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# Optimising glucocorticoid replacement therapy in severely adrenocorticoptropin deficient hypopituitary males.

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# Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of MD is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work.  Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

**Signed**

**RCSI Student Number 97009**

**Date** 1<sup>st</sup> March 2014

# List of publications and presentations arising from thesis

## Publications

Optimising glucocorticoid replacement therapy in severely adrenocorticotropin-deficient hypopituitary male patients.
**Behan LA**, Rogers B, Hannon MJ, O'Kelly P, Tormey W, Smith D, Thompson CJ, Agha A.
Clin Endocrinol (Oxf). 2011 Oct;75(4):505-13. doi: 10.1111/j.1365-2265.2011.04074.x.


Low-dose hydrocortisone replacement therapy is associated with improved bone remodelling balance in hypopituitary male patients.
**Behan LA**, Kelleher G, Hannon MJ, Brady JJ, Rogers B, Tormey W, Smith D, Thompson CJ, McKenna MJ, Agha A.
Eur J Endocrinol. 2013 Nov 29;170(1):141-50. doi: 10.1530/EJE-13-0596. Print 2014 Jan.


The modulation of corticosteroid metabolism by hydrocortisone therapy in patients with hypopituitarism increases tissue glucocorticoid exposure
Sherlock M*, **Behan LA**\*, Hannon MJ, Aragon Alonso A, Thompson CJ, Murray RD, Crabtree N, Hughes AB, Arlt W, Agha A, Toogood A, Stewart PM.
Submitted to J Clin Endocrinol Metab 2013, undergoing second review as of Feb 2014
*authors contributed equally to manuscript

# Oral Presentations

**Optimal glucocorticoid replacement in severely adrenocorticotropin (ACTH) deficient hypopituitary subjects.**

Behan LA, Rogers B, Hannon MJ, O'Kelly P, Smith D, Thompson CJ, Agha A

Oral presentation, Irish Endocrine Society, November 2009

Abstract published in Irish Journal Medical Science 2009


**Low dose hydrocortisone (HC) replacement therapy is associated with improved bone remodelling balance in hypopituitary subjects**

Behan LA, Kelleher G, Hannon MJ, Brady JJ, Rogers B, Tormey W, Smith D, Thompson CJ, McKenna MJ, Agha A.

Oral presentation, Irish Endocrine Society, November 2010

Abstract published Irish Journal Medical Science 2010


**The effect of different hydrocortisone (HC) replacement regimens on free serum cortisol and tissue cortisol exposure in hypopituitary men**

Behan LA, Sherlock M, Rogers B, Hannon MJ, O'Kelly P, Tormey W, Smith D, Thompson CJ, Stewart PM, Agha A.

Oral presentation, Irish Endocrine Society, November 2010

Abstract published Irish Journal Medical Science 2010


**Effect of cortisol dynamics on bone turnover in ACTH deficient hypopituitary patients receiving physiological hydrocortisone replacement.**

Behan LA, Kelleher G, Hannon MJ, Brady JJ, Rogers B, Tormey W, Smith D, Thompson CJ, McKenna MJ, Agha A.

Oral presentation, The Irish Endocrine Society, Kilkenny, November 2013

Abstract published Irish Journal Medical Science 2013

# Poster Presentations

**An assessment of patient quality of life (QoL) on 3 glucocorticoid replacement regimens in severely adrenocorticotropin (ACTH) deficient hypopituitary subjects.**

Behan LA, Rogers B, Hannon MJ, O'Kelly P, Smith D, Thompson CJ, Agha A.

Poster presentation, Irish Endocrine Society, Cork, November 2009

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**Optimal glucocorticoid replacement in severely adrenocorticotropin (ACTH) deficient hypopituitary subjects.**

Behan LA, Rogers B, Hannon MJ, O'Kelly P, Smith D, Thompson CJ, Agha A

Poster presentation, The Endocrine Society, San Diego, June 2010


**The effect of different doses of hydrocortisone (HC) replacement regimens on insulin sensitivity and blood pressure indices in hypopituitary men.**

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Poster presentation, The Irish Endocrine Society, Belfast, November 2011


**Low dose hydrocortisone (HC) replacement therapy is associated with improved bone remodelling balance in hypopituitary subjects**

Behan LA, Kelleher G, Hannon MJ, Brady JJ, Rogers B, Tormey W, Smith D, Thompson CJ, McKenna MJ, Agha A.

Poster presentation, The Endocrine Society, Boston, June 2011


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# List of abbreviations

| ACTH(D) | adrenocortiocotropin (deficiency) |
|---------|-----------------------------------|
| AI | adrenal insufficiency |
| APHD | anterior pituitary hormone deficiency |
| AVP | arginine vasopressin |
| BMD | bone mineral density |
| BMI | body mass index |
| BTM | bone turnover markers |
| bone ALP | bone alkaline phosphatase |
| BP | blood pressure |
| CBG | cortisol binding globulin |
| CDI | cranial diabetes insipidus |
| CRC | clinical research centre |
| CRH | corticotropin releasing hormone |
| CTX-I | C terminal cross-linking telopeptide |
| DBP | diastolic blood pressure |
| DM | diabetes mellitus |
| eGFR | estimated glomerular filtration rate |
| FSH | follicle stimulating hormone |
| GC | glucocorticoid |
| GH(D) | growth hormone (deficiency) |
| GST | glucagon stimulation test |
| HC | hydrocortisone |
| HOMA | homeostatic model assessment |
| HPA | hypothalamic-pituitary-adrenal |
| HR | hazard ratio |
| IGF-1 | insulin-like growth factor I |
| IQR | interquartile range |
| ITT | insulin tolerance test |
| KIMS | Pfizer international growth hormone metabolic database |
| LH | luteinising hormone |

| | |
|---|---|
| NHP | Nottingham health profile |
| NO | nitric oxide |
| NS | non-significant |
| OC | osteocalcin |
| OGIS | oral glucose insulin sensitivity index |
| OGTT | oral glucose tolerance test |
| PINP | pro collagen type 1 peptide |
| PTH | parathyroid hormone |
| QoL | quality of life |
| RR | relative risk |
| SBP | systolic blood pressure |
| SF36 | short form 36 |
| SMR | standardised mortality ratio |
| T2DM | type 2 diabetes mellitus |
| T4 | thyroxine |
| TBG | thyroid hormone binding globulin |
| THE | tetrahydrocortisone |
| THF | tetrahydrocortisol |
| TRACP5b | tartrate resistant acid phosphatase 5b |
| TSH | thyroid stimulating hormone |
| UFE | urinary free cortisone |
| UFF | urinary free cortisol |
| WCM | waist circumference |
| WDT | water deprivation test |
| WHO | World Health Organisation |
| Z score | standard deviation score |
| 11βHSD | 11 beta hydroxysteroid dehydrogenase |
| 25OHD | 25 hydroxy vitamin D |

# Summary of Thesis

Inappropriate cortisol replacement has been proposed as a contributing factor in the increased morbidity and mortality associated with hypopituitarism. The aim of this study is to examine the effect of three commonly prescribed hydrocortisone replacement regimens on predefined metabolic end points and quality of life (QoL) in male subjects with severe adrenocorticotropin (ACTH) deficiency with a view to identifying the most physiologic regimen.

10 hypopituitary men participated in a prospective, randomised, crossover, open protocol of 6 weeks on each of the following regimens: Dose A (20mg AM, 10mg PM), Dose B (10mg AM, 10mg PM), Dose C (10mg AM, 5mg PM). Results were compared between dose regimens and to healthy control subjects.

The lowest dose regimen, dose C (10mg/5mg), was associated with a 24 hour serum cortisol profile that most closely approximated that of the control subjects, without any difference in QoL between doses.

There were significant increases in serum markers of bone formation in the lower dose regimen dose C, with an 86% increase in procollagen type I N-propeptide (PINP), and a 56% increase in intact osteocalcin (OC[1-49]) compared to dose A, without concurrent increases in bone resorption markers. These findings reflect a more positive bone remodelling balance in the lowest dose regimen, resulting in net bone gain.

Tissue exposure to cortisol was assessed by examining 24hour urinary cortisol metabolites. Dose A (20mg/10mg) was associated with significantly higher total cortisol metabolites compared to the two other dose regimens and compared to healthy controls. We also demonstrated increased tissue cortisol exposure across all dose regimens compared to healthy controls; however it was highest with dose A.

There was no difference between dose regimens or compared to controls in glucose or insulin metabolism or in 24 hour blood pressure. Dose C (10mg/5mg) was associated with a reduction in the ambulatory arterial stiffness index (AASI) and a more physiological nocturnal blood pressure dip which may indicate a more favourable vascular status.

This study demonstrates a lower dose hydrocortisone replacement regimen in severe ACTH deficiency is associated with a more favourable metabolic profile without adversely affecting QoL. These findings suggest that physicians may safely aim for a reduction in prescribed hydrocortisone replacement doses.

# Chapter One: Introduction

## 1.1 Background

Cortisol, the primary glucocorticoid in humans, is produced in the zona fasiculata of the adrenal cortex in response to stimulus by adrenocorticotropin hormone (ACTH) from the anterior pituitary gland. ACTH release is modulated by corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus of the hypothalamus. The release of these peptides from the hypothalamus is controlled by a number of different factors including the sympathetic nervous system, stress, circadian rhythm and the negative feedback of cortisol on both the hypothalamus and the pituitary gland(1) (Figure 1.1).
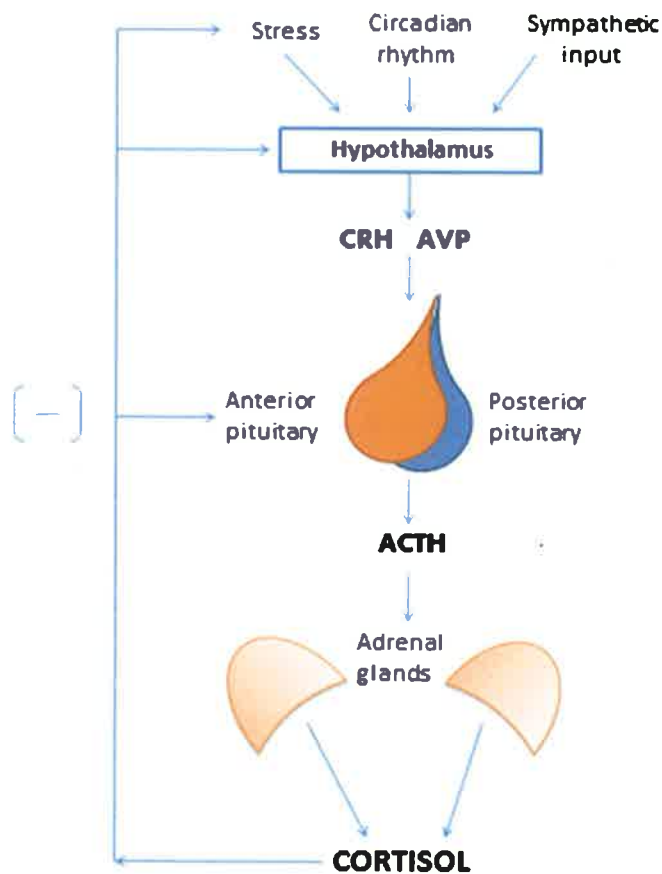
**Figure 1.1** Hypothalamic-pituitary-adrenal axis and cortisol production
CRH – corticotrophin releasing hormone, AVP – arginine vasopressin, ACTH –
adrenocorticotropin hormone

Glucocorticoids are necessary for life and well-being. The physiologic effects of

endogenous cortisol include anti-inflammatory effects through suppression of cytokines

and chemokines, and the maintenance of vascular responsiveness to sympathetic

stimulation, all of which serves to maintain cardiac output, vascular tone and avoid

excess inflammatory response in illness or trauma(2). Cortisol is also necessary to

facilitate appropriate diuresis.  Deficiency of this hormone may lead to a reduction in

vascular tone, which stimulates vasopressin release to cause excessive water retention and relative hyponatraemia with resultant clinical symptoms of nausea, vomiting, seizures, fatigue and ultimately may lead to coma and death. Adequate increases in cortisol production during times of physiologic stress are vital(1).

