolism, **60**, 464-471.

Raymond, L.W., Sode, J. & Tucci, J.R. (1972) Adrenocortical response to non-exhaustive muscular exercise. Acta Endocrinologica (Copenh), **70**, 73-80.

Reader, S.J.C., Robertson, W.R. & Diczfalusy, E. (1983) Microheterogeneity of luteinizing hormone in pituitary glands from women of pre- and post-menopausal age. Clinical Endocrinology, **19**, 355-363.

Reame, N.E., Sauder, S.E., Case, G.D., Kelch, R.P. & Marshall, J.C. (1985) Pulsatile gonadotropin secretion in women with hypothalamic amenorrhoea: evidence that reduced frequency of gonadotrophin-releasing hormone secretion is the mechanism of persistent anovulation. Journal of Clinical Endocrinology and Metabolism, **61**, 851-858.

Reame, N.E., Sauder, S.E., Kelch, R.P. & Marshall J.C. (1984) Pulsatile gonadotropin secretion during the human menstrual cycle: evidence for altered frequency of gonadotropin-releasing hormone secretion. Journal of Clinical Endocrinology and Metabolism, **59**, 328-337.

Refsum, H.E. & Strömme, S.B. (1979) Serum thyroxine, triiodothyronine and thyroid stimulating hormone after prolonged heavy exercise. Scandinavian Journal of Clinical and Laboratory Investigation, **39**, 455-459.

Reid, I.R., Ibbertson, H.K., France, J.T. & Pybas, J. (1985) Plasma testosterone concentrations in asthmatic men treated with glucocorticoids. British Medical Journal, **291**, 574.

Remes, K., Kuoppasalmi, K. & Adlercreutz, H. (1979) Effect of long-term physical training on plasma testosterone, androstenedione, luteinizing hormone and sex-hormone-binding globulin capacity. Scandinavian Journal of Clinical and Laboratory Investigation, **39**, 743-749.

Rivier, C. & Vale, W. (1989) In the rat, interleukin-1α acts at the level of the brain and the gonads to interfere with gonadotropin and sex steroid secretion. Endocrinology, **124**, 2105-2109.

Robertson, G.M., Moore, E.W., Switz, D.M., Sizemore, G.W. & Estep, H.L. (1976) Inadequate parathyroid response in acute pancreatitis. New England Journal of Medicine, **294**, 512-516.

Robertson, W.R., Lambert, A. & Loveridge, N. (1987) The role of modern bioassays in clinical endocrinology. Clinical Endocrinology, 27, 259-278.

Rocha, D.M., Santeusanio, F., Faloona, G.R. & Unger, R.H. (1973) Abnormal pancreatic alpha-cell function in bacterial infections. New England Journal of Medicine, 288, 700-703.

Rodger, R.S.C., Morrison, L., Dewar, J.H., Wilkinson, R., Ward, M.K. & Kerr, D.N.S. (1985) Loss of pulsatile luteinizing hormone secretion in men with chronic renal failure. British Medical Journal, 291, 1598-1600.

Rogol, A.D., Veldhuis, J.D., Williams, F.A. & Johnson, M.L. (1984) Pulsatile secretion of gonadotropins and prolactin in male marathon runners. Relation to the endogenous opiate system. Journal of Andrology, 5, 21-27.

Romijn, J.A. & Wiersinga, W.M. (1990) Decreased nocturnal surge of thyrotropin in nonthyroidal illness. Journal of Clinical Endocrinology and Metabolism, 70, 35-42.

Rose, R.M., Bourne, P.G., Poe, R.O., Mougey, E.H., Collins, D.R. & Mason, J.W. (1969) Androgen responses to stress. II. Excretion of testosterone, epitestosterone, androsterone and etiocholanolone during basic combat training and under threat of attack. Psychosomatic Medicine, 31, 418-436.

Rose, R.M., Poe, R.O. & Mason, J.W. (1968) Psychological state and body size as determinants of 17-OHCS excretion. Archives of Internal Medicine, 121, 406-413.

Rosner, W. (1972) A simplified method for the quantitative determination of testosterone-estradiol binding globulin activity in human plasma. Journal of Clinical Endocrinology and Metabolism, 34, 983-988.

