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A STUDY OF PITUITARY-ADRENAL HORMONAL RESPONSES IN PATIENTS TREATED FOR CHRONIC STRESS

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science in Medicine

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To my parents, Benjy and Jacki Adelsky, for their unconditional love
and steadfast support in all of my endeavours.

And

To Jonathan, Shmueli, Shimpa and Moshe Dov
Who survived



DECLARATION

I declare that this dissertation presented for the degree of MSc (Med) at the University of the Witwatersrand, Johannesburg, is my own work and has not been submitted for a degree or examination at any other university.



Shirra Leah Moch



Date

The study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand.

(Protocol No: M991116)

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ABSTRACT

Objectives: To document baseline pituitary-adrenal hormonal and related metabolic variables in 16 female patients with burnout. Then, following stress management intervention, to compare the changes with an equal number of untreated control subjects.

Methods: At monthly intervals for four months fasting blood samples were analysed for levels of cortisol, dehydroepiandrosterone sulphate, adrenocorticotrophic hormone, aldosterone and catecholamines. Other biochemical measurements included growth hormone, prolactin, insulin, glucose and lipid components. In addition, twenty-four hour urine samples were obtained for determination of free cortisol excretion. The Maslach Burnout Inventory, General Health Questionnaire-28 and Zung Depression Rating Scale were completed at each consecutive visit.

Results: The most striking finding was the reduction of urine free cortisol excretion in the patients compared with controls. Initial urinary free cortisol was significantly lower in the patients (Mean $\pm$ SEM= 47.2 $\pm$ 11.0 vs 79.0 $\pm$ 6.8 nmol/l, $p=0.02$) and remained significantly reduced at 4 months (Mean $\pm$ SEM= 44.0 $\pm$ 6.1 vs 91.1 $\pm$ 8.8 nmol/l, $p=0.0001$).

Conclusions: There is functional hypocortisolism in burnout, which is not immediately restored on stress management intervention despite clinical and psychological improvement.

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LIST OF ABBREVIATIONS

ACOD	- acyl-CoA oxidase
ACTH	- adrenocorticotrophic hormone
Acyl CS	- Acyl-Coenzyme A synthetase
acyl-CoA	- acyl-coenzyme A
AVP	- arginine-vasopressin
BMI	- body mass index
CNS	- central nervous system
CoA	- coenzyme A
COR	- conservation of resources
CPM	- counts per minute
CRH	- corticotrophin releasing hormone
DHEA	- dehydroepiandrosterone
DHEA-S	- dehydroepiandrosterone-sulphate
FFA	- free fatty acids
GAS	- general adaptation syndrome
GH	- growth hormone
GHQ-28	- general health questionnaire (28 statement version)
GR	- glucocorticoid "type II" receptors
GRE	- glucocorticoid-responsive elements
HDL-C	- high density lipoprotein cholesterol
HPA	- hypothalamic-pituitary-adrenal
HPLC	- high performance liquid chromatography

IGF-1	- insulin-like growth factor-1
IGFBP-1	- insulin-like growth factor-binding protein-1
LC	- locus coeruleus
LDL-C	- low density lipoprotein cholesterol
MB	- maximum binding
MBI	- maslach burnout inventory
MDD	- melancholic depressive disorder
mg	- milligram
mIMP	- monoclonal immunomagnetic particle
μL	- microlitres
mL	- millilitre
mmol/L	- millimoles per litre
MR	- mineralocorticoid “type I” receptors
NA	- noradrenaline
NADP	- nicotinamide adenine dinucleotide phosphate
NSB	- non-specific binding
PEG	- polyethylene glycol
PL	- prolactin
pmol/L	- pica-moles per litre
pNPP	- <i>p</i> -nitrophenyl phosphate
PTSD	- posttraumatic stress disorder
RIA	- radioimmunoassay
SNS	- sympathetic nervous system

SRIH	- somatotropin release-inhibiting hormone
TG	- triglycerides
ZDRS	- zung depression rating scale

CHAPTER 1

THE STRESS RESPONSE

1.1 Introduction

The term "stress" has been used to describe both the cause and the effect of pressure on biological systems. Although in common usage, the word has proved difficult to define scientifically since it has been applied in differing circumstances as a noun, a verb or an adjective (Kopin 1995). Jewell and Mylander (1988) wryly comment, "It seems as if stress, in addition to being itself and the result of itself, is also the cause of itself."

To rationalize terminology, Chrousos and Gold (1992) proposed the use of the terms "stressor" and "stress system response", where the stressor is the initiator, and the response is the consequence of exposure to the stressor. Stressors activate neural and hormonal mechanisms, which interact with each other to produce physiological and behavioural responses to stress, as well as adaptation to the stressful situation – the stress system response.

Current biological definitions of stress are based on the pioneering work of Walter Cannon and Hans Selye, who both initiated different

research pathways that have led to contemporary theories of the stress response. Cannon (1935) coined the term “homeostasis” to describe the tendency towards a stable equilibrium in the internal environment of an organism. He described a “critical stress” level as one that produced a “breaking strain” that resulted in failure to maintain homeostasis. He incorporated this idea in his work describing the “flight or fight” reactions produced by catecholamine secretion (1914, 1939).

Selye was inspired by Cannon's ideas on homeostasis and extended this concept to his work on the responses mediated by the hypothalamic-pituitary-adrenal-axis (HPA-axis). He proposed the theory that, in addition to specific homeostatic responses, the body responds to external stressors with a single, stereotypic “stress response” which he termed the “general adaptation syndrome” (GAS) (1946). The GAS described three stages of response: The alarm reaction was the immediate psycho-physiological response, the second stage was termed “resistance” to a continued stressor and the third stage was “exhaustion”. Selye (1956) also noted that not all stressors were harmful: some stressors stimulated the individual to accomplish goals and achieve success. This situation was called “eustress”, whilst he called detrimental stress “distress”.

Integrating elements of Cannon and Selye's theories with their own research, Chrousos and Gold (1992) defined stress as a state of

threatened homeostasis, to which the organism, in order to preserve its internal equilibrium, reacts with an array of adaptive responses. They reviewed the literature associated with stress research, including the neurobiology, endocrinology and behavioural aspects of stress. Their synopsis included a definition of stress and stressor, an explanation of the main components of the stress system, and the working hypothesis that dysregulation in the homeostatic responses of the stress system can lead to human disease.

McEwen and Stellar (1993) extended the stress definition to include the concept of allostatic load; the bodily cost of maintaining stability through change. "Stress may be defined as a threat, real or implied, to the psychological or physiological integrity of an individual; and it must be viewed in the long term as the wear and tear on the body produced by the body's reaction to life events."

To appreciate this definition in physiological terms, it is necessary to consider current research on the stress system response.

1.2 Response of the Stress System to Acute Stressors

In a review on the neuroanatomy of stress, Cullinan *et al.* (1995) clarified that the stress system in humans is located in both the central nervous system (CNS) and the periphery. In the CNS, the

hypothalamus releases corticotrophin-releasing hormone (CRH) and arginine-vasopressin (AVP), whilst the locus ceruleus (LC) and other noradrenergic (NA) cell groups in the medulla and pons initiate the sympathetic system response to stress (Stratakis and Chrousos, 1995a). The peripheral branches of the stress system include the hypothalamic-pituitary-adrenal (HPA) axis, as well as the peripheral sympathetic nervous system (SNS). Activation of the stress response, by either physical or psychological stressors, triggers CRH release from the hypothalamus, initiating the HPA cascade of adrenocorticotrophic hormone (ACTH) release from the pituitary, and cortisol release from the adrenal cortex (Dallman *et al.*, 2000). The LC/NA system prompts catecholamine release from nerves and the adrenal medulla (McCarty, 2000). The HPA-axis and LC/NA system positively regulate each other's action, so that activation of one of them promotes activation of the other as well. In addition, the stress response system is autoregulatory, with negative feedback exerted on stress mediator release both peripherally and centrally (Michelson *et al.*, 1995) (Figure 1). In initiating this biochemical response, the body equips itself to cope with the demands necessary either to deal with the stressor or retreat from the environment. Irrespective of the eventual action, once the threat of the stressor has passed, the stress system response in healthy individuals is terminated (Nesse and Young, 2000).

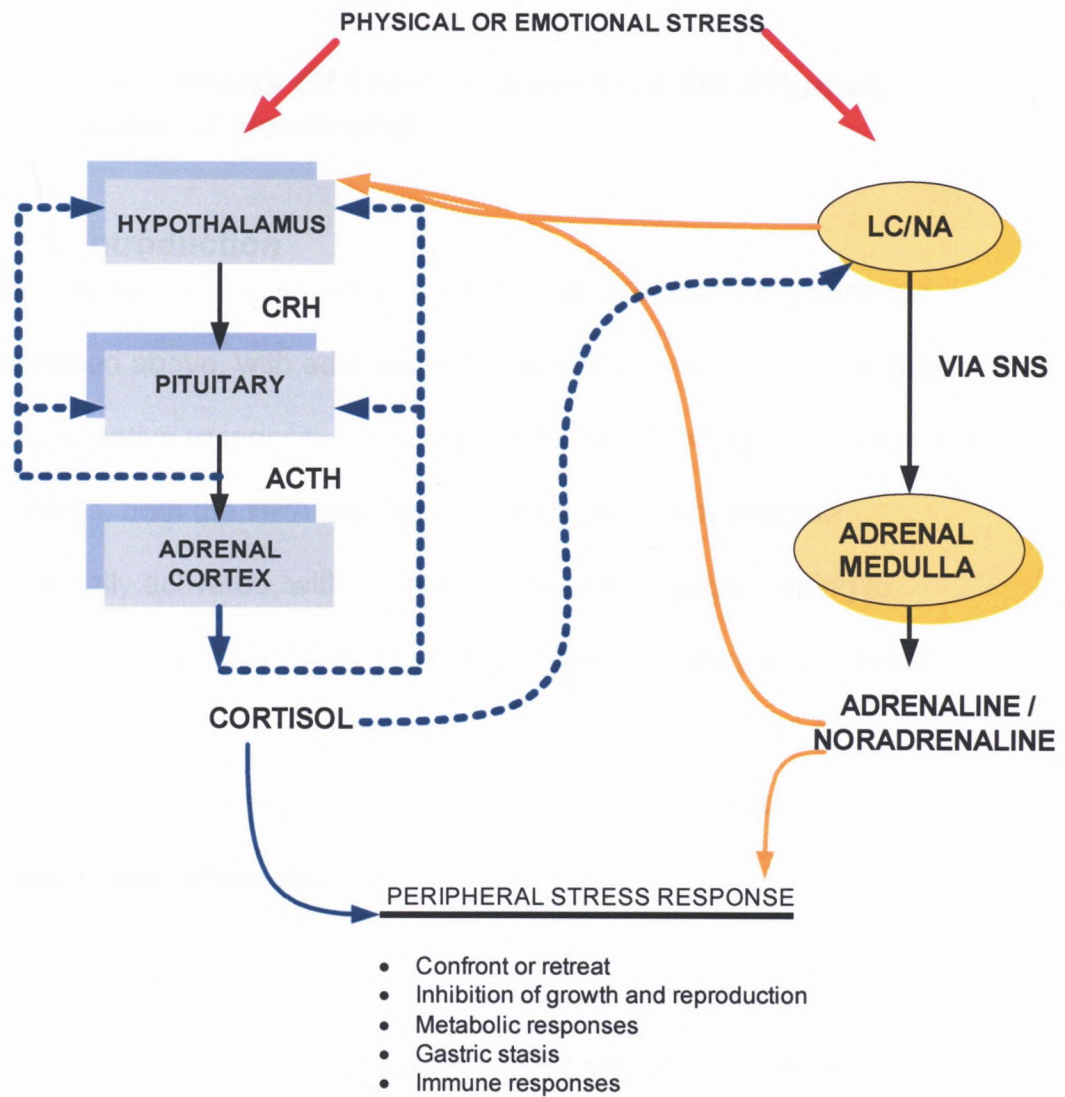


Figure 1. Diagrammatic representation of the HPA-axis and LC/NA system showing negative feedback effects (dotted lines) of cortisol at both the central and peripheral levels. Solid arrows show positive feed-forward effects on the stress system (Adapted from Stratakis and Chrousos, 1995a).

LC/NA =	locus coeruleus/noradrenergic system
SNS =	sympathetic nervous system
CRH =	corticotrophin-releasing hormone
ACTH =	adrenocorticotrophic hormone

1.3 The Effects Of Chronic Stressors On Pituitary-Adrenal Hormones

1.3.1 Introduction

The effects of an acute stressor on the stress system response are described above, with adrenaline and cortisol co-ordinating the short-term adaptive response. However, on repeated or long-term exposure to stress, both the HPA-axis and LC/NA system can become chronically activated, with excessive mediator release leading to receptor desensitisation and tissue damage (Sapolsky *et al.*, 2000).

In the following points, the consequences of exposure to chronic stressors are considered.

1.3.2 ACTH

In animal models of chronic stress it has been shown that the HPA-axis remained activated, with increased levels of ACTH (Kant *et al.*, 1987; Aguilera, 1994). In humans, CRH challenge-testing of patients with major depressive disorder (MDD) and anxiety disorders including posttraumatic stress disorder (PTSD), showed a blunted response of ACTH to CRH (Gold *et al.*, 1988a and 1988b; Buckingham *et al.*, 1992). In MDD, despite hypercortisolism, patients showed normal rather than elevated levels of ACTH. Gold *et al.* (1995) speculate that in MDD the adrenal cortex is hyperresponsive to ACTH, thus normal

circulating levels produce hypercortisolaemia. However patients with PTSD had lower than normal levels of the hormone (Yehuda, 2000). The disparity in these findings can be explained by considering the chronic stress paradigm. Although all the illnesses in the quoted studies have been used as models for chronic stress, their effects on the neuroendocrinology of the HPA-axis differ. For example, decreased ACTH response to CRH could be a sign of downregulation of pituitary CRH receptors in response to hypothalamic CRH hypersecretion (thought to be the case in MDD) (Holsboer *et al.*, 1984; Gold and Chrousos, 2002). In panic disorder, blunted response of ACTH to CRH challenge could occur consequent to increased negative feedback inhibition of the pituitary as a result of high cortisol levels (Yehuda and Nemeroff, 1994) and in PTSD, blunted ACTH response is attributed to hyperresponsivity of the pituitary to cortisol, as a direct result of an increased number of glucocorticoid receptors (GR) (Yehuda, 2002).

An interesting point has been made (Buckingham, 2000) concerning regulation of the HPA-axis in chronic stress as compared with acute stress. In animal models of chronic stress, AVP appears to be the primary driver of the HPA-axis (Scaccione *et al.*, 1991). In a human model of chronic stress (multiple sclerosis), patients displayed hypercortisolism relative to healthy control subjects. These patients also displayed a blunted response of ACTH to AVP, a finding consistent with the idea that the HPA-axis is driven by AVP in chronic

stress (Michelson *et al.*, 1994). Buckingham attributed this switch (from CRH to AVP as the driver of ACTH release in chronic stress) to disrupted feedback and compromised glucocorticoid receptor number during chronic stress episodes.

1.3.3 Cortisol

In healthy individuals cortisol secretion follows a circadian rhythm, with high levels at the end of the sleep phase in the early morning hours and a decrease over the day. The hormone is released in a pulsatile fashion in response to the short, intermittent release of ACTH from the anterior pituitary. The physiological and behavioural effects of cortisol depend on its ability to bind glucocorticoid receptors, thus alterations in the number and sensitivity of glucocorticoid receptors modulate the functioning of the HPA-axis (DeRijk *et al.*, 2002).

Two intracellular receptors for glucocorticoids have been identified: “Type I”, mineralocorticoid receptors (MR) have a high affinity for cortisol, as well as aldosterone. In contrast, “type II” glucocorticoid receptors (GR) bind strongly to synthetic steroids (e.g. dexamethasone) but have significantly lower affinity for endogenous hormones. On the basis of these differences, it is thought that MR play a primary role in mediating glucocorticoid effects under basal conditions, when hormone concentrations are low. As glucocorticoid levels rise, either in response to stress or as a function of the HPA circadian cycle, MR saturate, and GR become the chief transducer of

glucocorticoid activity and are thus the primary mediators of feedback inhibition of CRH and the HPA-axis (de Kloet, 1991).

In order to investigate aspects of HPA-axis feedback, the low-dose dexamethasone suppression test was used. In healthy subjects, administration of 1mg dexamethasone suppressed both ACTH and cortisol secretion since GR mediated negative feedback was enhanced (Kalin *et al.*, 1982). However, in chronic stress paradigms e.g. for patients with MDD, 50% failed to suppress cortisol release after dexamethasone administration (Kathol *et al.*, 1989; Gold *et al.*, 2002), indicating hyperresponsiveness in HPA-axis stress responses and concomitant increases in both ACTH and cortisol levels. According to Dallman and her colleagues (2000), the increase in HPA-axis hormones during chronic stress occurs as a result of decreases in the amplitude of the circadian rhythm through elevation of trough values.

1.3.4 Growth Hormone

The secretion of Growth Hormone (GH) is suppressed in rats exposed to repeated stressors such as immobilization, noise or exposure to ether. In addition, rats exposed to a repetitive stress respond to a novel acute stress with a reduction in GH secretion (Terry *et al.*, 1977; Aguila *et al.*, 1991).

In humans, children who are exposed to emotional deprivation or physical or psychological abuse can exhibit short stature and/or delayed puberty, known as psychosocial dwarfism. In these cases, GH secretion is reduced, but increases when the child is removed from the offending environment (Albanese *et al.*, 1994).

Long-term therapy with glucocorticoids is reported to depress the secretion of GH and to impair growth (Allen, 1996), however, the extent to which endogenous cortisol modulates the GH response to stress is unclear. In fact, acute elevation of plasma GH may occur at the onset of the stress response possibly as a result of direct stimulation of the GH gene by cortisol (through glucocorticoid-responsive elements (GREs) in its promoter region) (Burguera *et al.*, 1990).

Stratakis and Chrousos (1995b) proposed that the suppression of GH secretion resulting from prolonged activation of the HPA-axis occurs consequent to inhibition of the action of insulin-like growth factor-I (IGF-I) on target tissues. Another reported mechanism which reduced GH secretion during chronic stress was a CRH mediated increase in somatostatin (somatotropin release-inhibiting hormone SRIH) secretion (Reichlin, 1988).

1.3.5 Prolactin

Many animal studies have shown that prolactin (PL) secretion is affected by “stress” (Freeman *et al.*, 2000) – however, there is little consensus in the expected response, since different stressors provoked either an increase or decrease in PL secretion, depending on the nature of the stress and the endocrine status of the animal.

Stressors such as surgery, restraint, insulin-induced hypoglycaemia and cold precipitated a rapid, short lasting hypersecretion of PL (Gala, 1990). For ether anaesthesia, the PL response reportedly differed according to the reproductive state of the rat (Lookingland *et al.*, 1990). The literature concerning PL responses to acute immunological insults was also inconsistent. Some data indicated that exposure to endotoxin, IL-1 or IL-6 increased PL release, others showed that PL secretion was either unchanged or inhibited by these stimuli (Gala, 1990; Yirmiya *et al.*, 1991; Taylor *et al.*, 1995).

It has been suggested that acute stressors increased PL release via stimulation of prolactin releasing factors (e.g. vasoactive intestinal polypeptide) by the hypothalamus rather than suppression of the release of inhibitory agents e.g. dopamine. Also, central histaminergic and serotonergic pathways were implicated in driving the responses (Cooke *et al.*, 2004).

According to Freeman *et al.*, (2000) the significance of alterations in PL secretion observed in stress was unclear. Studies in the rat suggested that the physiological consequences were minimal, however PL was shown to have significant immune enhancing properties, (Matera and Mori, 2000) and it was theorized that the prompt changes in serum PL observed in stress served to adjust immune status and thus modulate the immunosuppressive actions of the corticosteroids (Buckingham *et al.*, 1997).

1.3.6 Dehydroepiandrosterone-sulphate

Dehydroepiandrosterone (DHEA) and DHEA-S, its sulphated metabolite, are steroids secreted by the adrenal cortex synchronously with cortisol. Both steroids can also be synthesized in the CNS. DHEA-S is more commonly measured than DHEA due to its much higher blood levels, long half-life and absence of diurnal variation (Hornsby, 1995). Some preliminary data suggested that DHEA might have antidepressant potential (Reus *et al.*, 1997; Wolkowitz *et al.*, 1999), since both DHEA and DHEA-S were reported to have antiglucocorticoid properties.

In a study on healthy volunteers in a military combat school, Morgan *et al.* (2004) reported that in response to an acute stressor, DHEA-S levels increased. They postulated that DHEA-S may afford beneficial

behavioural and neurotrophic effects during stress and that the DHEA-S –cortisol ratio may indicate the degree to which an individual exhibits resilience to the negative effects of a stressor.

When considering the situation of chronic stress, however, the data was less clear. Plasma levels of DHEA-S as well as the ratio DHEA-S to cortisol were found to be decreased in depression (Barrett-Connor *et al.*, 1999). Others failed to confirm these findings (Osran *et al.*, 1993) and increased plasma levels have also been reported (Heuser *et al.*, 1998).

In a study on premenopausal women with PTSD, Rasmusson *et al.* (2004) found that the peak DHEA –cortisol ratio was inversely correlated with negative mood symptoms, suggesting that an increased capacity for DHEA release in response to adrenal activation may mitigate the severity of PTSD.

Chronic inflammation is another characteristic paradigm of stress in which production of DHEA-S was decreased and the decline in levels was associated with neuronal dysfunction and degeneration (Straub *et al.*, 2002). Animal work has highlighted this neuroprotective propensity of DHEA and DHEA-S. For example, it was shown that DHEA-S protected rat hippocampal neurons against N-methyl-D-aspartate (NMDA)-induced excitotoxicity (Kimonides *et al.*, 1998). These findings

suggest that DHEA-S functions as a “prosurvival” agent against fluctuations in the local presence of growth factors. The decline of this steroid with aging or during chronic stress may leave the adrenal medulla unprotected against proapoptotic challenges and ultimately contribute to allostatic load (Schuurs and Verheul, 1990).

1.3.7 Aldosterone

Aldosterone is a hormone secreted from the zona glomerulosa of the adrenal cortex, which acts via mineralocorticoid receptors to promote transport of sodium across the epithelium. Secretion of aldosterone is mainly regulated by angiotensin II and plasma potassium concentrations. ACTH can also transiently raise aldosterone secretion, but its action is not persistent. (Funder, 2000)

Psychological or physical stressors that raise ACTH and adrenal catecholamine output do not lead to major or sustained elevations in aldosterone levels. However stressors in terms of fluid and electrolyte balance (e.g. diarrhoea, extreme sweating, pregnancy and lactation, or a low-sodium diet) raise aldosterone to act in a feedback loop to retain sodium and water.

The rationale for measuring aldosterone in this study was to demonstrate that the adrenal gland retained functional integrity, despite the paradoxically low output of glucocorticoids recorded in patients.

1.4. The Effects of Chronic Stressors on Carbohydrate and Lipid Metabolism and BMI

Acute activation of the HPA-axis causes adaptive metabolic changes in order to provide a source of energy for actions that improve the chances of an organism's survival. These changes include elevation of blood glucose, reduction in insulin secretion, metabolism of triglycerides into free fatty acids and glycerol, and the degradation of glycogen to glucose. Whilst all of these metabolic changes provide short-term energy for the organism, if they continue for a lengthy period, the allostatic load so incurred predisposes an individual to cardiac and metabolic illnesses (Björntorp and Rosmond, 2000).

1.4.1 Insulin

The effects of chronic stress on insulin concentrations were mediated by both glucocorticoids and catecholamines. Chronic stress caused insulin resistance, decreased insulin release from the pancreatic beta cells, increased hepatic glucose production by stimulation of both gluconeogenesis and glycogenolysis, and increased glucagon production and lipolysis, resulting in glucose intolerance and

hyperglycaemia (McCowen *et al.*, 2001). Hepatic insulin resistance was further characterized by elevated circulating levels of IGF-binding protein-1 (IGFBP-1)(Mizock, 1995).

1.4.2 Glucose

Cortisol stimulated gluconeogenesis in the liver by increasing transport of amino acids from extracellular fluids and muscle. The hormone also activated DNA transcription in liver cell nuclei to stimulate an increase in the production of enzymes required to convert amino acids into glucose (Rosmond *et al.*, 1998). In addition, cortisol decreased the rate of glucose utilization by cells, thus causing an increase in blood glucose concentrations. This response was considered acutely “protective” in the sense that raised blood glucose concentrations provided energy for the organism to respond to the stressful situation (Fain, 1979). However, in situations of chronic stress with repeated or sustained activation of the HPA-axis, increasing hyperglycaemia contributed to development of the metabolic syndrome (Friedman *et al.*, 1996), or to loss of glycaemic control in diabetic patients (Roy *et al.*, 1993; Taborsky and Porte, 1991).

1.4.3 Cholesterol

Chronic stress was associated with elevated total cholesterol and low-density lipoprotein cholesterol (LDL-C) (Brindley *et al.*, 1993). It was

suggested that these lipid changes could arise from stress-induced behavioural changes such as poor dietary choices and lack of exercise, however this was shown not to be the case - the elevation in lipids in response to chronic stress is maintained when controlling for variations in diet, exercise, sleep, medication use and general physical activity (Wolf *et al.*, 1962). In response to chronic stress, increased concentrations of adrenaline triggered very-low-density lipoprotein (VLDL) catabolism, which resulted in increased concentrations of circulating LDL-C. In addition insulin resistance and increased levels of cortisol suppressed hepatic LDL receptors and catabolism, thus delaying LDL clearance (Brindley *et al.*, 1993).

1.4.4 Triglycerides

In situations of chronic stress, noradrenaline inhibited the activity of both lipoprotein lipase and hepatic triglyceride lipase, causing a decline in triglyceride clearance, lower levels of high density lipoprotein cholesterol (HDL-C) and increased LDL-C. In addition, cortisol and free fatty acids (FFAs) stimulated the secretion of VLDL, increased the synthesis of hepatic triglycerides, and reduced insulin sensitivity in the tissues. Since insulin regulates triglyceride synthesis, stress-induced insulin resistance inhibited the regulation of triglycerides (Björntorp, 1996).

1.4.5 Free Fatty Acids

Activation of the sympathetic nervous system by stress or even exercise caused a rapid breakdown of triglycerides and mobilization of fatty acids, leading to a rapid rise in plasma level of FFA. Adrenaline-induced increases in FFA resulted from increased blood flow through adipose tissue or stimulation of adipose β -adrenoreceptors (Scheig, 1974). In the long term, increases in circulating FFAs eventually resulted in increased concentrations of serum LDL-C. Cortisol added to these effects by sensitizing adipose tissue to the lipolytic effects of adrenaline and increasing FFA concentrations (Brindley *et al.*, 1993). Elevated levels of FFA were implicated in the development of insulin resistance (Björntop and Rosmond, 1999).

1.4.6 Body Mass Index

Body mass index (BMI) is a tool for indicating weight status in adults (Garrow and Webster 1985). It is obtained by dividing weight in kilograms by the square of height in metres. There were no published longitudinal studies on the effects of chronic stress on BMI, however Grossi *et al.* (2003) recorded no significant difference in BMI between their subjects with low and high burnout scores at one time-point.

The rationale for considering the effects of chronic stress on BMI relates to the fact that, in addition to metabolic changes, chronic stress caused direct effects on appetite regulation. CRH suppressed appetite, an effect that is opposed by neuropeptide Y (NPY) (Glowa *et al.*, 1992). Leptin inhibited NPY and hence suppressed appetite and increased peripheral energy expenditure by activating the LC/NA system (Licinio *et al.*, 1997). Conversely, cortisol stimulated appetite (Tsigos and Chrousos, 2002). According to Sapolsky (1998), one of the reasons why some people overeat when they are stressed and others lose their appetite is related to the time-course of the stressor. He proposed that many short, stressful events lead to overeating, whilst exposure to a continuous stressor resulted in appetite suppression. This is based on the hypothesis that acute, stressful events provoked a short burst of CRH release, which was terminated within minutes. This was followed by release of cortisol, which persisted in the circulation for several hours after the stressful stimulus. In this case, the effects of cortisol predominated, and overeating ensued. In the case of a continuous stressor, circulating levels of CRH remained elevated and overrode the orexiogenic effects of cortisol, leading to loss of appetite.

1.5 The Effects Of Chronic Stressors On

Catecholamines

The catecholamines are synthesized from the amino acid tyrosine in brain and adrenal medullary chromaffin cells, sympathetic nerves and

sympathetic ganglia. Synthesis and release of catecholamines are regulated by feedback systems, neuronal depolarization, calcium dependent processes, prostaglandins, vasoactive amines, polypeptides and acetylcholine (Hoffman and Lefkowitz, 1996).

Upon recognition of a stressor, sympathetic nervous system (SNS) activity increased with a corresponding increase in catecholamine release and body activation. This initial reaction (e.g. rapid heart rate, increased sweating, GI distress) motivated the organism to act either to reduce the tension or eliminate its source (De Bellis *et al.*, 1999). In addition, SNS arousal led to increased availability of glucose and redistribution of blood to skeletal muscle, away from the skin, which supported further coping responses.

Circulating levels of catecholamines can be measured by assaying blood samples, but the half-life of adrenaline and noradrenaline is very short (Berne and Levy 1983), and biotransformation of circulating levels can occur within a minute or two. As a result, measures from blood usually reflect acute states (Ziegler, 1989).

In situations of chronic stress however, interpretation of catecholamine blood levels is difficult since, in these conditions, release of catecholamines can become sustained. Blood levels obtained from such patients may reflect acute stress system activation or a more chronic response which is indistinguishable from the acute response

(Goldstein, 2003). Also, people who have experienced chronic life stress reportedly show increased SNS reactivity to a laboratory stressor (Pike *et al.*, 1977).