Secondary adrenal insufficiency (AI) is a deficiency in adrenal glucocorticoids that is not due to a primary adrenal pathology and is due to disruption of hypothalamic-pituitary stimulation of cortisol release. While this can be a result of exogenous glucocorticoid use, for the purposes of this thesis I will refer to secondary adrenal insufficiency as a result of pituitary or hypothalamic pathology that leads to adrenocorticotrophic hormone (ACTH) deficiency. While ACTH deficiency can rarely occur in isolation, in the majority of cases it occurs in conjunction with other pituitary hormone deficiencies and is referred to as hypopituitarism. Secondary AI of this nature has an estimated prevalence of 290-455 per million(3). It is likely this is an underestimate as causes of hypopituitarism that were previously unrecognised are increasingly being investigated, including post-traumatic brain injury hypopituitarism(4, 5) and post cranial irradiation hypopituitarism(6); conditions which are thought to be underrepresented in available figures. ACTH deficiency leading to hypocortisolism can be fatal and requires careful glucocorticoid replacement(7).

Prior to the discovery of cortisone in 1935, Kendall's Compound E(8), and the subsequent production of cortisone as a pharmaceutical for clinical use in 1950(8, 9), adrenal insufficiency of any cause was a fatal condition. Since this life saving compound

has been produced for therapeutic use and for hormone replacement purposes, there has been much debate as to the most appropriate method of cortisol replacement for both primary and secondary adrenal insufficiency. Pituitary hormone replacement in hypopituitarism is challenging, and cortisol replacement in particular frequently presents the physician with some difficult challenges involving both how to provide an appropriate substitute and how to monitor patients for evidence of over or under replacement, both of which are associated with considerable morbidity(10-15)

## 1.1.1 There is currently no therapy available that closely mimics endogenous cortisol production.

Cortisol is produced in a pulsatile manner and has a distinct circadian rhythm that has been previously described in detail(16) (Figure 1.2).
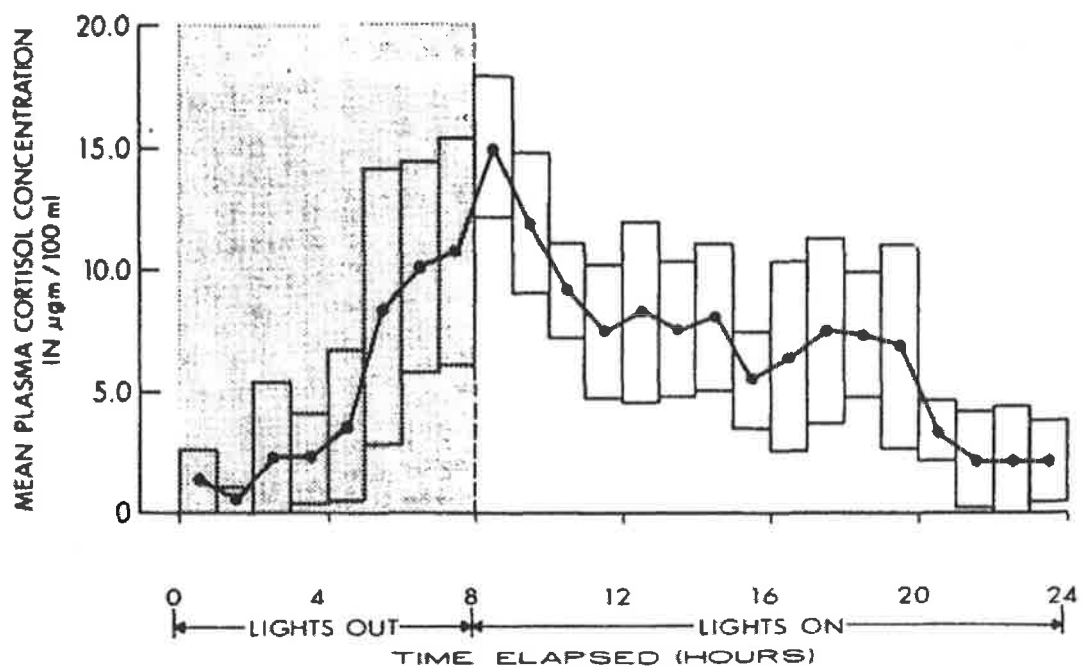
**Figure 1.2** Circadian rhythm of endogenous cortisol production
Mean and standard deviation plasma cortisol concentration micrograms/100ml based on serum sampling every 20 minutes from 7 healthy individuals. Adapted from Weitzman et al 1971(16)

As shown in Figure 1. 2 above, circadian cortisol secretion has two distinct peaks, the highest occurring in the early morning, usually starting from 2-4am and continuing until 6-8am when production starts to decrease until the second smaller peak that occurs in the early afternoon (most commonly between 12pm and 4pm), levels then gradually decline to low levels in the late afternoon and evening to a nadir near midnight. This pattern has been confirmed in 33 healthy subjects by Debono et al(17). No glucocorticoid replacement regimen currently available can mimic exactly this circadian rhythm of cortisol production. Hydrocortisone, which is active cortisol, is a short acting glucocorticoid and achieves a peak serum concentration approximately 1 hour after oral ingestion with a short half-life of 60-90 minutes and has over 95% oral bioavailability(18); while cortisone acetate, which is inactive cortisone and requires intrahepatic conversion

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to the active metabolite cortisol through the 11-β hydroxysteroid dehydrogenase (11-βHSD) enzyme system has more variable pharmacokinetics, yet it has been shown to have a similar peak onset and half-life as cortisol[19]. In order to mimic physiologic cortisol rhythm using these short acting preparations, patients would need to take short acting glucocorticoids sometime between 3am and 6am. Understandably this is not acceptable to the majority of patients and would be associated with significant sleep disruption, without proven benefit. Longer acting glucocorticoids such as dexamethasone and prednisolone are used as glucocorticoid replacement in situations where hydrocortisone or cortisone acetate are not readily available. They are potent glucocorticoids with a long half-life, approximately 3 and 4 hours respectively[18]; such pharmacodynamics lead to un-physiologic exposure to glucocorticoid, particularly at night and can be associated with adverse metabolic outcomes[20].

Traditionally hydrocortisone doses were prescribed as two thirds of the total daily dose in the morning upon waking and one third in the afternoon, (most commonly 20mg and 10mg doses), in order to mimic the peaks already discussed. However, this regime was based upon an over-estimation of the daily cortisol production of 12-15mg/m2/day[21, 22] and an underestimation of the oral bioavailability of HC[23]. Later studies using isotope dilution/mass spectrometry[24] and deconvolution analysis[25] have shown that the daily cortisol production rate is considerably lower at $5.7mg/m^2/day$, or 9.9mg/day. In view of these data efforts are being made to review the optimal dosing regimen for subjects with adrenal insufficiency in order to maintain health and well-being, while avoiding the chronic over-exposure inherent in the traditional replacement regimens detailed above.

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Although prescribing 30mg of cortisol daily is now considered excessive by many physicians it is clear that hypopituitary patients are still being exposed to a wide range of glucocorticoid replacement doses in clinical practice. Recent publications quote usage of glucocorticoid replacement equivalent to hydrocortisone doses of $\geq$ 30mg daily in 34%, 67% and 91% subjects(26-28) and $\geq$ 20mg but <30 daily in 39.4% and 80% of subjects(26, 29).

There is also much debate regarding the frequency of dosing with short acting glucocorticoids. Some physicians propose a thrice daily regimen over a twice daily regimen(30, 31), based on the desire to avoid supra-physiological peaks and prolonged sub-physiological trough levels. Others have recommended once daily dosing based upon concerns regarding compliance(1). On close examination, one of the main studies recommending thrice daily hydrocortisone compared subjects on a mean daily dose of 35mg hydrocortisone given at 0900, 1400 and 2000hours, with a twice daily regimen given at 0900 and 2000hours, eleven hours apart(30). In view of the pharmacodynamics of hydrocortisone described above, it is not surprising that by 2000hours the twice daily group had much lower levels of hydrocortisone recorded than the thrice daily group. No cortisol measurements were provided after 2000hours in this study, so it is not clear if the cortisol levels thereafter were supraphysiological, or if low trough levels were successfully avoided in the thrice daily regimen. While under-replacement in general is undesirable, there is no evidence that low trough levels during the day are harmful.

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In a retrospective uncontrolled study Howlett et al also compared thrice and twice daily regimens in subjects who had "HC day curves" as part of their routine care; samples after the morning dose at 0900hours and prior to the 1230 and /or the 1730hours dose(31). The cut off for optimal replacement was defined arbitrarily. The criticism of the twice daily regimen in this paper was again based on prolonged trough levels in a dose regimen where the second dose was scheduled for the evening, thus unsurprisingly predisposing to non-physiological pre-dose trough levels. There was no serum cortisol measurements taken after the 1730hour dose, but it is likely the evening levels were supra-physiological and no evidence was presented to show if low early morning cortisol levels were avoided using the thrice or twice daily regimens in this study.

Mah et al performed a pharmacokinetic study evaluating the impact of a weight-based thrice daily HC regimen in subjects with cortisol deficiency, the majority of whom had primary adrenal insufficiency. They examined a 10mg "fixed dose" of HC in the morning, compared to a 0.12mg/kg dose also in the morning, without assessing the impact of the other two doses later in the day. The weight-based regimen demonstrated less inter-individual variability in cortisol profiles compared to the fixed dose, however it did not result in lower peak cortisol levels compared to the fixed dose regimen(32). The potential benefits of thrice daily dosing may be more relevant in the setting of primary adrenal insufficiency, where suppression of endogenous ACTH production is desirable, but the potential metabolic cost of inappropriate glucocorticoid exposure should be considered. Overall there is little evidence to suggest that a thrice daily regimen is superior in the setting of secondary adrenal insufficiency and it is clear that the dynamics of a thrice

daily regimen do not mimic diurnal rhythm, with the added important consideration of potentially reduced compliance with increasing frequency of dose administration.

New formulations of modified release hydrocortisone are currently in development. Clinical trials are underway with a view to mimicking more closely the physiologic patterns of cortisol production(17, 33, 34) and the results will be of great interest. These formulations are likely to be more expensive than the currently available hydrocortisone and have yet to prove superiority in prospective, double-blind comparison studies of end organ metabolic effect and quality of life studies.