Ross, R.J.M., Goodwin, F.J., Houghton, B.J. & Boucher, B.J. (1985) Alteration of pituitary-thyroid function in patients with chronic renal failure treated by haemodialysis or continuous ambulatory peritoneal dialysis. Annals of Clinical Biochemistry, 22, 156-160.

Rubin, R.T., Miller, R.G., Clark, B.R., Poland, R.E. & Arthur, R.J. (1970) The stress of aircraft carrier landings. II. 3-methoxy-4-hydroxyphenylglycol excretion in naval aviators. Psychosomatic Medicine, 32, 589-597.

Rudman, D., Fleischer, A.S., Kutner, M.H. & Raggio, J.F. (1977) Suprahypophyseal hypogonadism and hypothyroidism during prolonged coma after head trauma. Journal of Clinical Endocrinology and Metabolism, 45, 747-754.

Sairam, M.R. & Bhargavi, G.N. (1985) A role for the glycosylation of the α subunit in transduction of biological signal in glycoprotein hormones. Science, 229, 65-67.

Samuels, H.H., Forman, B.M., Horowitz, Z.D. & Ye, Z-S. (1988) Regulation of gene expression by thyroid hormone. Journal of Clinical Investigation, 81, 957-967.

Sandberg, A.A., Eik-Nes, K., Migeon, C.J. & Samuels, L.T. (1956) Metabolism of adrenal steroids in dying patients. Journal of Clinical Endocrinology, 16, 1001-1016.

Santen, R.J. & Bardin, C.W. (1973) Episodic luteinizing hormone secretion in man. Pulse analysis, clinical interpretation, physiologic mechanisms. Journal of Clinical Investigation, 52, 2617-2628.

Schalch, D.S. (1967) The influence of physical stress and exercise on growth hormone and insulin secretion in man. Journal of Laboratory and Clinical Medicine, 69, 256-269.

Schalch, D.S., Gonzalez-Barcena, D., Kastin, A.J., Landa, L., Lee, L.A., Zamora, M.T. & Schally, A.V. (1975) Plasma gonadotrophins after administration of LH-releasing hormone in patients with renal or hepatic failure. Journal of Clinical Endocrinology and Metabolism, 41, 921-925.

Schally, A.V., Arimura, A., Kastin, A.J., Matsuo, H., Baba, Y., Nair, R.M.G. & Debeljuk, L. (1971) Gonadotropin-releasing hormone: one polypeptide regulates release of luteinizing and follicle-stimulating hormones. Science, 173, 1036-1038.

Schöffling, K., Federlin, K., Ditschuneit, H. & Pfeiffer, E.F. (1963) Disorders of sexual function in male diabetics. Diabetes, 12, 519-527.

Schürmeyer, T., Jung, K. & Nieschlag, E. (1984) The effect of an 1100 km run on testicular, adrenal and thyroid hormones. International Journal of Andrology, 7, 276-282.

Schussler, G.C., Schaffner, F. & Korn, F. (1978) Increased thyroid hormone binding and decreased free hormone in chronic active liver disease. New England Journal of Medicine, 299, 510-515.

Selgas, R., Albero, R., Beberide, J.M., Reiber, K., Gonzalez-Gomedo, P., Mendez, J. & Sunchez-Sicilia, L. (1983) Evaluation of thyroid function in patients treated with continuous ambulatory peritoneal dialysis (CAPD). Peritoneal Dialysis Bulletin, 3, 25-29.

Selye, H. (1936) A syndrome produced by diverse nocuous agents. Nature, 138, 32.

Selye, H. (1946) The general adaptation syndrome and the diseases of adaptation. Journal of Clinical Endocrinology, 6, 117-230.

Semple, C.G., Beastall, G.H., Henderson, I.S., Thomson, J.A. & Kennedy, A.C. (1982a) Thyroid function and continuous ambulatory peritoneal dialysis. Nephron, 32, 249-252.

Semple, C.G., Beastall, G.H., Henderson, I.S., Thomson, J.A. & Kennedy, A.C. (1982b) The pituitary-testicular axis of uraemic subjects on haemodialysis and continuous ambulatory peritoneal dialysis. Acta Endocrinologica (Copenh), 101, 464-467.

Semple, C.G., Gray, C.E. & Beastall, G.H. (1987a) Male hypogonadism - a non-specific consequence of illness. Quarterly Journal of Medicine, 64, 601-607.