1.5.1 Adrenaline

Adrenaline was the hormone most consistently shown to respond to stress, both in laboratory studies and people going about their everyday lives. However, it also responded to experiences not usually considered stressful, since it rose with mental arousal or effort, whether of a negative or a positive connotation (Dimsdale and Ziegler, 1991; Goldstein, 1995).

Once in the bloodstream, adrenaline stimulated α - and β -adrenergic receptors. Through stimulation of cardiac muscle β -receptors it caused an increased heart rate, force of contraction, and cardiac output. Its actions on vascular smooth muscle adrenergic receptors caused the shunting of blood away from the skin, mucosa, and kidneys to the coronary arteries, skeletal muscle and brain. Adrenaline also enhanced blood platelet adhesiveness and reduced clotting time. It promoted the role of glucagon in converting glycogen to glucose, leading to an increased secretion of glucose into the bloodstream and production of glucose in the liver. In addition, lipolytic activity in adipose tissues was

stimulated, with a resultant increase in levels of plasma free fatty acids, providing another source of energy (Goldstein, 2003).

It was thought that repeated adrenaline secretion, or chronically raised levels of adrenaline were likely to increase the risk of cardiovascular disease, partly by changing sympathetic tone and partly by inducing changes which accelerated the process of atherosclerosis (Chrousos and Gold, 1998; Wilhelmsen *et al.*, 2004).

1.5.2 Noradrenaline

Plasma levels of NA represented the ratio of the rate of release (spillover) of the substance into the plasma and the clearance of the substance from the plasma. This kinetic fact was especially important for NA because of the continuous release of NA into and the rapid removal of NA from the plasma (Goldstein *et al.*, 1983). Sympathetic nervous activity in the arm may not reflect sympathetic nervous activity elsewhere in the body, especially during stress, since a small proportion of NA released from sympathetic nerve endings reaches the circulation, the majority being removed back into the axonal cytoplasm by neuronal reuptake (Folkow *et al.*, 1983). Additional processes, including, heterogeneous changes in regional SNS traffic, and modulation of NA release by receptors on sympathetic nerve endings, complicate the relationship between plasma NA concentrations and

sympathetically mediated exocytosis (Cryer, 1980). Thus, measurements of antecubital venous NA levels should be interpreted with caution since the contribution of sympathoneuronal activation to the physiological responses should be taken into account (Goldstein *et al.*, 2003).

In animal models, acute stressors increased NA synthesis, release and metabolism in the CNS as well as in the periphery (Glavin, 1985). If the stress was severe, this translated to a reduction in tissue NA content as a result of increased turnover (Moore and Bloom, 1979).

Conversely, chronic exposure to a stressor increased tissue levels of NA (Roth *et al.*, 1982), after which the animal became tolerant to the stressor, with a decline in noradrenergic firing. Interestingly, when an animal was chronically exposed to a stressor and was then subjected to an acute, novel stressor, the noradrenergic response was enhanced. This phenomenon was referred to as “stress-evoked sensitization” (Adell *et al.*, 1990; Nisenbaum *et al.*, 1991).

In humans, NA was implicated in facilitating the planning and execution of behaviours important for survival (Svensson, 1987). However, Charney *et al.* (1995) postulated that severe stressors could cause hyperresponsiveness of the LC/NA system similar to animal sensitization, and that subsequent exposure to further stressors could result in chronic anxiety-related symptoms.

1.6 *The Link Between Chronic Stress and Disease*

Selye (1976) recognized the paradox that the physiological systems activated by stress not only protected and restored, but could also damage the body. He used the association between stress and increased cortisol secretion as a model to explain the development of disease as a result of dysregulation in the homeostatic responses of the body's stress system, where repeated or chronic activation of the stress system could produce cumulative adverse effects as a result of prolonged exposure to glucocorticoids or catecholamines.

Chrousos (1998) listed the states in which hyperarousal or chronic activation of the HPA-axis has been implicated: Individuals who were sensitive to chronic stressors could present with maladaptive symptoms including anxiety disorders, depression, substance abuse, personality and conduct disorders, eating disorders and "psychosomatic" diseases such as irritable bowel syndrome, low back pain, insomnia, headaches, eczema and others. In addition, susceptible people have shown accumulation of visceral fat leading to hyperinsulinism, insulin resistance, hypertension, hyperlipidemia, osteoporosis, increased blood platelet reactivity, increased risk for cardiovascular disease, immunosuppression and consequent

increased risk of infection or malignant tumours (Wolkowitz *et al.*, 2001).

McEwen (1998a) postulated that two major factors determined individual responses to potentially stressful situations: the way a person perceived a situation and a person's general state of physical health – where health was determined not only by genetic factors, but also by behavioural and lifestyle choices. In support of this theory, Nicolosi *et al.* (1999) noted that the effects of chronic stress may be exacerbated by a diet high in fats and simple carbohydrates, the use of tobacco, alcohol, prescription and illicit drugs, and can be reduced by moderate exercise. Cognitive behavioural psychotherapy to reduce the perception of threats also reduced stress-related illness (Rippe *et al.*, 1999). McEwan's model, utilizing the paradigm of allostatic load, thus accounted for the variation in vulnerability to stress-related diseases among people with similar life experiences.

Four situations were associated with increased allostatic load that could predispose a person to developing a stress-related illness (McEwen and Seeman, 1999):

- a. Frequent exposure to stressors.
- b. Lack of adaptation to repeated stressors of the same type.
- c. Inability to shut off allostatic responses after a stress is terminated.

- d. Inadequate responses by some allostatic systems trigger compensatory increases in others. When one system did not respond adequately to a stressful stimulus, the activity of other systems increased, because the underactive system was not providing the usual counterregulation. For example, if cortisol secretion did not increase in response to stress, secretion of inflammatory cytokines (which are counterregulated by cortisol) increased (Munck *et al.*, 1984).

The first three conditions caused increased allostatic load as a result of prolonged exposure to stress hormones. The link between hypercortisolism and stress-related illness is well established (Sapolsky, 1998). However, consideration of the last type of allostatic load was the basis for examining the condition of hypocortisolism in stress, which, initially, seemed an inconsistent and contradictory finding.

CHAPTER 2

HYPOCORTISOLISM

2.1 *Introduction*

In a review of central non-glucocorticoid inhibitors of the HPA-axis, Jessop (1999) stated, “Failure to terminate a response to acute stress can result in the syndrome of chronic stress which is associated with inappropriately elevated secretion of glucocorticoids...”.

The concept as proposed by Selye (1950) that a wide variety of stressors consistently activated the HPA-axis, resulting in cortisol release (in humans), gave rise to acceptance of the theory that hypercortisolaemia was responsible for the biological and psychological symptoms of excessive or chronic stress (Chrousos and Gold, 1998). The “glucocorticoid cascade” hypothesis was an example of this position, where it was proposed that excessive glucocorticoid production damaged hippocampal structures which were essential for HPA-axis restraint. This damage lead to a cycle whereby ongoing

stressors drove the glucocorticoid overproduction indefinitely, giving rise to illness (Sapolsky *et al.*, 1986).

Although isolated studies through the years reported contrary findings (Friedman *et al.*, 1963; Bourne *et al.*, 1967, 1968; Mason *et al.*, 1968; Caplan *et al.*, 1979), it was only recently, with the work of Yehuda *et al.* (1990), that this evidence was viewed cohesively. Yehuda and her colleagues described the phenomenon of hypocortisolism in patients with posttraumatic stress disorder (PTSD), leading to a re-examination of the earlier studies reporting hypocortisolism. Taken together, the evidence from these studies led to the conclusion that in some severely or chronically stressed patients, there was a relatively decreased rather than increased cortisol secretion. In a review on hypocortisolism and stress, Heim and her colleagues stated, "The phenomenon of hypocortisolism..... challenges, or virtually reverses, prevailing concepts on the neuroendocrinology of stress." (Heim *et al.*, 2000).

2.2 Stress Syndromes Associated With Low Cortisol

In this review, Heim and her colleagues (2000) commented that several studies have provided convincing evidence that the adrenal cortex is hypoactive in some stress-related states. The phenomenon of functional hypocortisolism as described by Yehuda (2002) mainly

referred to patients who experienced a traumatic event and subsequently developed post-traumatic stress disorder. However, hypocortisolism was also reported in patients with chronic fatigue syndrome (Demitrack *et al.*, 1991), fibromyalgia (Crofford *et al.*, 1994), rheumatoid arthritis (Chikanza *et al.*, 1992), asthma (Holgate, 1996), and atypical depression (Gold and Chrousos, 1999). The pathophysiology of many of these disorders was related to stress, with fatigue being a central component of the symptomatology.

2.3 Functional Definitions of Hypocortisolism

In reporting hypocortisolism as a feature of the disease states/syndromes listed above, various criteria were applied. Heim *et al.* (2000) defined hypocortisolism as any state in which the signalling capacity of glucocorticoids was inadequate to restrain relevant stress-responsive systems. This definition implicated no specific mechanism or absolute deficiency, but focused instead on the end point of glucocorticoid activity. Utilizing these criteria there was a possibility that even in the case of glucocorticoid hypersecretion, glucocorticoid insufficiency could exist, if reduced glucocorticoid sensitivity in relevant target tissues outweighed excess circulating hormone.

Raison and Miller (2003) applied a similar definition as summarized in Figure 2. According to this classification, hypocortisolism resulted from

either decreased hormone bioavailability or attenuated glucocorticoid responsiveness (e.g. secondary to reduced glucocorticoid receptor sensitivity).

2.3.1 Decreased hormone bioavailability

Within the HPA-axis, a decrease in cortisol bioavailability could occur as a result of decreased production of CRH and ACTH, as well as from a primary deficit in adrenal hormone production and/or release. Further factors affecting cortisol bioavailability included alterations in:

- i. binding proteins (Torpy *et al.*, 2004),
- ii. enzymes such as 11 β -hydroxysteroid dehydrogenase, which metabolised cortisol intracellularly (Krozowski, 1999), and
- iii. the multidrug resistance pump, which extruded cortisol from the cell (Karssen *et al.*, 2001).

2.3.2 Decreased receptor mediated signal transduction

As with the production of hormones, decreased receptor-mediated signal transduction might have occurred as a result of alterations in receptor number, binding affinity, or functional capacity at any level of the HPA-axis, including CRH and ACTH receptors, in addition to GR and MR (Bamberger *et al.*, 1996; Sapolsky *et al.*, 2000).

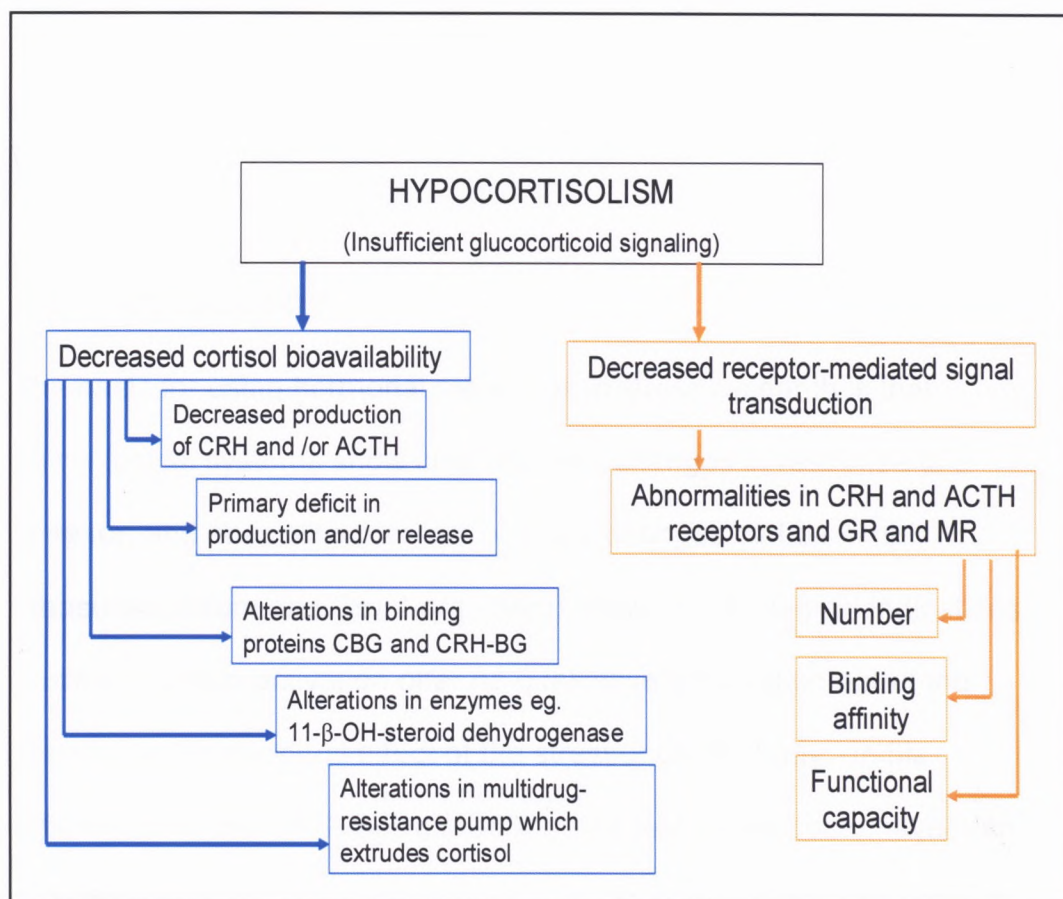


Figure 2. A flow diagram illustrating classification of functional hypocortisolism according to Raison and Miller (2003).

CHAPTER 3

PSYCHOLOGICAL MEASURES OF CHRONIC STRESS

3.1 *Introduction*

The basis for using hormonal measures in stress research is that physiological systems show characteristic changes in response to a stressor, and endocrine markers of these changes can be simply measured (Baum and Grunberg, 1995). However, biological indications of stress system activation offer no qualitative information about the stressor or the eventual effect of the stressor on the body. In his reflections on the GAS, Selye (1950) noted that stress could have both negative and positive consequences. i.e. stress could be a motivator to growth, development and adaptation or it could lead to exhaustion and death. He therefore distinguished between stressors that cause distress and those that result in positive eustress. Several psychological models of stress, developed from these observations, are briefly detailed below.

3.2 *Psychological models of Stress*

3.2.1 Stimulus Based Model

This model considered stress as an independent variable, where environmental conditions comprised the stressors, which increased the risk of impaired health and disease. This model applied the engineering analogy, where stress was defined as a force exerted, which resulted in a demand or load, which caused distortion. Both inorganic and organic substances have tolerance levels. If these were exceeded, temporary or permanent damage occurred (Sutherland and Cooper, 1990).

3.2.2 Response-Based Model

In this model, stress was considered the dependent variable, i.e. a response to a stressor. Response-based observations were classically from a physiological perspective, where symptoms were recognized, but not necessarily the causes (Biondi and Picardi, 1999). Selye took this approach in much of his work: "stress is the non-specific response of the body to any demand made upon it..." (1956). Response to stress was deemed to be invariant to the nature of the stressor and followed a universal pattern (Sutherland and Cooper, 1990).

3.2.3 Interactive Model

An interactive model of stress, which considered the stressor source, the perception of the situation, and the response, was a useful model in psychology (Lazarus, 1966). The way a situation was perceived depended on familiarity with the situation, previous exposure, learning and training. Successful coping restored stress-induced imbalance, but unsuccessful coping resulted in symptoms of distress. It was apparent from this model that situations were not inherently stressful, but were potentially stressful, depending on one's perception, and that response to a stressor was a dynamic psychological (as well as biological) process (Lazarus, 1977).

In addition, the interactive model acknowledged that the pattern of responses to a stressor was dependent on the nature, intensity and duration of the stress, as well as the nature and current state of health of the individual. Failure to mount an appropriate response to the stressor increased the risk of adverse health outcomes (Cooper and Dewe, 2004).

3.3 *Chronic Stress Syndromes*

As mentioned in 1.6, constant physiological efforts to respond to a stressor could result in increased neuroendocrine activation and haemodynamic activity that might influence the onset or maintenance

of diseases (Chrousos and Gold, 1992). Disease models of chronic stress demonstrating excessive stress-system activity included cardiovascular disease (Hjemdahl, 2000) with contributory hypertension (Steptoe A, 2000) atherosclerosis (Ketterer and Randall, 2000) and insulin resistance (Keltikangas-Järvinen, 2000) and MDD (Negrão and Gold, 2000).

In psychological terms, chronic stressors also impose significant allostatic load. When exposed to repeated stressors, previously healthy individuals may develop a chronic stress syndrome (Noshpitz, 1990). Symptoms included psychosomatic complaints (e.g.: low back pain, headaches, irritable bowel syndrome, etc) neurovegetative signs (eating and sleeping disturbances, lack of energy), feelings of hopelessness, helplessness, sadness and emptiness, including lack of coping and irritability.

In addition to the direct negative effects of chronic stressors on the body, there could be indirect or secondary effects that resulted from individuals' efforts to cope. According to the cost of coping model (Cohen *et al.*, 1986) coping efforts that were designed to alleviate distress caused by stressors, can impact negatively on health. The allostatic load incurred through prolonged and active coping with a stressor could take the form of psychological fatigue, resulting in inattention, confused thoughts, poor decision-making, and reduced

performance (Cohen, 1980), that could make people more prone to accidents. Also, repeated and failed attempts to cope with a chronic stressor could induce a sense of helplessness and concomitant psychological distress (Seligman, 1975). Inappropriate forms of coping which are self-destructive and escapist (e.g. alcohol and drug use) could have harmful bodily effects if they became routine and excessive – as they might in the face of chronic stressors. Finally, some chronic stressors could cause people to become socially avoidant, thereby diminishing their social support resources (which are potentially beneficial to health) (Lepore *et al.*, 1991a). All of these secondary effects of exposure to a chronic stressor may pose threats to health that are equal to or even greater than the direct effects of the chronic stressor (Lepore *et al.*, 1991b).

These data suggest that chronic stressors may increase psychological vulnerability to acute stressors by stripping people of their biological, psychological and social coping resources. A psychologically defined chronic stress syndrome which highlights these aspects is that of burnout.

3.4 Definitions of Burnout

Burnout has been defined as a syndrome in which depletion of emotional, cognitive, and physical energy resulted in symptoms (Maslach, 1982): Emotional depletion led to emotional exhaustion,

tearfulness, apathy, perceived meaninglessness, reduced self-esteem, lack of coping, helplessness, irritability and detachment from work. Cognitive depletion was evidenced by reduced attention span and a reduction in professional efficacy. Depletion of physical functioning included overwhelming fatigue, headaches, low back pain, non-specific pain syndromes, as well as eating and sleeping disturbances. Table 1 lists documented consequences of burnout (Cordes and Dougherty, 1993).

Table 1. Consequences of Burnout. (Adapted from Cordes and Dougherty, 1993.)

Physical and Emotional
Depression
irritability
helplessness
anxiety
insomnia
headaches
poor appetite
chest pain
gastrointestinal disturbances
depersonalization
Interpersonal
deterioration in social and family relationships
withdrawal from interaction with clients
increase impatience and moodiness in interactions with others
lower marital satisfaction
Job attitudes
lower job satisfaction
lower organizational commitment
Behavioural
higher levels of drug, alcohol and tobacco use
higher level of absenteeism
negative communication with co-workers about the management
poor job performance
Turnover intentions
thinking about or planning to leave a job
Actual turnover
leaving a job because of burnout

The aetiology of the syndrome was originally linked with the unremitting emotional stress experienced by social service providers and caregiving professionals. Freudenberger (1974) was the first clinician to ascribe the term 'burnout' to the cluster of symptoms he observed in stressed health care workers in free clinics in America. He postulated that the physical and mental exhaustion experienced by these workers was directly related to their conditions of work: long hours, poor salary, demanding patients and meager resources. His eventual definition of burnout linked depleted personal capacity with poor organizational conditions (1977).

Since then, there have been many other approaches in defining burnout. Rather than focusing on an externally stressful environment (as did Freudenberger), Lyall (1989) and Selder and Paustian (1989) considered individual responses to the working environment and concluded that burnout arises in people who have unrealistic expectations of themselves and others. This theme is continued in the work of Cherniss (1980) and Meier (1983) who both proposed that burnout occurs in response to diminished confidence associated with lack of expected job recognition and reward. Field, McCabe and Schneiderman (1985) describe an "expected lowering of self-esteem" in people whose work was constantly subjected to public professional scrutiny. Similarly, Wallace and Brinkerhoff (1991) base their definition of burnout on the inability of workers to respond adequately to

perceived demands, and their resultant dread of negative consequences of their failure to perform.

In their review of burnout in 1982, Perlman and Hartman synthesised their own definition of burnout by clarifying the common themes from 48 separate burnout definitions. They described burnout as a response to chronic emotional stress with three major components:

- emotional and/or physical exhaustion
- lowered job productivity
- overdepersonalisation

This definition was very similar to the "attributional /environmental" model proposed by Maslach and Jackson (1982), where burnout was identified as a multidimensional process with three central constructs:

1. emotional exhaustion - having no capacity left to offer psychological support to one's self or others
2. depersonalization - a negative, cynical or callous attitude towards others
3. reduced sense of personal accomplishment - the tendency to evaluate oneself negatively, particularly with regard to one's work and past achievements.

Maslach and Jackson's definition of burnout has achieved wide usage over the past 20 years since it not only includes the general

classification thought to underlie the condition but, in addition, these researchers developed a measurement instrument utilizing these three constructs. As investigations into burnout have progressed, the validity of their measurement tool, the Maslach Burnout Inventory (MBI) was extended to include occupations without direct-personal contact. Thus their definition of burnout has expanded to acknowledge that burnout can occur across the spectrum of all workers, not specifically those in service - oriented occupations (Maslach *et al.*, 1996).

3.4.1 Theories of Burnout

Hobfoll and Freedy, (1993) conceptualized burnout as a unique disorder amongst the spectrum of stress-related illnesses, that occurred in response to stressful work conditions. Their burnout model utilized conservation of resources (COR) as a basis for development of the disorder. In this model, individuals strove to obtain and keep what they valued – these being called resources. Examples are “conditions” such as having a job, or a quality marriage; “energies” such as money, stamina and knowledge, “personal characteristics” such as skills, good health or high self-esteem; and “objects” such as clothing and furniture. The model predicted that allostatic load was incurred when circumstances threatened a person’s obtaining and keeping resources. Three conditions resulted in allostatic load:

1. When resources were threatened or inadequate to meet demands
2. When resources were lost, and
3. When individuals invested in resources and did not receive the expected return.

In the workplace, examples of demands included heavy workload and pressure, stressful events, role conflict and role ambiguity (Lee and Ashforth, 1996).

Maslach and her colleagues (1986) suggested a process model of burnout similar to Hobfoll and Freedy's COR model where emotional exhaustion and depersonalization arose from work demands and conflicts, while reduced personal accomplishment occurred as a consequence of lack of social support and reduced chances for professional development. This process model is illustrated in Figure 3. In summary, the risk of burnout increased when there was prolonged occupational stress, such as work overload or unmet expectations, and inadequate resources in meeting these stressors, including limited control over one's work, exclusion from important decisions made at the workplace, and lack of a sense of community in the workplace.

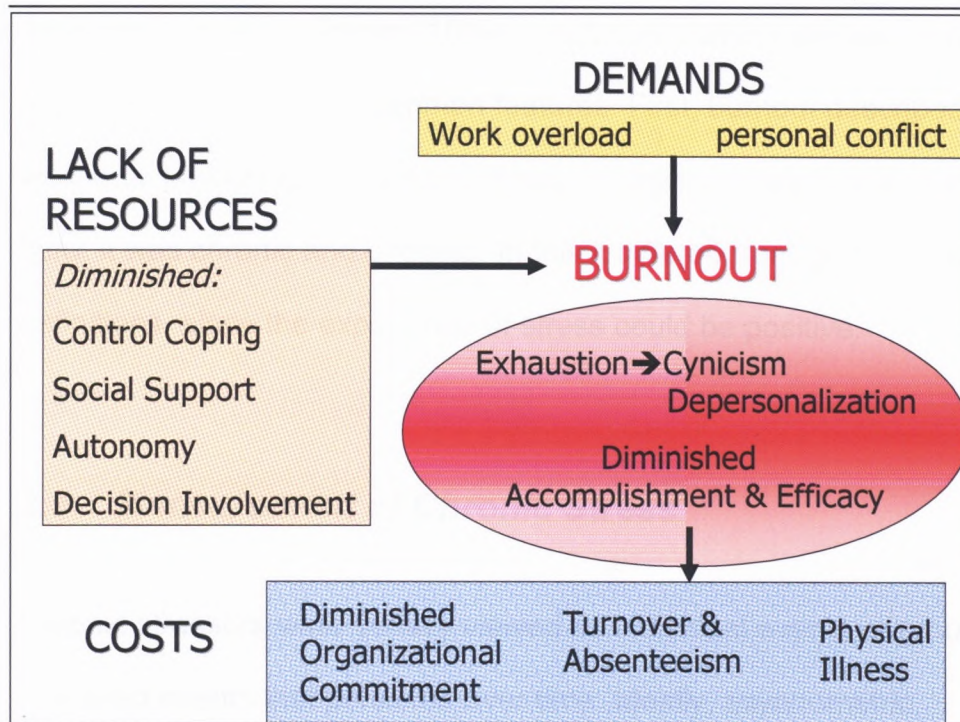


Figure 3 . A Process Model of Burnout Indicating Predictors for the 3 Maslach Burnout Inventory Subscales. (Adapted from Maslach *et al.*, 1996).

Although Maslach's three-factor model was the most widely used, there were other operational definitions cited in the literature (Pines and Aronson, 1988; Schaufeli et al., 1993; Shirom, 2003). In a review of work organizations, Shirom (1989) concluded that the various models of burnout shared three common features. First, burnout described an individual phenomenon, second, it was a negative experience, and third, it was chronic and ongoing. In this it differed from general stress definitions, since the experience of stress could be positive.

3.5 *Measurement of Chronic Stress*

Chronic stressors were usually viewed as individual events or groups of related events that persisted over time. Ideally, psychometric measurement of chronic stressors should be free of subjective bias, and produce consistent results when used by different investigators or in different populations. According to these criteria, it was possible to measure several stressors e.g. environmental stressors such as crowding, noise and air pollution (Evans and Cohen 1987) and economic stressors such as unemployment or inability to pay bills (Dooley and Catalano 1988). However, examples such as these, where direct measurements could be made, were sparse. Therefore, subjective social-science techniques, which involve self-report observations of the subject (e.g. questionnaires, interviews) or of

another human observer (e.g. naturalistic observation, laboratory observation, informants) have been used (Brown, 1989).

The following self-report questionnaires have been used in the measurement of chronic stress:

3.6 Psychometric Measures of Chronic Stress

Copies of the psychometric questionnaires are appended (p197).

3.6.1 Maslach Burnout Inventory

The Maslach Burnout Inventory (MBI) has been used extensively in burnout research and is the 'gold-standard' in measuring burnout (Carson and Hardy, 1998). The full MBI includes 22 questions, 9 evaluating emotional exhaustion (EE), 5 quantitating depersonalization (DP), and 8 assessing personal accomplishments (PA). A subscale is calculated for each of the 3 components. Values of ≥ 27 for EE, ≥ 13 for DP and ≤ 31 for PA are indicative of burnout.

In attempting to correlate physical symptoms with the MBI, the EE subscale showed the strongest relationship, being more predictive of stressor-related illness than DP or PA (Cox *et al.*, 1993).

3.6.2 General Health Questionnaire 28

The General Health Questionnaire 28 (GHQ-28) is a measure of general psychopathology with 4 subscales containing 7 questions in a

Likert scale format. These differentiate “somatic” symptoms, anxiety and insomnia, social dysfunction, and depression, but the cumulative GHQ score enables the identification of psychiatric ‘cases’. Goldberg and Williams (1988) noted that the sub-scales are not independent of each other and that these sub-scores represented “dimensions of symptomatology” rather than directly corresponding with psychiatric diagnosis.

3.6.3 Zung Depression Rating Scale

The Zung Depression Rating Scale (ZDRS) is a symptom-based checklist for assessing depression. It comprises 20 questions in a Likert scale format, with half the questions asked in a positive format and half asked in a negative fashion. Scores range from 1 (minimal) to 4 (maximum) per question giving a possible total of 80. Raw scores are converted to a 100-point scale by multiplication by 1.25. Scores of 50 – 59 indicated mild depression, 60-69 moderate depression, 70-79 severe depression and >80 extreme depression (Zung *et al.*, 1965).

Advantages of using the ZDRS over other measures of depression e.g. the Hamilton Depression Rating Scale or the Beck Depression Inventory, included its self-rating design- as opposed to administration by a health care professional, and its length – it was short in

comparison with other measures, and thus patient compliance in completing the questionnaire was improved (Shumway *et al.*, 2004).

CHAPTER 4

A STUDY OF PITUITARY-ADRENAL HORMONAL RESPONSES IN PATIENTS TREATED FOR CHRONIC STRESS

4.1 Introduction

Clinical reports have shown that some patients exposed to chronic stress exhibit hypocortisolism. No studies to date have hypothesised a mechanism for the origin of this finding, or the effects that such a finding would have on other hormones in the HPA-axis. In addition, current psychopharmacological management of chronic stress is non-specific, relying on suppression of symptoms to improve the clinical picture. Further elucidation of the HPA-axis hormonal profile in chronically stressed patients could suggest novel areas of pharmacological intervention in the treatment of these patients.

4.2 Aims Of The Study

To chart “baseline” pituitary-adrenal hormonal and biochemical values for chronically stressed patients. Then to compare the change in these hormonal and biochemical levels as the patients clinically improve, following both pharmacological and non-pharmacological intervention.