### 1.1.2 When on glucocorticoid replacement, how do we monitor patients for over or under-replacement?

There is no reliable biomarker to monitor cortisol replacement objectively in either primary or secondary adrenal insufficiency. Measurement of ACTH cannot be used to assess adequacy of glucocorticoid supplementation in ACTH deficiency, nor is it reliable in primary adrenal insufficiency since ACTH rises before the morning dose. Given the limitations of oral HC replacement, "normalisation" of ACTH requires high dose glucocorticoid replacement at inappropriate time points, which will lead to chronic over-replacement(35). Serial serum cortisol measurements or "cortisol day-curves" have been proposed as an alternative method to assess cortisol replacement(31, 36). However frequency of serum sampling varied in these studies from every 90 minutes for 12 hours(36) to 3 hourly for 9 hours(31). Interpretation of cortisol day curves depends on timing of dose administration in conjunction with timing of serum sampling, laboratory assay used and consideration of locally generated reference curves from the healthy

population. Arlt et al demonstrated a poor correlation between clinical assessment and 3 timed cortisol measurements in 46 subjects with primary and secondary adrenal insufficiency(28). Serial serum cortisol measurements are time consuming for both patients and healthcare staff and are likely to be only truly of benefit in the research setting where more frequent sampling is possible, or for patients who may have altered cortisol metabolism, such as those on medications that induce the cytochrome P450 pathway(37).

Urinary free cortisol (UFC) measurement has also been proposed as a method to assess patients for over or under-replacement of cortisol(31, 38). This requires diligent collection of urine for a 24 hour period with reference to an established normative range and has been shown to be associated with considerable inter-individual variability(10). Assessment of UFC reflects only the total 24 hour dose and does not account for over exposure at certain time points or under exposure at other time points nor does it quantify overall tissue exposure to cortisol.

In view of the issues described above with it is clear that in the clinical setting physicians must evaluate the success, or otherwise, of the cortisol replacement regimen based on a somewhat subjective clinical assessment. The effects of under-replacement include fatigue and electrolyte abnormalities; however fatigue may be a result of other pituitary hormone deficiencies, other co-morbidities or psychological issues and hence is not a reliable marker for under replacement. Electrolyte abnormalities may also be a manifestation of inappropriate ADH replacement, anti-hypertensive therapies, or

anticonvulsant therapies; when truly due to adrenal insufficiency such abnormalities are late manifestations of inadequate replacement. Subjects with secondary adrenal insufficiency due to hypothalamic-pituitary disease usually have intact adrenal glands and mineralocorticoid axis. Therefore, even in the setting of inadequate cortisol replacement, they do not usually complain of postural hypotension, although extreme cases of cortisol deficiency may result in reduced cardiac output and hypotension(39). This would be a late clinical finding and is generally associated with non-compliance with glucocorticoid replacement or concomitant illness and adrenal crisis.

In the clinical setting physicians assess patients for evidence of over-replacement based on blood pressure measurements, fasting glucose levels, weight gain and occasionally the presence of osteoporosis based on the knowledge of adverse outcomes associated with cortisol over-exposure in Cushing's disease(40). Unfortunately in the setting of hypopituitarism and adrenal insufficiency these complications may only occur after years of chronic over exposure to cortisol and thus, while all of these complications should be considered on routine out-patient assessment, prevention of their development is vital.

### 1.1.3 Why is appropriate replacement important?

Patients with hypopituitarism, independent of subjects with Cushing's disease or Acromegaly, have higher morbidity and mortality compared to the general population with an all cause standardised mortality ratio (SMR) ranging between 0.83 to 3.36 for men, and in general a higher SMR for women ranging between 1.3 to 4.54 (41-47). A recent meta-analysis concluded that the overall SMR associated with hypopituitarism in

men is 2.06 (95% confidence interval (CI), 1.94-2.20) and a significantly higher SMR in women of 2.80 (95% CI, 2.59-3.02, p<0.0001)(47). Although the aetiology of the elevated SMR in hypopituitarism is likely multifactorial and may relate to underlying diagnosis, type of treatment received, recurrence of pituitary pathology or other malignancy, it may in part be related to inappropriate pituitary hormone replacement therapy. A recent study by Sherlock et al(27) of patients with acromegaly and ACTH deficiency, demonstrated an increase in relative risk (RR) of mortality in patients receiving daily HC doses between 25mg-30mg (RR, 1.6, 95% CI 1.1-2.4), and in those receiving doses >30mg daily an RR of 2.9 (95% CI 1.4-5.9, p=0.003). The increased risk of mortality seen was independent of age, sex, calendar period and radiotherapy treatment. Over 30% of deaths were due to cardiovascular causes, with an increase in cardiovascular deaths as hydrocortisone dose increased from 10% in those on ≤ 20mg up to 44.4% in those on >30mg daily. A study of 105 subjects with non-functioning pituitary adenomas also demonstrated an increased mortality rate, independent of underlying cardiometabolic risk factors with increasing doses of hydrocortisone, resulting in a hazard ratio (HR) of 2.03 for those on 20mg-29mg hydrocortisone daily to an HR of 4.0 in those on ≥30mg daily, p=0.039(48).


### 1.1.3.1 Cardiovascular morbidity and mortality

The primary causes of morbidity and mortality in hypopituitarism are cardiovascular or cerebrovascular in origin. Rosen and Bengtsson retrospectively analysed 333 patients who were treated for hypopituitarism between 1956 and 1987 and demonstrated 60 vascular deaths compared to 30.8 expected, resulting in an SMR of 1.95 in the group combined; 1.7 in men and 2.7 in women(41). Tomlinson et al demonstrated a significant

increase in cardiovascular deaths and cerebrovascular deaths with an SMR of 1.62 and 2.55 respectively(46). Those studies previously described do not specifically assess the role of individual hormone replacement, or indeed how that replacement is undertaken and although the observed increased morbidity and mortality, is likely multifactorial, recent studies in subjects with acromegaly and non-functioning pituitary adenomas have linked high dose glucocorticoid replacement as an independent risk factor for increased mortality(48, 49). The reason for this increased vascular morbidity and mortality is unclear.

## A) What is the role of blood pressure?

There is conflicting data regarding hypertension in hypopituitarism. Rosen et al retrospectively evaluated 104 hypopituitary subjects compared to a control group from a population based study and noted that while absolute levels of systolic or diastolic blood pressure (BP) were not different between patients and controls, the prevalence of treated hypertension was higher in hypopituitary patients and the difference between groups remained significant after controlling for BMI(50). Subsequent studies have not found a difference in blood pressure between patients and healthy controls(51, 52). Amato et al evaluated 7 hypopituitary patients before and after growth hormone replacement therapy compared to controls and there was no difference in systolic or diastolic BP between patients and controls at any time point. The authors provide no comment on whether or not these subjects were taking anti-hypertensive medication or if this was an exclusion criterion(51). Markussis et al examined 34 hypopituitary patients matched for age, sex, BMI and smoking status and found that neither systolic nor diastolic BP differed between the patients and the controls. In this case "pre-existing cardiovascular disease" was an exclusion criterion. 25 of the patients studied were on

glucocorticoid replacement but GC dose was not reported in the publication(52) and therefore it is difficult to assess whether glucocorticoid replacement was adequate or inappropriate. Using 24 hour ambulatory BP measurements Dunne et al demonstrated a lower mean BP in 13 GH deficient hypopituitary subjects, not treated with GH, compared to age and BMI matched controls; however this difference in BP was only statistically significant for the male patients and there was no difference in BP between patients on the different dose regimens of 30mg daily versus 15 mg daily of hydrocortisone(53). Such conflicting data might suggest that cardiovascular and cerebrovascular mortality is possibly unrelated to hypertension in this population group, and no sound inference can be made regarding the impact of glucocorticoid replacement on blood pressure parameters. However, apart from the study by Dunne et al(53), these studies used clinic BP recordings and patients had varying degrees of pituitary insufficiency.

## B) Does vascular dysfunction contribute?

Carotid intima media thickness (IMT), as measured by ultrasound, has been shown to predict cardiovascular and cerebrovascular accidents in adults over 65 years of age(54) and data regarding IMT in hypopituitarism is again conflicting, with little emphasis on the role of inappropriate replacement. Markussis et al in the study described in section a) above also examined carotid IMT in male hypopituitary subjects and found a significant increase in carotid IMT in patients compared to matched controls. The difference was not significant in subjects under 40 years of age and was greates in those aged over 60 years(52). Pfeifer et al demonstrated that carotid IMT was 20-30% higher in 11 hypopituitary growth hormone deficient (GHD) subjects compared to 12 matched controls and that there was a reduction in IMT, although it was not statistically significant following GH replacement(55). Endothelium-dependent dilation of blood vessels was

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reduced at baseline in the hypopituitary males; however this was not statistically significant(55). These findings were not replicated in other studies. Leonsson et al found carotid IMT to be higher in 34 hypopituitary GHD patients, not replaced with GH, 20 of who had ACTH deficiency compared to non-obese aged matched controls, but this increase in IMT did not persist on comparison with BMI-matched controls(56). Baseline BP and smoking status were not different between patients and either control group, but heart rate was higher in the patient population. Elhadd et al failed to demonstrate any difference in carotid IMT in 52 hypopituitary GHD patients compared to 54 control subjects(57). They found that biochemical markers of endothelial cell activation were higher and endothelium-derived dilation was lower in the patient group, (non-significant in males, significant in females) compared to controls that were matched for age and smoking status but not BMI(57). Vascular disease is associated with increasing stiffness of the vascular tree with failure to dilate appropriately in response to the pressure wave(58) This process is dependent on nitric oxide (NO) generation(59). Reduced endothelium derived dilation is a reflection of abnormal vascular function in and has been demonstrated in hypopituitary patients(55, 57), along with a reduction in large vessel reactivity and impaired NO generation(59).

## C) Hypopituitary subjects have adverse cardiometabolic profiles

A number of studies have demonstrated adverse fasting lipid profiles in hypopituitary patients with low levels of high-density lipoprotein (HDL) cholesterol, elevated triglycerides (TG) and reduced low density-lipoprotein (LDL) particle size(29, 56, 60, 61), with a significant proportion having an increased BMI; 32% of 2,589 hypopituitary patients were noted by Abs et al to be obese (BMI >30kg/m2)(60). However, few of these studies have assessed the role of inappropriate glucocorticoid replacement on

adverse cardiometabolic profile. Filipsson et al demonstrated an adverse metabolic profile in hypopituitary GHD subjects being treated for ACTH deficiency, compared to ACTH sufficient patients, which included an elevated haemoglobin A1c and waist circumference. In that study ACTH deficient subjects on hydrocortisone equivalent doses less than 20mg daily did not differ in these metabolic outcomes compared to their ACTH sufficient counter-parts. The adverse metabolic profile was associated with doses $\geq$20mg and was more pronounced with doses $\geq$25mg daily(29). Therefore, an adverse metabolic profile may contribute to the increased vascular mortality in this group and is possibly related to glucocorticoid replacement.

In general the studies discussed above demonstrate potential mechanisms for increased cardiovascular and cerebrovascular morbidity and mortality seen in hypopituitary subjects, although the exact pathophysiology is unclear inappropriate glucocorticoid replacement in now known to be an independent risk factor for increased cardiovascular mortality and it may play a role in the induction of these abnormalities.

### 1.1.3.2 Impaired quality of life (QoL)

Subjects with pituitary adenomas have been shown to have impaired QoL compared to the general population in a number of studies(47, 62-65). This is speculated to be due to several factors, including radiotherapy(64), transcranial surgery(47) and hypopituitarism(64, 65). Van der Kalaauw et al compared 403 pituitary patients with treated acromegaly (n=118), treated Cushing's disease (n=58), prolactinomas (n=128) and non-functioning pituitary adenoma (NFPA) (n=99) to 82 subjects with paraganglionoma and to 440 healthy controls from similar socio-economic backgrounds.