Semple, C.G., Gray, C.E. & Beastall, G.H. (1987b) Adrenal androgens and illness Acta Endocrinologica (Copenh), 116, 155-160.

Semple, C.G., Gray, C.E. & Beastall, G.H. (1988b) Androgen levels in men with diabetes mellitus. Diabetic Medicine, 5, 122-125.

Semple, C.G., Gray, C.E. , Borland, W., Espie C. & Beastall, G.H. (1988). Endocrine effects of examination stress. Clinical Science, **74**, 255-259.

Semple, C.G., Robertson, W.R., Mitchell, R., Gordon, D., Gray, C.E., Beastall, G.H. & Reid, W.H. (1987c) Mechanisms leading to hypogonadism in men with burns injuries. British Medical Journal, **295**, 403-407.

Semple, C.G., Thomson, J.A. & Beastall, G.H. (1985) Endocrine responses to marathon running. British Journal of Sports Medicine, **19**, 148-151.

Semple, C.G., Thomson, J.A., Stark, A.N., McDonald, M. & Beastall, G.H. (1982c) Trilostane and the normal hypothalamic-pituitary-adrenocortical axis. Clinical Endocrinology, **17**, 569-575.

Semple, C.G., Weir, S.W., Thomson, J.A. & Beastall, G.H. (1982d) Trilostane and the normal hypothalamic-pituitary-testicular axis. Clinical Endocrinology, **17**, 99-102.

Semple, P.d'A., Beastall, G.H., Watson, W.S. & Hume, R. (1981) Hypothalamic-pituitary dysfunction in respiratory hypoxia. Thorax, **36**, 605-609.

Semple, P.d'A., Semple, C.G., Beastall, G.H., Brown, T.M., Watson, W.S. & Hume, R. (1985) Endocrine studies in cyanotic congenital heart disease. Scottish Medical Journal, **30**, 25-29.

Shahwan, M.M., Spathis, G.S., Fry, D.E., Wood, P.J. & Marks, V. (1978) Differences in pituitary and testicular function between diabetic patients on insulin and oral anti-diabetic agents. Diabetologia, **15**, 13-17.

Silverberg, D.S., Ulan, R.A., Fawcett, D.M., Dossetor, J.B., Grace, M. & Bettcher, K. (1973) Effects of chronic hemodialysis on thyroid function in chronic renal failure. Canadian Medical Association Journal, **109**, 282-286.

Simmonian, M.H. & Gill, G.N. (1981) Regulation of the fetal human adrenal cortex: effects of adrenocorticotrophin on growth and function of monolayer cultures of fetal and definitive zone cells. Endocrinology, **108**, 1769-1779.

Slag, M.F., Morley, J.E., Elson, M.K., Crowson, T.W., Nuttall, F.Q. & Shafer, R.B. (1981) Hypothyroxinemia in critically ill patients as a predictor of high mortality. Journal of the American Medical Association, **245**, 43-45.

Smeds, S., Kagedal, B., Liedén, G. & Liljedahl, S-O. (1981) Thyroid function after thermal trauma. Scandinavian Journal of Plastic and Reconstructive Surgery, **15**, 141-148.

Snyder, P.J., Bashey, H.M., Montecinos, A., Odell, W.D. & Spitalnik, S.L. (1989) Secretion of multiple forms of luteinizing hormone by cultured fetal human pituitary cells. Journal of Endocrinology and Metabolism, **68**, 1033-1038.

Spector, D.A., Davis, P.J., Helderman, J.H., Bell, B. & Utiger, R.D. (1976) Thyroid function and metabolic state in chronic renal failure. Annals of Internal Medicine, **85**, 724-730.

St-Arnaud, R., Lachance, R., Kelly, S.J., Belanger, A., Dupont, A. & Labrie, F. (1986) Loss of luteinizing hormone bioactivity in patients with prostatic cancer treated with an LHRH agonist and a pure antiandrogen. Clinical Endocrinology, **24**, 21-30.

Stewart-Bentley, M., Gans, D. & Horton, R. (1974) Regulation of gonadal function in uremia. Metabolism, 23, 1065-1072.

Strollo, F., Harlin, J., Hernandez-Montes, H., Robertson, D.M., Zaidi, A.A.& Diczfalusy, E. (1981) Qualitative and quantitative differences in the isoelectrofocussing profile of biologically active lutropin in the blood of normally menstruating and post-menopausal women. Acta Endocrinologica (Copenh), 97, 166-175.