4.3 Patients And Control Groups

Twenty female patients (mean age $\pm$ SEM = 38.1 $\pm$ 2.9 years; range 25-59 years) were enrolled in a multipoint study; two patients were postmenopausal. During the course of the study, two patients moved away from the Johannesburg area and so did not complete the full series of 5 visits. Although eighteen patients completed the series, two patients became pregnant during the course of the study and, because of the effects of pregnancy on the HPA-axis and biochemical measurements, these data were excluded from the final sets. Recruitment of patients into the study lasted for 12 months, after which time it was decided to proceed with analysis. Although the eventual number of patients participating in the study was small, one cannot underestimate the achievement of attaining full participation from those who agreed to contribute; the nature of burnout is such that patients experience fatigue, amotivation, and great difficulty in making decisions. Patients required continual motivation to keep the monthly appointments, to make decisions such as what day they should choose

to attend the follow-up sessions, and to complete all the psychometric forms in their entirety.

On initial clinical and psychological assessment patients were diagnosed by a psychiatrist as suffering from stress burnout. This diagnosis was based on a standardised clinical examination in addition to a series of psychometric rating scales and symptom checklists (WHO publication 1998). These included the MBI, the GHQ-28, and the ZDRS. Patients with any psychiatric diagnosis other than major depression or subsyndromal depression were excluded from the study. All patients were biochemically euthyroid.

Sixteen age-matched women (mean age $\pm$ SEM = 37.3 $\pm$ 2.9 years; range 19-58 years) were recruited as control subjects; three control subjects were postmenopausal. In order for comparisons to reflect meaningful differences, the control population was drawn from the working hospital environment. In this way, the stress experienced by the controls paralleled the stresses of daily living in both the working and greater environment, but the control subjects did not show any symptoms of burnout .

Patients and control subjects gave written, informed consent to participate in the study, which was approved by the Ethics Committee

For Research On Human Subjects (Medical) of the University of the Witwatersrand.

4.4 *Experimental Design*

A summary of the experimental design followed is illustrated in Figure 4.

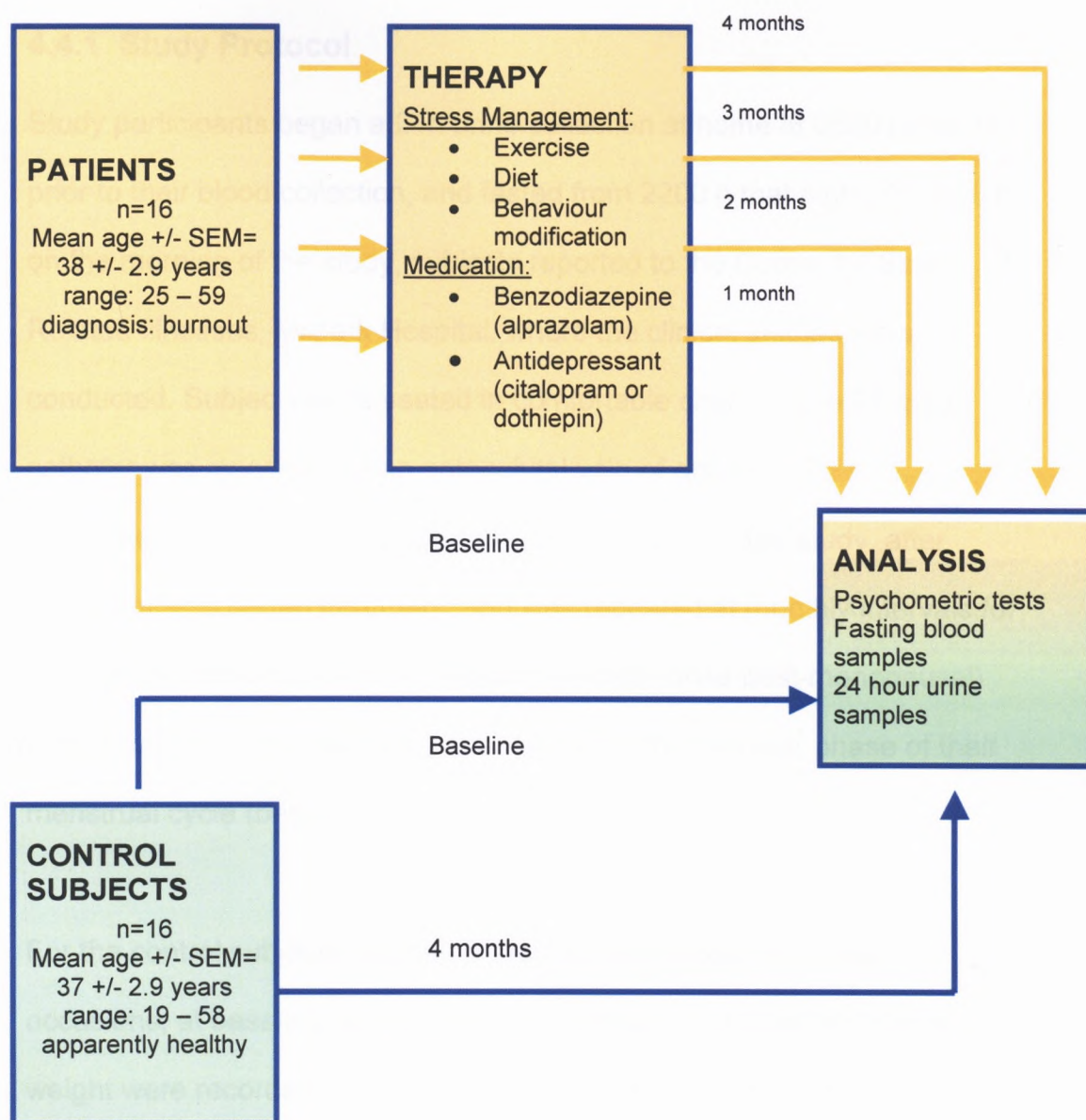


Figure 4. Flow diagram illustrating the experimental design of the study.

Baseline psychometric, blood and urine tests were taken for both patients and control subjects. Patients were then enrolled in a treatment programme, which included stress management and medication. Psychometric, blood and urine tests were carried out on the patients every month for four months, and for controls at four months.

4.4.1 Study Protocol

Study participants began a 24h urine collection at home at 0800 h the day prior to their blood collection, and fasted from 2200 h that night. At 0800 h on the morning of the study, subjects reported to the Centre for Stress-Related Illnesses, Milpark Hospital, where the clinical studies were conducted. Subjects were seated in comfortable chairs and a 21-gage catheter was inserted into an antecubital vein of one arm. This experimental procedure was performed at the start of the study, after which patients began treatment, and was repeated at monthly intervals for four further consecutive visits. Patients (except those post-menopausal) scheduled their blood tests to be taken during the follicular phase of their menstrual cycle (between days 6 – 10).

For the control subjects, the same protocol was followed on two occasions, at baseline and at 4 months. Measurements of height and weight were recorded at baseline and 4 months for both patients and controls, and body mass index (BMI) was calculated.

4.4.2 Clinical Ratings

At each visit according to the above protocol, patients and controls completed the MBI, GHQ28 and ZDRS. For the MBI, values of ≥ 27 for

emotional exhaustion, ≥ 13 for depersonalisation and ≤ 31 for personal accomplishment were indicative of burnout. For the GHQ28, scores of 5 and above were used as the indicator. In the ZDRS, scores of 50 – 59 indicated mild depression, 60-69 moderate depression, 70-79 severe depression and >80 extreme depression.

4.4.3 Interventions

Patients participated in a stress management program from 0800 h to 14:00 h for 5 - 10 consecutive days, which included exercise, diet and behaviour modification. The day was divided into three main sessions: The first session included relaxation and breathing exercises, visualization, awareness training, meditation and autogenic training. The second session focused on personal mission development, emotional intelligence, goal setting, time management, communication, cope-demand relationships, optimal nutrition and other specialised topics. The last session of the day was an exercise programme individually tailored for each patient. Typical exercise included aqua-aerobics, treadmill walking, cycling and the development of a home exercise programme.

Medication consisted of a benzodiazepine (alprazolam) and antidepressants (citalopram or dothiepin). The benzodiazepine was initiated at a dose of 1mg qid for 2 days, then tapered over 10 days to 1mg

nocte when necessary. Antidepressant therapy was initiated at the start of the study, and patients continued with this treatment throughout the study. Patients were started on 20mg citalopram daily; however 2 patients could not tolerate this drug, and they were given dothiepin 25mg mane and 75mg nocte.

4.5 *Hormone Assays and Biochemical Measurements*

Analytical methods used in the study are summarized in Table 2 below and reported in further detail in Appendix A.

After insertion of the butterfly needle into the arm, patients and controls were required to rest in comfortable chairs for 30 minutes. Blood samples were then collected in iced tubes and centrifuged; plasma/serum aliquots were separated and stored at -20⁰ C, except for catecholamines, which were analysed immediately. Adrenaline and noradrenaline concentrations were measured using high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA). (Respective normal fasting ranges: 0-366 pmol/L; 560-2636 pmol/L). Plasma glucose levels were determined by a standard glucose hexokinase method (normal fasting range: 3.0-6.0 mmol/L). Insulin, growth hormone (GH) and prolactin (PL) concentrations were measured by immunoassay using direct chemiluminometric

technology and reagents supplied by Diagnostic Products Corporation, Los Angeles, CA. (Respective normal fasting ranges: 6.0-27.0 mIU/L; 0.16-13.0 mIU/L; 2.8-29.2 µg/L [premenopause] and 1.8-20.3 µg/L [postmenopause]). Radioimmunoassay kits were used to measure adrenocorticotrophic hormone (ACTH), cortisol, dehydroepiandrosterone-sulphate(DHEA-S) (Diagnostic Systems Laboratories, Inc., Webster, Texas) and aldosterone (Diagnostic Products Corporation, Los Angeles, CA). (Respective normal fasting ranges: 2.2-19.8 pmol/L; 138-690 nmol/L [7.00-9.00 am]; 1.0-7.3 µmol/L [female]; 28-444 pmol/L [recumbent]). Free fatty acids (FFA), triglycerides, total cholesterol and high-density-lipoprotein cholesterol (HDL-C) levels were determined by enzymatic, colorimetric methods using reagents supplied by Roche Diagnostics, Basel, Switzerland. (Respective normal fasting ranges: 0.3-0.8 mmol/L; 0.0-2.0 mmol/L; <6.2 mmol/L; >1.6 mmol/L). Low-density-lipoprotein cholesterol (LDL-C) concentration was calculated according to the formula of Friedewald *et al.* (1972), (normal fasting range: <4.1 mmol/L). Urine free cortisol concentrations were measured by a competitive immunoassay (Bayer Corporation, Tarrytown, NY). (Normal range: 55-250 nmol/24hr). Intra-assay coefficients of variation for these assays were all < 5%.

Table 2. Summary Of Normal Fasting Ranges And Analytical Methods Used To Determine Serum And Urine Levels Of The Listed Variables In The Study

	METHOD	NORMAL FASTING RANGE
ACTH	Radioimmunoassay	2.2 – 19.8 pmol/l
Cortisol (serum)	Radioimmunoassay	138 – 690 nmol/l (7.00 – 9.00 a.m.)
Cortisol (24 hour urine)	Competitive immunoassay	55 – 250 nmol/24hrs
Growth Hormone	Immunometric assay	0.16 – 13.0 mIU/l
Prolactin	Chemiluminometric assay	2.8 – 29.2 µg/l (premenopause) 1.8 – 20.3 µg/l (postmenopause)
DHEA-S	Radioimmunoassay	1.0 – 7.3 µmol/l (female)
Aldosterone	Radioimmunoassay	28 – 444 pmol/l (recumbent)
Insulin	Immunometric assay	6.0 – 27.0 mIU/l
Glucose	Glucose hexokinase assay	3.0 – 6.0 mmol/l
Total cholesterol	Enzymatic colorimetric assay	<6.2 mmol/l
HDL-cholesterol	Enzymatic colorimetric assay	>1.6 mmol/l
LDL-cholesterol	Friedewald formula	<4.1 mmol/l
Triglycerides	Enzymatic colorimetric assay	0.0 – 2.0 mmol/l
Free Fatty Acids	Enzymatic colorimetric assay	0.3 – 0.8 mmol/l
Adrenaline	High-performance liquid chromatography	0.0 – 366 pmol/l
Noradrenaline	High-performance liquid chromatography	560 – 2636 pmol/l

4.6 Statistical Analysis

Results are expressed as mean $\pm$ SEM except for catecholamines, where the medians with ranges are stated due to the non-parametric distribution of the data. Statistical significance of non-parametric data was assessed by the Friedman repeated measures test, Mann-Whitney “U” test and the Wilcoxon Signed Rank test; parametric data were assessed by the Student’s t- test (two-tailed) for paired and unpaired samples. Repeated measures analysis of variance was used to examine differences in patients’ psychometric rating scales from baseline to 4 months. The Bonferroni Multiple Comparisons test was used to determine significances at each timepoint. A value of $p < 0.05$ was considered significant.

CHAPTER 5

RESULTS: HORMONAL AND BIOCHEMICAL DATA

5.1 *Pituitary-Adrenal Hormones*

Results of fasting 0800h serum and 24-hour urine concentrations of pituitary and adrenal hormones before and during 4 months of therapy for chronic stress in patients and untreated control subjects are summarized in Table 3, and individually detailed in Appendix B (Tables B1-B7).

Table 3. Fasting 8.00 am Serum Concentrations of Pituitary and Adrenal Hormones, Before and During 4 months of Therapy for Stress Burnout in Patients, and Untreated Control Subjects (Mean $\pm$ SEM)							
	PATIENTS					CONTROL SUBJECTS	
	Baseline	1 month	2 months	3 months	4 months	Baseline	4 months
Number of Subjects	16	16	16	16	16	16	16
ACTH (pmol/L)	40.0 $\pm$ 10.0	36.6 $\pm$ 8.4	44.9 $\pm$ 14.5	22.2 $\pm$ 2.7	42.6 $\pm$ 9.3	71.0 $\pm$ 14.7 ^a	40.8 $\pm$ 7.1
Urine free cortisol (nmol/24hr)	47.2 $\pm$ 11 ^b	45.8 $\pm$ 5.4	49.9 $\pm$ 6.7	48.4 $\pm$ 7.0	44.0 $\pm$ 6.1 ^c	79.0 $\pm$ 6.8	91.1 $\pm$ 8.8
Cortisol (nmol/L)	621 $\pm$ 92	686 $\pm$ 102	790 $\pm$ 135	658 $\pm$ 125	524 $\pm$ 71 ^d	894 $\pm$ 129	1031 $\pm$ 122
Growth hormone (mIU/L)	3.7 $\pm$ 2.1	6.1 $\pm$ 1.8	4.5 $\pm$ 1.4	5.0 $\pm$ 1.2	8.7 $\pm$ 2.2	7.7 $\pm$ 2.3	8.7 $\pm$ 2.9
Prolactin (μ g/L)	8.1 $\pm$ 1.9	7.7 $\pm$ 1.6	7.2 $\pm$ 1.4	8.0 $\pm$ 1.3	8.9 $\pm$ 2.5	8.6 $\pm$ 1.1	8.8 $\pm$ 1.3
DHEA-S (μ mol/L)	2.6 $\pm$ 0.4	2.7 $\pm$ 0.5	2.6 $\pm$ 0.5	2.6 $\pm$ 0.5	2.8 $\pm$ 0.6	2.0 $\pm$ 0.3	2.0 $\pm$ 0.3
Aldosterone (pmol/L)	277 $\pm$ 45	311 $\pm$ 60	373 $\pm$ 112	265 $\pm$ 46	406 $\pm$ 80	345 $\pm$ 46	358 $\pm$ 69

^a significance of values in the control group at baseline compared with four months: $p = 0.01$

^b significance of values in the patient group compared with control subjects at baseline: $p = 0.022$

^c significance of values in the patient group compared with control subjects at four months: $p = 0.002$

^d significance of values in the patient group compared with control subjects at four months: $p = 0.001$

5.1.1 ACTH

Apart from significant elevation of serum adrenocorticotrophic hormone (ACTH) at baseline in control subjects as compared with four months ($p=0.01$), mean fasting serum concentrations of ACTH were similar in both patients and controls. This trend is reflected in Figure 5.

5.1.2 Urine Cortisol

Compared with control subjects, mean urine free cortisol levels in the patients were significantly lower both at baseline ($p=0.02$) and at four months ($p=0.0001$), consistently below the lower level of the clinically quoted normal adult range (Figure 5 and 6).

5.1.3 Serum Cortisol

As shown in Figure 5, mean serum cortisol levels in patients were lower than controls. This trend only achieved statistical significance when comparing patients and control subjects at 4 months ($p=0.001$).

5.1.4 Growth Hormone

In control subjects, serum GH at baseline was raised compared with patients, however this difference was not statistically significant. After 4 months, mean fasting serum concentrations of GH in patients compared with untreated controls were similar.

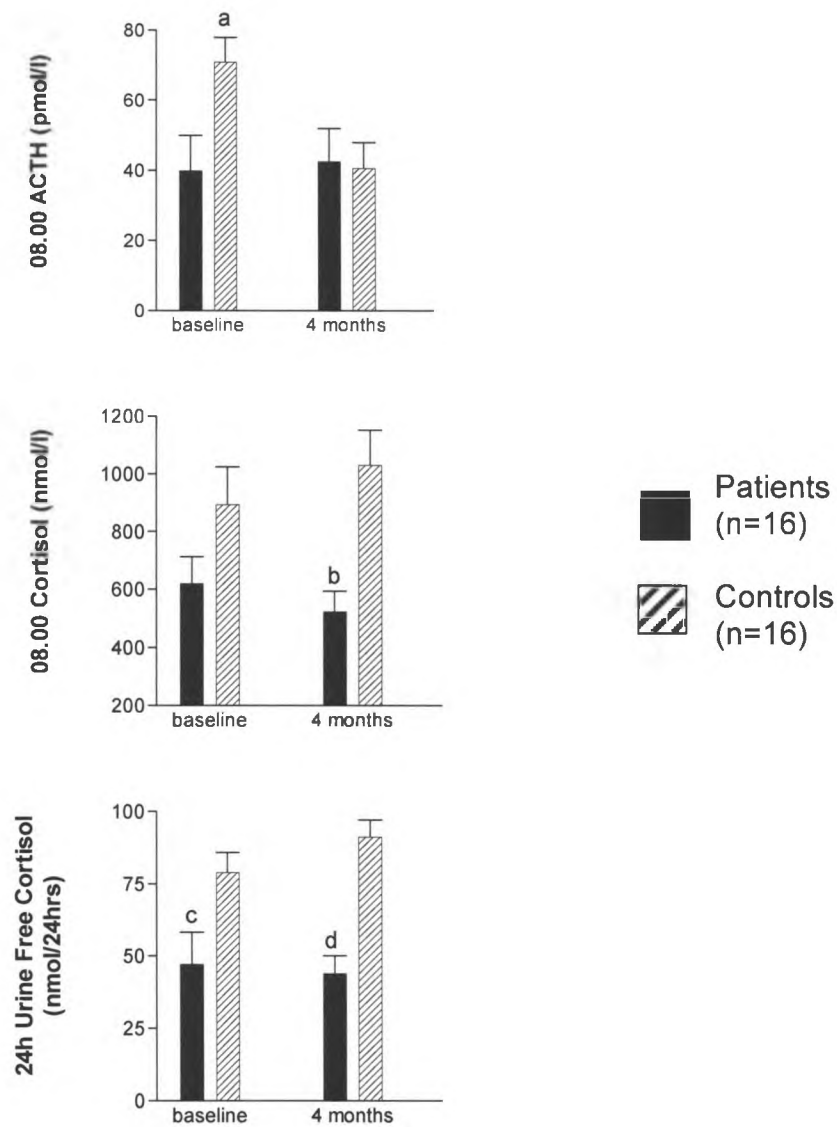


Figure 5. Panel highlighting the pituitary-adrenal responses to chronic stress before, and during four months of therapy in patients, and in untreated controls.

Mean fasting serum ACTH (upper), cortisol (middle) and 24-hour urine free cortisol (lower) concentrations in patients and control subjects at baseline and at 4 months. Vertical bars show SEM.

^aSignificant difference in serum ACTH concentrations between control subjects at baseline and 4 months ($p=0.01$)

^bSignificant difference in serum cortisol concentrations between patients and control subjects at 4 months ($p=0.01$)

^cSignificant difference in urine free cortisol concentrations between patients and controls at baseline ($p=0.02$)

^dSignificant difference in urine free cortisol concentrations between patients and controls at 4 months ($p=0.0001$)

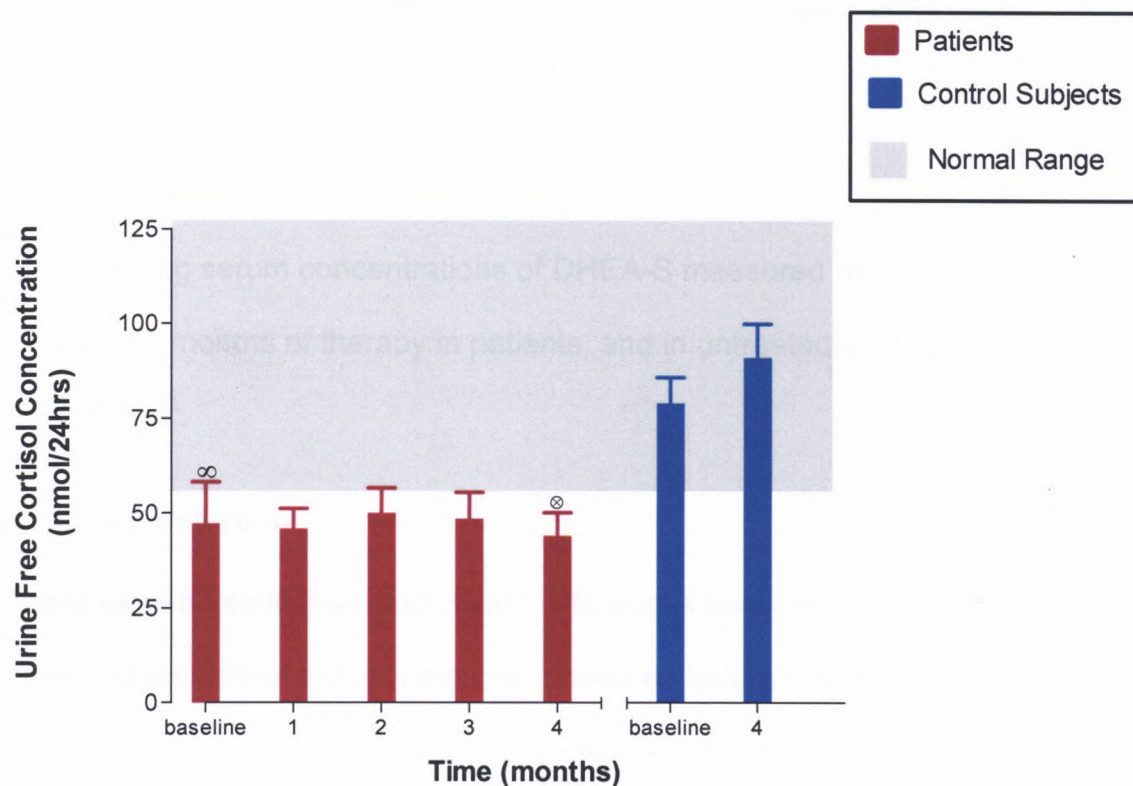


Figure 6. Mean 24-hour urine free cortisol concentration in patients and control subjects at baseline and four months including detail of patients' monthly results. Vertical bars show SEM.

- ∞Significant difference in urine free cortisol concentrations between patients and control subjects at baseline ($p=0.02$)
- ⊗Significant difference in urine free cortisol concentrations between patients and control subjects at 4 months ($p=0.0001$)

5.1.5 Prolactin

Mean fasting serum concentrations of prolactin measured before and during four months of therapy in patients, and in untreated controls were similar.

5.1.6 DHEA-S

Mean fasting serum concentrations of DHEA-S measured before and during four months of therapy in patients, and in untreated controls were similar.

5.1.7 Aldosterone

There were no statistically significant differences between mean fasting serum concentrations of aldosterone measured before and during four months of therapy in patients, and in untreated controls.

5.2 *Carbohydrate And Lipid Metabolism And BMI*

Results of fasting 8.00 am serum biochemical concentrations before and during 4 months of therapy for chronic stress in patients, and in untreated control subjects are summarized in Table 4, and individually detailed in Appendix B (Tables B8-B16).

Table 4. Fasting Serum Biochemical Concentrations, Before and During 4 months of Therapy for Chronic Stress in Patients, and Untreated Control Subjects (mean $\pm$ SEM).

	PATIENTS					CONTROL SUBJECTS	
	Baseline	1 month	2 months	3 months	4 months	Baseline	4 months
Number of Subjects	16	16	16	16	16	16	16
Insulin (mIU/L)	8.3 $\pm$ 1.3	10.1 $\pm$ 1.9	9.5 $\pm$ 1.5	10.4 $\pm$ 1.2	9.0 $\pm$ 1.2	7.3 $\pm$ 0.8	8.0 $\pm$ 1.2
Glucose (mmol/L)	4.8 $\pm$ 0.1	5.0 $\pm$ 0.1	4.9 $\pm$ 0.1	4.7 $\pm$ 0.1	4.6 $\pm$ 0.2	4.8 $\pm$ 0.2	5.0 $\pm$ 0.2
Total cholesterol (mmol/L)	4.6 $\pm$ 0.3	4.7 $\pm$ 0.2	4.9 $\pm$ 0.3	4.8 $\pm$ 0.2	4.7 $\pm$ 0.3	4.9 $\pm$ 0.2	5.1 $\pm$ 0.2
HDL-cholesterol (mmol/L)	1.4 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	1.6 $\pm$ 0.1	1.6 $\pm$ 0.1
LDL-cholesterol (mmol/L)	2.8 $\pm$ 0.2	2.7 $\pm$ 0.2	3.0 $\pm$ 0.3	2.8 $\pm$ 0.2	2.7 $\pm$ 0.2	2.8 $\pm$ 0.2	3.0 $\pm$ 0.2
Triglycerides (mmol/L)	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1
Free Fatty Acids (mmol/L)	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1

5.2.1 Insulin

Although patients showed a slightly raised trend in mean fasting serum insulin concentrations before and during four months of therapy compared with untreated control subjects, there were no statistically significant differences.

5.2.2 Glucose

There were no statistically significant differences in mean fasting serum concentrations before and during four months of therapy, in patients versus untreated control subjects for glucose.

5.2.3 Total Cholesterol

Mean fasting serum concentrations of total cholesterol, before and during four months of therapy, in patients versus untreated control subjects were similar, exhibiting no statistically significant differences.

5.2.4 HDL-cholesterol

Although mean fasting HDL-C serum concentrations were fractionally lower in the patient group at baseline and during four months of therapy, this difference did not achieve statistical significance when compared with untreated control subjects.

5.2.5 LDL-cholesterol

There were no statistically significant differences in mean fasting serum concentrations before and during four months of therapy, in patients versus untreated control subjects for LDL-C.

5.2.6 Triglycerides

Mean fasting serum concentrations of triglycerides, before and during four months of therapy, in patients versus untreated control subjects were similar, exhibiting no statistically significant differences.

5.2.7 Free Fatty Acids

There were also no statistically significant differences in mean fasting serum concentrations before and during four months of therapy, in patients versus untreated control subjects for free fatty acids.

5.2.8 BMI

There was no significant difference in mean BMI between patients and controls at baseline (mean $\pm$ SEM=23.9 $\pm$ 0.9 vs. 24.8 $\pm$ 0.9 kg/m², p=N.S.) and at 4 months (mean $\pm$ SEM=23.3 $\pm$ 0.8 vs. 24.6 $\pm$ 0.8 kg/m², p=N.S.) These data are detailed in Appendix B (Tables B15 and B16).

5.3 Catecholamines

Results of fasting 8.00 am plasma concentrations of catecholamines before and during 4 months of therapy for chronic stress in patients, and in untreated control subjects are summarized in Table 5, and individually detailed in Appendix B (Tables B17 and B18). Because of the non-parametric nature of these data, median and ranges were quoted in place of mean $\pm$ SEM.

5.3.1 Adrenaline

There were no differences in median levels of plasma adrenaline between patients and controls.

5.3.1 Noradrenaline

The median plasma noradrenaline levels at baseline were significantly higher in the control group than in the patients ($p=0.03$).

Table 5

Fasting Plasma Concentrations of Catecholamines Before and During 4 months of Therapy for Stress Burnout in Patients, and Untreated Control Subjects (Median and Range)

	Patients					Control Subjects			
	Subjects (Median and Range)								
	Baseline	1 month	2 months	3 months	4 months	Baseline	4 months	4 months	4 months
number of subjects	16	16	16	16	16	16	16	16	16
Adrenaline (pmol/L)	222 (49-4036)	563 (42-6361)	334 (76-4431)	206 (10-3144)	151 (14-2211)	206 (15-1672)	114 (0-454)		
Noradrenaline (pmol/L)	1271 (395-5436) ^a	1570 (244-4960)	1442 (87-7203)	1816 (619-3325)	2313 (699-7538)	2338 (452-3974)	2704 (1351-4337)		

^aSignificance of values in the patient group compared with control subjects at baseline: p = 0.03

CHAPTER 6

RESULTS: PSYCHOMETRIC TESTING

Results of monthly psychometric tests before and during 4 months of therapy for chronic stress in patients, and in untreated control subjects are summarized in Table 6, and individually detailed in Appendix B (Tables B19 - B27).