ACTH deficiency was present in 33% of the pituitary patients overall, however this varied from 12% in prolactinomas to 63% in the NFPA group. Overall quality of life and all QoL subscales were reduced in patients compared to controls and the presence of hypopituitarism negatively influenced the total QoL score. When QoL measures in pituitary patients were compared to the paraganglionoma subgroup, but with no pituitary disease, there was no difference in overall QoL score. However, patients with pituitary adenomas experienced greater impairment in the following subscales of quality of life: role functioning due to emotional and physical problems on the Short Form 36 (SF-36), more pain and impairment in physical ability in the Nottingham Health Profile (NHP); the authors suggest that hypopituitarism and imperfect endocrine replacement may be a contributing factor to these altered QoL findings(64).

Nielsen et al examined 109 patients with a history of NFPA, 27% of whom were panhypopituitary and 46% with less severe hypopituitarism. They were unable to demonstrate any decrease in QoL compared to healthy controls, except in those who had undergone craniotomy(47). In contrast Dekkers et al found multiple pituitary hormone deficiency to be an independent predictor of impaired QoL, especially with respect to social and physical functioning and increased general fatigue in a group of 99 subjects with a history of NFPA. Interestingly radiotherapy and GHD were not independent predictors for impaired QoL(65). The difference in findings between these studies may be due to the higher rate of hypopituitarism in Dekkers' study where 93% had some degree of hypopituitarism and 48% were panhypopituitary compared to 73% and 27% in Nielsen's group respectively.

It is clear that health related quality of life is reduced in primary adrenal insufficiency(66, 67). However, apart from the above-mentioned studies assessing QoL in pituitary disease in general, there is a paucity of data regarding the effect of secondary adrenal insufficiency and GC replacement on QoL. Hahner et al studied 132 subjects with primary adrenal insufficiency, compared to 78 subjects with secondary adrenal insufficiency(67). Hydrocortisone equivalent replacement doses ranged from 10mg daily to 60mg daily in primary AI or 5mg to 50mg daily in secondary AI. Quality of life was reduced for all patients with adrenal insufficiency irrespective of age, sex, concomitant disease or type of adrenal insufficiency. However those with secondary AI had a more severe impairment compared to primary AI, in the domains of bodily pain ($p=0.011$) and physical functioning ($p=0.015$) on the SF 36. Notably, age and glucocorticoid dose were inversely correlated with subjective health status for the group as a whole. This was an observational study and no further analysis of glucocorticoid dose effect was provided for the secondary AI group.

There are few prospective, controlled studies examining quality of life in hypopituitary subjects with respect to glucocorticoid replacement doses. It has been suggested that a thrice daily HC regimen is a more desirable dose schedule, compared to twice daily HC with respect to patient well-being. This is based on a study by Groves et al that examined patient well-being through a visual analogue scale ranging from "washed out" at the lower end of the scale to "on top of the world" at the higher end of the scale. 7 patients were assessed on thrice daily HC and twice daily HC at least one month apart, however doses were not standardised and no placebo was administered to the twice daily group, therefore the subjects were fully aware of which treatment they were

receiving. Subjects were asked to complete the visual analogue scale at 4 time-points per day for 3 days prior to attending the hospital for biochemical assessment on each regimen. It was noted that well-being was lowest in the morning before first dose of HC for both regimens and although not statistically significant, well-being appeared higher in the thrice daily group compared to the twice daily group at 1600hours. This was the time-point at which the thrice daily group were receiving a dose of HC that the twice daily group were not(30). The weaknesses of that study included the lack of a placebo for the third time point to blind the subjects to whether they were receiving twice or thrice daily HC, they did not use a validated tool for assessing quality of life and lastly the twice daily regimen involved HC doses that were given 12 hours apart which is not an appropriate comparison in view of the short half-life of hydrocortisone, which is not prescribed at 12 hourly intervals in clinical practice.

In a prospective randomised double blind study of 5 men and 4 women with hypopituitarism Wichers et al examined the effect of altering glucocorticoid doses in a random manner from 15mg to 20mg to 30mg daily on quality of life. Although no difference in quality of life between dose regimens was identified, it is worth noting that patients were on each dose schedule for only 2 weeks and it may not be reliable to assess quality of life over such a period of time(68). In an open prospective study 11 panhypopituitary subjects, not replaced with growth hormone, were asked to reduce their dose by a mean of 55% (range 25% to 75%) from approximately 26.36mg/day to 13.1mg/day. They were followed for a mean of 10 months and quality of life, as quantified by the QoL-Adult Growth Hormone Deficiency Assessment (AGHDA) questionnaire, was significantly higher following the dose reduction. 4 subjects had no

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change at all in QoL-AGHDA score and the improvement in QoL appears to have been driven by 2 patients who had dramatic reductions in their score(69). It was not clarified if they were the subjects who had their GC dose reduced by 25% or 75% or how the individual dose reduction was chosen.

The best available study regarding quality of life in secondary adrenal insufficiency is a prospective, randomised, placebo-controlled, double-blind, crossover protocol comparing 3 different glucocorticoid dose schedules in 18 patients with varying degrees of hypopituitarism, between doses and compared to healthy controls(70). In this study subjects were underwent 4 weeks on each dose schedule, dose A hydrocortisone 10mg-placebo-5mg-placebo, dose B hydrocortisone 10mg-5mg-placebo-5mg and lastly dose C, prednisolone 5mg – placebo-placebo-placebo with each tablet to be taken at 0770hours-1200hours-1500hours and 1800hours. This group demonstrated better quality of life in several domains of the Short Form 36 quality of life questionnaire compared to the other dose regimens, although quality of life was in general lower in the patient group compared to controls. The is a well-designed study, however the severity of ACTH deficiency was not clear and 83% of patients were growth hormone deficient, however only 50% of those subjects were replaced with GH.  Lastly 66% of subjects had previously been on dose A and although it was a randomised crossover protocol it is possible the patients were habituated to that dose.

### 1.1.3.3 Abnormal glucose metabolism

Diabetes mellitus is associated with increased vascular mortality(71-74) and impaired glucose tolerance is also a well-established risk factor for cardiovascular disease(75,

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76). Subjects with asymptomatic hyperglycaemia have been shown to have excess cardiovascular mortality with a relative risk of 1.8(77) while insulin resistance is also associated with increased mortality(78).

Glucocorticoids have a vital role in regulation of carbohydrate, fat and protein metabolism(79) and cortisol has long been described as a counter-regulatory hormone, the purpose of which is to respond to stress, illness or hypoglycaemia(80). Deficiency of cortisol is associated with insulin sensitivity(81, 82) and exposure to excess GC is associated with insulin resistance(83-86). Indeed, abnormal glucose metabolism is characteristic of Cushing's syndrome and may occur in over 80% of cases(87). The pathophysiology leading to increased insulin resistance is complex and is not completely understood. A number of studies evaluating the effect of cortisol infusions on healthy subjects have demonstrated increased hepatic and peripheral insulin resistance, leading to reduced glucose utilisation and increased gluconeogenesis; the mechanism appears to involve reduced glucose utilisation by peripheral tissues and increased hepatic gluconeogenesis leading to elevated glucose levels(85, 88-90). It is worth noting that the doses of hydrocortisone used in these studies were supraphysiological. Both Rizza et al(85) and Rooney et al(90) infused 2mcg/kg/minute which in each study resulted in serum cortisol concentrations over 1000mmol/L, compared to cortisol concentrations less than 350nmol/L in the healthy subjects on a placebo saline infusion. Dinneen et al(89) aimed for a more physiological infusion that increased from 0.3mcg/kg/min up to a maximum of 1.4mcg/kg, in order to mimic the early morning rise in cortisol and demonstrated a peak cortisol concentration of approximately 550nmol/L while confirming similar findings regarding insulin resistance to the former 2 studies. In healthy volunteers

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the oral consumption of 100mg hydrocortisone compared to placebo resulted in peak cortisol levels of 1013nmol/L and 298nmol/L respectively which resulted in reduced insulin sensitivity that manifested 4-6 hours after ingestion of the HC and appears to persist for over 16 hours following ingestion. As with the previous studies discussed, these findings reflect the effects of supraphysiological doses of glucocorticoid which should not be used for standard pituitary hormone replacement. The evidence regarding the effect of lower doses of HC replacement on insulin sensitivity and impaired glucose tolerance is less clear cut. A randomised, prospective, crossover study of 20mg oral hydrocortisone replacement (15mg at 0800hours and 5mg at 1700hours) compared to a physiological hydrocortisone infusion found no difference in fasting glucose and insulin levels between regimens and there was no evidence of an increase in hepatic or peripheral insulin resistance(91).

Hypopituitary patients are reported to have an increased prevalence of IGT and DM compared to age and sex matched controls(92, 93) although these studies have been undertaken in subjects with varying degrees of hormone deficiency and replacement. In a study of 45 ACTH deficient hypopituitary adults, 33 of whom were GHD without GH replacement, with a mean BMI of 29 $\pm$ 5kg/m2, McConnell et al performed a 75g oral glucose tolerance test on all subjects taking 20mg hydrocortisone daily, divided as 15mg in the morning and 5mg in the late afternoon(92). Using the WHO criteria for diagnosis of Diabetes, 2% had previously undiagnosed DM and 18% had IGT. There was no significant difference in HbA1c or fasting insulin; however the area under the insulin curve (AUC) during the OGTT was increased in those diagnosed with IGT, without a significant difference in AUC for serum cortisol concentration between those with normal

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glucose tolerance and those with IGT. Since a pre-existing diagnosis of DM was an exclusion criterion this study likely underestimates the true prevalence of DM in the hypopituitary population.

In contrast, a study by Krzyzanowska and colleagues of GH deficient adults compared to healthy matched controls found that 13% of 61 hypopituitary subjects had type 2 DM, while 16% had IGT(93). In that study 24 subjects had only 1 anterior pituitary hormone deficiency (APHD), 11 had 2 APHD, 15 had 3 APHD and 11 had 4; replacement status was not defined although the authors stated there was no effect of hormone replacement on glycaemic status. More recently Dullaart et al examined 165 adult hypopituitary GHD subjects on growth hormone therapy for 1 year, 48 of whom were ACTH sufficient and 117 were ACTH deficient on a mean HC equivalent dose of 20mg daily(26). 9.4% of ACTH deficient patients had hyperglycaemia or type 2 DM, while none of the ACTH sufficient subjects had abnormal glucose (p=0.03). It is interesting to note that 34.2% of patients in that study were on HC equivalent doses over 30mg daily(26).


A small number of other studies comparing glucocorticoid replacement regimens in hypopituitary subjects have failed to demonstrate a difference in fasting glucose or insulin levels between doses or compared to ACTH sufficient hypopituitary subjects(53, 69, 94). In those studies GHD subjects were not on GH replacement, and as GH deficiency is associated with insulin sensitivity, this may have affected those results. In a cohort of 9 subjects, 8 with primary adrenal insufficiency, those who were replaced with 0.1mg/15kg body weight dexamethasone had a non-significant trend towards higher insulin values than subjects on HC 20mg in daily divided doses(95).