Surks, M.I., Hupart, K.H., Pan, C. & Shapiro, L.E. (1988) Normal free thyroxine in critical nonthyroidal illnesses measured by ultrafiltration of undiluted serum and equilibrium dialysis. Journal of Clinical Endocrinology and Metabolism, 67, 1031-1039.

Sutton, J.R., Coleman, M.J. & Casey, J.H. (1978) Testosterone production rate during exercise. In 3rd International Symposium on Biochemistry of Exercise, Book 3, eds. Landry, F. & Orban, W.A.R., pp 227-234. Symposia Specialists: Miami.

Sutton, J.R., Coleman, M.J., Casey, J. & Lazarus, L. (1973) Androgen responses during physical exercise. British Medical Journal, 1, 520-522.

Szpunar, W.E., Blair, A.J. & McCann, D.S. (1977) Plasma androgen concentrations in diabetic women. Diabetes, 26, 1125-1129.

Szyfelbein, S.K., Drop, L.J. & Martyn, J.A.J. (1981) Persistent ionized hypocalcemia during resuscitation and recovery phases of body burns. Critical Care Medicine, 9, 454-458.

Taché, Y., Ducharme, J.R., Charpenet, G., Haouz, F., Saej, J. & Collu, R. (1980) Effect of chronic intermittent immobilization stress on hypophyso-gonadal function of rats. Acta Endocrinologica (Copenh), 93, 168-174.

Talbot, J.A., Rodger, R.S.C., Shalet, S.M., Littley, M. & Robertson, W.R. The pulsatile secretion of bioactive luteinising hormone in normal adult men. Acta Endocrinologica (Copenh), (in press).

Talwar, K.K., Sawhney, R.C. & Rastogi, G.K. (1977) Serum levels of thyrotropin, thyroid hormones and their response to thyrotropin releasing hormone in infective febrile illnesses. Journal of Clinical Endocrinology and Metabolism, 44, 398-403.

Taylor, B., Sibbald, W.J., Edmonds, M.W., Holliday, R.L. & Williams, C. (1978) Ionized hypocalcemia in critically ill patients with sepsis. Canadian Journal of Surgery, 21, 429-433.

Terjung, R.L. & Tipton, C.M. (1971) Plasma thyroxine and thyroid-stimulating hormone levels during submaximal exercise in humans. American Journal of Physiology, 220, 1840-1845.

Thysen, B., Gatz, M., Freeman, R., Alpert, B.E. & Chartyan, C. (1983) Serum thyroid hormone levels in patients on continuous ambulatory peritoneal dialysis and regular hemodialysis. Nephron, 33, 49-52.

Tsatsoulis, A., Shalet, S.M., Talbot, A. & Robertson, W.R. (1989) Quantitative and qualitative changes in LH secretion following pulsatile GnRH therapy in a man with idiopathic hypogonadotrophic hypogonadism. Clinical Endocrinology, 30, 167-175.

Tsatsoulis, A., Shalet, S.M., Richardson, P. & Robertson, W.R. (1990) Testosterone reduces the bioactivity of luteinizing hormone (LH) in man. Clinical Endocrinology, 32, 73-82.

Vagenakis, A.G., Burger, A., Portnay, G.I., Rudolph, M., O'Brian, J.T., Azizi, F., Arky. R.A., Nicod, P., Ingbar, S.H. & Braverman, L.E. (1975) Diversion of peripheral thyroxine metabolism from activating to inactivating pathways during complete fasting. Journal of Clinical Endocrinology and Metabolism, 41, 191-194.

Vagenakis, A.G., Downs, P., Braverman, L.E., Burger, A. & Ingbar, S.H. (1973) Control of thyroid hormone secretion in normal subjects receiving iodides. Journal of Clinical Investigation, 52, 528-532.

Vaitukaitis, J.L., Dale, S.L. & Melby, J.C. (1969) Role of ACTH in the secretion of free dehydroepiandrosterone and its sulfate ester in man. Journal of Clinical Endocrinology and Metabolism, 29, 1443-1447.

Van Damme, M-P., Robertson, D.M. & Diczfalusy, E. (1974) An improved in vitro bioassay method for measuring luteinizing hormone (LH) activity using mouse leydig cell preparations. Acta Endocrinologica (Copenh), 77, 655-671.