6.1 *Maslach Burnout Inventory*

As summarized in Table 6 and illustrated in Figure 7, regarding the MBI, mean scores of emotional exhaustion declined significantly over the 4 months ($p < 0.0001$) as compared with baseline measurements. There were significant differences between patients and controls at baseline in emotional exhaustion ($p < 0.0001$), depersonalisation ($p = 0.001$) and personal accomplishment ($p = 0.0001$). Patients varied significantly from controls at four months in their scores of emotional exhaustion ($p = 0.001$) and depersonalisation ($p = 0.03$).

Table 6

**Monthly Psychometric Tests (MBI, GHQ-28 and ZSDS) Before And During 4 Months Of Therapy For Chronic Stress
In Patients, And In Untreated Control Subjects (Mean ± SEM).**

Test	PATIENTS				CONTROL SUBJECTS	
	Baseline	1 month	2 months	3 months	4 months	Baseline
Maslach Burnout Inventory						
Emotional Exhaustion	39.81± 2.26	28.19±2.78	25.94± 3.80	23.50±2.93	23.38±2.55	11.88± 1.86
Depersonalization	11.00± 2.11	7.38± 1.26	9.25± 1.73	7.13± 1.27	8.31± 1.61	2.81± 0.73
Personal Accomplishment	27.56± 1.68	29.81± 2.18	29.38± 2.20	31.88± 1.83	32.38± 1.65	37.56± 1.56
GHQ-28 composite score	55.25± 4.35	28.63± 4.32	28.50±4.08	26.19± 3.97	24.69± 3.74	12.38±1.62
ZDRS score	71.75± 1.55	58.81±2.65	54.44±2.60	55.44±2.44	54.25± 2.70	41.81± 1.54

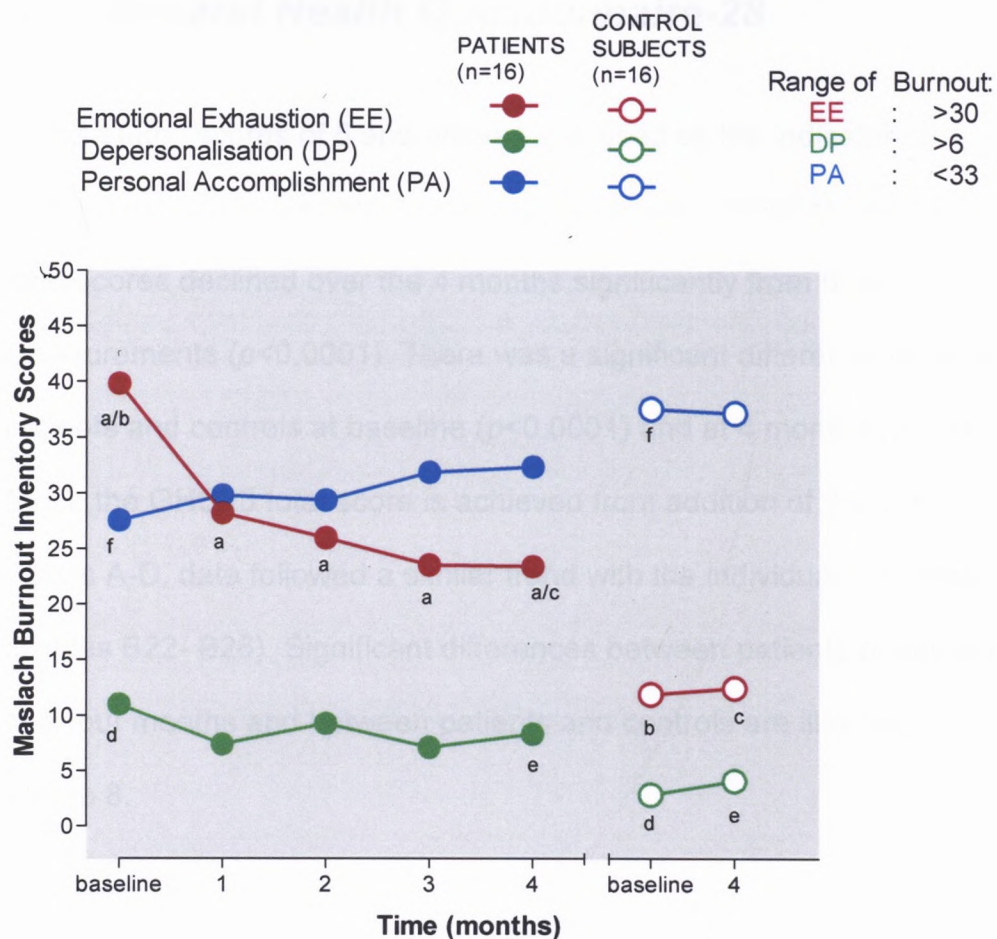


Figure 7. Mean Maslach Burnout Inventory (MBI) sub- scores i.e. Emotional Exhaustion (EE), Depersonalisation (DP) and Personal Accomplishment (PA) in patients and control subjects at baseline and 4 months.

^aSignificant difference in EE between patients at baseline vs 1,2,3 and 4 months ($p<0.001$)

^bSignificant difference in EE between patients and controls at baseline ($p<0.0001$)

^cSignificant difference in EE between patients and controls at 4 months ($p=0.001$)

^dSignificant difference in DP between patients and controls at baseline ($p<0.001$)

^eSignificant difference in DP between patients and controls at 4 months ($p=0.03$)

^fSignificant difference in PA between patients and controls at baseline ($p=0.0001$)

6.2 General Health Questionnaire-28

In this study, scores of 5 and above were used as the indicator of “caseness” for the General Health Questionnaire (GHQ28). Patients’ total scores declined over the 4 months significantly from baseline measurements ($p < 0.0001$). There was a significant difference between patients and controls at baseline ($p < 0.0001$) and at 4 months ($p < 0.01$). Since the GHQ28 total score is achieved from addition of the sub-scales A-D, data followed a similar trend with the individual sub-scales (Tables B22- B26). Significant differences between patients at baseline and four months and between patients and controls are illustrated in Figure 8.

6.3 Zung Depression Rating Scale

Table 6 and Figure 8 illustrate that the ZDRS also showed a declining trend, with patients scoring significantly higher at baseline than in the subsequent 4 months ($p<0.0001$). In comparison with controls, patients' scores were significantly different at baseline ($p<0.0001$) and at 4 months ($p=0.0002$).

The following composite figure 8, showing a similar trend for the results of the clinical ratings, illustrates the overall change in patients' perception of their illness compared with healthy controls over the four months. In the interest of clarity, since emotional exhaustion is regarded as the most stable burnout dimension of the MBI (Carson and Hardy 1998), only this subscale of the MBI was included in this graphical depiction.

Patients (n=16)	Controls (n=16)
ZSDS	ZSDS
GHQ-28	GHQ-28
EE	EE

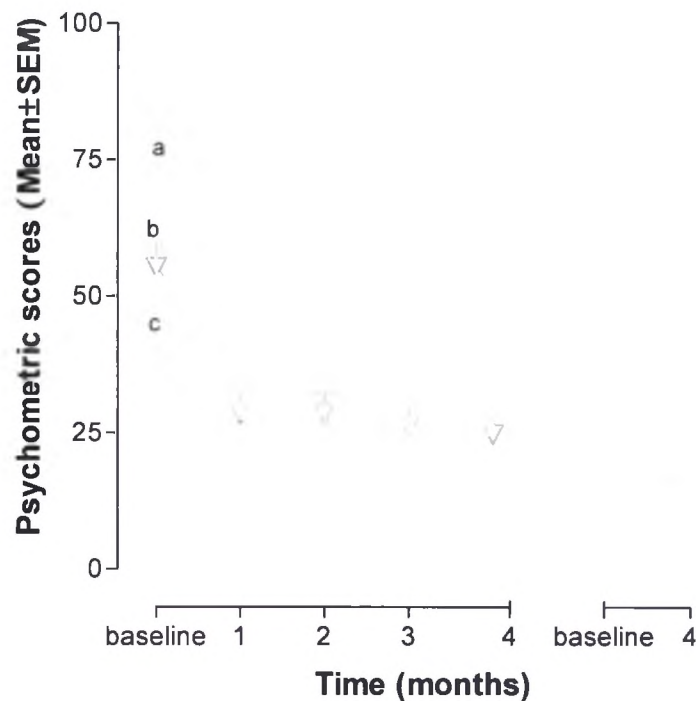


Figure 8. Mean Zung Depression Rating Scale (ZDRS), General Health Questionnaire-28 (GHQ28) and Maslach Burnout Inventory Emotional Exhaustion (EE) scores in patients and control subjects at baseline and 4 months. Vertical bars show SEM.

^aSignificant difference in ZDRS scores for patients between baseline and 1,2,3 and 4 months ($p<0.0001$) and for patients versus controls at baseline ($p<0.0001$) and at 4 months ($p=0.0002$).

^bSignificant difference in GHQ28 scores for patients at baseline and 1,2,3 and 4 months ($p<0.0001$) and for patients versus controls at baseline ($p<0.0001$) and at 4 months ($p<0.01$).

^cSignificant difference in emotional exhaustion scores for patients at baseline and 1,2,3 and 4 months ($p<0.0001$) and for patients versus controls at baseline ($p<0.0001$) and at 4 months ($p=0.001$).

CHAPTER 7

DISCUSSION AND CONCLUDING COMMENTS

7.1 Pituitary Adrenal Findings in Context

In this investigation, the most consistent endocrine finding was a reduction of urine free cortisol excretion in the patient group, suggesting a situation of functional hypoadrenalism in burnout. No significant recovery of cortisol excretion occurred during the study, implying an increased predisposition to future burnout episodes.

The 0800 h serum cortisol levels in patients were, however, within the stated normal clinical range. This finding, in the face of low urinary cortisol excretion, seems contradictory. Since all the patients had normal creatinine clearance, it was improbable that impaired renal function influenced cortisol excretion. Crofford *et al.* (1994) postulated that in fibromyalgia the low 24-hour levels may reflect reduced cortisol pulsatility during the 24-hour period, with preservation of the early morning peak. This is a possible explanation for the preserved cortisol 0800 h peak. Pruessner *et al.* (1999) noted that patients with burnout exhibited an exaggerated cortisol response to awakening, which could further explain the 0800 h cortisol levels in this study.

Serum levels of ACTH in the normal range in the patients cast doubt as to the central origin of the functional hypoadrenalism. Unfortunately, the severity of illness of patients enrolled in this study precluded a protocol involving challenge testing. Consequently, patients were not subjected to dexamethasone suppression of cortisol, ACTH challenge testing with CRH stimulation, or insulin-induced hypoglycemia. Therefore, the functional integrity of the HPA pathway in burnout remains uncertain and warrants further study.

The observation that, despite control subjects resting for 30 minutes prior to blood sampling, mean ACTH and NA levels exceeded those of patients also seems inconsistent. This finding may reflect a stress response to the needle-stick, or could highlight high “background” levels of stress in this group. In the South African context, chronic, unavoidable stress factors include the high incidence of violent crime and trauma (McKendrick and Hoffman, 1990), and the impact of AIDS, both socially and economically on the developing population (Panz and Joffe, 1999). Despite these biochemical anomalies, the control subjects showed no burnout symptoms.

Interestingly these findings are congruent with data presented recently by Jezova *et al.*, (2004) who studied subjects with high trait anxiety vs non-anxious subjects. When subjected to a laboratory psychosocial stress test, the non-anxious subjects showed statistically significantly

raised plasma levels of adrenaline, NA, ACTH and cortisol. The authors concluded that the intensity of physiological responses during stress appears higher in “stress controllers” than in people with a higher level of anxiety.

In the context of burnout, although trait anxiety was not measured in our study, anxiety is a well-documented feature of the syndrome (Grossi *et al.*, 2003; Michels *et al.*, 2003), and our patients with burnout exhibited a similar hormonal profile to that of the high trait anxiety patients in Jezova and colleagues’ experiments – reduced plasma cortisol, ACTH and NA. It is apparent that inability to respond with adequate hormone release when challenged by stressors is a feature common to burnout and high trait anxiety patients. In view of this idea, the relatively raised levels of ACTH and NA in control subjects in our study may merely reflect their resilience in anxiety control.

7.2 Implications of Hypocortisolism

The findings of this study resemble the reduced adrenocortical hormonal secretion that has been documented for other stress-related illnesses e.g. PTSD (Yehuda, 2002), chronic fatigue syndrome (Demitrack *et al.*, 1991) and fibromyalgia (Crofford *et al.*, 1994).

Hypocortisolism may be a relevant factor in the pathogenesis of all these disorders, since the lack of cortisol availability is associated with

increased SNS tone, vulnerability to autoimmune disorders, inflammation, chronic pain, asthma and allergies (Chrousos, 1998).

More specifically, as a consequence of McEwen's (1998b) fourth instance of allostatic load, a deficiency in cortisol secretion has been linked with increased concentrations of pro-inflammatory cytokines. These mediators amplify the risk of adverse health consequences (McEwen and Seeman, 1999). Interleukin-6 (IL-6), tumour necrosis factor alpha (TNF- α) and interleukin-1 (IL-1) are mediators of peripheral immune system activation. They have been implicated in the "sickness response", in which reported symptoms included fatigue, pain, cognitive disturbances, depressed mood and sleep disorders (Watkins *et al.*, 1995). Grossi *et al.* (2003) documented enhanced inflammatory responses and oxidative stress in women with burnout. In addition, the increase in cytokines as a result of reduced cortisol counterregulation, may contribute to the behavioural features of PTSD, chronic fatigue syndrome and fibromyalgia. (Rohleder *et al.*, 2004; Raison and Miller, 2003; Watkins *et al.*, 1995).

Since hypocortisolism contributed to allostatic load, it was postulated that reversal of hypocortisolism could alleviate the "sickness response" symptoms. There have been 2 randomized controlled trials testing specifically the hypothesis that low dose hydrocortisone in replacement doses might be therapeutic in chronic fatigue syndrome (McKenzie *et*

al., 1998; Cleare *et al.*, 1999). Although useful in selected cases, the rapid loss of efficacy on discontinuation and high rates of adrenal suppression suggested that this was not a practical therapeutic option.

What remains unclear is why, in some cases of chronic stress, the initial activation of the HPA-axis following stimulation by an acute stressor reverses to a relative deficiency of cortisol at the time of obvious signs of psychological and physical disturbances (e.g. burnout). Possibly, these patients show inadequate coping strategies, genetic risk factors for HPA-axis dysregulations or developmental risk factors like prenatal or early life stress that may favour maladaptation to traumatic experiences (Heim *et al.*, 2000; Ehler *et al.*, 2001).

7.3 Potential Effect of Drug Therapy

The rationale for using antidepressant medication in the treatment of burnout was multifactorial and included:

- evidence for the successful use of antidepressants in the treatment of other hypocortisolaemic states e.g. chronic fatigue syndrome, PTSD, (Goodnick and Sandoval, 1993; Asnis *et al.*, 2004)
- sleep disturbance
- mood disturbance
- Zung depression rating scale measures.

In MDD, HPA-axis hyperresponsiveness could result in hypercortisolism as a consequence of down-regulation of GRs in the hippocampus and hypothalamus (Barden *et al.*, 1995; Holsboer, 2000). The putative action of antidepressants includes a direct effect of the drugs in improving GR function, leading to “normalization” of the HPA-axis (Pariante *et al.*, 2004). Complimentary to this hypothesis is the theory that cortisol blocks expression of brain-derived neurotrophic factor (BDNF) in MDD by prevention of phosphorylation of cAMP response element binding protein (CREB) (Duman *et al.*, 1997). It was postulated that antidepressants could act either through enhancement of BDNF expression or blocking the stress-induced decrease of BDNF. However, these theories do not account for the reported efficacy of antidepressants in patients with hypocortisolaemic syndromes (McGrath *et al.*, 2000) and the molecular mechanisms of action of antidepressants in these syndromes remains elusive.

Interestingly, the response rates of patients with MDD to CRH-1 antagonists were lower than those to selective serotonin reuptake inhibitors (Seymour *et al.*, 2003). This raises the question of the impact of chronic stressors on the drivers of HPA activation, where AVP may predominate in situations of repeated or ongoing stress.

The question of whether the antidepressants used in this study affected the HPA-axis results in patients should be addressed: There was no

evidence in the literature of the direct effect of dothiepin on the HPA-axis. However, dothiepin is a tricyclic antidepressant that is structurally related to amitriptyline and has similar therapeutic efficacy to this drug (Lancaster and Gonzalez, 1989), and so the effect of amitriptyline on the HPA-axis was considered.

Pariante and his colleagues (2004) commented on the literature describing the effects of antidepressant drugs in animal and cellular models. The consensus of this work implicated antidepressants in reducing serum cortisol, increasing GR function and inhibiting steroid transporters. Interestingly, these effects were not borne out in human studies, where Rota *et al.* (2005) published results showing no effect of amitriptyline on normalizing cortisol in patients with MDD, and Deuschle *et al.* (2003) showed a decrease in salivary cortisol only in responders to amitriptyline, not in SSRI responders or non-responders.

For citalopram, there were differences between acute and chronic effects of the medication, as well as differences in the reported HPA-axis responses. In animal models, a single injection of citalopram increased plasma levels of ACTH and corticosterone, whilst repeated administration led to attenuation of the HPA-axis response to the SSRI (Raap and Van de Kar, 1999). It was proposed that the HPA-axis activation following acute citalopram administration resulted from

hypothalamic stimulation, whereas long-term citalopram treatment desensitized the HPA-axis at the pituitary level (Jensen *et al.*, 1999).

In another animal study, Hesketh *et al.* (2005) noted that acute citalopram treatment prolonged HPA-axis activation in response to the stress of acute restraint, whilst chronic citalopram treatment attenuated the ACTH response to this stress. Of interest in this study, however, is the observation that chronic treatment with citalopram did not alter basal plasma levels of corticosterone or ACTH.

In humans, acute treatment (1-6 days) with citalopram in healthy volunteers significantly enhanced the increase in salivary cortisol produced by waking, however there was no overall change in basal salivary cortisol levels sampled in a standard pattern throughout the day (Harmer, 2003). In a study on obese males, Ljung *et al.* (2001) reported a normalization of hypocortisolaemia during 6 months of treatment with citalopram. Cortisol concentrations after stimulation with CRH or stress were elevated by citalopram treatment, but urinary cortisol excretion was unchanged.

In our study, where patients were chronically treated with either citalopram or dothiepin, levels of ACTH were low-normal, whilst levels of urine free cortisol remained low. These results are in concordance with Ljung *et al.*'s findings that chronic citalopram treatment did not

influence urine free cortisol. The literature on human studies supports the view that chronic antidepressant therapy did not influence levels of ACTH and cortisol. However, since no molecular work was undertaken in our study (on expression of HPA-axis drivers and products), we cannot rule out the possibility that continued low levels of urine free cortisol in the face of the patients' clinical recovery could be attributed to a reduction in HPA-axis responsiveness as a result of antidepressant therapy.

Alprazolam suppressed CRH release and therefore inhibited the HPA-axis in both animals and humans (Arvat *et al.*, 2002). In our study, alprazolam was used by all patients on initiation of treatment, but was phased out (by graded dose reduction) before the first follow-up visit at 1 month. Pre-treatment baseline levels of urine free cortisol, serum ACTH and serum cortisol were lower than in untreated controls and subsequent serum levels appeared unaffected by alprazolam use.

7.4 *Link Between Burnout and Depression*

The possible link between burnout and depression deserves comment. All patients enrolled in the study were assessed to be depressed at baseline (according to the ZDRS). Gold and Chrousos (2002) recently reviewed HPA-axis functioning in two diagnostic subtypes of

depression and clarified that the biochemical “signature” of melancholic depression was typified by hyperactivity of the HPA-axis, with increased CRH, ACTH and cortisol secretion, and decreased sensitivity of the negative feedback system. In contrast, neuroendocrinologic features of atypical depression highlighted underactivity of the HPA-axis (with decreased CRH, ACTH and cortisol secretion and a supersensitised negative feedback system) (Rubin *et al.*, 1987). Symptomatically, patients with atypical depression present with the antithesis of melancholia: they are lethargic, hypersomnic and hyperphagic. In burnout patients we found ACTH levels in the low-normal range with low cortisol secretion that may reflect an “alternate or mixed” type of depression – which is also distinct both symptomatically and biochemically from PTSD.

7.5 Study Limitations

Certain limitations of our study should be noted:

- The number of patients enrolled in the study was relatively small.
- It would have been an advantage to document cortisol over the full circadian cycle, however the illness of the patients precluded 24 hour plasma cortisol profiling.
- Challenge testing with insulin, dexamethasone, CRH stimulation, exogenous ACTH or metyrapone would have

provided a better indication of HPA-axis functioning. However, the patients were judged to be too ill to participate in challenge testing.

- The period of follow-up observations was restricted to four months.

7.6 *Future Studies*

The aim of this study was to determine baseline levels of pituitary-adrenal hormones and metabolic variables in patients with burnout. Having completed this phase it would be of value to continue this work by further investigation of the principal finding – hypocortisolaemia in burnout patients. Suggested studies include:

- 24 hour serum cortisol profiling in order to determine cortisol kinetics in these patients. Salivary cortisol levels could also be used to obtain adequate results for comparison.
- Challenge testing with low-dose dexamethasone , ovine-CRH metyrapone and exogenous ACTH - in order to clarify HPA-axis responses in these patients.
- Investigation of lymphocyte MR and GR number and activity – in order to infer functionality of negative feedback systems.
- Determination of cytokine levels in burnout patients – in order to test the hypothesis that in the absence of counterregulatory cortisol, cytokine levels in these patients are elevated.

- Determination of melatonin levels over the circadian cycle in these patients – since low melatonin levels are implicated in insomnia, and burnout patients present with insomnia and excessive fatigue.

In addition, a closer examination of the similarities and differences between burnout patients and those with atypical depression should be undertaken – with particular emphasis on resolving the question of how antidepressants improve the clinical picture in these patients, since current theories of the molecular mechanism of antidepressants focus on correction of hypercortisolaemia and exclude pre-existing hypocortisolaemia.

7.7 Conclusion

After undergoing an integrated stress-management programme, patient recovery was significant over the 4-month period when charted on the MBI, the GHQ28 and the ZDRS. Interestingly, during this period, the decrease in emotional exhaustion, psychiatric symptoms and depression was not accompanied by a corresponding rise in urine free cortisol levels. This suggests that clinical improvement following stress-management measures precedes any accompanying change in biochemical measurements.

Since responses to chronic stress and its management are multifactorial and dependent on varied aspects e.g. genetic vulnerability, coping skills, intrinsic personality, and previous exposure to stress, inter-individual variability in biochemical responses is to be expected (McEwen 1998a).

Although stressful events have been studied primarily as risk factors for disease, it is becoming increasingly clear that confronting and adapting to stressful events can result in positive outcomes such as personal growth, reprioritization of life goals, increased feelings of self esteem and self-efficacy, and strengthening of social networks (Briner, 2000). In the face of acute traumatic stressors (e.g. the events of September 11, 2001 in America), acute and ongoing stressors (e.g. the Tsunami in South East Asia and its consequences) and situations of chronic stress (e.g. American military personnel in Iraq), a greater emphasis on the “re-framing” for successful adaptation to stress is desirable – and would broaden our understanding of psychological as well as biological aspects of the stress process in a positive, rather than a negative sense.

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APPENDIX A

RESEARCH METHODOLOGY

The methodologies utilized to determine blood levels of ACTH, cortisol, growth hormone, prolactin, DHEA-S, aldosterone, insulin, glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, free fatty acids, adrenaline, and noradrenaline, and 24-hour urine levels of cortisol are briefly described in this appendix.

All tests were run in laboratories with defined quality assurance protocols – where daily (or per batch) standard curves (as appropriate) were constructed during machine calibration. These standard curves were thus omitted from this appendix.

A.1 Determination of Serum ACTH

Serum ACTH concentrations were determined using a radioimmunoassay kit supplied by Diagnostic Systems Laboratories, Inc., Webster, Texas. Normal fasting range was 2.2-19.8 pmol/L.

A.1.1 Principle of the Assay

The general principle of radioimmunoassay (RIA) is illustrated in Figure A1. The sample (or standard) is mixed with a known amount of radiolabelled antigen and a highly specific antibody. The unlabelled (sample) and labelled antigens compete for the free binding sites on the antibody and soluble antigen-antibody complexes are formed. The amount of bound labelled antigen is inversely proportional to the amount of unlabelled antigen. Once equilibrium has been attained, the antibody-bound and free antigen are separated and the radioactivity of one of the fractions is measured. In this instance, separation is effected by precipitation of the antibody-bound antigen using a second antibody capable of reacting specifically with the first antibody.

In the case of the ACTH test, there is competition between a radioactive and non-radioactive antigen for a fixed number of antibody binding sites. The amount of ^{125}I -labelled ACTH bound to the antibody is inversely proportional to the concentration of the unlabelled ACTH present. The separation of free and bound antigen is achieved by using a pre-reacted double antibody system.

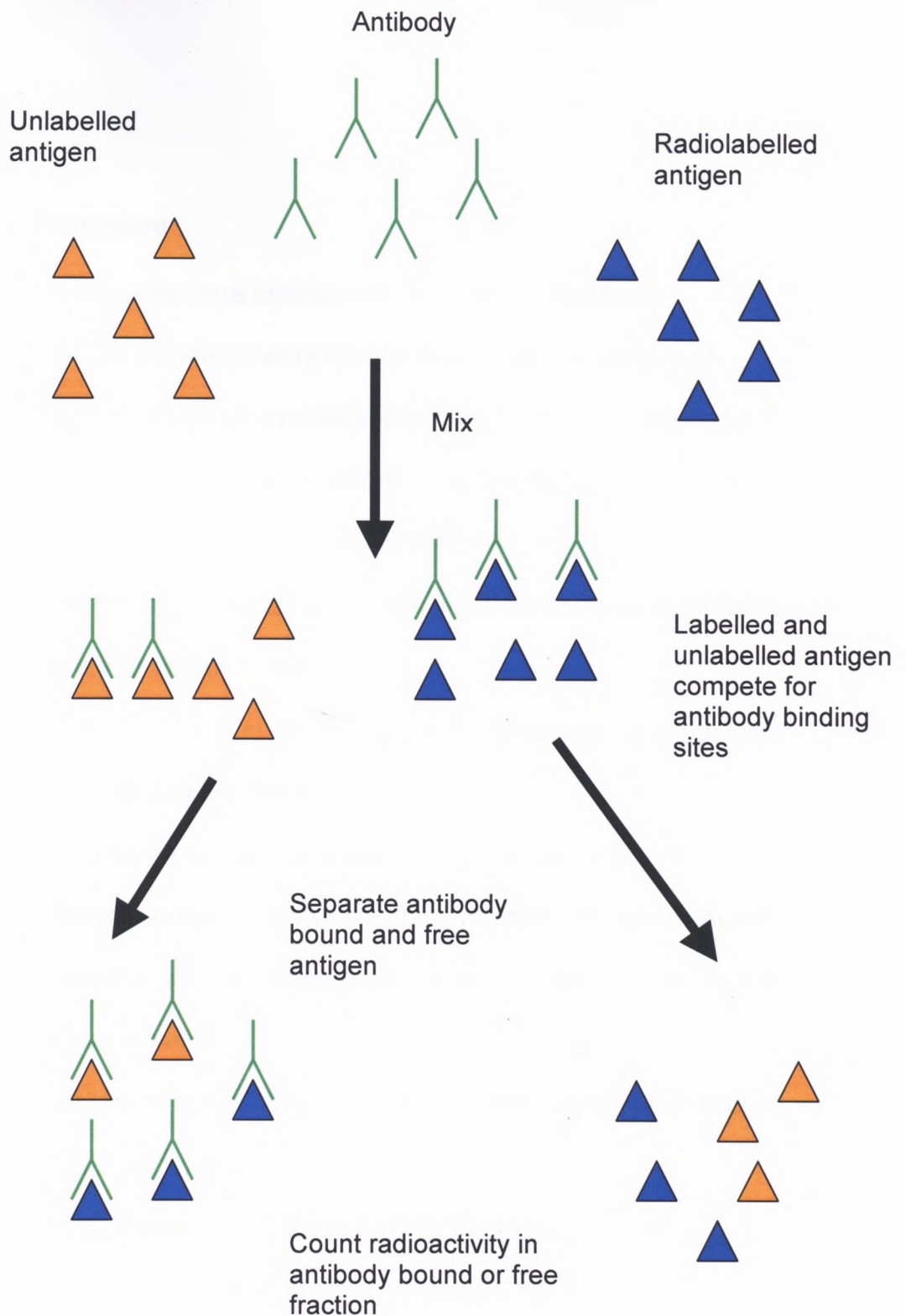


Figure A.1 Principles of Radioimmunoassay

The unlabelled and labelled antigen compete for the free binding sites on the antibody. Soluble antibody-antigen complexes are formed and equilibrium is attained. The antibody-bound and free antigen are separated and radioactivity in one of the fractions is measured (adapted from Walker, 1977).

A.1.2 Procedure

1. Test tubes were labelled and arranged in duplicate.
2. ACTH standards were reconstituted in ACTH buffer with sodium azide as a preservative. Standards were labelled A-F, and contained concentrations of approximately 0, 25, 100, 200, 500 and 1000 pg/mL respectively.
3. 200 μ L of the standards, controls or unknowns were added to the appropriate tubes.
4. 300 μ L of 0 pg/mL ACTH standard (A) was added to the non-specific binding (NSB) tubes.
5. The ACTH antiserum was reconstituted with 10mL of deionised water. The vial contained rabbit anti-human ACTH serum in a buffer with a protein stabilizer and sodium azide as a preservative.
6. 100 μ L of the ACTH antiserum was added to all tubes except NSB and total count tubes.
7. Tubes were incubated at 2 - 8 $^{\circ}$ C for 4 hours.
8. 100 μ L of 125 I-ACTH reagent was added to all tubes.
9. All tubes were vortexed.
10. Tubes were incubated at 2 - 8 $^{\circ}$ C for 16-24 hours.
11. One mL of precipitating reagent was added to all tubes except total count tubes.