It is very likely that cortisol dynamics, with respect to endogenous circadian rhythm effects, type and timing of glucocorticoid replacement, play an important role in the response of insulin and glucose to glucocorticoids. In healthy subjects it has been shown that glucose tolerance is optimal in the early morning, impaired in the afternoon and worsens as the evening progresses (96-98)- suggesting an inverse relationship between the normal circadian rhythm of cortisol and glucose tolerance. In a placebo controlled study Plat et al examined the effect of 50mg hydrocortisone given to healthy volunteers in order to compare the effect of timing of the dose on measures of glucose and insulin metabolism(99). Endogenous cortisol production was suppressed with metyrapone and 2 separate studies were performed, 50mg HC given at 0500hours compared to 50mg HC administered at 1700hours. Although both morning and evening HC administration were associated with hyperglycaemia, the onset of which was 4-6 hours after the dose was administered, the elevation following the morning dose was minimal, whereas there was a significantly more pronounced elevation in glucose following the evening dose. Glucose levels were 20% higher than placebo following evening administration of HC and insulin clearance was approximately 30% lower than insulin clearance following the morning dose. It was also noted in this study that hydrocortisone clearance was slower in the evening by approximately 50% (99). This difference in hydrocortisone clearance in the evening hours has previously been demonstrated(100).

Al-Shoumer studied 8 hypopituitary adults who underwent oral glucose tolerance testing on two separate occasions, 1 week apart to assess the effect of administering their usual HC dose (mean 26mg, range 15mg to 30mg daily) 1 hour before OGTT or after

completion of the OGTT. Although the fasting insulin and glucose levels were not different between the two studies, AUC was higher for both glucose and insulin in the group that took their HC 1 hour before the OGTT. There was a significant positive correlation between the glucose AUC and maximum cortisol concentrations. Interestingly 3 subjects were classified as IGT if HC was taken 1 hour before OGTT, whereas only 1 remained IGT when HC was taken after OGTT was completed(101). This was a small study in which neither subjects nor investigators were blinded to the timing of HC administration, however it serves to emphasise that the timing of GC administration may affect the outcome of an OGTT.

These studies demonstrate that optimising glucocorticoid replacement with respect to overall dose and also timing of administration is vital in order to minimise metabolic complications.

### 1.1.3.4 Abnormal bone metabolism

Approximately 10% of human bone mass is renewed each year by the activity of two opposing, yet coupled processes at cellular level in the bone remodelling unit; resorption by osteoclasts and formation by osteoblasts(102). Disease states or pharmacologic interventions can lead to an alteration in bone turnover with increased and/or dissociated bone turnover(102, 103) that may adversely affect the bone milieu. Glucocorticoid excess has long been recognised as a cause of osteoporosis(87), and recent data have shown in healthy individuals that endogenous cortisol/cortisone levels are correlated with bone mineral density and influence the rate of bone loss(104, 105). Glucocorticoids

(GC) induce a negative calcium balance, evidenced by reduced intestinal calcium absorption and increased renal calcium excretion with reduced bone formation through suppression of osteoblast activity(106). There are conflicting data regarding GC effects on bone resorption, but it has been shown that GC administration maintains osteoclasts in the active phase of the resorption cycle(107) which, in combination with the changes described above, leads to increased bone fragility, reduced bone quality and increased fracture risk that can predate decreases in bone mineral density (BMD)(106, 108). These adverse effects have been clearly demonstrated in studies using grossly supraphysiological doses of glucocorticoid for anti-inflammatory therapeutic effect(109-111), however an increased relative risk of 1.55 (95% CI 1.2-2.01) for vertebral fractures has also been seen with long term use of lower doses of prednisolone <2.5mg/day(112).

Although glucocorticoid replacement therapy for primary and secondary adrenal insufficiency aims to use more physiological dose regimens than those used for anti-inflammatory effect, there is conflicting evidence that glucocorticoid replacement doses are also associated with deleterious effects on bone. The majority of available studies are in subjects with primary adrenal failure, with some studies finding a reduced BMD in men with increasing hydrocortisone (HC) equivalent doses(113, 114), and lower femoral neck BMD that correlated with weight adjusted dose(115), while others found no difference in BMD compared to the control population(20, 28), except in subjects on prednisolone who had a significant decrease in BMD(116).

A small number of studies done in subjects with hypopituitarism have demonstrated an increased prevalence of fractures compared to the general population(117-119). It is difficult to delineate the exact cause of this increase, as pituitary hormone replacement itself may contribute, as may concomitant medications such as anti-epileptics, which are known to affect bone metabolism(120). There are few data on the effect of glucocorticoid replacement on bone metabolism in patients with ACTH deficiency. Peacey et al demonstrated in a cohort of 32 patients, 20 of whom had secondary adrenal insufficiency, that a reduction in GC dose by 30% (to 20mg daily) was associated with a 19% increase in osteocalcin, a marker of bone formation. They also found a weak but significant negative correlation between absolute BMD and dose of hydrocortisone (HC) replacement(121). This finding has been replicated by Chikada et al in an observational study of primary (n=10) and secondary (n=5) hypoadrenal patients who demonstrated a negative correlation between HC dose and BMD and also between duration of therapy, cumulative HC dose and BMD(122). These correlations were lost when only those subjects taking less than 13.6mg/m2/day were analysed. In this study, one half of patients were treated with hydrocortisone and dexamethasone and 1 patient with prednisolone, 6 patients were post-menopausal (5 of whom were not on oestrogen therapy) and no other details were provided regarding other pituitary hormone status in the 5 subjects with secondary AI. Wichers, in a cross-over double blind study in 9 hypopituitary patients with severe ACTH deficiency, found an increase in the bone formation marker osteocalcin with a reduction in glucocorticoid dose, significant for reduction from 30mg to 20mg (p<0.05) and from 30mg to 15mg (p<0.01) after only two weeks on each treatment schedule(68). They were unable to demonstrate any alterations in other bone turnover markers including markers of bone resorption. However there was no control group and no details regarding other pituitary hormone

replacement were provided, except that patients were GH deficient but not receiving GH replacement. Suliman et al examined the effect of 3 glucocorticoid replacement schedules (S) on bone turnover markers in 9 subjects (1 ACTH deficient). S1 was hydrocortisone 10mg mane, 5mg tarde, S2 was 10mg mane, 5mg tarde and 5mg at 1600hours and S3 was dexamethasone at 0.1mg/15kg body weight daily with comparison of findings to unmatched, younger, healthy controls(95). They demonstrated a lower ionised calcium and a higher 25 (OH) D across all replacement schedules compared to controls with no difference in PTH between doses or compared to controls. Except for a reduced resorption marker, urinary free deoxypyridinoline (FDPD), in those receiving dexamethasone compared to hydrocortisone, there were no other differences in markers of bone formation or resorption between schedules. Pro Collagen Type 1 Peptide (PINP) another marker of bone formation was shown to be reduced 3 hours after administration of glucocorticoid; however this was also seen on a control day in which no GC was given and therefore likely reflected the diurnal pattern of PINP rather than GC effect. Cortisol dynamics are likely to be as important as the overall prescribed dose since studies on bone turnover and mineral density have demonstrated more pronounced adverse effects in those on synthetic glucocorticoids (prednisolone and dexamethasone) compared to those on hydrocortisone or cortisone acetate(95, 115, 116).

### 1.1.3.5 Complications of under-replacement in secondary adrenal insufficiency

The solution to concerns regarding the adverse effects of higher doses of steroids is not simply to reduce the dose of glucocorticoid replacement, as such a reduction may not be safe or necessary for patients. The risk of adrenal crisis must be borne in mind. In a

retrospective case note analysis examining mortality in GHD subjects, on GH replacement from 1963 - 1985, 23% of whom were also ACTH deficient, concomitant adrenal insufficiency had a relative risk of mortality of 7.1; of 35 subjects found dead, 30 were "probably" secondary to adrenal crisis(11). The authors of that study acknowledge that due to the retrospective observational nature, the exact cause of death would have been difficult to ascertain. Omori et al found in a retrospective case note analysis that 30% of 115 secondary adrenal insufficient subjects had adrenal crises and that the relative risk of an adrenal crisis was increased in the presence of untreated hypogonadism (RR 3.70,CI 1.75 – 7.98), however there was no detail provided in this study as to the cause of adrenal crisis and the true contribution of under-replacement with glucocorticoid cannot be ascertained(123). In a retrospective questionnaire-based study, Hahner and colleagues evaluated the epidemiology of adrenal crises in both primary (n = 254) and secondary adrenal insufficiency (n=190). 35% of subjects with secondary AI experienced at least 1 adrenal crisis equivalent to 5.8 crises/100 patient years, compared to 6.6crises/100 patient years in primary AI(124). The study relied on subjects' recollection of events and reporting of each crisis retrospectively and in the secondary AI group 12.7% of crises were "unexplained" and therefore may potentially have been related to under-replacement with glucocorticoid; however it is noteworthy that the odds ratio of adrenal crisis was not related to GC dose/body surface area. It is somewhat reassuring that other studies have demonstrated a reduction in glucocorticoid dose from 30mg daily to 15mg daily without adverse event(53, 69). Druce et al assessed the need for increased doses of glucocorticoid secondary to illness in a cohort of primary and secondary adrenal insufficient patients(15). Over the preceding year approximately 50% of subjects with pituitary disease needed to increase their GC dose during illness; only 30% needed to receive an intramuscular injection and only 4% were hospitalised for

adrenal crisis. This is similar to data from Arlt et al in which subjects with primary adrenal insufficiency had 3.8 hospital admissions per 100 replacement years compared to 2.5 per 100 replacement years in subjects with secondary AI(10).

Previous work by my colleagues has shown that patients with partial ACTH deficiency (basal cortisol >200nmol/L, peak cortisol on insulin tolerance test <500nmol/L) have HC day curves similar to healthy controls and are generally over treated by conventional replacement therapy(125). This is likely to also be the case in subjects with severe ACTH deficiency (basal cortisol <100nmol/L and peak cortisol <400nmol/L).

**Rationale for further investigation:**

In view of the findings that subjects with partial ACTH deficiency are generally over replaced with conventional glucocorticoid replacement doses (ref) I was keen to assess if this was also the case for subjects with severe ACTH deficiency while assessing the impact of glucocorticoid replacement on metabolic and psychologic parameters. The studies I have described in this introductory chapter that have assessed metabolic or quality of life outcome have primarily been retrospective or observational, or in a mixed cohort of primary and secondary adrenal insufficiency without reference to severity of ACTH deficiency. In the prospective studies I have described subjects with ACTH deficiency again had varying degrees of hypopituitarism and the majority, if GHD, were not replaced with GHD. For the majority of studies, at most one or two metabolic outcomes were assessed, for example Peacey et al evaluated serum cortisol and bone turnover, Dunne et al evaluated glucose metabolism and blood pressure and Suliman et

al examined bone and glucose metabolism. Of the studies I have reviewed the study by Danilowicz et al is the nearest to achieving the majority of these parameters. They described 11 panhypopituitary subjects on glucocorticoid replacement and evaluated the effect of dose reduction on weight, fat mass, markers of insulin resistance, bone mineral density and quality of life over a 6-12 month period. The issue with that study relate to the arbritary reduction in dose that ranges from 25% to 75% of the original dose, details regarding severity of ACTH deficiency are not provided and the subjects are GHD without replacement. Pituitary patients are still exposed to a wide range of glucocorticoid replacement regimens in clinical practice and in the absence of a reliable biochemical or clinical marker of replacement it is vital to further understand the metabolic outcomes of inappropriate replacement and to use this knowledge to aim for the most physiologic glucocorticoid replacement available.