Van Hall, E.V., Vaitukaitis, J.L., Ross, G.T., Hickman, J.W. & Ashwell, G. (1971) Immunological and biological activity of HCG following progressive desialylation. Endocrinology, 88, 456-464.

Van Thiel, D.H., Lester, R. & Sherins, R.J. (1974) Hypogonadism in alcoholic liver disease: evidence for a double defect. Gastroenterology, 67, 1188-1199.

Van Thiel, D.H. & Loriaux, D.L. (1979) Evidence for an adrenal origin of plasma estrogens in alcoholic men. Metabolism, 28, 536-541.

Vaughan, G.M., Becker, R.A., Allen, J.P., Goodwin, C.W., Pruitt, B.A. & Mason, A.D. (1982) Cortisol and corticotrophin in burned men. Journal of Trauma, 22, 263-272.

Vaughan, G.M., Mason, A.D., McManus, W.F. & Pruitt, B.A. (1985) Alterations of mental status and thyroid hormones after thermal injury. Journal of Clinical Endocrinology and Metabolism, 60, 1221-1225.

Veldhuis, J.D. & Dufau, M.L. (1987) Estradiol modulates the pulsatile secretion of biologically active luteinizing hormone in man. Journal of Clinical Investigation, 80, 631-638.

Veldhuis, J.D., Evans, W.S., Demers, L.M., Thorner, M.O., Wakat, D. & Rogol, A.D. (1985) Altered neuroendocrine regulation of gonadotropin secretion in women distance runners. Journal of Clinical Endocrinology and Metabolism, 61, 557-563.

Veldhuis, J.D., Evans, W.S., Johnson, M.L., Wills, M.R. & Rogol, A.D. (1986) Physiological properties of the luteinizing hormone pulse signal: impact of intensive and extended venous sampling parodigms on its characterisation in healthy men and women. Journal of Clinical Endocrinology and Metabolism, 62, 881-891.

Veldhuis, J.D., Johnson, M.L. & Dufau, M.L. (1987) Preferential release of bioactive luteinizing hormone in response to endogenous and low dose exogenous gonadotropin-releasing hormone pulses in man. Journal of Clinical Endocrinology and Metabolism, 64, 1275-1282.

Veldhuis, J.D., King, J.C., Urban, R.J., Rogol, A.D., Evans, W.S., Kolp, L.A. & Johnson, M.L. (1987) Operating characteristics of the male hypothalamic-pituitary-gonadal axis: pulsatile release of testosterone and follicle stimulating hormone and their temporal coupling with luteinizing hormone. Journal of Clinical Endocrinology and Metabolism, 65, 929-941.

Veldhuis, J.D., Rogol, A.D. & Johnson, M.L. (1983) Endogenous opiates modulate the pulsatile secretion of biologically active luteinizing hormone in man. Journal of Clinical Investigation, 72, 2031-2040.

Vermeulen, A., Verdonck, L., Van der Straeten, M. & Orie, N. (1969) Capacity of the testosterone-binding globulin in human plasma and influence of specific binding of testosterone on its metabolic clearance rate. Journal of Clinical Endocrinology , 29, 1470-1480.

Vogel, A.V., Peake, G.T. & Rada, R.T. (1985) Pituitary-testicular dysfunction in burned men. Journal of Clinical Endocrinology and Metabolism, 60, 658-665.

Wade, C.E., Lindberg, J.S., Cockrell, J.L., Lamiell, J.M., Hunt, M.M., Ducey, J. & Jurney, T.H. (1988) Upon-admission adrenal steroidogenesis is adapted to the degree of illness in intensive care unit patients. Journal of Clinical Endocrinology and Metabolism, 67, 223-227.

Wallace, E.Z., Rosman, P., Toshav, N., Sacerdote, A. & Balthazar, A. (1980) Pituitary-adrenocortical function in chronic renal failure: studies of episodic secretion of cortisol and dexamethasone supprressibility. Journal of Clinical Endocrinology and Metabolism, 50, 46-51.

Wallenstein, S., Zucker, C.L. & Fleiss, J.L. (1980) Some statistical methods useful in circulation research. Circulation Research, 47, 1-9.