12. All tubes were vortexed.
13. Tubes were incubated at room temperature
(approx 18 – 25 °C) for 15-20 minutes.
14. All except total count tubes were centrifuged for 15-20 minutes
at 3000 rpm.
15. All except total count tubes were decanted by simultaneous
inversion with a sponge rack. Tubes were allowed to drain on
absorbent material for 30 seconds, then blotted to remove any
droplets adhering to the rim before turning them upright.
16. All tubes were counted in a gamma counter for one minute.

A.1.3 Calculation of Results

For each standard, control and unknown, the mean counts per minute (CPM) were determined and the corrected counts were obtained by subtracting the CPM for the NSB tubes from all the counts. The percentage of radioactive-labelled ACTH bound for each standard, control and unknown was calculated as follows:

$$\% \text{ Bound} = \frac{\text{mean sample counts} - \text{NSB counts}}{\text{mean total counts}} \times 100$$

On semi-log paper a graph was plotted of %bound for the standards against the ACTH concentration. The concentrations of the unknowns were determined by interpolation from the means of the duplicate counts plotted onto the standard curve.

A.2 *Determination of Serum Cortisol*

Serum cortisol concentrations were determined using a radioimmunoassay kit supplied by Diagnostic Systems Laboratories, Inc., Webster, Texas. The normal fasting range was 138-690 nmol/L.

A.2.1 Principle of the Assay

(See A.1.1 above for the general principle of radioimmunoassay).

The Diagnostic Systems Laboratories kit uses a specific rabbit anti-cortisol antibody, and does not require prior sample extraction from the serum. Cross-reactivity to other naturally occurring steroids is low. In this test there is competition between a radioactive and non-radioactive antigen for a fixed number of antibody binding sites. The amount of ¹²⁵I- labelled cortisol bound to the antibody is inversely proportional to the concentration of the unlabelled cortisol present. The separation of free and bound antigen is achieved by using a pre-reacted double antibody system.

A.2.2 Procedure

1. Tubes for total count, standards, controls and unknowns were labelled and arranged in duplicate.
2. 25 µL of the standards, controls or unknowns were pipetted to the bottom of the tubes.

3. 500 µL of the cortisol (I-125) reagent was immediately added to each tube.
4. 500 µL of the cortisol antiserum complex was added to all tubes except total count tubes.
5. All tubes were vortexed
6. tubes were incubated in a waterbath at 37+/- two degrees C. for 45 minutes.
7. All except total count tubes were centrifuged for 15-20 minutes at 3000 rpm in a refrigerated centrifuge.
8. All tubes except total count tubes were decanted by simultaneous inversion with a sponge rack. They were drained on absorbent material for 30 seconds, then tubes were blotted to remove any droplets adhering to the rim, before turning them to the upright position.
9. All tubes were counted in a gamma counter for one minute.

A.2.3 Calculation of Results

For each standard, control and unknown, the mean counts per minute (CPM) were calculated. The percentage of radioactive-labelled cortisol bound for each standard, control and unknown was calculated as follows:

$$\% \text{ Bound} = \frac{\text{mean sample counts}}{\text{mean total counts}} \times 100$$

A curve was plotted of %bound for the standards (on the y-axis) against the cortisol concentration (on the x-axis) on semi-log graph paper. The concentrations of the unknowns were determined from the means of the duplicate counts plotted onto the standard curve.

A.3 *Determination of Urine Free Cortisol*

Urine free cortisol concentrations were measured by a competitive immunoassay (Bayer Corporation, Tarrytown, NY). The normal range was 55-250 nmol/24hr.

A.3.1 Principle of the Assay

Preliminary extraction of free cortisol from the urine specimen is required because of the large number of cortisol metabolites and conjugates in urine that cross-react with the cortisol antibody in the assay. The extraction relies on the principle that most interfering substances are more soluble than cortisol in the urine, thus the cortisol can be extracted with an organic solvent (e.g. dichloromethane).

The principle of competitive immunoassay is similar to radioimmunoassay, however an enzyme conjugate is used to label the antigen rather than a radioisotope. The unlabelled antigen is thus incubated with a known amount of enzyme and a specific antibody. The enzyme-conjugated antigen and unlabelled antigen compete for the free binding sites on the antibody and soluble antibody-antigen complexes are formed. In the cortisol assay, the soluble complexes are reacted with a monoclonal immunomagnetic particle which separates the complexes from any unbound antigen. Enzymatic activity of the

complex is determined with *p*-nitrophenyl phosphate (pNPP) as the substrate. The alkaline phosphatase in the antibody conjugate reacts with the pNPP to form para-nitrophenoxide and phosphate. Increasing absorbance, due to the formation of para-nitrophenoxide, is monitored at 405 nm. A sample having no cortisol will have the maximum bound, while samples containing high cortisol concentrations will have the minimum bound. Thus, the dose-response curve is inversely proportional to the cortisol concentrations in the sample.

A.3.2 Procedure

A.3.2.1 Extraction:

1. For each unknown sample, 500 μ L of well mixed urine from the 24 hour collection was pipetted in duplicate into 12mm glass tubes.
2. 2.5 ml of cold (4⁰C) dichloromethane was added to the tubes using a positive displacement pipette.
3. Tubes were capped, vortexed vigorously, then centrifuged at 2000rpm for 5 minutes.
4. The upper aqueous layer was removed by suction
5. 1.0 ml of the lower dichloromethane layer was removed and pipetted into fresh glass tubes.
6. This extract was evaporated to dryness using rotary vacuum evaporation.

7. The extract was reconstituted with 250 μ L of zero calibrator buffer, capped and left to stand overnight in the fridge (at 4⁰C).
8. The following morning, extracts were vortexed and then analysed in the automated Boehringer Mannheim/Hitachi 917 analyzing system using the Immuno 1 cortisol channel.
9. Extraction efficiency was demonstrated by performing the extraction procedure five times each on two samples with known cortisol concentrations (high and low). The percentage recovery in all these tests was between 90 - 110%, deemed acceptable for the determination of cortisol.

A.3.2.2 Cortisol Determination:

1. The reagent cassette was loaded with Cortisol Antibody Conjugate (R1) and Cortisol Enzyme Conjugate (R2).
2. Both unknown samples and known calibration samples were inserted in the reaction tray and loaded into the system. The following steps then occurred within the system:
3. R1 and R2 were reacted with samples in the reaction tray and incubated in the system at 37⁰C.
4. The monoclonal ImmunoMagnetic Particle (mIMP) Reagent was added and a second incubation occurred.
5. The mIMP/antibody complex was washed and the pNPP substrate was added.

6. The absorbances of the samples in the reaction tray were read at 405nm.
7. Calibration of the system was performed using Technicon SETpoint Cortisol Calibrators (Bayer Product No. T03-3252-01), which contained six cortisol calibrator levels in a range between high and low levels.
8. A standard calibration curve was constructed from these known concentrations, from which the concentration of free cortisol in the unknown samples was calculated.

A.4 Determination of Serum Growth Hormone

Growth Hormone (GH) concentrations were measured using an immunometric assay supplied by Diagnostic Products Corporation, Los Angeles, CA. Normal fasting ranges were 0.16-13.0 mIU/L.

A.4.1 Principle of the Assay

This immunometric assay is a solid-phase, two-site chemiluminescent assay. The solid phase, a polystyrene bead enclosed within the test unit, is coated with a rabbit monoclonal antibody specific for GH. The patient sample is incubated together with the alkaline phosphatase-conjugated rabbit monoclonal anti-GH antibody and GH in the sample binds to form an antibody “sandwich” complex. Unbound enzyme conjugate is removed by a centrifugal wash, after which substrate is added and the test unit is incubated further. The chemiluminescent substrate, a phosphate ester of adamantyl dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of the intermediate results in the sustained emission of light, thus providing a window for multiple readings and thereby improving precision. The bound complex (and thus also the photon output, as measured by the luminometer) is proportional to the concentration of GH in the sample.

A.4.2 Procedure

1. This assay was conducted using the Immulite ® automated system (Diagnostic Products Corporation [DPC], Los Angeles, California, USA).
2. The machine was calibrated according to the specifications, using the Con 6 Tri-level 1,2 and 3 controls (DPC) in duplicate. The Con 6 controls include known high and low levels of GH.
3. All frozen samples were allowed to reach room temperature.
4. The test units were allowed to come to room temperature before opening to prevent moisture condensation.
5. The reagent wedge was inserted into the reagent carousel tray.
6. Patient samples in duplicate were inserted into the labelled sample cup holder storage rack and the GH test unit was placed behind the sample cup holder.
7. The immulite system:
 - a. added patient sample and alkaline phosphatase-conjugated rabbit polyclonal anti-GH antibody to a test unit and incubated for 30 minutes at 37⁰C with intermittent agitation,
 - b. removed unbound enzyme conjugate by means of a centrifugal wash,
 - c. added substrate and incubated the test unit for a further 10 minutes,

- d. read the photon output in the luminometer at 425-500nm.
 - e. printed the results.
8. The immulite system calculated test results for patients and controls from the observed signal, using a stored master curve.

A.5 *Determination of Serum Prolactin*

Prolactin (PL) concentrations were measured by immunoassay using direct chemiluminometric technology and reagents supplied by Diagnostic Products Corporation, Los Angeles, CA. Normal fasting ranges: 2.8-29.2 µg/L [premenopause] and 1.8-20.3 µg/L [postmenopause].

A.5.1 Principle of the Assay

This assay is a two-site chemiluminometric “sandwich” immunoassay, which uses constant amounts of two antibodies to prolactin. The first antibody is a polyclonal goat antiprolactin antibody labelled with acridinium ester (labelled “Lite Reagent”). The second antibody (Solid Phase) is a monoclonal mouse anti-prolactin antibody, which is covalently coupled to paramagnetic particles. A direct relationship exists between the prolactin in the sample and the relative light units detected by the Bayer systems detector.

A.5.2 Procedure

1. This assay was conducted using the Advia ® Centaur automated system (Bayer Diagnostics, Berkshire, UK).

2. The machine was calibrated according to the specifications, using the Tri-level Ligand Plus.1,2 and 3 controls in duplicate.
3. All frozen samples were allowed to reach room temperature.
4. Samples were then clearly labelled (by barcode) and loaded in duplicate into the rack for the automated test run. This rack was placed in the sample entry queue
5. The prolactin pack was dated and loaded into the reagent refrigerator compartment.
6. At the “start” command, the Centaur system:
 - a. dispensed 25 μ L of sample into a cuvette,
 - b. dispensed 100 μ L of Lite Reagent and incubated it for 5 minutes at 37⁰C,
 - c. dispensed 450 μ L of Solid Phase and incubated it for 2.5 minutes at 37⁰C,
 - d. separated, aspirated and washed the cuvettes with reagent water,
 - e. dispensed 300 μ L each of Acid and Base Reagent to initiate the chemiluminescent reaction,
 - f. printed the results.
7. Samples were removed from the racks.
8. Results were reported.

A.6 *Determination of Serum DHEA-S*

Serum DHEA-S concentrations were determined using a radioimmunoassay kit supplied by Diagnostic Systems Laboratories, Inc., Webster, Texas. The normal fasting range for females was 1.0-7.3 $\mu\text{mol/L}$.

A.6.1 Principle of the Assay

(See A.1.1 for the general principle of radioimmunoassay).

In this test there is competition between a radioactive and non-radioactive antigen for a fixed number of antibody binding sites. The amount of ^{125}I -labelled DHEA-S bound to the antibody is inversely proportional to the concentration of the unlabelled DHEA-S present. The separation of free and bound antigen is achieved by decanting the tubes, since these are pre-coated with anti-DHEA-S antibody.

A.6.2 Procedure

1. Two plain tubes (uncoated) were labelled for total counts.
2. Anti-DHEA-S coated tubes were labelled and arranged in duplicate for standards, controls and unknowns.

3. 10 µL of the standards, controls and unknowns were pipetted to the bottom of the appropriate tubes.
4. 500 µL of the DHEA-S (¹²⁵I) reagent was immediately added to each tube.
5. Tubes were mixed by shaking the test tube rack gently.
6. All tubes were incubated in a water bath at 37+/- two degrees C. for 45 minutes.
7. All except total count tubes were decanted by simultaneous inversion with a sponge rack. They were drained on absorbent material for two minutes, then blotted to remove the any droplets adhering to the rim. Tubes were then returned to the upright position.
8. All tubes were counted in a gamma counter for one minute.

A.6.3 Calculation of Results

For each standard, control and unknown, the mean counts per minute (CPM) was calculated. The percentage of radioactive-labelled DHEA-S bound for each standard, control and unknown was calculated as follows:

$$\% \text{ Bound} = \frac{\text{mean sample counts}}{\text{mean total counts}} \times 100$$

On semi-log paper a graph was plotted of %bound for the standards against the DHEA-S concentration. The concentrations of the unknowns were determined from the means of the duplicate counts plotted onto the standard curve.

A.7 Determination of Serum Aldosterone

A.7.1 Principle of the Assay

(See A.1.1 for the general principle of radioimmunoassay).

The Coat-A-Count procedure is a solid-phase radioimmunoassay, based on aldosterone-specific antibody immobilized to the wall of a polypropylene tube. ^{125}I -labelled aldosterone competes for a fixed time with aldosterone in the patient sample for antibody sites. The tube is then decanted, to separate bound from free, and counted in a gamma counter. The amount of aldosterone present in the patient sample is determined from a calibration curve.

The antiserum for the Coat-A-Count aldosterone kit is very specific for aldosterone, with an extremely low cross-reactivity to other compounds that may be found in patient samples. The specificity of this test is therefore correspondingly high.

A.7.2 Procedure

1. Four plain (uncoated) 12x75mm polypropylene tubes were labelled for total counts (T) and non-specific binding (NSB) in duplicate.
2. Fourteen aldosterone antibody-coated tubes were labelled A (maximum binding) to G in duplicate to construct a calibration curve

3. Sixty-four aldosterone antibody-coated tubes were labelled for control and patient samples in duplicate.
4. 200 μ L of the zero calibrator (A) was pipetted into the NSB and A tubes.
5. 200 μ L of the calibrators B-G, the control samples and the patient samples were pipetted into the appropriately labelled tubes, changing the disposable-tip of the micropipet between each sample..
6. 1.0ml of 125 I aldosterone was added to each tube and the tubes were vortexed gently.
7. The tubes were incubated for 18 hours at room temperature.
8. The tubes were then decanted thoroughly using a foam decanting rack and draining any residual droplets onto absorbent paper.
9. All tubes were then counted for 1 minute in a gamma counter

A.7.3 Calculation of Results

To determine corrected counts per minute (CPM), the average NSB CPM were subtracted from the average recorded CPM.

To determine the binding of each pair of tubes as a percent of the maximum binding (MB), the NSB –corrected counts of the A tubes (containing no aldosterone) were taken as 100%. Percentage binding

was then calculated as the ratio of net counts to net MB counts multiplied by 100.

Using logit-log graph paper, the percent bound was plotted on the vertical axis against concentration on the horizontal axis for each of the known calibrators, and a straight line was drawn from these points.

Results for the unknowns were then read off from the line by interpolation.

A.8 Determination of Serum Insulin

Serum insulin concentrations were measured by radioimmunoassay using reagents supplied by Diagnostic Products Corporation, Los Angeles, CA. Normal fasting range was 6.0-27.0 mIU/L.

A.8.1 Principle of the Assay

The method is a solid phase radioimmunoassay where ^{125}I -labelled insulin competes for a fixed time with insulin in the patient sample for binding sites on insulin-specific antibody. The insulin concentration is measured comparing its competitive capacity with that of insulin standards of known concentration. Because the antibody is immobilized to the wall of a polypropylene tube, decanting the supernatant terminates the competition and isolates the antibody-bound fraction of the radiolabelled insulin. The amount of bound radioactivity is inversely proportional to the quantity of insulin present in the standards and the samples.

A.8.2 Procedure

1. Samples were allowed to reach room temperature before use.
2. Four plain (uncoated) tubes were labelled for total counts (T) and non-specific binding (NSB) in duplicate.

3. Fourteen insulin antibody-coated tubes were labelled from A maximum binding (MB) to G, where approximate $\mu\text{IU/mL}$ for each calibrator was as follows:

A(MB)	0
B	5
C	15
D	50
E	100
F	200
G	350

4. Sixty four insulin antibody-coated tubes were labelled according to patient samples and controls in duplicate.
5. 200 μL of the zero calibrator A was pipetted directly into the bottom of the NSB and A tubes.
6. 200 μL of each remaining calibrator, control and patient sample was pipetted directly into the bottom of the prepared tubes using a disposable –tip micropipette.
7. 1 mL of ^{125}I insulin was added to every tube and these were vortexed.
8. Tubes were incubated for 18-24 hours at room temperature.
9. Except for the T tubes, all tubes were decanted thoroughly using a foam decanting rack. They were allowed to drain for 3 minutes and all visible moisture was removed.
10. Tubes were counted for 1 minute in a gamma counter.

A.8.3 Calculation of Results

For each pair of tubes, the average NSB-corrected counts per minute was calculated as follows:

Net counts = average CPM minus average NSB CPM.

Then, the binding of each pair of tubes was determined as a percent of maximum binding (MB) with the NSB-corrected counts of the A tubes taken as 100%:

$$\text{Percent bound} = \frac{\text{net counts}}{\text{net MB counts}} \times 100$$

Using logit-log graph paper, the percentage bound was plotted on the y-axis against concentration on the (logarithmic) x-axis for each of the non-zero calibrators. A straight line graph was obtained, from which results of insulin concentrations in the samples were determined.

A.9 Determination of Serum Glucose

Serum glucose concentrations were determined using a standard glucose phosphorylation method, employing a Roche/Hitachi 917 Analyzer (Roche Diagnostics Corporation, Indianapolis, USA).

The normal fasting range was 3.0-6.0 mmol/L.

A.9.1 Principle of the Assay

The Roche/Hitachi 917 Analyzer measures glucose concentration by an enzymatic method. Hexokinase catalyses the phosphorylation of glucose to glucose-6-phosphate by ATP. The resultant glucose-6-phosphate is oxidised by glucose-6-phosphate dehydrogenase to gluconate-6-phosphate with concomitant reduction of nicotinamide adenine dinucleotide phosphate (NADP). No other carbohydrate is oxidised. The amount of NADPH formation during the reaction is directly proportional to the glucose concentration and can be measured photometrically (at 340nm).

A.9.2 Procedure

1. The reagent cassette was loaded with Buffer/ATP/NADP solution (R1) and hexokinase/G-6-PDH solution (R2).

2. Both unknown samples and known calibration samples were labelled by barcode, inserted in the reaction tray and loaded into the system. The following steps then occurred within the system:
3. 180 μ L of R1 and 36 μ L of R2 were reacted with samples in the reaction cells and incubated in the system for 5 minutes.
4. The absorbances of the samples in the reaction tray were read at 505nm, and concentrations of glucose were computer calculated.

A.10 Determination of Serum Total Cholesterol

Total cholesterol (TC) concentrations were determined by an enzymatic, colorimetric assay using a reagent kit from Roche Diagnostics Corporation, Indianapolis, USA. The normal fasting range was <6.2 mmol/L.

A.10.1 Principle of the Assay

Cholesterol esters are hydrolysed by cholesterol esterase to produce free cholesterol. The free cholesterol is oxidised by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide formed in this reaction combines with 4-aminophenazone and phenol in the presence of peroxidase to form a red dye. The dye colour intensity is directly proportional to the concentration of cholesterol and can be measured on a spectrophotometer.

A.10.2 Procedure

1. The reagent cassette was loaded with cholesterol reagent (R1).
2. Both unknown samples and known calibration samples were labelled by barcode, inserted in the reaction tray and loaded into the system. The following steps then occurred within the system:
3. 180 μ L of R1 was reacted with samples in the reaction cells and incubated in the system for 10 minutes.

4. The absorbances of the samples in the reaction tray were read at 505nm, and concentrations of cholesterol were computer calculated.

A.11 Determination of Serum High-Density Lipoprotein Cholesterol

High density lipoprotein-cholesterol (HDL-C) levels were determined by an enzymatic, colorimetric method using a reagent kit supplied by Roche Diagnostics Corporation, Indianapolis, USA. The normal fasting range was >1.6 mmol/L

The concentration of HDL-C was determined by direct measurement in the serum with polyethylene glycol-modified enzymes and sulphated α -cyclodextrin, according to the method of Sugiuchi *et al.* (1995).

A.11.1 Principle of the Assay

Modification of cholesterol esterase and cholesterol oxidase enzymes by polyethylene glycol (PEG) causes their catalytic activity to become selective, with the reactivity towards lipoprotein fractions increasing in the order: LDL < VLDL $\approx$ chylomicrons < HDL.

In the presence of magnesium ions, sulphated α -cyclodextrin selectively forms water soluble complexes with LDL, VLDL and chylomicrons which are resistant to PEG-modified enzymatic breakdown. Using this method, there is thus no necessity to remove the other lipoproteins by precipitation.

HDL-C-esters are broken down by PEG-modified cholesterol esterase to form free cholesterol and fatty acids. In the presence of oxygen, this

cholesterol is oxidised by cholesterol oxidase to Δ^4 -cholestenone and hydrogen peroxide. The hydrogen peroxide formed during this reaction reacts with 4-aminophenazone and an aniline substance in the presence of peroxidase to form a purple blue dye. The colour intensity of the dye is directly proportional to the HDL-C concentration and can be measured using a spectrophotometer.

A.11.2 Procedure

1. The reagent cassette was loaded with buffer/ α -cyclodextrin solution (R1) and PEG-enzymes/4-aminophenazone/buffer solution (R2).
2. Both unknown samples and known calibration samples were labelled by barcode, inserted in the reaction tray and loaded into the system. The following steps then occurred within the system:
3. 180 μ L of R1 and 60 μ L of R2 were reacted with samples in the reaction cells and incubated in the system for 10 minutes.
4. The absorbances of the samples in the reaction tray were read at 600nm, and concentrations of HDL-C were computer calculated.

A.12 Calculation of Low-Density Lipoprotein Cholesterol

Low density lipoprotein –cholesterol (LDL-C) was calculated according to the formula of Friedewald *et al.* (1972). This method gives an approximation of the LDL-C, provided that the triglyceride (TG) level is below 5.6 mmol/L.

$$\text{LDL-C (mmol/L)} = \text{TC} - \text{TG} - \frac{\text{HDL-C}}{2.2}$$

The normal fasting range for LDL-C was <4.1 mmol/L.

A.13 Determination of Serum Triglyceride Concentration

Triglycerides (TG) were determined by an enzymatic, colorimetric method using a reagent kit supplied by Roche Diagnostics Corporation, Indianapolis, USA. The normal fasting range was 0.0-2.0 mmol/L.

A.13.1 Principle of the Assay

The method utilizes a reagent containing microbial lipoprotein lipases which rapidly hydrolyse TG to glycerol. The glycerol is oxidised to dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide then reacts with 4-aminophenazone and 4-chlorophenol to form a red dye. The amount of colour produced is proportional to the concentration of TG and can be measured photometrically.

A.13.2 Procedure

1. The reagent cassette was loaded with buffer/4-chlorophenol/enzymes solution (R1).
2. Both unknown samples and known calibration samples were labelled by barcode, inserted in the reaction tray and loaded

into the system. The following steps then occurred within the system:

3. 180 μ L of R1 was reacted with samples in the reaction cells and incubated in the system for 10 minutes.
4. The absorbances of the samples in the reaction tray were read at 505nm, and concentrations of triglycerides were computer calculated.

A.14 Determination of Serum Free Fatty Acids

Free fatty acid (FFA) levels (nonesterified) were determined by an enzymatic, colourimetric method using a reagent kit supplied by Roche Diagnostics Corporation, Indianapolis, USA. The normal fasting range was 0.3-0.8 mmol/L.

A.14.1 Principle of the Assay

In the presence of the enzyme acyl-CoA synthetase (Acyl CS) and coenzyme A (CoA), FFA are converted utilising energy from the dephosphorylation of ATP to AMP into acyl-coenzyme A (acyl-CoA) and pyrophosphate. In the presence of oxygen, the enzyme acyl-CoA oxidase (ACOD) catalyses the oxidation of acyl-CoA to 2,3-enoyl-coenzyme A and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide converts 2,4,6-tribromo-3-hydroxy-benzoic acid and 4-aminoantipyrine to a red dye. The red dye is measured at 546nm wavelength on a spectrophotometer and the amount of colour produced is proportional to the concentration of FFA in the sample or standard.

A.14.2 Procedure

1. The reagents and samples were allowed to reach RT and were mixed before use.
2. The reagent cassette was loaded with buffer/ATP/coA/Acyl CS solution (Reaction mixture A) -solution I, N-ethyl-maleinimide – solution II, and ACOD solution (Reaction mixture B) - solution III.
3. Both unknown samples and known calibration samples were labelled by barcode, inserted in the reaction tray and loaded into the system. The following steps then occurred within the system:
 4. 500 μ L of Reaction mixture A was added to samples in the reaction cells and incubated in the system for 10 minutes.
 5. 30 μ L of solution II was added and the computer recorded absorbances for the solutions (A_1).
 6. 30 μ L of reaction mixture B was added to samples in the reaction cells and incubated in the system for 15 minutes.
7. The absorbances of the samples in the reaction tray were read again (A_2), and concentrations of FFA were computer calculated.

A.14.3 Calculation Of Results

For both the blank and the samples, absorbance differences were calculated by subtracting A_1 from A_2 . The final reading (ΔA) was obtained by subtracting the absorbance difference from the blank (ΔA_b) from the absorbance difference of the sample (ΔA_s).

$$\Delta A = \Delta A_s - \Delta A_b$$

These results were computer generated.

A.15 Determination of Plasma Adrenaline and Noradrenaline

Adrenaline and noradrenaline concentrations were measured using high-performance liquid chromatography (HPLC) (Bio-Rad Laboratories, Hercules, CA). Respective normal fasting ranges were 0-366 pmol/L and 560-2636 pmol/L.

A.15.1 Principle of the Assay

HPLC is a separation technique which is well suited to analyse the low picogram concentrations of catecholamines found in plasma. The HPLC system (Figure A.2) consists of 4 major components: the solvent delivery system (pump), an injection system, a column and a detector. In this method, a mixture of components in a liquid(mobile) phase is injected at the top of the column which is packed with a solid (stationary) phase. As the mixture of solutes travels down the column it is separated into its component molecules, with the rate of migration of each component through the column being proportional to its distribution coefficient.

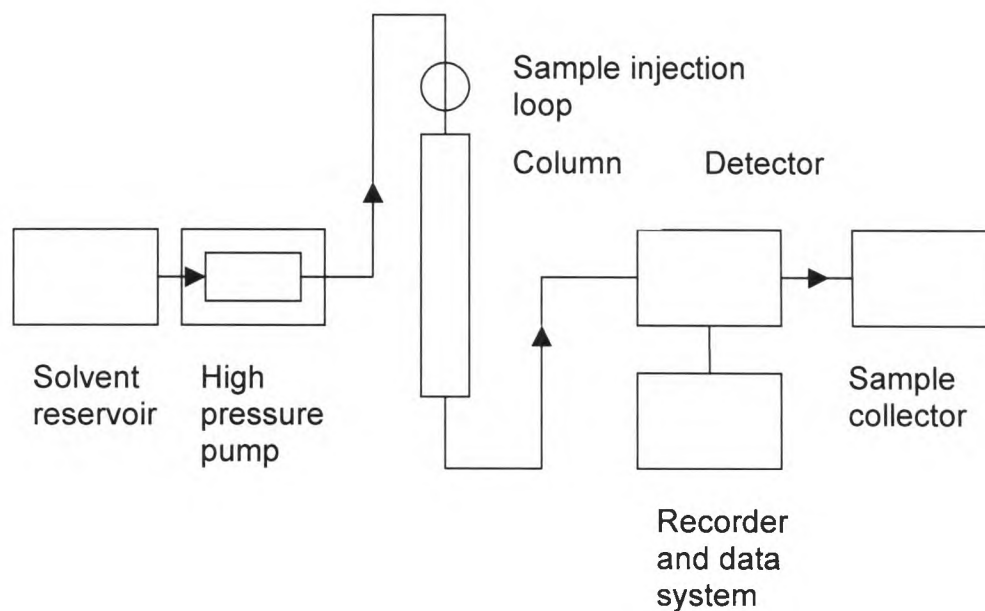


Figure A.2. Schematic diagram of an HPLC system

The HPLC system consists of 4 major components: the solvent delivery system (pump), an injection system, a column and a detector. A mixture of components in a liquid(mobile) phase is injected at the top of the column which is packed with a solid (stationary) phase. The column effects separation of the mixture, according to the distribution coefficients of its constituents.

A.15.2 Procedure

A.15.2.1 Alumina extraction:

1. Five extraction tubes were labelled for the calibrator, 2 tubes for each control and 2 tubes for each patient sample.
2. Approx. 35 mg of alumina was placed into each tube.
3. 2 ml calibrator, control or patient sample was added to the series of labelled tubes.
4. 200 μ L of working internal standard was added, followed by 1 ml TRIS buffer (25mM at pH 7.4). The tubes were capped, then held in a horizontal position and shaken vigorously by hand for 5 minutes.
5. Tubes were then centrifuged at 2500 rpm for 5 minutes.
6. As much liquid as possible was removed using disposable transfer pipettes, while care was exercised not to disturb the alumina.
7. 1ml HPLC grade water was added, then each tube was vortexed for 15 seconds.
8. Tubes were then centrifuged at 2500 rpm for 5 minutes, following which as much liquid as possible was again removed.
9. The alumina was washed again by repeating steps 7 and 8.
10. 150 μ L 0.1M phosphoric acid was added to the alumina and each tube was vortexed for 30 seconds.