## 1.2 Aim of study

The aims of this study are as follows:

1. To examine the effects of three commonly prescribed regimens of hydrocortisone replacement in male hypopituitary patients with severe ACTH deficiency, fully replaced on all other pituitary hormones including GH, on the following end points as markers of physical and psychological effects of long term replacement therapy:
   a. 24 hour serum cortisol day curves
   b. Quality of life
   c. 24 hour ambulatory blood pressure

d. Glucose homeostasis

e. Bone turnover/metabolism

f. 24 hour tissue cortisol exposure through urinary cortisol metabolite measurement

2. To compare results between dose regimens and to healthy matched control subjects with a view to identifying the most physiologic replacement regimen, and the least association with adverse outcome in order to simplify clinical decision making regarding cortisol replacement in the setting of severe ACTH deficiency and hypopituitarism.

# Chapter Two: Study design and methods

## 2.1 Methodology

This chapter contains a description of the methods used for each study performed and will compliment detail provided in subsequent chapters.

## 2.2 Identification and Recruitment of study subjects

Male patients who had a history of treatment for organic pituitary disease leading to hypopituitarism and ACTH deficiency were identified from the Beaumont Hospital pituitary register and a chart review was performed to assess initial eligibility criteria for inclusion in the study. When eligible patients attended Beaumont Hospital pituitary out-patient clinic for routine scheduled follow up appointments, they were informed of the study aims and procedures, if they were willing to be assessed for eligibility they were then assessed for inclusion and exclusion criteria described below. If the patient was deemed an appropriate candidate they were given a detailed patient information leaflet and asked to consider participating in the study. Unless the subjects had denied permission to contact them again about the study, we followed them up with a phone call no less than 1 week later to answer any questions and to discuss whether they were willing to participate.

## 2.2.1 Inclusion criteria

- Adult male patients
- Organic pituitary disease leading to hypopituitarism previously confirmed on dynamic pituitary testing
- Appropriate, stable treatment of all pituitary hormone deficiencies
- Severe adrenocorticotropin hormone (ACTH) deficiency, as defined below

## 2.2.2 Exclusion criteria

- Age less than 18 years
- Acute medical/surgical illness, advanced cardiac/pulmonary disease, terminal illness
- Conditions associated with altered bone turnover such as Paget's disease, osteoporosis or fracture within the previous 1 year
- Patients on glucocorticoids for purposes other than ACTH deficiency
- Those on agents that interfere with corticosteroid metabolism and/or bone metabolism such as anti-epileptic medications
- Patients with known Type 1 or 2 diabetes mellitus
- Patients with uncontrolled hypertension
- Female patients were excluded because of the variable effects of oestrogen status/menstrual cycle on corticosteroid binding globulin (CBG) levels, thus affecting total cortisol concentrations and kinetics(126) and on bone turnover marker measurement(102)

## 2.2.3 Definition of hormone abnormalities

All subjects previously had dynamic pituitary hormone testing at the time of their original diagnosis and treatment for organic pituitary disease. In the event that patients had multiple treatments and repeat dynamic testing, the most recent results were used to define hormone abnormalities.

### 2.2.3.1 Anterior Pituitary Function:

ACTH and growth hormone (GH) secretion were assessed by measurement of cortisol and growth hormone in response to stimulation by hypoglycaemia with the insulin tolerance test (ITT)(127). In those in whom the ITT was contraindicated the glucagon stimulation test (GST) was used(5). Basal samples were taken for measurement of free T4, TSH, gonadotropins, testosterone and Insulin-like growth factor-I (IGF-I).

Severe ACTH deficiency was defined as a fasting morning total serum cortisol <100nmol/l and a stimulated peak serum cortisol <400nmol/l in response to ITT or <450nmol/l in response to GST based on local normative data(5). Severe adult GH deficiency was diagnosed if the peak GH was <3ng/ml in response to ITT or GST also based on previously established local normative data(5). Gonadotropin deficiency was defined by a low sex steroid concentration with inappropriately normal or low FSH and LH. TSH deficiency was defined by a low serum free T4 with inappropriately normal or low TSH.

### 2.2.3.2 Posterior Pituitary Dysfunction

The diagnosis of cranial diabetes insipidus (CDI) was made by the water deprivation test (WDT). CDI was defined by a failure to achieve a peak urine osmolality >700mOsm/kg or a peak urine to plasma osmolality ratio >2 based on local normative data. In some cases formal WDT was not carried out and CDI was diagnosed in the appropriate clinical setting (post neurosurgery) in conjunction with hypernatraemia (Na >145mmol/l), polyuria >3.5litres/24hours and an early morning fasting urine osmolality <300mOsm/kg.

## 2.3 Study design

This was a prospective clinical trial wherein all 10 patients were randomised in a randomised cross-over protocol to take 3 commonly prescribed doses of hydrocortisone replacement

- **dose A** (20mg at 0800hours, 10mg at 1600hours),
- **dose B** (10mg at 0800hours and at 1600hours)
- **dose C** (10mg at 0800hours and 5mg at 1600hours)

Each dose regimen was taken for 6 weeks and at the end of each treatment schedule patients were admitted at to our clinical research centre (CRC) for a 28 hour period, to undergo a series of metabolic and endocrine assessments.

All patients were on stable appropriate pituitary hormone replacement, including growth hormone replacement, without alteration in dose for at least 3 months prior to the study. Hormone replacement therapy regimens were not adjusted during the study period, except for hydrocortisone dose, as per study protocol.

10 healthy male controls, matched for age, body mass index (BMI) and waist circumference (WCM) were enrolled to undergo the same biochemical investigations and clinical examination as the patient group in order to provide information on normal physiology for comparison with each dose regimen.

## 2.4 Study Procedures

At the end of each 6 week treatment schedule patients were admitted to the CRC for a 28 hour period. Therefore each patient underwent admission and the following study procedures on 3 separate occasions, while the control patients underwent this same process once only.

Subjects presented in the non-fasting state to the CRC at 0730 hours on the day of admission and underwent a physical examination that included blood pressure, weight, height, and waist circumference measurement. Following this examination an 18g cannula (Optiva 2, Medex Medical Ltd, Hastingdon, England) was placed in the antecubital fossa under aseptic technique. Basal samples were taken for cortisol, cortisol binding globulin (CBG), free T4, TSH, testosterone, gonadotropins, prolactin and insulin like growth factor-I (IGF-I), parathyroid hormone (PTH), 25-hydroxyvitamin D (25 [OH] D), calcium, albumin and renal function. The cannula was then flushed with 10mls of a heparinised solution (heparin sodium, 100 units diluted in 100mls of 0.9% normal saline) in order to maintain patency for the full 28 hour period. The use of diluted heparin in this manner does not affect any of the laboratory sampling techniques used subsequently(127) and since the first 5 mls of blood withdrawn at each time point was

discarded there was little chance of contamination with such dilute levels of heparin. During this period the subjects underwent the following assessments:

## 2.4.1 Assessment of Cortisol Dynamics

Serum cortisol samples were taken hourly through the indwelling cannula from the time of admission until midnight and were taken two hourly from midnight until 0800 hours the following morning. The first 5mls of blood withdrawn from the cannula was discarded and a further 5mls was withdrawn for cortisol analysis. The cannula was flushed after each sample aspiration with 10mls of the heparinised solution described above. Patients took the designated hydrocortisone dose that they had been taking for the preceding 6 weeks at 0800hours just after admission, at 1600hours and at 0800hours the next morning. Control subjects underwent the same sampling intervals but did not take exogenous cortisol. Cortisol binding globulin (CBG) was measured upon admission. These samples were allowed to stand at room temperature for 30 minutes in order to facilitate clotting prior to being centrifuged at 3,000 rpm for 15 minutes, stored in 1ml aliquots and frozen at -20 and at -80degrees centigrade for cortisol and CBG respectively until analysis.

## 2.4.2 Assessment of Tissue Cortisol Exposure

During the admission period patients and control subjects were advised to discard the first early morning urine sample and collect all urine thereafter for 24 hours during this admission. Once the urine collection was completed, the total urine volume was recorded and two 5ml aliquots were preserved for storage at -80degrees until analysis for quantitative data on the urinary excretion of individual cortisol metabolites could be performed.

### 2.4.3 Assessment of bone turnover

During the 24hour admission to the CRC all subjects fasted from midnight and underwent venous sampling between 0700hours and 0800hours for collection of serum bone turnover markers bone-specific alkaline phosphatase (bone ALP), procollagen type I N-propeptide (PINP), intact osteocalcin (OC[1-49]), C-terminal cross-linking telopeptide (CTX-I) and tartrate resistant acid phosphatase 5b (TRACP5b). Samples were centrifuged immediately at 3,000 rpm for 15 minutes and stored in 1ml aliquots at -80 °C until analysis.

### 2.4.4 Assessment of glucose and insulin homeostasis

At 0800hours following an overnight 10 hour fast patients were asked to take the designated hydrocortisone dose with a small sip of water. The oral glucose tolerance test was then performed 1 hour later in order to standardise the testing conditions with respect to hydrocortisone administration and mimic daily living. 75g of anhydrous glucose was dissolved in 300mls of water to be consumed as an oral glucose challenge. Paired samples for insulin and glucose were taken at Time 0, before consumption of the glucose load and at 30, 60, 90 and 120 minutes (Time 30, 60, 90 and Time 120) post oral consumption of glucose. Insulin samples were centrifuged at 3,000 rpm for 15 minutes and stored in 1ml aliquots at -20 °C until analysis, while glucose samples were processed immediately. Healthy controls also underwent an oral glucose challenge without taking exogenous hydrocortisone.

### 2.4.5 Assessment of 24 hour ambulatory blood pressure

On admission between 0730hours and 0800hours patients were fitted with validated oscillometric devices to record 24 hour blood pressure, (SpaceLabs 90202 or 90207).

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The recorders were programmed to obtain blood pressure readings at 30-minute intervals for 24 hours throughout each 28 hour admission period. Failed recordings automatically triggered a repeat recording within a 2 minute interval. All of the recorded clinical data were transferred into the dabl Cardiovascular software package (dabl Ltd) in order to produce a report for each event.

## 2.4.6 Assessment of quality of life

During each admission subjects were administered the Short Form 36 (SF36) questionnaire(128) and the Nottingham Health Profile (NHP)(129) at 0900 hours on the first day of study admission in order to assess quality of life (QoL) on each dose regimen and in healthy controls. In order to improve the statistical robustness for analysis of quality of life an additional 20 healthy matched controls from similar socio-economic background to our patient population completed the QoL assessment (as well as the 10 healthy matched controls that underwent the full biochemical assessment).

### The Short Form 36 Questionnaire

The SF 36 aims to assess general well-being over the preceding 4 weeks by evaluating responses to statements through 8 domains of health; 1. physical functioning, 2. social functioning, 3.role physical – limitations in role activities due to physical health problems, 4. role emotional – limitations in role activities because of emotional problems, 5. mental health, 6. vitality, 7. pain and 8. general health . Scores are coded and transformed to a scale of 0-100 with higher scores indicating a better quality of life(128) (Appendix 1.)