Wang, C., Chan, V., Tse, T.F. & Yeung, R.T.T. (1978a) Effect of acute myocardial function on pituitary-testicular function. Clinical Endocrinology, 9, 249-253.

Wang, C., Chan, V. & Yeung, R.T.T. (1978b) Effect of surgical stress on pituitary-testicular function. Clinical Endocrinology, 9, 255-266.

Warner, B.A., Dufau, M.L. & Santen, R.J. (1985) Effects of ageing and illness on the pituitary testicular axis in men: qualitative as well as quantitative changes in luteinizing hormone. Journal of Clinical Endocrinology and Metabolism, 60, 263-268.

Warren, M.P., Sirris, E.S. & Petrovich, C. (1977) The influence of severe illness on gonadotrophin secretion in the postmenopausal female. Journal of Clinical Endocrinology and Metabolism, 45, 99-104.

Webb, R., Baxter, G., McBride, D., Nordblom, G.D. & Shaw, M.P.K. (1985) The measurement of testosterone and oestradiol-17β using iodinated tracers and incorporating an affinity chromatography extraction procedure. Journal of Steroid Biochemistry, 23, 1043-1051.

Weber, H.G., Schildknecht, J. & Kesselring, P. (1971) A new equilibrium dialysing system. American Laboratories (Fairfield, Connecticut), Oct, 15-21.

Wehman, R.E., Gregerman, R.I., Burns, W.H., Saral, R. & Santos, G.W. (1985) Suppression of thyrotropin in the low-thyroxine state of severe nonthyroidal illness. New England Journal of Medicine, 312, 546-552.

Wheatley, T., Clark, P.M.S., Clark, J.D.A., Raggatt, P.R., Evans, D.B. & Holder, R. (1987) Pulsatility of luteinising hormone in men with chronic renal failure: abnormal rather than absent. British Medical Journal, 294, 482.

Wheeler, G.D., Wall, S.R., Belcastro, A.N., Conger, P. & Cumming, D.C. (1986) Are anorexic tendencies prevalent in the habitual runner? British Journal of Sports Medicine, 20, 77-81.

Wheeler, G.D., Wall, S.R., Belcastro, A.N. & Cumming, D.C. (1984) Reduced serum testosterone and prolactin levels in male distance runners. Journal of the American Medical Association, 252, 514-516.

Wiersinga, W.M., Lie, K.I. & Touber, J.L. (1981) Thyroid hormones in acute myocardial infarction. Clinical Endocrinology, 14, 367-374.

Williams, G.R., Franklyn, J.A., Neuberger, J.M. & Sheppard, M.C. (1989) Thyroid hormone receptor expression in the "sick euthyroid" syndrome. Lancet, II, 1477-1481.

Wilmore, D.W., Orcutt, T.W., Mason, A.D. & Pruitt, B.A. (1975) Alterations in hypothalamic function following thermal injury. Journal of Trauma, 15, 697-703.

Wilson, H., Lovelace, J.R. & Hardy, J.D. (1955) The adrenocortical response to extensive burns in man. Annals of Surgery, 141, 175-184.

Wilson, R.C., Kesner, J.S., Kaufman, J-M., Uemara, T., Akema, T. & Knobil, E. (1984) Central electrophysiologic correlates of pulsatile luteinizing hormone secretion in the rhesus monkey. Neuroendocrinology, 39, 256-260.

Winer, R.L., Rajudin, M.M., Skowsky, W.R. & Parker, L.N. (1982) Preservation of normal adrenal androgen secretion in end stage renal disease. Metabolism, 31, 269-273.

Winters, S.J. & Troen, P. (1983) A reexamination of pulsatile luteinizing hormone secretion in primary testicular failure. Journal of Clinical Endocrinology and Metabolism, 57, 432-435.

Winters, S.J. & Troen, P. (1984) Altered pulsatile secretion of luteinizing hormone in hypogonadal men with hyperprolactinaemia. Clinical Endocrinology, 21, 257-263.

Woeber, K.A. & Maddux, B.A. (1981) Thyroid hormone binding in nonthyroidal illness. Metabolism, 30, 412-416.

Wong, E.T., Bradley, S.G. & Schultz, A.L. (1981) Elevations of thyroid-stimulating hormone during acute non-thyroidal illness. Archives of Internal Medicine, 141, 873-875.