11. Tubes were then centrifuged at 2500 rpm for 2 minutes.
12. Extracts were carefully removed from the alumina and transferred to an appropriately labelled automatic sampler vial.

A.15.2.2 HPLC Analysis

1. The mobile phase (a Bio-Rad proprietary preparation) was pumped at 1.1 ml/min for 60 minutes with the heater on at 30⁰C and the electrochemical detector cell in the “on” position to ensure that the column was fully calibrated. Settings on the Electrochemical Detector (ED) were adjusted to: potential 0.50 V, sensitivity 2 nA/V full scale, filter (time constant) 5 seconds. The output was adjusted to between 0.1 and 0.2 V.
2. The injected sample volume was 50 µL. The test solution was injected twice, then compared with a previous chromatogram. Similar retention times confirmed that the system was at equilibrium.
3. The calibrators, containing specified concentrations of adrenaline, noradrenaline and dopamine, were reconstituted with de-ionised water.
4. The first calibrator extract was then injected and when all the peaks had eluted, the second calibrator extract was injected. These chromatograms were used for calibration.
5. Controls followed by unknown samples were then injected in turn. At the end of the run, controls were again injected to

ensure that column or detector performance had not altered during the run. Two control samples were used, one containing “low” levels of catecholamines (range: 50-100 pg) and one containing a “high” level of hormone (range: 300-400pg).

6. The mobile phase flow rate was lowered to 0.1 ml/min until calculation of results was complete, then the HPLC system was rinsed with 30 ml HPLC wash reagent prior to shutdown.

A.15.3 Calculation of Results

Adrenaline (A), noradrenaline (NA) and internal standard (IS) peaks were identified for each chromatogram by their retention times. Peak heights were measured and the unknown concentrations were calculated using the following formula:

$$\text{Unknown concentration} = \frac{\text{calibrator A or NA concentration (pg/ml)}}{\text{A or NA /IS peak height ratio for calibrator}}$$

The final concentration was converted to pmol/L by division of the pg/ml result by a factor of 183.15 for adrenaline and 169.20 for noradrenaline.

APPENDIX B: RESULTS

INDIVIDUAL DATA FOR PATIENTS AND CONTROL SUBJECTS

B.1 Pituitary and Adrenal Hormones

The results of the pituitary and adrenal hormone studies in patients and controls are documented in tables B1-7, and summarised in Table 3, Chapter 5.

B.1.1 ACTH

Table B1

Fasting 8.00 AM Serum Concentrations of **ACTH (pmol/L)**, Before and During 4 months of Therapy for
Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	39	41.4	40.7	37.9	24.7	C1	137.6	58.7
2	34.8	43.8	53.0	35.1	48.2	C2	52.1	38.4
3	49.2	41.1	33.6	27.6	29.9	C3	19.4	49.3
4	25.8	18.6	15.4	11.7	30.0	C4	161.6	56.7
5	123.8	29.6	22.0	22.9	152.0	C5	18.7	38.2
6	23.9	60.5	31.2	23.9	30.1	C6	54.3	47.0
7	41.5	40.1	33.4	41.1	53.2	C7	42.8	28.9
8	21.1	16.8	21.0	20.2	70.8	C8	24.3	33.9
9	11.8	148.6	13.3	15.1	31.3	C9	43.3	22.1
10	14.3	7.0	11.2	10.4	12.5	C10	135.0	26.7
11	18.9	12.1	33.9	13.4	100.3	C11	28.0	23.2
12	151.4	28.7	19.7	19.6	15.4	C12	73.3	34.8
13	10.9	10.8	256.4	9.5	14.0	C13	53.6	10.0
14	32.3	46.7	54.6	37.7	34.1	C14	25.6	25.9
15	12.5	17.7	55.4	16.1	15.8	C15	50.1	23.3
16	28.1	21.5	23.6	13.0	19.0	C16	215.7	135.6
Mean ±	40.0±10.0	36.6±8.4	44.9±14.5	22.2±2.7	42.6±9.3		71.0±14.7	40.8±7.1
SEM								

B.1.2 Urine Free Cortisol

TableB 2

24 Hour Concentrations of **Urine free cortisol (nmol/24hr)**, Before and During 4 months of Therapy
for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	59.4	62.2	59.6	30.9	16.6	C1	104.8	30.6
2	67.6	56.6	55.6	48.9	69.9	C2	133.5	94.6
3	72.7	77.1	52.7	46.2	39.9	C3	92.0	70.3
4	16.2	26.2	17.9	64.4	69.8	C4	98.5	67.9
5	89.9	71.3	51.5	136.6	79.7	C5	61.3	92.7
6	26.5	27.3	47.0	56.0	4.9	C6	88.9	113.1
7	22.1	25.7	69.5	32.1	30.0	C7	31.0	32.4
8	18.2	34.2	57.5	31.1	20.5	C8	65.8	141.4
9	30.8	55.0	9.8	5.8	2.1	C9	62.4	98.5
10	185	35.2	102.6	58.9	63.2	C10	34.8	121.0
11	37.6		73.2	61.5	58.8	C11	70.4	137.4
12	18.6	22.4	11.5	43.4	32.4	C12	116.6	122.1
13	8.2	8.5	9.9	22.0	41.7	C13	66.8	96.5
14	45.1	70.3	41.0	39.5	61.3	C14	93.0	45.4
15	10.0	32.1	83.2	48.1	61.3	C15	71.9	122.6
16	47.3	70.3	55.4	48.3	51.5	C16	71.6	70.9
Mean ±	47.2±11	45.8±5.4	49.9±6.7	48.4±7.0	44.0±6.1		79.0±6.8	91.1±8.8
SEM								

B.1.3 Cortisol

Table B3

Fasting 8.00 AM Serum Concentrations of **Cortisol (nmol/L)**, Before and During 4 months of Therapy
for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	273	315	254	546	337	C1	566	1049
2	177	298	453	582	420	C2	933	1063
3	994	646	461	571	580	C3	1479	1413
4	1341	1648	1816	2277	367	C4	1943	2095
5	403	834	627	384	1054	C5	770	1236
6	972	1129	878	665	254	C6	505	1212
7	450	259	276	257	138	C7	469	740
8	1104	858	1926	571	858	C8	751	1046
9	1165	1297	900	872	1074	C9	574	640
10	466	654	1181	508	696	C10	834	938
11	328	370	132	505	496	C11	756	442
12	662	936	811	1264	618	C12	298	1546
13	392	464	1372	166	127	C13	1317	1576
14	381	395	436	400	508	C14	952	846
15	331	580	447	522	364	C15	259	367
16	491	290	676	431	486	C16	1893	284
Mean ±	621±92	686±102	790±135	658±125	524±71d		894±129	1031±122
SEM								

B.1.4 Growth Hormone

Table B4

Fasting 8.00 AM Serum Concentrations of **Growth Hormone (mIU/L)**, Before and During 4 months of Therapy for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	7.0	3.1	12.2	15.0	25.8	C1	0.2	0.6
2	1.5	0.2	0.6	0.2	0.3	C2	2.1	1.8
3	1.5	1.5	0.6	0.4	0.6	C3	1.9	0.8
4	0.8	11.9	0.8	0.6	26.0	C4	12.4	36.2
5	0.3	0.9	1.7	5.5	16.2	C5	9.1	0.6
6	2.2	17.4	8.9	13.0	14.7	C6	10.0	25.5
7	0.3	18.8	18.0	9.8	0.7	C7	7.3	5.3
8	0.6	7.7	1.5	0.3	7.3	C8	16.1	14.3
9	2.1	1.4	0.5	8.4	5.9	C9	8.6	6.3
10	0.2	9.5	4.5	3.6	11.8	C10	37.0	28.8
11	1.3	1.7	0.6	5.6	0.8	C11	0.3	1.2
12	4.2	0.3	6.6	1.6	9.2	C12	3.1	0.5
13	1.0	1.7	0.6	0.6	1.0	C13	4.1	2.9
14	0.2	0.2	0.3	0.2	0.2	C14	0.5	2.5
15	0.3	0.7	0.4	8.7	2.1	C15	7.4	0.3
16	35.0	21.1	13.9	5.9	17.2	C16	2.9	12.3
Mean ±								
	3.7±2.1	6.1±1.8	4.5±1.4	5.0±1.2	8.7±2.2		7.7±2.3	8.7±2.9
SEM								

B.1.5 Prolactin

Table B5

Fasting 8.00 AM Serum Concentrations of **Prolactin (µg/L)**, Before and During 4 months of Therapy
for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	10.4	8.9	6.8	10.1	7.9	C1	4.9	4.5
2	5.2	4.9	8.7	3.0	5.8	C2	4.4	4.5
3	2.3	5.4	3.2	4.8	7.4	C3	6.0	6.3
4	7.3	7.3	6.5	18.2	14.8	C4	3.9	5.7
5	5.1	6.3	11.3	6.1	5.4	C5	7.4	9.5
6	3.5	4.0	5.5	3.1	2.6	C6	6.6	3.9
7	33.5	8.2	5.2	6.0	3.4	C7	10.1	12.3
8	3.9	3.7	4.1	7.1	3.7	C8	7.0	8.7
9	5.7	7.4	3.4	6.2	4.0	C9	16.8	23.9
10	6.6	6.0	10.7	5.6	4.7	C10	4.1	5.7
11	4.8	3.5	0.9	6.5	6.3	C11	14.5	14.7
12	14.9	29.7	26.0	21.8	44.3	C12	9.1	5.0
13	5.1	2.2	4.0	3.6	8.4	C13	10.8	11.5
14	5.1	5.5	5.6	6.5	6.3	C14	4.4	6.7
15	7.3	6.9	4.9	8.5	5.1	C15	13.6	11.1
16	8.6	12.5	8.7	10.1	12.9	C16	14.1	7.0
Mean ±	8.1±1.9	7.7±1.6	7.2±1.4	8.0±1.3	8.9±2.5		8.6±1.1	8.8±1.3
SEM								

B.1.6 DHEA-S

Table B6

Fasting 8.00 AM Serum Concentrations of **DHEA-S** ($\mu\text{mol/L}$), Before and During 4 months of Therapy
for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	4.62	4.15	2.79	3.84	3.78	C1	1.27	1.61
2	4.49	6.26	6.50	6.41	6.96	C2	1.73	0.94
3	2.31	4.55	1.22	1.95	2.02	C3	0.56	0.60
4	5.21	6.47	4.34	5.04	7.54	C4	0.80	0.70
5	0.95	0.78	0.82	0.98	0.91	C5	3.65	3.51
6	0.30	0.78	0.59	0.92	0.31	C6	1.73	1.42
7	2.73	1.45	1.25	1.85	1.54	C7	0.80	0.54
8	2.18	2.32	2.46	2.09	2.09	C8	2.18	2.65
9	1.01	0.47	0.38	0.39	0.33	C9	1.73	1.92
10	3.02	2.38	2.63	2.65	3.68	C10	1.14	0.93
11	3.42	3.22	3.63	2.83	3.52	C11	3.08	2.95
12	0.17	0.21	0.23	0.22	0.11	C12	4.53	2.58
13	2.09	1.42	3.62	2.09	1.55	C13	3.08	4.01
14	1.78	2.48	2.57	2.48	2.99	C14	1.74	1.34
15	4.86	3.89	6.12	5.61	6.16	C15	1.70	1.67
16	2.09	1.81	2.57	1.54	1.70	C16	2.80	4.91
Mean $\pm$	2.6$\pm$0.4	2.7$\pm$0.5	2.6$\pm$0.5	2.6$\pm$0.5	2.8$\pm$0.6		2.0$\pm$0.3	2.0$\pm$0.3
SEM								

B.1.7 Aldosterone

Table B7

Fasting 8.00 AM Serum Concentrations of **Aldosterone (pmol/L)**, Before and During 4 months of Therapy for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	134.0	77.2	168.0	137.0	126.0	C1	119.0	50.0
2	184.0	57.6	200.0	112.0	92.7	C2	209.0	261.0
3	51.9	53.0	136.0	257.0	89.0	C3	174	278
4	169.0	321.0	579.0	102.0	240.0	C4	428	1240
5	79	346	176	246	577	C5	361	238
6	205	237	154	78.3	263	C6	287	587
7	135.0	305.0	374	570	499	C7	398	331
8	196.0	204.0	212	117	323	C8	305	441
9	373.0	1070	392.0	641.0	1148.0	C9	228	394
10	818.0	120.0	212.0	191.0	695.0	C10	627	307
11	214.0	353	137.0	209.0	245	C11	509	290
12	51.3	352.0	182.0	183.0	109.0	C12	111	115
13	223.0	414.0	1974.0	228.0	134.0	C13	750	581
14	256.0	481.0	389	541.0	467.0	C14	526	233
15	278.0	200.0	159.0	145.0	520.0	C15	269	170
16	271.0	379.0	518.0	476.0	971.0	C16	224	208
Mean ±	277±45	311±60	373±112	265±46	406±80		345±46	358±69
SEM								

B.2 Biochemical Data and BMI

The results of studies on serum glucose, insulin and lipids in patients and control subjects are documented in tables B8-16 and summarised in Table 4, Chapter 5.

B.2.1 Insulin

Table B8

Fasting Serum Concentrations of **Insulin (mIU/L)**, Before and During 4 months of Therapy for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	7.7	6.1	8.9	6.6	8.3	C1	6.7	5.4
2	8.8	8.1	7.9	9.7	10.0	C2	6.1	6.2
3	14.7	32.6	13.4	10.9	9.9	C3	7.4	6.7
4	4.4	7.5	4.1	12.5	5.3	C4	6.3	7.1
5	6.0	6.4	5.4	5.1	7.2	C5	10.9	10.7
6	9.4	13.5	11.7	8.8	13.5	C6	2.1	3.7
7	10.9	8.7	5.8	6.9	6.6	C7	6.1	5.0
8	6.2	5.6	6.2	6.7	7.0	C8	8.4	6.8
9	23.1	23.2	21.3	19.9	24.0	C9	16.6	14.5
10	6.0	4.2	4.0	9.5	9.0	C10	6.7	5.1
11	7.4	8.2	8.8	8.6	9.6	C11	7.5	7.9
12	4.1	3.9	7.3	23.2	3.0	C12	7.1	6.4
13	11.5	15.1	25.8	14.2	9.6	C13	8.1	21.9
14	2.6	5.1	5.7	6.2	5.7	C14	6.6	5.7
15	4.9	5.5	6.7	9.7	8.7	C15	5.5	4.6
16	5.0	7.6	8.3	8.0	7.3	C16	5.2	10.5
Mean ±								
	8.3±1.3	10.1±1.9	9.5±1.5	10.4±1.2	9.0±1.2		7.3±0.8	8.0±1.2
SEM								

B.2.2 Glucose

Table B9

Fasting Serum Concentrations of **Glucose (mmol/L)**, Before and During 4 months of Therapy for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	4.6	4.8	4.6	5.0	3.9	C1	5.4	5.2
2	4.7	5.3	5.1	5.0	5.0	C2	4.3	4.7
3	5.0	4.8	4.4	4.8	4.4	C3	5.5	5.5
4	5.4	5.2	4.8	4.9	4.4	C4	5.3	5.1
5	4.6	5.1	5.2	4.5	4.9	C5	4.9	4.2
6	4.4	4.7	4.6	4.4	4.3	C6	4.8	4.7
7	4.9	4.6	4.1	4.5	4.1	C7	4.6	4.5
8	4.9	4.8	5.0	4.6	4.7	C8	4.7	5.2
9	5.6	6.0	5.8	4.6	6.5	C9	6.5	6.8
10	4.3	4.9	4.3	4.5	4.8	C10	4.2	4.4
11	4.7	4.6	4.9	5.2	4.9	C11	4.9	4.7
12	5.1	5.1	6.0	5.2	4.4	C12	4.7	4.6
13	5.3	5.1	5.4	4.4	3.7	C13	4.5	6.6
14	4.0	4.4	4.4	3.6	4.0	C14	4.2	4.4
15	4.5	5.1	4.9	5.5	5.0	C15	4.2	4.7
16	4.9	5.0	4.2	4.0	4.5	C16	4.6	4.6
Mean ±SEM	4.8±0.11	5.0±0.09	4.9±0.14	4.7±0.12	4.6±0.16		4.8±0.15	5.0±0.19

B.2.3 Total Cholesterol

Table B10

Fasting Serum Concentrations of **Total Cholesterol (mmol/L)**, Before and During 4 months of Therapy
for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	5.2	5.2	4.4	4.9	4.3	C1	7.1	7.6
2	5.3	5.4	5.8	6.4	6.9	C2	5.2	5.6
3	2.9	3.9	4.3	4.4	4.1	C3	5.4	5.5
4	5.0	5.1	4.8	5.3	5.3	C4	5.0	5.0
5	5.9	5.8	6.3	5.4	5.9	C5	4.4	4.7
6	5.2	5.3	6.5	6.2	4.8	C6	4.5	4.7
7	4.3	4.0	3.9	4.0	3.6	C7	4.7	4.8
8	4.6	5.0	4.4	4.7	4.7	C8	4.5	4.7
9	4.2	3.8	3.7	3.8	3.8	C9	4.3	4.4
10	2.2	2.3	2.9	2.7	2.6	C10	4.6	5.5
11	3.8	3.6	4.8	4.4	4.5	C11	4.1	4.4
12	5.5	6.0	4.6	4.6	4.5	C12	4.4	4.8
13	6.2	5.5	6.6	4.9	5.2	C13	5.7	4.9
14	4.7	4.9	4.6	5.2	5.3	C14	5.8	5.8
15	5.3	4.9	6.4	6.0	5.2	C15	4.8	4.5
16	3.9	3.7	4.1	3.5	3.8	C16	3.9	4.9
Mean ±	4.6±0.3	4.7±0.2	4.9±0.3	4.8±0.2	4.7±0.3		4.9±0.2	5.1±0.2
SEM								

B.2.4 HDL-Cholesterol

Table B11

Fasting Serum Concentrations of **HDL-Cholesterol (mmol/L)**, Before and During 4 months of Therapy for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	1.2	1.2	1.1	1.1	1.2	C1	1.3	1.4
2	1.2	1.5	1.3	1.5	1.5	C2	1.3	1.3
3	1.0	1.3	1.4	1.4	1.3	C3	2.2	2.0
4	1.7	1.9	1.9	1.9	1.7	C4	2.2	2.4
5	1.7	2.0	1.7	2.0	2.1	C5	1.0	1.2
6	1.4	1.4	1.5	1.6	1.3	C6	2.2	2.3
7	1.6	1.6	1.8	1.8	1.8	C7	1.7	1.8
8	2.0	2.0	1.9	1.9	2.0	C8	1.6	1.9
9	1.4	1.4	1.3	1.1	1.2	C9	1.4	1.2
10	0.9	1.2	1.2	1.2	1.4	C10	1.7	1.6
11	1.6	1.4	1.6	1.6	1.7	C11	1.4	1.3
12	1.4	1.5	1.5	1.5	1.5	C12	1.2	1.6
13	1.6	1.4	1.9	1.4	1.4	C13	1.2	1.2
14	1.2	1.7	1.5	1.7	1.9	C14	1.8	1.9
15	1.1	1.0	1.2	1.2	1.1	C15	1.7	1.5
16	1.4	1.5	1.5	1.3	1.3	C16	1.4	1.5
Mean ±								
	1.4±0.1	1.5±0.1	1.5±0.1	1.5±0.1	1.5±0.1		1.6±0.1	1.6±0.1
SEM								

B.2.5 LDL-Cholesterol

Table B12

Fasting Serum Concentrations of **LDL-Cholesterol (mmol/L)**, Before and During 4 months of Therapy for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	3.8	3.7	3.0	3.5	2.7	C1	5.2	5.1
2	3.6	3.6	4.1	4.5	4.9	C2	3.5	3.7
3	1.7	2.4	2.6	2.9	2.5	C3	2.6	2.6
4	2.6	2.5	2.3	2.6	3.1	C4	1.9	2.0
5	3.8	3.6	4.2	3.1	3.4	C5	3.2	3.2
6	3.2	3.3	4.4	4.0	2.6	C6	2.0	2.1
7	2.4	2.1	1.8	2.0	1.8	C7	2.7	2.8
8	2.1	2.6	2.1	2.4	2.3	C8	2.5	2.6
9	2.4	2.0	2.1	2.3	1.9	C9	2.5	2.8
10	1.1	0.8	1.5	1.3	0.6	C10	2.5	3.6
11	1.9	1.9	2.7	2.4	2.4	C11	2.0	2.8
12	3.5	3.7	2.4	2.4	2.5	C12	2.6	2.8
13	3.8	3.0	4.4	2.9	3.2	C13	3.8	3.3
14	2.9	2.8	2.7	3.0	2.9	C14	3.4	3.5
15	3.7	3.5	4.8	4.3	3.7	C15	2.8	2.8
16	2.2	2.0	2.3	1.9	2.1	C16	2.1	3.0
Mean ±	2.8±0.2	2.7±0.2	3.0±0.3	2.8±0.2	2.7±0.2		2.8±0.2	3.0±0.2
SEM								

B.2.6 Triglycerides

Table B13

Fasting Serum Concentrations of **Triglycerides (mmol/L)**, Before and During 4 months of Therapy for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	0.5	0.7	0.6	0.6	0.8	C1	1.4	2.3
2	1.1	0.6	0.8	0.8	1.0	C2	0.8	1.2
3	0.5	0.5	0.7	0.3	0.7	C3	1.4	2.0
4	1.5	1.5	1.2	1.8	1.1	C4	1.9	1.2
5	0.9	0.8	0.8	0.7	0.8	C5	0.4	0.6
6	1.2	1.3	1.3	1.2	1.9	C6	0.6	0.7
7	0.6	0.6	0.6	0.5	0.4	C7	0.6	0.5
8	1.0	0.8	0.9	0.9	0.9	C8	0.9	0.7
9	0.9	0.9	0.6	0.9	1.5	C9	0.8	1.3
10	0.5	0.7	0.5	0.5	1.3	C10	0.8	0.8
11	0.6	0.7	1.1	0.8	0.9	C11	1.5	0.8
12	1.4	1.8	1.5	1.5	1.1	C12	1.2	1.5
13	1.8	2.5	0.7	1.2	1.3	C13	1.6	1.2
14	1.2	0.9	0.8	1.1	1.0	C14	1.1	1.0
15	1.1	0.8	0.8	1.1	0.9	C15	0.7	0.8
16	0.7	0.5	0.7	0.7	0.8	C16	0.8	0.9
Mean ±								
	1.0±0.1	1.0±0.1	0.9±0.1	0.9±0.1	1.0±0.1		1.0±0.1	1.1±0.1
SEM								

B.2.7 Free Fatty Acids

Table B14

Fasting Serum Concentrations of **Free Fatty Acids (mmol/L)**, Before and During 4 months of Therapy
for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	1.03	0.17	0.12	0.23	0.13	C1	1.26	0.77
2	0.51	0.14	0.16	0.17	0.07	C2	0.89	0.50
3	0.28	0.10	0.12	0.01	0.11	C3	0.63	0.75
4	0.87	0.71	0.27	0.14	0.83	C4	0.98	1.03
5	0.25	0.20	0.32	0.26	0.10	C5	0.45	0.79
6	0.31	0.38	0.25	0.46	0.88	C6	0.45	0.40
7	0.25	0.79	0.55	0.40	0.49	C7	0.75	0.53
8	0.30	0.49	0.52	0.72	0.66	C8	0.49	0.69
9	0.53	0.57	1.12	0.23	0.01	C9	0.58	0.17
10	0.34	0.47	0.63	0.23	0.10	C10	0.97	1.05
11	0.31	0.68	0.93	0.55	0.76	C11	0.27	0.23
12	0.42	0.89	0.77	0.33	1.10	C12	0.58	0.19
13	0.65	0.58	0.89	0.71	0.24	C13	0.48	0.64
14	0.63	0.67	0.47	1.12	0.60	C14	0.31	0.25
15	0.70	0.74	1.12	0.39	0.35	C15	0.26	0.30
16	0.60	0.73	0.50	0.27	0.53	C16	0.29	0.41
Mean ±								
	0.5±0.1	0.5±0.1	0.5±0.1	0.4±0.1	0.4±0.1		0.6±0.1	0.5±0.1
SEM								

B.2.8 Age, Height and Weight

Table B15

Age (Yrs) and Height(m) of Patients and Control Subjects at Baseline, and weight (kg) of Patients and Control Subjects at Baseline and 4 Months

PATIENTS					CONTROL SUBJECTS				
number	Age	Height	Baseline Weight	4 month Weight	number	Age	Height	Baseline Weight	4 month Weight
1	29	1.65	61.4	59.9	C1	54	1.64	74.4	71.6
2	48	1.53	73.6	75.5	C2	36	1.70	75.4	71.2
3	28	1.55	56.5	55.2	C3	56	1.57	60.1	64.2
4	25	1.73	65.1	64.3	C4	43	1.60	65.1	63.4
5	58	1.60	68.7	67.8	C5	30	1.55	58.2	55.3
6	39	1.66	79.0	71.1	C6	42	1.69	60.3	60.1
7	33	1.70	68.2	63.7	C7	38	1.60	61.7	61.3
8	59	1.60	52.6	53.4	C8	36	1.65	65.8	66.6
9	46	1.63	64.1	62.6	C9	30	1.50	80.1	75.2
10	28	1.45	47.8	49.2	C10	31	1.55	51.4	53.2
11	30	1.75	61.7	63.9	C11	44	1.64	75.8	80.2
12	49	1.63	63.3	59.3	C12	19	1.52	49.8	49.1
13	30	1.58	49.8	52.5	C13	25	1.68	63.2	58.4
14	45	1.60	52.3	51.2	C14	58	1.53	58.6	59.5
15	25	1.53	69.6	58.4	C15	28	1.50	51.1	50.7
16	37	1.68	62.7	64.1	C16	26	1.60	59.2	60.4
Mean ± SEM	38.06± 2.85					37.25± 2.89			

B.2.9 BMI

Table B16

Calculated **Body Mass Index (BMI)** kg/m² of Patients and Control Subjects at Baseline and 4 Months

PATIENTS			CONTROL SUBJECTS		
number	baseline BMI	4 month BMI	number	baseline BMI	4 month BMI
1	22.6	22.0	C1	27.7	26.6
2	31.4	32.3	C2	26.1	24.6
3	23.5	23.0	C3	24.4	26.0
4	21.8	21.5	C4	25.4	24.8
5	26.8	26.5	C5	24.2	23.0
6	28.7	25.8	C6	21.1	21.0
7	23.6	22.0	C7	24.1	23.9
8	20.5	20.9	C8	24.2	24.5
9	24.1	23.6	C9	35.6	33.4
10	22.7	23.4	C10	21.4	22.1
11	20.1	20.9	C11	28.2	29.8
12	23.8	22.3	C12	21.6	21.3
13	19.9	21.0	C13	22.4	20.7
14	20.4	20.0	C14	25.0	25.4
15	29.7	24.9	C15	22.7	22.5
16	22.2	22.7	C16	23.1	23.6
Mean ± SEM	23.86 ± 0.88	23.30 ± 0.75		24.83 ± 0.89	24.58 ± 0.83

B.3 *Catecholamines*

The results of the catecholamine studies in patients and controls are documented in Tables B 17 and B18 and summarized in Table 5, Chapter 5.

B.3.1 Adrenaline

Table B17

Fasting Plasma Concentrations of **adrenaline (pmol/L)**, Before and During 4 months of Therapy for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	2255	354	496	563	355	C1	49	92
2	88	1906	917	115	16	C2	476	184
3	284	1151	1213	49	545	C3	15	221
4	464	42	100	60	80	C4	41	217
5	4036	720	265	--	816	C5	246	429
6	721	5046	560	199	165	C6	46	133
7	1114	6361	4431	810	14	C7	24	94
8	700	148	2056	657	136	C8	219	45
9	159	157	651	330	2211	C9	72	454
10	97	810	76	3144	98	C10	70	76
11	49	251	119	21	96	C11	317	304
12	135	445	212	97	235	C12	407	163
13	135	682	170	159	57	C13	1672	94
14	754	733	199	10	129	C14	192	40
15	60	213	402	212	225	C15	241	28
16	118	81	94	493	186	C16	729	--
Median	222	563	334	206	151		206	114
(range)	(49-4036)	(42-6361)	(76-4431)	(10-3144)	(14-2211)		(15-1672)	(0-454)

B.3.2 Noradrenaline

Table B18

Fasting Plasma Concentrations of **noradrenaline (pmol/L)**, Before and During 4 months of Therapy for Chronic Stress in Patients, and Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	2311	244	617	619	1352	C1	2765	2641
2	2039	1808	1090	3325	1779	C2	2360	1351
3	5436	1431	961	1702	1925	C3	3552	4337
4	633	357	87	1762	669	C4	3118	2767
5	1446	2316	3608	-	2227	C5	2349	2220
6	395	963	898	2521	1724	C6	1519	1573
7	682	971	4883	2247	7538	C7	912	1965
8	986	4960	3727	3176	2535	C8	1971	3519
9	3309	1038	7203	1992	2398	C9	452	3071
10	1265	2247	1424	1015	2051	C10	2327	2575
11	2143	1285	841	867	3802	C11	2084	1965
12	2317	1963	2752	3308	3663	C12	1903	3361
13	1276	1708	2187	1086	2598	C13	2857	3946
14	746	1911	936	1775	2446	C14	3974	3752
15	1248	840	1460	881	1042	C15	2084	2962
16	834	4085	2841	1856	5024	C16	2352	1916
Median	1271	1570	1442	1816	2313		2338	2704
(range)	(395-5436) a	(244-4960)	(87-7203)	(619-3325)	(699-7538)		(452-3974)	(1351-4337)

B.4 Psychometric Tests

The results of the psychometric tests in patients and controls are documented in Tables B19-27 and summarized in Table 6, Chapter 6.