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## *The Nottingham Health Profile (NHP) Questionnaire*

The NHP contains 38 yes/no questions over 6 health domains; 1. energy (three items), 2. pain (eight items), 3. emotional reaction (nine items), 4. sleep (five items), 5. social isolation (five items) and physical mobility/functioning (eight items). Each question is weighted and the sum of each domain is 0-100 with higher scores indicating worse quality of life in this case(129) (Appendix 2)

### 2.4.7 Study Procedures Proforma

The proforma used for each subject admission is in appendix 3.

## 2.5 Laboratory Techniques

This study involves extensive biochemical analysis of end points. In this section I will describe in detail the assays that were not routinely performed in our department at the time of this study, such as cortisol binding globulin, bone turnover markers and the assessment of urinary cortisol metabolites. All other assays are not novel and are routinely performed in the department of chemical pathology in Beaumont Hospital and will be described in less detail.

### 2.5.1 Estimation of serum cortisol binding globulin

Serum CBG was measured using a radioimmunoassay, BioSource Europe S.A, Nivelles, Belgium. This assay was not available in our Endocrine laboratory; therefore under the supervision and guidance of Ms Patricia Barrett from our Chemical Pathology

department I set up and validated the assay. Samples were removed from storage at – 80°C and allowed to thaw at room temperature and kept at 2-8°C until assayed. They were mixed by repeated inversion and centrifuged at 3000 rpm for 20 minutes prior to assay. Kit components were kept at 2-8°C and brought to room temperature before use. The assay was performed in duplicate.

**Steps:**

Disposable polystyrene tubes were labelled in duplicate as follows:

    a. Tubes 1 + 2 – Total Counts

    b. Tubes 3 + 4 – Zero Calibrator

    c. Tubes 5 – 16 – Calibrators/Standards 1-6

    d. Tubes 17 – 20 – high and low controls from manufacturer

    e. Tubes 21 – 60 – patient samples on hydrocortisone

    f. Tubes 61 – 100 – healthy matched control samples

Zero calibrator and standards of CBG were provided lyophilised in phosphate buffer with bovine serum albumin and azide (<0.1%) and reconstituted with 3ml and 1ml of distilled water respectively. Specific antibody for anti-human CBG in a phosphate buffer and capture antibody from anti-mouse antiserum linked to microcrystalline cellulose were provided by the manufacturer ready for use. The Tracer was [125]Iodine labelled CBG in phosphate buffer with bovine serum and was ready for use. The controls supplied by the manufacturer were reconstituted with 0.5mls of distilled water. Serum samples were diluted 1:25 in dilution buffer (100μl serum +2.4mls dilution buffer). All reagents were mixed on a roller-mixer for at least 30 minutes before use.

All calibrators, controls and diluted serum samples were briefly vortexed prior to being dispensed into relevant tubes. 100 µl of the zero calibrator was dispensed into tubes 3+4 for non-specific binding determination. 100 µl of calibrators 1-6 were dispensed into tubes 5-16, while 100 µl of commercial control 1 + 2 were dispensed into tubes 17-20 to assess intra-assay consistency. 100 µl diluted serum sample was dispensed into tubes 21-100. A reverse pipetting technique was employed to improve pipetting accuracy.

Following the addition 100 µl of $^{125}$Iodine labelled CBG into each tube, including tubes1+2 for total count, 100 µl of CBG antiserum was dispensed into each tube, except the total counts and the zero calibrators. The rack was then shaken gently to release any trapped air bubbles. Following incubation for 2 hours at room temperature, during which time the tubes were then all covered with plastic, 100 µl of the anti-mouse antiserum immunoadsorbent was dispensed into each tube, except the total counts. The contents were mixed by gentle shaking for 20 seconds and incubated for a further 20 minutes at room temperature. 3mls of working wash solution was then added to all tubes, except the total counts and centrifuged for 15minutes at 1500g. Tubes 1+2 (total counts) were removed from the rack and the liquid supernatant in the remaining tubes were drained by careful decantation. The tubes were rested for 10minutes upside down on blotting paper prior to all tubes, including the total counts being placed in the gamma counter for measurement of radioactivity.

Standard curve and result calculation was performed with a commercial computer data reduction program (Gamma Wizard), as follows:

Binding value B was calculated from the average counts of each pair of standards, B0 was the average counts of tubes 3+4. Percent bound = B/B0 x 100. The percent bound

versus the concentration of the standards was plotted on a semi-logarithmic scale. Percentage bound of the zero standard = B0/total counts x 100 (which should be> 20%).

The curve generated by Gamma counter shown in Figure 2.1. The concentrations on the calibration curve had to be multiplied by 25, which was the dilution factor. Calculation of co-efficient of variation (CV) was performed as follows: CV = (standard deviation / mean) x 100, where standard deviation = $\ddot{O}(1/n\text{-}1).S\ (x_i - mean)^2$. The interassay CV is 5.5% and 2.4% at serum CBG concentrations of 23.8 and 108 mg/l respectively while the intra-assay CV is 3.9% and 2.9% at 0.98 and 4.26mg/l respectively.
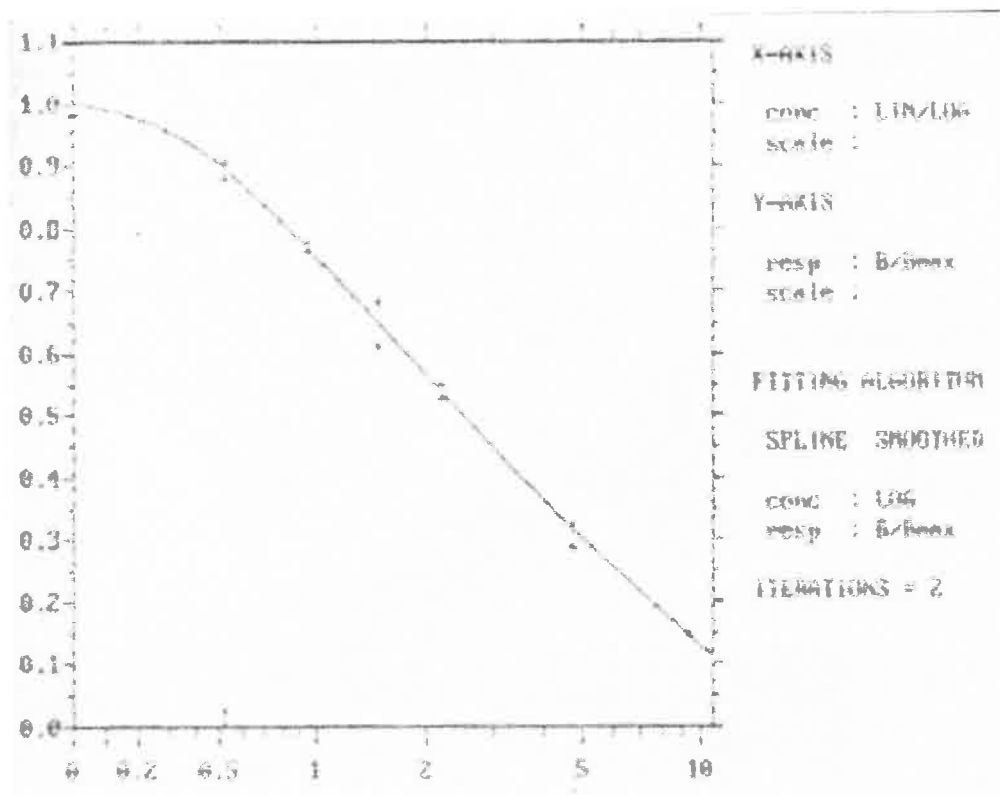


Figure 2.1 Standard curve for cortisol binding globulin assay
Radioimmunoassay generated by GammaWizard.,
X-axis shows the concentration of standards 1-6, Y-axis shows the
percentage bound for each standard.

## 2.5.2 Estimation of serum bone turnover markers

At the time of this study bone turnover markers were not routinely being performed in Beaumont Hospital, however the department of chemical pathology was in the process of establishing protocols for doing select bone markers within the department. Therefore, I assisted in performing the assays for procollagen type I N-propeptide (PINP), intact osteocalcin (OC[1-49]) and C-terminal cross-linking telopeptide (CTX-I) in Beaumont Hospital laboratory with the supervision and assistance of Ms Grainne Kelleher; while the assays for bone-specific alkaline phosphatase (bone ALP) and tartrate resistant acid phosphatase 5b (TRACP5b) were performed by laboratory staff in the Metabolism Laboratory, St Vincent's University Hospital under the supervision of Dr Jennifer Brady and in collaboration with Dr Malachi McKenna.

### 2.5.2.1 Bone turnover marker (BTM) assays performed in Beaumont Hospital

The assays for PINP, OC[1-49] and CTX-I were all performed using an automated electrochemiluminescence immunoassay on the Elecsys 2010 analyser (Roche Diagnostics, Mannheim, Germany) using a sandwich technique as follows:

- 1st incubation: 20 µL of sample and a biotinylated monoclonal P1NP or OC[1-49] or CTX-I-specific antibody are incubated together.
- 2nd incubation: After addition of streptavidin labelled microparticles and a monoclonal P1NP, or OC[1-49] or CTX-I-specific antibody labelled with a ruthenium complex, a sandwich complex is formed which becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound

substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- The results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

In our assays there was an intra-assay CV of 1.8%, 2.1%, 2.9% and interassay CVs of 2.3%, 2.4%, 3.7% at P1NP concentrations of 274ng/ml, 271ng/mL and 799ng/mL respectively and a lower limit of detection of 5ng/mL; an intra-assay CV of 4.0%, 3.3%, 1.4% and an interassay CV of 6.5%, 3.8% and 1.8% at OC[1-49] concentrations of 15.5ng/mL, 13.7ng/mL and 68.3ng/mL respectively and a lower limit of detection of 0.500ng/ml For CTX-I there was an intra-assay CV of 4.6%, 1.8% and 10.% and interassay CVs of 4.7%, 4.3% and 1.6% at concentrations of 0.08ng/mL, 0.39ng/ml and 3.59ng/mL respectively. The lower detection limit was 0.01ng/mL. In the normal population the reference range for these markers are dependent on age and sex.

### 2.5.2.2 BTM assays undertaken in St Vincent's Hospital Metabolism Laboratory

Bone ALP and TRACP5b were both measured by immunoenzymatic assay (Immunodiagnostic Systems Ltd, Boldon, UK) on an automated enzyme linked immunosorbent assay platform with an inter- and intra-assay coefficient of variation for bone ALP of 5.8% (at 8.4μg/L) and 6.5% (at 7μg/L) respectively and an inter- and intra-assay coefficient of variation for TRACP5b of 4.25% (at 3.20u/L) and 4.7% (at 5u/L) respectively.

## 2.5.3 Estimation of urinary corticosteroid metabolites

Gas Chromatography/Mass Spectrometry (GC/MS) urinary steroid analysis was carried out by Ms Beverley Hughes at the Institute for Biomedical Research, Centre for Endocrinology, Diabetes and Metabolism (CEDAM), University of Birmingham, in collaboration with Dr Mark Sherlock and Professor Paul Stewart. The GC/MS was based on the method described by Palermo et al(130). The methods of sample work-up for GC/MS analysis (conjugate hydrolysis, extraction and derivatization) and their methodologies have been published in detail in several manuscripts(131, 132) previously. GC/MS was used to analyse the metabolites of steroid hormones and their precursors.