Woolf, P.D., Hamill, R.W., McDonald, J.V., Lee, L.A. & Kelly, M. (1985) Transient hypogonadotrophic hypogonadism caused by critical illness. Journal of Clinical Endocrinology and Metabolism, 60, 444-450.

Woolf, P.D., Hamill, R.W., McDonald, J.V., Lee, L.A. & Kelly, M. (1986) Transient hypogonadotropic hypogonadism after head trauma: effects on steroid precursors and correlation with sympathetic nervous system activity. Clinical Endocrinology, 25, 265-274.

Yamaji, T. & Ibayashi, H. (1969) Plasma dehydroepiandrosterone sulfate in normal and pathological conditions. Journal of Clinical Endocrinology, 29, 273-278.

Yamaji, T., Ibayashi, M., Takaku, F., Itabashi, A., Katayama, S. & Ishii, J. (1987) Serum dehydroepiandrosterone sulfate concentrations in secondary adrenal insufficiency. Journal of Clinical Endocrinology and Metabolism, 65, 448-451.

Young, R.J., Ismail, A.H., Bradley, A. & Corrigan, D.L. (1976) Effect of prolonged exercise on serum testosterone levels in adult men. British Journal of Sports Medicine, 10, 230-235.

Zipser, R.D., Davenport, M.W., Martin, K.L., Tuck, M.L., Warner, N.E., Swinney, R.R., Davis, C.L. & Horton, R. (1981) Hyperreninemic hypoaldosteronism in the critically ill: a new entity. Journal of Clinical Endocrinology and Metabolism, 53, 867-873.

Zuckerman, M., Persky, H., Hopkins, T.R., Murtaugh, T., Basu, G.K. & Schilling, M. (1966) Comparison of stress effects of perceptual and social isolation. Archives of General Psychiatry, 14, 356-365.

Zumoff, B., Bradlow, H.L., Gallagher, T.F. & Hellman, L. (1971) Decreased conversion of androgens to normal 17-ketosteroid metabolites: a non-specific consequence of illness. Journal of Clinical Endocrinology and Metabolism, 32, 824-832.

Zumoff, B., Walter, L., Rosenfeld, R.S., Strain, J.J., Degan, K., Strain, G.W., Levin, J. & Fukushima, D. (1980) Subnormal plasma androgen levels in men with uremia. Journal of Clinical Endocrinology and Metabolism, 51, 801-805.

Zung, W.W.K. (1971) A rating instrument for anxiety disorders. Psychosomatics, 12, 371-379.

PUBLICATIONS RESULTING FROM WORK INCLUDED IN THIS THESIS

Semple, C.G., Beastall, G.H., Henderson, I.S., Thomson, J.A. & Kennedy, A.C. (1982) The pituitary-testicular axis of uraemic subjects on haemodialysis and continuous ambulatory peritoneal dialysis. Acta Endocrinologica (Copenh), 101, 464-467.

Semple, C.G., Beastall, G.H., Henderson, I.S., Thomson, J.A. & Kennedy, A.C. (1982) Thyroid function and continuous ambulatory peritoneal dialysis. Nephron, 32, 249-252.

Semple, C.G., Thomson, J.A. & Beastall G.H. (1985) Endocrine responses to marathon running. British Journal of Sports Medicine, 19, 148-151.

Semple, C.G. (1986) Regular review: Hormonal changes in non-endocrine disease. British Medical Journal, 293, 1049-1052.

Semple, C.G., Gray, C.E. & Beastall G.H. (1987) Adrenal androgens and illness. Acta Endocrinologica (Copenh), 116, 155-160.

Semple, C.G., Gray, C.E. & Beastall, G.H. (1987) Male hypogonadism - a non-specific consequence of illness.Quarterly Journal of Medicine, 64, 601-607.

Semple, C.G., Robertson, W.R., Mitchell, R., Gordon, D., Gray, C.E., Beastall, G.H. & Reid, W.H. (1987) Mechanisms leading to hypogonadism in men with burns injuries. British Medical Journal, 295, 403-407.

Semple, C.G., Gray, C.E., Borland, W., Espie, C. & Beastall, G.H. (1988) Endocrine effects of examination stress. Clinical Science, 74, 255-259.

Semple, C.G., Gray, C.E. & Beastall, G.H. (1988) Androgen levels in men with diabetes mellitus. Diabetic Medicine, 5, 122-125.