B.4.1 MBI-Emotional Exhaustion

Table B19

Monthly MASLACH BURNOUT INVENTORY – Emotional Exhaustion Before And During 4 Months Of Therapy For Chronic Stress In Patients And Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	53	22	16	15	10	C1	9	8
2	40	38	36	36	35	C2	17	17
3	27	17	16	20	21	C3	12	11
4	47	26	16	24	22	C4	9	11
5	25	12	9	12	17	C5	6	8
6	38	34	39	30	30	C6	15	11
7	32	21	10	4	9	C7	16	11
8	28	21	11	8	12	C8	11	12
9	40	36	45	21	20	C9	0	3
10	46	12	6	20	11	C10	8	8
11	47	27	32	20	34	C11	4	7
12	49	30	20	23	33	C12	29	20
13	49	45	51	45	32	C13	5	4
14	39	45	37	35	31	C14	8	16
15	30	21	22	20	17	C15	22	30
16	47	44	49	43	40	C16	19	22
Mean ±								
	39.81± 2.26	28.19±2.78	25.94± 3.80	23.50±2.93	23.38±2.55		11.88± 1.86	12.44± 1.77
SEM								

B.4.2 MBI-Depersonalisation

Table B20

Monthly **MASLACH BURNOUT INVENTORY – Depersonalisation** Before And During 4 Months Of
Therapy For Chronic Stress In Patients And Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	26	12	6	4	15	C1	2	2
2	16	14	14	12	11	C2	0	0
3	9	11	9	10	13	C3	0	0
4	25	10	8	10	8	C4	8	8
5	0	1	10	1	1	C5	0	0
6	13	10	15	9	11	C6	4	2
7	2	0	2	2	1	C7	3	0
8	0	1	0	0	1	C8	2	6
9	22	13	17	6	4	C9	2	13
10	4	3	0	2	2	C10	1	0
11	15	14	14	5	9	C11	1	1
12	11	5	5	15	24	C12	10	7
13	8	6	24	15	14	C13	2	7
14	2	5	6	8	7	C14	1	9
15	9	2	2	2	2	C15	6	8
16	14	11	16	13	10	C16	3	2
Mean ± SEM	11.00± 2.11	7.38± 1.26	9.25± 1.73	7.13± 1.27	8.31± 1.61		2.81± 0.73	4.06± 1.04

B.4.3 MBI-Personal Accomplishment

Table B21

Monthly Maslach Burnout Inventory – Personal Accomplishment Score Before And During 4
Months Of Therapy For Chronic Stress In Patients And Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	20	32	39	34	30	C1	43	42
2	24	22	26	22	21	C2	44	40
3	25	15	11	20	34	C3	43	44
4	26	25	26	35	31	C4	39	36
5	26	22	33	30	22	C5	32	30
6	32	29	24	33	32	C6	34	31
7	38	43	33	35	25	C7	38	34
8	24	25	36	40	38	C8	33	33
9	25	36	28	42	38	C9	39	34
10	30	41	41	33	33	C10	20	21
11	45	42	39	41	40	C11	33	38
12	28	25	14	25	27	C12	42	44
13	20	31	36	23	38	C13	42	47
14	32	42	35	42	45	C14	39	41
15	20	25	28	30	34	C15	36	36
16	26	22	21	25	30	C16	44	44
Mean ± SEM	27.56± 1.68	29.81± 2.18	29.38± 2.20	31.88± 1.83	32.38± 1.65		37.56± 1.56	37.19± 1.68

B.4.4 GHQ-28 A Scale

Table B22

Monthly General Health Questionnaire – 28 A-scale Score Before And During 4 Months Of Therapy
For Chronic Stress In Patients And Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	20	5	7	8	5	C1	1	2
2	20	12	9	11	10	C2	2	3
3	18	10	11	9	9	C3	2	2
4	9	17	1	4	6	C4	4	5
5	19	1	4	2	13	C5	3	2
6	12	6	4	7	11	C6	7	4
7	5	3	0	8	6	C7	4	6
8	12	9	3	4	3	C8	2	4
9	11	10	13	3	0	C9	4	5
10	20	1	9	0	0	C10	4	7
11	4	7	7	7	11	C11	3	2
12	18	13	14	14	13	C12	3	1
13	5	7	8	15	0	C13	1	3
14	9	17	4	6	11	C14	1	2
15	14	3	9	6	5	C15	6	7
16	16	14	11	11	3	C16	2	10
Mean ±	13.25±	8.44±	7.13±	7.19±	6.63±		3.06±	4.06±
SEM	1.43	1.30	1.04	1.04	1.15		0.43	0.62

B.4.5 GHQ-28 B Scale

Table B23

Monthly General Health Questionnaire – 28 B-scale Score Before And During 4 Months Of Therapy
For Chronic Stress In Patients And Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	20	6	6	7	6	C1	6	4
2	20	7	7	10	12	C2	2	2
3	15	13	8	8	6	C3	2	2
4	21	10	6	5	2	C4	4	3
5	14	5	4	6	11	C5	1	1
6	14	7	7	9	10	C6	7	7
7	3	3	1	5	4	C7	5	5
8	18	13	4	5	5	C8	0	2
9	21	5	19	3	0	C9	0	1
10	18	0	1	1	1	C10	8	7
11	17	7	10	6	13	C11	3	0
12	16	15	8	8	11	C12	2	1
13	5	8	14	19	5	C13	0	1
14	11	17	7	7	9	C14	5	3
15	15	2	12	16	24	C15	9	8
16	20	17	14	14	11	C16	2	5
Mean ±	15.50±	8.44±	8.00±	8.06±	8.13±		3.50±	3.25±
SEM	1.34	1.31	1.21	1.19	1.47		0.72	0.62

B.4.6 GHQ-28 C Scale

Table B24

Monthly General Health Questionnaire – 28 C-scale Score Before And During 4 Months Of Therapy
For Chronic Stress In Patients And Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	17	0	0	0	1	C1	5	3
2	17	4	8	10	13	C2	4	4
3	18	14	13	13	11	C3	3	3
4	19	5	4	2	4	C4	6	7
5	12	7	10	6	9	C5	7	5
6	11	9	3	6	9	C6	7	7
7	8	7	7	7	9	C7	7	7
8	16	10	2	2	2	C8	7	7
9	15	1	16	6	1	C9	0	4
10	19	2	6	3	0	C10	7	8
11	8	4	5	6	8	C11	7	5
12	19	18	16	10	14	C12	2	2
13	4	2	13	18	0	C13	4	4
14	9	9	7	7	8	C14	5	5
15	9	4	7	7	6	C15	5	12
16	14	11	11	7	8	C16	4	3
Mean ±	13.44±	6.69±	8.00±	6.88±	6.44±		5.00±	5.34±
SEM	1.20	1.24	1.20	1.11	1.15		0.52	0.63

B.4.7 GHQ-28 D Scale

Table B25

Monthly General Health Questionnaire – 28 D-scale Score Before And During 4 Months Of Therapy
For Chronic Stress In Patients And Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	14	4	0	0	0	C1	0	1
2	12	1	1	3	2	C2	1	0
3	16	14	12	10	9	C3	1	0
4	21	11	1	2	4	C4	0	1
5	2	0	0	0	2	C5	0	1
6	7	3	5	3	11	C6	0	0
7	4	5	0	3	8	C7	0	0
8	14	5	0	2	2	C8	0	0
9	20	1	9	0	0	C9	0	0
10	16	0	0	0	0	C10	9	1
11	7	6	7	0	10	C11	0	1
12	14	9	9	8	9	C12	0	0
13	6	1	12	14	1	C13	0	0
14	18	18	5	2	1	C14	1	0
15	14	1	13	10	8	C15	1	3
16	14	12	12	8	8	C16	0	0
Mean ±	12.44±	5.69±	5.38±	4.06±	4.69±		0.81±	0.50±
SEM	1.41	1.39	1.29	1.12	1.03		0.56	0.20

B.4.8 GHQ-28 Composite Score

Table B26

Monthly General Health Questionnaire – 28 Composite Score Before And During 4 Months Of
Therapy For Chronic Stress In Patients And Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	71	15	13	15	12	C1	12	9
2	69	24	25	34	37	C2	9	9
3	67	51	44	40	35	C3	8	7
4	70	43	12	13	16	C4	14	16
5	47	13	18	14	35	C5	11	9
6	44	25	19	25	41	C6	21	18
7	20	18	8	23	27	C7	16	18
8	60	37	9	13	12	C8	9	13
9	67	17	57	12	1	C9	4	10
10	73	3	16	4	1	C10	28	23
11	36	24	29	19	42	C11	13	8
12	67	55	47	40	47	C12	7	4
13	20	18	47	66	6	C13	5	8
14	57	51	23	22	29	C14	12	10
15	52	10	41	39	24	C15	21	30
16	64	54	48	40	30	C16	8	18
Mean ±	55.25±	28.63±	28.50±	26.19±	24.69±		12.38±	13.13±
SEM	4.35	4.32	4.08	3.97	3.74		1.62	1.72

B.4.9 Zung Depression Rating Scale Score

Table B27

Monthly Zung Depression Rating Scale Score Before And During 4 Months Of Therapy For Chronic Stress In Patients And Untreated Control Subjects

PATIENTS						CONTROL SUBJECTS		
number	Baseline	1 month	2 months	3 months	4 months	number	Baseline	4 months
1	74	52	46	51	40	C1	36	41
2	76	69	65	65	69	C2	40	46
3	80	76	61	63	66	C3	41	40
4	68	64	44	54	50	C4	34	36
5	58	60	51	51	52	C5	31	34
6	66	66	63	61	72	C6	51	48
7	75	43	39	45	50	C7	50	47
8	75	65	44	44	47	C8	45	41
9	76	53	70	43	38	C9	46	30
10	75	37	39	49	42	C10	51	51
11	76	51	45	56	65	C11	39	32
12	76	57	61	53	65	C12	42	37
13	61	59	61	82	50	C13	36	30
14	66	63	57	56	53	C14	40	41
15	71	52	57	52	47	C15	48	53
16	75	74	68	62	62	C16	39	39
Mean ±								
	71.75± 1.55	58.81± 2.65	54.44± 2.60	55.44± 2.44	54.25± 2.70		41.81± 1.54	40.38± 1.79
SEM								

APPENDIX C: Forms

C.1 Information Sheet for patients.

Hello! My name is Shirra Moch and together with other researchers at Wits Medical School, I am conducting a project on the effects of prolonged emotional stress.

People who have been subjected to stress over a prolonged period of time may develop a condition known as psychological burnout and/or breakdown.

Since you have been assessed to be amongst this group, you are invited to participate in the study.

Purpose of the research:

The purpose of the investigation is to find out the effects that burnout has on certain chemicals (e.g. cortisol, adrenaline) in the blood and urine. This will broaden our knowledge of how the condition occurs, and in turn may ultimately lead to the development of newer treatments for this condition.

Participation:

Your participation in the study is voluntary and you may refuse to take part or withdraw your consent at any time. If you choose not to participate, your therapy will not be disadvantaged in any way.

Procedure:

You will be asked to fill in several written questionnaires - which should take about half an hour to complete. Your height, weight and blood pressure will be recorded and you will be supplied with a large sample bottle and asked to collect all urine passed in a 24 hour period. The next day before breakfast you will be requested to give a fasting blood sample. You will need to lie down for half an hour before the blood sample is taken. The whole procedure will be repeated 1, 2, 3 and four months after starting therapy. At each of these visits a total of 45 mls of blood (about three tablespoons) will be taken for analysis. The results of the blood tests will be made available to you on your request.

Discomfort / Risks:

No risks are expected and the only discomfort you may feel is having a small needle inserted into a vein in your arm to take the blood sample. This may be relieved by compression and the application of an Elastoplast dressing.

Confidentiality:

All records are strictly confidential. The results may be published in a medical journal but your name will not be revealed.

Costs:

There will be no costs to you for the urine and blood tests as well as the extra appointments necessitated by the study. The normal costs of the stress reduction programme will still be in place.

Your help with this research project is much appreciated. Should you have any queries at any stage, please contact me:

SHIRRA MOCH

Tel: (011) 717-2372 (morning) or (011) 648-2181 afternoon /evening

Fax: (011) 648-9280

e-mail: mochs@therapy.wits.ac.za

C.2 Information sheet for study participants (controls).

Hello! My name is Shirra Moch and together with other researchers at Wits Medical School, I am conducting a project on the effects of prolonged emotional stress.

People who have been subjected to stress over a prolonged period of time may develop a condition known as psychological burnout and/or breakdown.

You are invited to participate in the study **as a control**, since you are not suffering from either burnout or breakdown.

Purpose of the research:

The purpose of the investigation is to find out the effects that burnout has on certain chemicals (e.g. cortisol, adrenaline) in the blood and urine. This will broaden our knowledge of how the condition occurs, and in turn may ultimately lead to the development of newer treatments for this condition.

Participation:

Your participation in the study is voluntary and you may refuse to take part or withdraw your consent at any time

Procedure:

You will be asked to fill in several written questionnaires - which should take about half an hour to complete. Your height, weight and blood pressure will be recorded and you will be supplied with a large sample bottle and asked to collect all urine passed in a 24 hour period. The next day before breakfast you will be requested to give a fasting blood sample. You will need to relax for half an hour before the blood sample is taken. A total of 45 mls of blood (about three tablespoons) will be taken for analysis. The results of the blood tests will be made available to you on your request. The whole procedure will be repeated 3 months later to assess if you are still in "ideal" and coping well with stress.

Discomfort / Risks:

No risks are expected and the only discomfort you may feel is having a small needle inserted into a vein in your arm to take the blood sample. This may be relieved by compression and the application of an Elastoplast dressing.

Confidentiality:

All records are strictly confidential. The results may be published in a medical journal but your name will not be revealed.

Costs:

There will be no costs to you for the urine and blood tests necessitated by the study.

Your help with this research project is much appreciated. Should you have any queries at any stage, please contact me:

SHIRRA MOCH

Tel: (011) 717-2372 (morning) or (011) 648-2181 afternoon /evening

Fax: (011) 648-9280

e-mail: mochs@therapy.wits.ac.za

C.2 Informed Consent Form

INFORMED CONSENT

UNIVERSITY OF THE WITWATERSRAND

HOSPITAL

I(insert name of subject) aged
years, consent to the following procedure being performed on myself namely
Fasting blood sample and 24 hour urine collection. (give details of procedure)

I(insert name of guardian/spouse) consent to the
above procedure being performed on the said.....
(insert name of subject).

Dr. has fully explained the procedure and its purpose,
and I/we understand and appreciate:

- a. such possible harmful effects including pain or discomfort as may according to medical knowledge be involved;
- b. the degree of likelihood according to medical knowledge of the occurrence of any such effects; and
- c. to what extent (if any) the procedure may be considered of benefit to the subject.

I/we also appreciate that my/our consent may be withdrawn at any time.

Witnesses:

1. (signature of subject)

2. (signature of guardian/spouse)

I, Dr., declare that I have fully explained the
above procedure, its purpose, such harmful effects including pain or discomfort as may
~~according to medical knowledge be involved, the degree of likelihood according to~~
medical knowledge of the occurrence of any such effects, and to what extent (if any) the
procedure may be considered of benefit to the subject.

I verily believe my explanation to have been fully understood by the consenting parties*

..... (signature of doctor)

*Where an interpreter is used, the doctor should take special care to satisfy himself that this requirement
has been met.

C.3 Psychometric tests

ZUNG SELF-RATING DEPRESSION SCALE

Patient's Initials _____

Date of Assessment _____

Please read each statement and decide how much of the time the statement describes how you have been feeling during the past several days.

Make check mark (✓) in appropriate column.	A little of the time	Some of the time	Good part of the time	Most of the time
1. I feel down-hearted and blue				
2. Morning is when I feel the best				
3. I have crying spells or feel like it				
4. I have trouble sleeping at night				
5. I eat as much as I used to				
6. I still enjoy sex				
7. I notice that I am losing weight				
8. I have trouble with constipation				
9. My heart beats faster than usual				
10. I get tired for no reason				
11. My mind is as clear as it used to be				
12. I find it easy to do the things I used to				
13. I am restless and can't keep still				
14. I feel hopeful about the future				
15. I am more irritable than usual				
16. I find it easy to make decisions				
17. I feel that I am useful and needed				
18. My life is pretty full				
19. I feel that others would be better off if I were dead				
20. I still enjoy the things I used to do				

Adapted from Zung, A self rating depression scale, *Arch Gen Psychiatry*, 1965;12:63-70.

MBI—General Survey

The purpose of this survey is to assess how staff members view their job and their reactions to their work.

On the following page there are 16 statements of job-related feelings. Please read each statement carefully and decide if you ever feel this way about your job. If you have never had this feeling, write a "0" (zero) in the space before the statement. If you have had this feeling, indicate how often you feel it by writing the number (from 1 to 6) that best describes how frequently you feel that way. An example is shown below.

Example

How often:	0	1	2	3	4	5	6
	Never	A few times a year or less	Once a month or less	A few times a month	Once a week	A few times a week	Every day

How Often

0–6

Statement:

I. _____ I feel depressed at work.

If you never feel depressed at work, you would write the number "0" (zero) under then heading "How often." If you rarely feel depressed at work (a few times a year or less), you would write the number "1." If your feelings of depression are fairly frequent (a few times a week, but not daily) you would write a "5."

Note to Researchers

We would appreciate your contribution to establishing occupational and national norms for the MBI—GS. If you wish to contribute, please send survey data on computer disks. Include on the disks item by item data files on all subjects along with whatever demographic data you have collected. Include on the disk a text file identifying the researchers, describing the nature of the sample, and outlining the structure of the data file. Your contribution will be acknowledged in the next edition of the MBI Manual.

Send files to:

Michael P. Leiter, Ph.D.

Centre for Organizational Research & Development

Acadia University

Wolfville, NS, Canada B0P 1X0.



Consulting Psychologists Press, Inc.

3803 E. Bayshore Road • Palo Alto, CA 94303

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Maslach Burnout Inventory (2nd Edition)

HOW OFTEN:	0	1	2	3	4	5	6
	Never	A few times a year or less	Once a month or less	A few times a month	Once a week	A few times a week	Every day

HOW OFTEN
0 - 6

Statements:

recipients = clients and / or colleagues

1. _____ I feel emotionally drained from my work.
2. _____ I feel used up at the end of the workday.
3. _____ I feel fatigued when I get up in the morning and have to face another day on the job.
4. _____ I can easily understand how my recipients feel about things.
5. _____ I feel I treat some recipients as if they were impersonal objects.
6. _____ Working with people all day is really a strain for me.
7. _____ I deal very effectively with the problems of my recipients.
8. _____ I feel burned out from my work.
9. _____ I feel I'm positively influencing other people's lives through my work.
10. _____ I've become more callous toward people since I took this job.
11. _____ I worry that this job is hardening me emotionally.
12. _____ I feel very energetic.
13. _____ I feel frustrated by my job.
14. _____ I feel I'm working too hard on my job.
15. _____ I don't really care what happens to some recipients.
16. _____ Working with people directly puts too much stress on me.
17. _____ I can easily create a relaxed atmosphere with my recipients.
18. _____ I feel exhilarated after working closely with my recipients.
19. _____ I have accomplished many worthwhile things in this job.
20. _____ I feel like I'm at the end of my rope.
21. _____ In my work, I deal with emotional problems very calmly.
22. _____ I feel recipients blame me for some of their problems.

(Administrative use only)

cat.

cat.

cat.

EE: _____ DP: _____ PA: _____

THE GENERAL HEALTH QUESTIONNAIRE

GHQ 28

David Goldberg

Please read this carefully.

We should like to know if you have had any medical complaints and how your health has been in general, *over the past few weeks*. Please answer ALL the questions on the following pages simply by underlining the answer which you think most nearly applies to you. Remember that we want to know about present and recent complaints, not those that you had in the past.

It is important that you try to answer ALL the questions.

Thank you very much for your co-operation.

Have you recently

A1 – been feeling perfectly well and in good health?	Better than usual	Same as usual	Worse than usual	Much worse than usual
A2 – been feeling in need of a good tonic?	Not at all	No more than usual	Rather more than usual	Much more than usual
A3 – been feeling run down and out of sorts?	Not at all	No more than usual	Rather more than usual	Much more than usual
A4 – felt that you are ill?	Not at all	No more than usual	Rather more than usual	Much more than usual
A5 – been getting any pains in your head?	Not at all	No more than usual	Rather more than usual	Much more than usual
A6 – been getting a feeling of tightness or pressure in your head?	Not at all	No more than usual	Rather more than usual	Much more than usual
A7 – been having hot or cold spells?	Not at all	No more than usual	Rather more than usual	Much more than usual

B1 – lost much sleep over worry?	Not at all	No more than usual	Rather more than usual	Much more than usual
B2 – had difficulty in staying asleep once you are off?	Not at all	No more than usual	Rather more than usual	Much more than usual
B3 – felt constantly under strain?	Not at all	No more than usual	Rather more than usual	Much more than usual
B4 – been getting edgy and bad-tempered?	Not at all	No more than usual	Rather more than usual	Much more than usual
B5 – been getting scared or panicky for no good reason?	Not at all	No more than usual	Rather more than usual	Much more than usual
B6 – found everything getting on top of you?	Not at all	No more than usual	Rather more than usual	Much more than usual
B7 – been feeling nervous and strung-up all the time?	Not at all	No more than usual	Rather more than usual	Much more than usual

Please turn over

C1 – been managing to keep yourself busy and occupied?	More so than usual	Same as usual	Rather less than usual	Much less than usual
C2 – been taking longer over the things you do?	Quicker than usual	Same as usual	Longer than usual	Much longer than usual
C3 – felt on the whole you were doing things well?	Better than usual	About the same	Less well than usual	Much less well
C4 – been satisfied with the way you've carried out your task?	More satisfied	About same as usual	Less satisfied than usual	Much less satisfied
C5 – felt that you are playing a useful part in things?	More so than usual	Same as usual	Less useful than usual	Much less useful
C6 – felt capable of making decisions about things?	More so than usual	Same as usual	Less so than usual	Much less capable
C7 – been able to enjoy your normal day-to-day activities?	More so than usual	Same as usual	Less so than usual	Much less than usual

D1 – been thinking of yourself as a worthless person?	Not at all	No more than usual	Rather more than usual	Much more than usual
D2 – felt that life is entirely hopeless?	Not at all	No more than usual	Rather more than usual	Much more than usual
D3 – felt that life isn't worth living?	Not at all	No more than usual	Rather more than usual	Much more than usual
D4 – thought of the possibility that you might make away with yourself?	Definitely not	I don't think so	Has crossed my mind	Definitely have
D5 – found at times you couldn't do anything because your nerves were too bad?	Not at all	No more than usual	Rather more than usual	Much more than usual
D6 – found yourself wishing you were dead and away from it all?	Not at all	No more than usual	Rather more than usual	Much more than usual
D7 – found that the idea of taking your own life kept coming into your mind?	Definitely not	I don't think so	Has crossed my mind	Definitely has

A		B		C		D		TOTAL	
---	----------------------	---	----------------------	---	----------------------	---	----------------------	-------	----------------------

C.4 Clearance certificate from the Committee for Research on

Human Subjects, Protocol No: M991116

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)

Ref: R14/49 Moch

CLEARANCE CERTIFICATE

PROTOCOL NUMBER M991116

PROJECT

A Study of Pituitary-Adrenal Hormonal
Responses In Patients Treated For Chronic
Stress

INVESTIGATORS

Mrs S Moch

DEPARTMENT

Exp. & Clinical Pharmacology, Wits Medical School

DATE CONSIDERED

991126

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 00/01/31

CHAIRMAN..........(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof I Havlik

Dept of Exp.& Clinical Pharmacology, Wits Medical School

Works2\lawn0015\HumEth97.wdb\W 991116

=====

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATESIGNATURE

PROTOCOL NO.: M 991116

200

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX D: Presentations and Publications associated with this work

D.1 Poster Presentation

Data included in this dissertation were presented at the 32nd Annual meeting of the International Society of Psychoneuroendocrinology (ISPNE) held in Quebec City, Canada from August 5-8, 2001 (see attached copy).

D.2 Journal Article

Data included in this dissertation were published in the Journal *Endocrine*:

Moch SL, Panz VR, Joffe BI, Havlik I, Moch JD, 2003. Longitudinal changes in pituitary-adrenal hormones in South African women with burnout. *Endocrine* 21(3): 267-72 (see attached copy).

THE NEUROENDOCRINOLOGY OF BURNOUT

SL Moch¹, VR Panz²,
BI Joffe², I Havlik¹, JD Moch³

¹Department of Pharmacy and Pharmacology, and ²Carbohydrate and Lipid Metabolism Research Group, Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand, ³Centre for Stress-Related Illnesses, Milpark Hospital, Johannesburg, South Africa.

INTRODUCTION

A chronic stress syndrome termed burnout may develop in healthy individuals when they are exposed to repeated stressors. Stressors may be physical, psychological or social in nature, and may be accompanied by a personal or social accompaniment (1). Dysregulation in the homeostatic responses of the body's stress system can lead to disease (2).

The stress system is composed of the central nervous system (CNS) and the periphery (Figure 1). In the CNS, the hypothalamus releases corticotrophic hormone (CRH), while the locus ceruleus (LC) and other noradrenergic (NA) cell groups in the medulla and pons initiate the stress response. The peripheral branches comprise the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. Activation of the stress response triggers CRH release from the hypothalamus, inducing the HPA cascade of growth hormone, prolactin and ACTH secretion from the pituitary, and cortisol release from the adrenal cortex. The LC/NA system prompts the discharge of catecholamines from the nerves and the adrenal medulla. The system is autoregulatory, with negative feedback exerted on stress mediators to prevent the onset of a stressor abuse.

This model explains the pathogenesis of many stress-related illnesses in which disease develops as a result of prolonged exposure to raised levels of stress mediators [3]. Some patients, however, exhibit hypersecretion of the stress system in conditions such as post-traumatic stress disorder, chronic fatigue syndrome, fibromyalgia, rheumatoid arthritis, and asthma [4]. Since the pathophysiology of these disorders has been related to stress, this study was unfortunatly to determine if a similar biochemical status exists in patients with stress burnout.

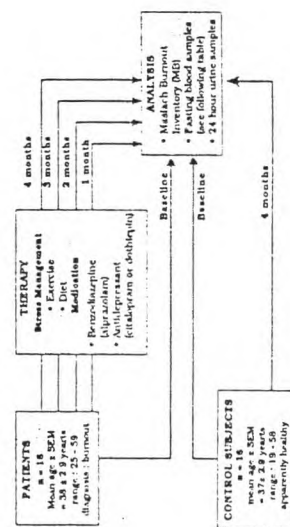
AIMS OF STUDY

- Measure baseline pituitary-adrenal hormones and related metabolic variables in patients with stress burnout.
- Compare changes in these variables longitudinally following therapy and stress management.

PATIENTS AND METHODS

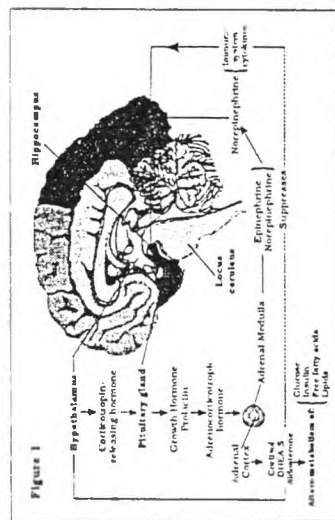
Statistical analysis

Results are expressed as mean $\pm$ SEM except for catecholamines, where the medians with ranges are stated due to the non-parametric distribution of data. Statistical significance of non-parametric data was assessed by the Friedman repeated measures test, Mann-Whitney 'U' test and the Wilcoxon Signed Rank test; parametric data were assessed by the Student's *t*-test for paired and unpaired samples. A value of $p < 0.05$ was considered significant.

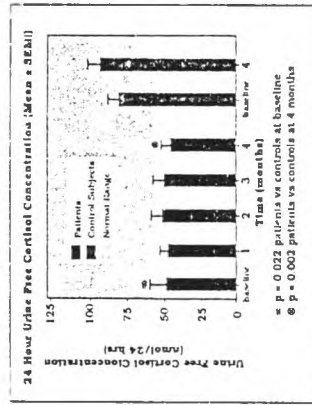
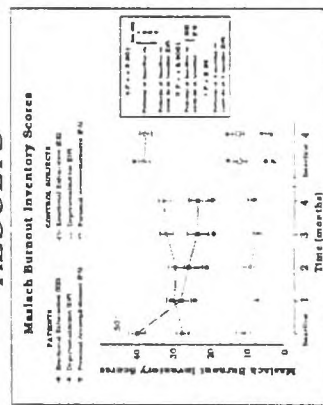


NORMAL FASTING RANGE

METHOD		NORMAL FASTING RANGE	
Glucose	Glucose oxidase	3.0 - 6.0 mmol/l	
Insulin	Immunometric assay	6.0 - 27.0 mIU/l	
Free Fatty Acids	Enzymatic colorimetric assay	0.3 - 0.8 mmol/l	
Total cholesterol	Enzymatic colorimetric assay	< 6.2 mmol/l	
LDL cholesterol	Enzymatic colorimetric assay	> 1.6 mmol/l	
HDL cholesterol	Friedewald formula	< 4.1 mmol/l	
Triglycerides	Enzymatic colorimetric assay	0.0 - 2.0 mmol/l	
Growth Hormone	Immunometric assay	0.16 - 13.0 mIU/l	
Prolactin	Chemiluminometric assay	2.8 - 29.2 μ g/l (premenopausal) 1.8 - 20.3 μ g/l (postmenopausal)	
ACTH	Radioimmunoassay	2.2 - 19.6 pmol/l	
Cortisol (serum)	Radioimmunoassay	138 - 690 nmol/l (17.00 - 90.00 nm)	
Cortisol (24 hour urine)	Competitive immunoassay	55 - 250 nmol/24hrs	
DHEA-S	Radioimmunoassay	1.0 - 7.3 μ mol/l (female)	
Alutatorone	Radioimmunoassay	28 - 414 μ mol/l (recumbent)	
Euphorine	High pressure liquid chromatography	0.0 - 356 pmol/l	
Norepinephrine	High pressure liquid chromatography	560 - 2636 pmol/l	
Dopamine	High pressure liquid chromatography	0.0 - 890 pmol/l	



RESULTS



CONCLUSIONS

- The reduction of urine free cortisol excretion in the patient group suggests functional hypocortisolism in burnout.
- High levels of ACTH were not observed, suggesting an increased predisposition to future burnout episodes.
- High levels of ACTH indicate that the functional hypocortisolism is not mediated centrally.
- The reduction of urinary cortisol levels in patients with low urinary cortisol excretion may reflect reduced cortisol pulsatility during the 24-hour period, with conservation of the early peak.
- Patient recovery was significant over the 4-month period when charted on the Maslach Burnout Inventory.
- These findings resemble stress disorders for other stress-related illnesses e.g. fibromyalgia, chronic fatigue syndrome and post-traumatic stress disorder.

	PATIENTS (n = 16)					CONTROL SUBJECTS (n = 16)		
	Baseline	1 month	2 months	3 months	4 months	Baseline	4 months	4 months
Glucose mmol/L	4.8 $\pm$ 0.1	5.0 $\pm$ 0.1	4.9 $\pm$ 0.1	4.7 $\pm$ 0.1	4.6 $\pm$ 0.2	4.8 $\pm$ 0.2	5.0 $\pm$ 0.2	5.0 $\pm$ 0.2
Insulin mU/L	8.5 $\pm$ 1.3	10.1 $\pm$ 1.9	9.5 $\pm$ 1.5	10.4 $\pm$ 1.2	9.0 $\pm$ 1.2	7.3 $\pm$ 0.8	8.0 $\pm$ 1.2	8.0 $\pm$ 1.2
Free Fatty Acids mmol/L	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.8 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1
Total Cholesterol mmol/L	4.8 $\pm$ 0.2	4.7 $\pm$ 0.2	4.8 $\pm$ 0.2	4.8 $\pm$ 0.2	4.7 $\pm$ 0.2	4.8 $\pm$ 0.2	5.1 $\pm$ 0.2	5.1 $\pm$ 0.2
HDL-Cholesterol mmol/L	1.4 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	1.6 $\pm$ 0.1	1.8 $\pm$ 0.1	1.8 $\pm$ 0.1
LDL-Cholesterol mmol/L	3.6 $\pm$ 0.2	2.7 $\pm$ 0.2	3.2 $\pm$ 0.2	3.3 $\pm$ 0.2	3.2 $\pm$ 0.2	2.8 $\pm$ 0.2	3.0 $\pm$ 0.2	3.0 $\pm$ 0.2
Triglycerides mmol/L	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	0.8 $\pm$ 0.1	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1

Fasting plasma concentrations of catecholamines before and during 4 months of therapy for stress burnout in patients and untreated control subjects (Median & Range)

[illegible]

Fasting serum concentrations of pituitary and adrenal hormones before and during 4 months of therapy for stress burnout in patients and untreated control subjects (Mean $\pm$ SEM)

		PATIENTS (n = 16)				CONTROL SUBJECTS (n = 16)	
		Baseline	1 month	2 months	4 months	Baseline	4 months
Growth hormone		3.7 ± 2.1	6.1 ± 1.6	4.3 ± 1.4	5.0 ± 1.2	7.7 ± 2.3	6.7 ± 2.9
Prolactin		8.1 ± 1.6	7.7 ± 1.6	7.1 ± 1.4	8.0 ± 1.3	8.6 ± 1.1	8.8 ± 1.3
ACTH		40.0 ± 10.0	36.6 ± 9.4	44.3 ± 14.5	23.2 ± 2.7	42.6 ± 9.3	71.0 ± 14.7
ACTH	(nmol/24 hr)	47.2 ± 11.0*	45.8 ± 3.4	49.9 ± 6.7	46.4 ± 7.0	44.0 ± 6.1*	91.1 ± 8.8
Urine free cortisol							
Cortisol	(nmol/24 hr)	621 ± 92	656 ± 102	760 ± 135	638 ± 123	534 ± 71*	894 ± 126
DHEA-S		2.8 ± 0.4	2.7 ± 0.5	2.8 ± 0.5	2.8 ± 0.5	2.0 ± 0.3	2.0 ± 0.3
Adosterone		22.7 ± 4.5*	31.1 ± 6.0	37.8 ± 11.2	26.8 ± 4.8	34.3 ± 4.8	35.8 ± 6.9

* p < 0.01; * control subjects at baseline vs 4 months.
 * p < 0.05; * patients at baseline vs baseline.
 * p < 0.02; * patients at 4 months vs control subjects at 4 months.
 * p < 0.01; * patients at 4 months vs control subjects at 3 months.

ACTH = adrenocorticotrophic hormone
 DHEA-S = dehydroepiandrosterone sulphate

0.675

- REFERENCES**
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Longitudinal Changes in Pituitary-Adrenal Hormones in South African Women With Burnout

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The authors' goal was to document baseline pituitary-adrenal hormonal and related metabolic variables in 16 female patients with burnout. Then, following stress management intervention, to compare the changes with an equal number of untreated control subjects. At monthly intervals for 4 mo, 24-h urine samples were obtained for determination of free cortisol excretion. In addition, fasting blood samples were analyzed for levels of cortisol, dehydroepiandrosterone sulfate (DHEA-S), ACTH, aldosterone, and catecholamines. Other biochemical measurements included growth hormone, prolactin, insulin, glucose, and lipid components. The Maslach Burnout Inventory, General Health Questionnaire-28, and Zung depression rating scale were completed on each consecutive visit. The most striking finding was the reduction of urine free-cortisol excretion in the patients compared with controls. Initial urinary free cortisol was significantly lower in the patients (mean $\pm$ SEM = 47.2 ± 11.0 vs 79.0 ± 6.8 nmol/L, $p = 0.02$) and remained significantly reduced at 4 mo (mean $\pm$ SEM = 44.0 ± 6.1 vs 91.1 ± 8.8 nmol/L, $p = 0.0001$). There were no significant changes in the other hormonal and biochemical data. We conclude that there is functional hypocortisolism in burnout, which is not immediately restored on stress management intervention despite clinical and psychological improvement.

Key Words: Burnout; HPA axis; cortisol; ACTH; rating scales, depression.

Introduction

Burnout has been defined as a syndrome in which depletion of emotional, cognitive, and physical energy results in symptoms (1):

1. Emotional depletion leads to emotional exhaustion, tearfulness, apathy, perceived meaninglessness, and detachment from work.
2. Cognitive depletion is evidenced by reduced attention span and a reduction in professional efficacy.
3. Depletion of physical functioning includes overwhelming fatigue (with concomitant insomnia), headaches, and non-specific pain.

It can be measured using the Maslach Burnout Inventory (MBI) in which high levels of emotional exhaustion and depersonalization, coupled with low levels of personal accomplishment, are indicative of burnout (2).

The etiology of the syndrome was originally linked with the unremitting emotional stress experienced by social service providers and caregiving professionals (3). However, it is now recognized that all workers are exposed to job and life stressors that may precipitate the phenomenon (4). Additional predictors of burnout include lack of decision-making involvement at work, deficient coping skills, and poor social support (5).

Since Selye's (6) pioneering work on stress in the 1930s, the association between stress and increased cortisol secretion has been used as a model to explain the development of disease as a result of dysregulation in the homeostatic responses of the body's stress system (7). While activation of the hypothalamic-pituitary-adrenal (HPA) axis and the resultant increase in the secretion of cortisol from the adrenal cortex is considered a normal response to stress, the allostatic load incurred during prolonged or excessive HPA axis activation may lead to illness (8). An example of this is the melancholia subtype of major depressive disorder, where the stress response appears hyperactive and patients commonly experience dysphoric arousal with symptoms such as anxiety, insomnia, and decreased appetite (9).

In direct contrast to this model, however, a number of studies have now provided convincing evidence that the adrenal cortex is hypoactive in some stress-related states (10). This phenomenon, termed "functional hypocortisolism" (11), has mainly been applied to patients who experienced a traumatic event and subsequently developed post-traumatic stress disorder (12). However, hypocortisolism has also been reported in patients with chronic fatigue syndrome

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Table 1
Fasting 8.00 AM Serum Concentrations of Pituitary and Adrenal Hormones
Before and During 4 mo of Therapy for Stress Burnout in Patients, and Untreated Control Subjects (Mean $\pm$ SEM)

	Patients					Control Subjects	
	Baseline	1 mo	2 mo	3 mo	4 mo	Baseline	4 mo
Number of Subjects	16	16	16	16	16	16	16
Growth hormone (mIU/L)	3.7 $\pm$ 2.1	6.1 $\pm$ 1.8	4.5 $\pm$ 1.4	5.0 $\pm$ 1.2	8.7 $\pm$ 2.2	7.7 $\pm$ 2.3	8.7 $\pm$ 2.9
Prolactin (μ g/L)	8.1 $\pm$ 1.9	7.7 $\pm$ 1.6	7.2 $\pm$ 1.4	8.0 $\pm$ 1.3	8.9 $\pm$ 2.5	8.6 $\pm$ 1.1	8.8 $\pm$ 1.3
ACTH (pmol/L)	40.0 $\pm$ 10.0	36.6 $\pm$ 8.4	44.9 $\pm$ 14.5	22.2 $\pm$ 2.7	42.6 $\pm$ 9.3	71.0 $\pm$ 14.7	40.8 $\pm$ 7.1 ^a
Urine free cortisol (nmol/24hr)	47.2 $\pm$ 11 ^b	45.8 $\pm$ 5.4	49.9 $\pm$ 6.7	48.4 $\pm$ 7.0	44.0 $\pm$ 6.1 ^c	79.0 $\pm$ 6.8	91.1 $\pm$ 8.8
Cortisol (nmol/L)	621 $\pm$ 92	686 $\pm$ 102	790 $\pm$ 135	658 $\pm$ 125	524 $\pm$ 71 ^d	894 $\pm$ 129	1031 $\pm$ 122
DHEA-S (μ mol/L)	2.6 $\pm$ 0.4	2.7 $\pm$ 0.5	2.6 $\pm$ 0.5	2.6 $\pm$ 0.5	2.8 $\pm$ 0.6	2.0 $\pm$ 0.3	2.0 $\pm$ 0.3
Aldosterone (pmol/L)	277 $\pm$ 45	311 $\pm$ 60	373 $\pm$ 112	265 $\pm$ 46	406 $\pm$ 80	345 $\pm$ 46	358 $\pm$ 69

^aSignificance of values in the control group at baseline compared with four months: $p = 0.01$.

^bSignificance of values in the patient group compared with control subjects at baseline: $p = 0.02$.

^cSignificance of values in the patient group compared with control subjects at four months: $p = 0.002$.

^dSignificance of values in the patient group compared with control subjects at four months: $p = 0.001$.

Table 2
Fasting Serum Biochemical Concentrations
Before and During 4 mo of Therapy for Stress Burnout in Patients, and Untreated Control Subjects (Mean $\pm$ SEM)

	Patients					Control Subjects	
	Baseline	1 mo	2 mo	3 mo	4 mo	Baseline	4 mo
Number of Subjects	16	16	16	16	16	16	16
Glucose (mmol/L)	4.8 $\pm$ 0.1	5.0 $\pm$ 0.1	4.9 $\pm$ 0.1	4.7 $\pm$ 0.1	4.6 $\pm$ 0.2	4.8 $\pm$ 0.2	5.0 $\pm$ 0.2
Insulin (mIU/L)	8.3 $\pm$ 1.3	10.1 $\pm$ 1.9	9.5 $\pm$ 1.5	10.4 $\pm$ 1.2	9.0 $\pm$ 1.2	7.3 $\pm$ 0.8	8.0 $\pm$ 1.2
Free fatty acids (mmol/L)	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1
Total cholesterol (mmol/L)	4.6 $\pm$ 0.3	4.7 $\pm$ 0.2	4.9 $\pm$ 0.3	4.8 $\pm$ 0.2	4.7 $\pm$ 0.3	4.9 $\pm$ 0.2	5.1 $\pm$ 0.2
HDL-cholesterol (mmol/L)	1.4 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	1.6 $\pm$ 0.1	1.6 $\pm$ 0.1
LDL-cholesterol (mmol/L)	2.8 $\pm$ 0.2	2.7 $\pm$ 0.2	3.0 $\pm$ 0.3	2.8 $\pm$ 0.2	2.7 $\pm$ 0.2	2.8 $\pm$ 0.2	3.0 $\pm$ 0.2
Triglycerides (mmol/L)	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1

(13), fibromyalgia (14), rheumatoid arthritis (15), asthma (16), and atypical depression (17). The pathophysiology of many of these disorders has been related to stress, with fatigue being a central component of the symptomatology.

Because fatigue is a key symptom of burnout, and this symptom is shared by other syndromes in which hypocortisolism is a feature, this study was undertaken to determine if a similar biochemical status exists in burnout patients and to assess the longitudinal changes during a treatment period of 4 mo.

Results

Pituitary and Adrenal Hormones (Table 1)

Compared with control subjects, mean urine free-cortisol levels in the patients were significantly lower both at

baseline ($p = 0.02$) and at 4 mo ($p = 0.0001$), consistently below the lower level of the normal adult range. However, the mean serum cortisol level in patients was significantly lower than controls only at 4 mo ($p = 0.001$). Apart from significant elevation of serum adrenocorticotrophic hormone (ACTH) at baseline in control subjects as compared with 4 mo ($p = 0.01$), and raised serum growth hormone (GH) at baseline in controls compared with patients ($p = \text{NS}$), all the other mean fasting serum concentrations of pituitary and adrenal hormones measured before and during 4 mo of therapy were similar.

Biochemical Data (Table 2) and BMI

There were also no statistically significant differences in mean fasting serum concentrations before and during 4 mo of therapy, in patients versus control subjects for glucose,

Table 3

Fasting Plasma Concentrations of Catecholamines
Before and During 4 mo of Therapy for Stress Burnout in Patients, and Untreated Control Subjects (Median and Range)

	Patients					Control Subjects	
	Baseline	1 mo	2 mo	3 mo	4 mo	Baseline	4 mo
Number of Subjects	16	16	16	16	16	16	16
Epinephrine (pmol/L)	222 (49–4036)	563 (42–6361)	334 (76–4431)	206 (10–3144)	151 (14–2211)	206 (15–1672)	114 (0–454)
Norepinephrine (pmol/L)	1271 (395–5436) ^a	1570 (244–4960)	1442 (87–7203)	1816 (619–3325)	2313 (699–7538)	2338 (452–3974)	2704 (1351–4337)

^aSignificance of values in the patient group compared with control subjects at baseline: $p = 0.03$.

insulin, free fatty acids (FFA), total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, and triglycerides. In addition, there was no significant difference in mean body mass index (BMI) between patients and controls at baseline (mean $\pm$ SEM = 23.9 ± 0.9 vs 24.8 ± 0.9 kg/m², $p = \text{NS}$) and at 4 mo (mean $\pm$ SEM = 23.3 ± 0.8 vs 24.6 ± 0.8 kg/m², $p = \text{NS}$).

Catecholamines (Table 3)

There were no differences in median levels of plasma epinephrine between patients and controls. The median plasma norepinephrine level at baseline, however, was significantly higher in the control group than in the patients ($p = 0.03$).

Clinical Ratings

A composite graph showing a similar trend for the results of the clinical ratings is shown (Fig. 1). Because emotional exhaustion is regarded as the most stable burnout dimension of the MBI (18), only this subscale of the MBI was graphically depicted.

Regarding the MBI, mean scores of emotional exhaustion declined significantly over the 4 mo ($p < 0.0001$) as compared with baseline measurements. There were significant differences between patients and controls at baseline in emotional exhaustion ($p < 0.0001$), depersonalization ($p = 0.001$), and personal accomplishment ($p = 0.0001$). Patients varied significantly from controls at four mo in their scores of emotional exhaustion ($p = 0.001$) and depersonalization ($p = 0.03$).

For the General Health Questionnaire (GHQ28), patients' scores declined over the 4 mo significantly from baseline measurements ($p < 0.0001$). There was a significant difference between patients and controls at baseline ($p < 0.0001$) and at 4 mo ($p < 0.01$).

The Zung depression rating scale (ZDRS) also showed a declining trend, with patients scoring significantly higher at baseline than in the subsequent 4 mo ($p < 0.0001$). In comparison with controls, patients' scores were significantly different at baseline ($p < 0.0001$) and at 4 mo ($p = 0.0002$).

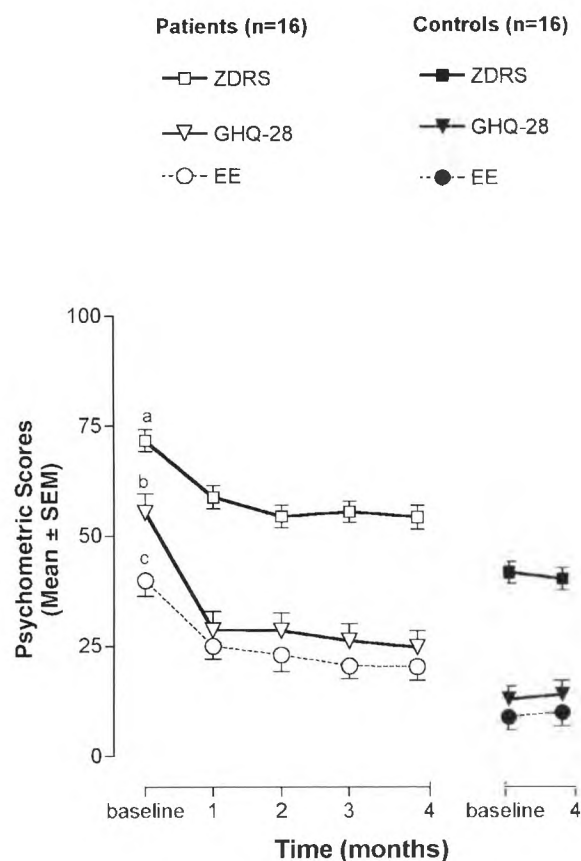


Fig. 1. Mean Zung depression rating scale (ZDRS), General Health Questionnaire-28 (GHQ-28), and Maslach Burnout Inventory Emotional Exhaustion scores in patients and control subjects at baseline and 4 mo. Vertical bars show SEM. ^aSignificant difference in ZDRS scores for patients between baseline and 1, 2, 3, and 4 mo ($p < 0.0001$) and for patients vs controls at baseline ($p < 0.0001$) and at 4 mo ($p = 0.0002$). ^bSignificant difference in GHQ-28 scores for patients at baseline and 1, 2, 3, and 4 mo ($p < 0.0001$) and for patients vs controls at baseline ($p < 0.0001$) and at 4 mo ($p < 0.01$). ^cSignificant difference in emotional exhaustion scores for patients at baseline and 1, 2, 3, and 4 mo ($p < 0.0001$) and for patients vs controls at baseline ($p < 0.0001$) and at 4 mo ($p = 0.001$).

Discussion

Pituitary Adrenal Function

In this investigation, the most consistent endocrine finding was a reduction of urine free-cortisol excretion in the patient group, suggesting a situation of functional hypoadrenalism in burnout. No significant recovery of cortisol excretion occurred during the study, implying an increased predisposition to future burnout episodes.

The normal 0800 h serum cortisol levels in patients in the face of low urinary cortisol excretion is difficult to explain. Because all the patients had normal creatinine clearance, it is improbable that impaired renal function influenced cortisol excretion. We concur with Crofford et al. (14) who postulate that in fibromyalgia the low 24-h levels may reflect reduced cortisol pulsatility during the 24-h period, with preservation of the early morning peak.

Serum levels of ACTH above the normal range in the patients cast doubt as to the central origin of the functional hypoadrenalism. However, as patients were not subjected to dexamethasone suppression of cortisol, ACTH challenge testing with corticotropin-releasing-hormone (CRH) stimulation, or insulin-induced hypoglycemia, the functional integrity of the HPA pathway in burnout remains uncertain and warrants further study.

In addition, although control subjects rested for 30 min prior to blood sampling, mean ACTH (and norepinephrine) levels also exceeded normal ranges. This may reflect a stress response to the needle-stick, or could highlight high “background” levels of stress in this group. In the South African context, chronic, unavoidable stress factors include the high incidence of violent crime and trauma (19) and the impact of AIDS, both socially and economically on the developing population (20). Despite these biochemical anomalies, the controls showed no burnout symptoms.

Our findings resemble the reduced adrenocortical hormonal secretion that has been documented for other stress-related illnesses, e.g., post-traumatic stress disorder (PTSD) (12), chronic fatigue syndrome (13), and fibromyalgia (14). Hypocortisolism may be a relevant factor in the pathogenesis of all these disorders, since the lack of cortisol availability may cause symptoms common to these conditions: vulnerability to autoimmune disorders, inflammation, chronic pain, asthma, and allergies (21).

Potential Effect of Drug Therapy

The putative action of chronic psychotropic medication on the HPA axis is inhibitory, probably by suppression of hypothalamic CRH and/or arginine vasopressin release (22). Despite these central inhibitory effects, in studies on alprazolam, citalopram, and imipramine (a tricyclic antidepressant similar to dothiepin), treatment did not affect basal plasma ACTH and cortisol levels, nor did it influence urinary free cortisol excretion (22–24).

Link Between Burnout and Depression

The possible link between burnout and depression deserves comment. All patients enrolled in the study were assessed to be depressed at baseline (according to the ZDRS). According to Arana, up to 45% of depressives exhibit HPA axis hyperarousal (25). Gold and Chrousos (26) recently reviewed HPA axis functioning in two diagnostic subtypes of depression and clarify that the biochemical “signature” of melancholic depression is typified by hyperactivity of the HPA axis, with increased CRH, ACTH, and cortisol secretion, and decreased sensitivity of the negative feedback system. In contrast, neuroendocrinologic features of atypical depression highlight underactivity of the HPA axis (with decreased CRH, ACTH, and cortisol secretion and a supersensitized negative feedback system) (22). Symptomatically, patients with atypical depression present with the antithesis of melancholia: they are lethargic, hypersomnic, and hyperphagic. In burnout patients we found high ACTH levels but low cortisol secretion, which may reflect an “alternate or mixed” type of depression—which is also distinct both symptomatically and biochemically from PTSD.

Study Limitations

Certain limitations of our study should be noted: The relatively small number of patients and controls involved, the lack of 24-h plasma cortisol profiling, and the restricted period of follow-up observations. Notwithstanding these limitations, after undergoing an integrated stress management program, patient recovery was significant over the 4-mo period when charted on the MBI, the GHQ28, and the ZDRS. Interestingly, during this period, the decrease in emotional exhaustion, psychiatric symptoms and depression was not accompanied by a corresponding rise in urine free-cortisol levels. This suggests that clinical improvement following stress management measures precedes any accompanying change in biochemical measurements.

In Conclusion

Because responses to chronic stress and its management are multifactorial and dependent on varied aspects, e.g. genetic vulnerability, coping skills, intrinsic personality, and previous exposure to stress, interindividual variability in biochemical responses is to be expected (8). In highlighting hypocortisolism as a feature of burnout, this study indicates possible therapeutic strategies for treatment-resistant patients. For example, low-dose cortisol supplementation could be considered as an interim measure, as is the case in patients with chronic fatigue syndrome (27).

In view of the intense interest currently being focused on the functioning of the HPA axis following acute and chronic stress after recent tragic international catastrophes (28), our findings provide insight into the hormonal changes associated with burnout.

Patients and Methods

Patients

Sixteen female patients (mean age $\pm$ SEM = 38.1 ± 2.9 yr; range 25–59 yr) were enrolled in a multipoint study; two patients were postmenopausal. On initial clinical and psychological assessment they were diagnosed by a psychiatrist (J.D.M.) as suffering from stress burnout. This diagnosis was based on a standardized clinical examination in addition to a series of psychometric rating scales and symptom checklists (29). These included the MBI, the GHQ-28, and the ZDRS. Patients with any psychiatric diagnosis other than major depression or subsyndromal depression were excluded from the study. All patients were biochemically euthyroid. Sixteen age-matched women (mean age $\pm$ SEM = 37.3 ± 2.9 yr; range 19–58 yr) were recruited as control subjects; three control subjects were postmenopausal. In order for comparisons to reflect meaningful differences, the control population was drawn from the working hospital environment. In this way, the stress experienced by the controls paralleled the stresses of daily living in both the working and greater environment, but they had not developed any burnout symptoms. Patients and control subjects gave written, informed consent to participate in the study, which was approved by the Ethics Committee For Research On Human Subjects (Medical) of the University of the Witwatersrand.

Study Protocol

Study participants began a 24-h urine collection at home at 0800 h the day prior to their blood collection, and fasted from 2200 h that night. At 0800 h on the morning of the study, subjects reported to the Centre for Stress-Related Illnesses, Milpark Hospital, where the clinical studies were conducted. Subjects were seated in comfortable chairs and a 21-gauge catheter was inserted into an antecubital vein of one arm. This experimental procedure was performed at the start of the study, after which patients began treatment, and was repeated at monthly intervals for four further consecutive visits. For the control subjects, the same protocol was followed on two occasions, at baseline and at 4 mo. Measurements of height and weight were recorded at baseline and 4 mo for both patients and controls, and BMI was calculated.

Clinical Ratings

At each visit according to the above protocol, patients and controls completed the MBI, GHQ-28, and ZDRS. The MBI comprises three subscales, which measure the three main components of burnout: emotional exhaustion, depersonalization or cynicism, and reduced personal accomplishment. Values of ≥ 27 for emotional exhaustion, ≥ 13 for depersonalization, and ≤ 31 for personal accomplishment were indicative of burnout. The GHQ-28 is a measure of

general psychopathology with four subscales. These differentiate “somatic” symptoms, anxiety and insomnia, social dysfunction, and depression, but the cumulative GHQ score enables the identification of psychiatric “cases.” In this study, scores of 5 and above were used as the indicator. The ZDRS is a symptoms-based checklist for assessing depression. Scores of 50–59 indicate mild depression, 60–69 moderate depression, 70–79 severe depression, and >80 extreme depression.

Interventions

Patients participated in a stress management program from 0800 h to 1400 h for 5–10 consecutive days, which included exercise, diet, and behavior modification.

Medication consisted of a benzodiazepine (alprazolam) and antidepressants (citalopram or dothiepin). The benzodiazepine was initiated at a dose of 1 mg qid for 2 d, then tapered over 10 days to 1 mg nocte when necessary. Antidepressant therapy was initiated at the start of the study, and patients continued with this treatment throughout the study. Patients were started on 20 mg citalopram daily; however, two patients could not tolerate this drug, and they were given dothiepin 25 mg mane and 75 mg nocte.

Hormone Assays and Biochemical Measurements

After a 30-min resting period, blood samples were collected in iced tubes and centrifuged; plasma/serum aliquots were separated and stored at -20°C , except for catecholamines, which were analyzed immediately. Epinephrine and norepinephrine concentrations were measured using high-pressure liquid chromatography (Bio-Rad Laboratories, Hercules, CA) according to the method of Koch and Polzin (30) (respective normal ranges: 0–366 pmol/L; 560–2636 pmol/L). Plasma glucose levels were determined by a standard glucose oxidase method (normal range: 3.0–6.0 mmol/L). Insulin, GH, and prolactin concentrations were measured by immunoassay using direct chemiluminometric technology and reagents supplied by Diagnostic Products Corporation, Los Angeles, CA (respective normal ranges: 6.0–27.0 mIU/L; 0.16–13.0 mIU/L; 2.8–29.2 $\mu\text{g/L}$ [premenopause] and 1.8–20.3 $\mu\text{g/L}$ [postmenopause]). Radioimmunoassay kits were used to measure ACTH, cortisol, dehydroepiandrosterone sulfate (DHEA-S) (Diagnostic Systems Laboratories, Inc., Webster, Texas), and aldosterone (Diagnostic Products Corporation, Los Angeles, CA) (respective normal ranges: 2.2–19.8 pmol/L; 138–690 nmol/L [7:00–9:00 AM]; 1.0–7.3 $\mu\text{mol/L}$ [female]; 28–444 pmol/L [recumbent]); FFA triglycerides, total cholesterol, and HDL-cholesterol levels were determined by enzymatic, colorimetric methods using reagents supplied by Roche Diagnostics, Basel, Switzerland (respective normal ranges: 0.3–0.8 mmol/L; 0.0–2.0 mmol/L; <6.2 mmol/L; >1.6 mmol/L). LDL-cholesterol was calculated according to the formula of Friedewald et al. (31) (normal range: <4.1 mmol/L). Urine free-cortisol concen-

trations were measured by a competitive immunoassay (Bayer Corporation, Tarrytown, NY) (normal range: 55–250 nmol/24 h). Intraassay coefficients of variation for these assays were all <5%.

Statistical Analysis

Results are expressed as mean $\pm$ SEM except for catecholamines, where the medians with ranges are stated due to the non-parametric distribution of the data. Statistical significance of non-parametric data was assessed by the Friedman repeated measures test, Mann–Whitney “U” test, and the Wilcoxon signed rank test; parametric data were assessed by the Student’s *t*-test (two-tailed) for paired and unpaired samples. Repeated measures analysis of variance was used to examine differences in patients’ psychometric rating scales from baseline to 4 mo. The Bonferroni multiple comparisons test was used to determine significances at each time-point. A value of $p < 0.05$ was considered significant.

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FACULTY OF HEALTH SCIENCES

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