In brief the major route of cortisol metabolism comprises the interconversion of cortisol (Kendall's compound F) to cortisone (Kendall's compound E) through the activity of 11 β-HSD isozymes or reduction of the C4-5 bond by either 5α-reductase or 5β-reductase to yield 5α-THF (allo THF) and 5β-THF respectively(133). THF, allo THF and tetrahydrocortisone (THE) are rapidly conjugated with glucuronic acid and excreted in the urine(133). Downstream, cleavage of the THF and THE to the C19 steroids 11 hydro or 11-oxo-androsterone or etiocholanolone occur. Alternatively reduction of the 20-oxo group by 20α or 20β hydroxysteroid dehydrogenases yield α and β cortols and cortolones, respectively, with the subsequent oxidation at the C21 position to form the extremely polar metabolites cortolic and cortolonic acids. Approximately 50% of secreted cortisol appears in the urine as THF, allo THF and THE, 25% appears as cortols/cortolones, 10% as C19 steroids, 10% cortolic/cortolonic acids and remaining are free unconjugated steroids. In the CEDAM, at the University of Birmingham,

approximately 40 steroids are targeted for selected-ion-monitoring analysis, which cover all disorders of steroid synthesis and metabolism. Within CEDAM normal ranges have been developed from a large number of healthy controls. A sample GC/MS profile of an individual patient is plotted for each determined parameter against the normal reference population (Figure 2.2 below) and a two sample profiles in hypopituitarism is plotted in a patient with GHD but an intact HPA axis (Figure 2.3a) and for a hypopituitary patient GHD and ACTHD on hydrocortisone replacement (Figure 2.3b below).
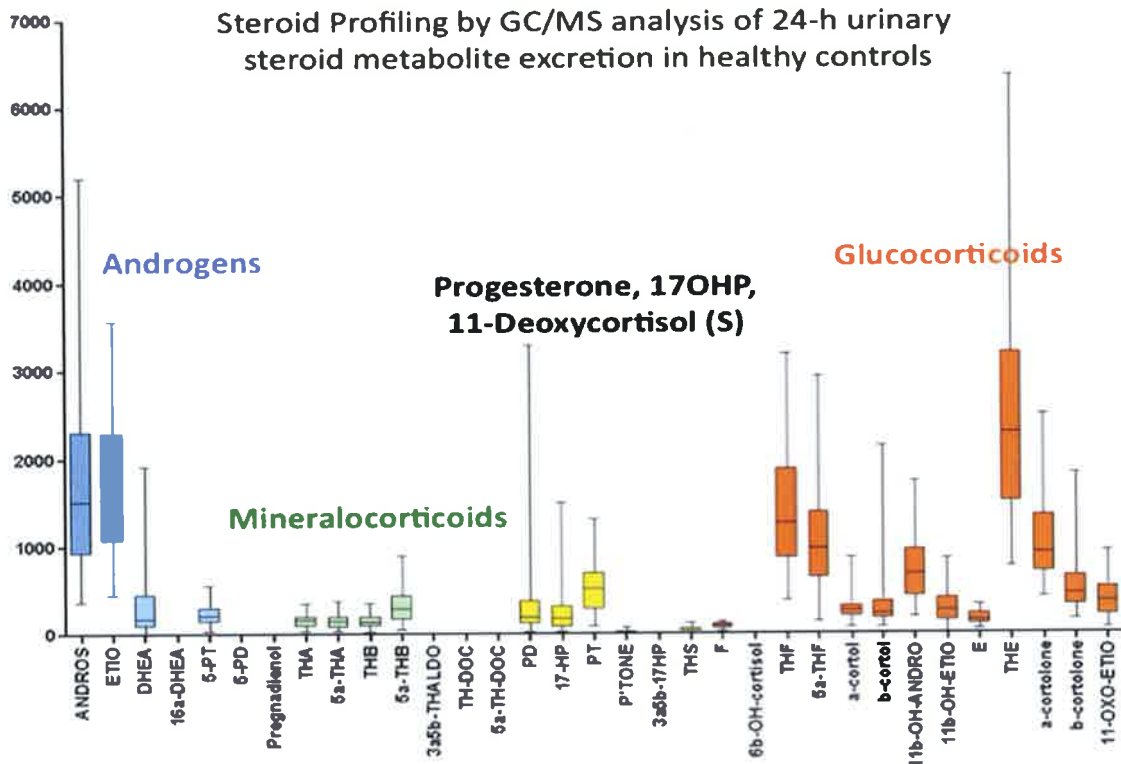
**Figure 2.2** Representative graph of steroid metabolite excretion (μg/ 24 hours) assessed by 24 hour urinary gas chromatography/ mass spectrometry in healthy adults.
Results divided into metabolites of androgens, mineralocorticoids and progesterone (17-OHP and 11-deoxycortisol) and glucocorticoids. Box and whisker plots represent mean and 5th and 95th percentile. Courtesy of CEDAM.

**Figure 2.3** Representative graph of steroid metabolite excretion (μg/ 24 hours) assessed by 24 hour urinary GC/MS in hypopituitary patients

2.3a) with growth hormone deficiency but not ACTH deficiency and 2.3b) ACTH deficiency on hydrocortisone (HC) replacement. Metabolites of glucocorticoids are particularly increased in patients receiving HC therapy (b, blue box). Box and whisker plots represent mean and 5th and 95th percentile of healthy control group. Symbols represent individual patient results. Courtesy of CEDAM

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Such profiles allow for an immediate overview of the complete set of metabolites(134). Most hormonal imbalances caused by enzyme deficiencies or "blocks" cause depletion of a steroid product and build-up of the upstream precursors. Thus, a ratio of metabolites of the substrate to metabolites of the product should indicate if there is such a block. In this thesis ratios of glucocorticoid metabolites are used to determine the relative activity of 11 β-HSD1 and 11 β-HSD2.

The following isotope labelled internal standards were used; (9,11,12,12-$^2$H) cortisol and (9,12,12-$^2$H) cortisone. The standards were calibrated by high performance liquid chromatography (HPLC) analysis of solubilised, non-labelled standard on known weight. Free steroid was extracted using Sep-pak C18 cartridges (104). Labelled steroid $d_4$-cortisol (0.18μg), and $d_3$-cortisone (0.12μg), as well as internal standards (stigmasterol and cholesteryl butyrate), 200μg were then added. The samples were then derived using 100μl of 2% methoxyamine hydrochloride in pyridine and 50μl of trimethylsilylimidzole. Lipidex chromatography was then used to purify the steroid derivative.

GC/MS was carried out using a Hewlett Packard 5970 mass spectometer and 15m fused-silica capillary column, 0.25mmID, 0.25μm film thickness (J&B Scientific, Folsom CA, USA) using 2μl of sample. Steroids were quantified by comparing individual peak area to the peak area of the internal standards, for cortisol fragment 605m/z compared to 609 m/z and for cortisone fragment 531 m/z compared to 534 m/z. The relative peak area was calculated and the metabolite concentration expressed as μg/24hr. A quality control (QC) was analysed with each batch. The intra and inter-assay co-efficient of variance was <10%.

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## 2.5.4 Estimation of serum cortisol

Serum total cortisol was measured in Beaumont Hospital Chemical Pathology Department using a chemiluminescent immunoassay with the Beckman Coulter Unicell DXI 800 with intra-assay coefficients of variation (CV) of 8.3%, 5% and 4.6% at serum cortisol concentrations of 76, 438 and 865nmol/l respectively.

## 2.5.5 Estimation of renal and bone indices

Serum 25 hydroxy(OH) D was measured in St Vincent's Hospital Metabolism Laboratory by a competitive radioimmunoassay (Immunodiagnostic Systems Ltd, Boldon, UK). The inter-assay coefficient of variation was 6.2% (at 28.8nmol/l) and 7.7% (at 104.5nmol/l); intra-assay coefficient of variation was 3.0% (at 28.9nmol/l) and 2.7% (at 73.9nmol/l). Parathyroid Hormone (PTH) was measured in Beaumont Hospital using an electrochemiluminescent immunoassay on the Elecsys 2010 analyser (Roche Diagnostics, Mannheim, Germany) with intra-assay co-efficients of variation (CV) of 2.7%, 1.6%, 1.5% and an interassay CV of 6.5%, 3.9%, 3.0% at PTH concentrations of 26.7pg/ml, 52.5pg/ml and 261pg/ml respectively. Normal reference range was 15-65pg/ml (1.6-6.9pmol/l) based on manufacturer's guidelines. Renal function, albumin and calcium were measured using the Beckman Coulter AU5400 by standard laboratory protocols.

## 2.5.6 Estimation of serum glucose and insulin

Glucose was estimated using the hexokinase method, an enzymatic UV test, on an automated Olympus analyser, calibrated to the Olympus System Calibrator Cat. no. 66300, which is traceable to the National Institute of Standards and Technology Standard Reference Material 965. Serum samples were taken for insulin, allowed to clot

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and centrifuged for separation. Haemolysed samples were not used for estimation of insulin, to minimise insulinase action and false low estimation of insulin results. Insulin levels were estimated used a chemiluminescent immunoassay on the Beckman Coulter™ Access$^R$ Immunoassay System. This was a simultaneous one-step immunoenzymatic sandwich assay. These assays were performed in the Department Chemical Pathology in Beaumont Hospital.

### 2.5.7 Estimation of pituitary hormones

Serum total testosterone, free T4, TSH, gonadotropins and prolactin were measured using the competitive inhibition binding principle of fluoroimmunoassay (FIA), AutoDelfia (Perkin Elmer, Turku, Finland). IGF-I was measured using the one step sandwich chemiluminescent immunometric assay on Immulite 2000 (Siemens Medical Solutions, Los Angeles, USA).

## 2.6 Statistical analysis

Statistical analysis was performed using Prism for Windows version 5.0 (GraphPad Software Inc, San Diego, CA, USA). Elaborations on statistical tests used are presented in the relevant chapters.

## 2.7 Ethics

This study was approved by the Beaumont Hospital Medical Ethics and Research Board in conjunction with approval from the Irish Medicines Board, Clinical Trial Number – CT900/459/1, EudraCT Number – 2007-005018-37. All subjects gave written informed consent.

## Chapter Three: The effect of 3 different hydrocortisone regimens on 24 hour serum cortisol dynamics and quality of life in hypopituitary men

## 3.1 Introduction

It has been postulated that inappropriate glucocorticoid replacement may contribute to the excess morbidity and mortality seen in hypopituitarism(29, 46). The hydrocortisone dose used for adrenal replacement in hypopituitary patients remains largely empiric and physician-dependent. This reflects the lack of reliable biological or biochemical markers to assess the adequacy of replacement(135) and the paucity of evidence in favour of any particular replacement regimen. A recent study in acromegaly patients found excess cardiovascular mortality when doses of hydrocortisone $\geq$ 25 mgs per day are used, an association which seems to be independent of other risk factors(49). The observational nature of available studies and the many confounding factors preclude the establishment of a causal link between glucocorticoid replacement and morbidity and/or mortality. Although it is acknowledged that ideal glucocorticoid replacement should mirror the normal healthy state as much as possible, the reproduction of physiological cortisol dynamics remains very challenging. Clinical practice varies widely from replacement therapy only in the case of intercurrent illness in mild ACTH deficiency(125), up to daily doses of 30 mgs or more in more severe cases(28, 29, 49, 136, 137).

Clearly both over and under replacement with glucocorticoids is undesirable. The primary aim of this part of the study was to assess which of three commonly used

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hydrocortisone replacement regimens used in severely adrenocorticotropin (ACTH) deficient hypopituitary subjects would replicate most closely the biochemical cortisol profile of healthy matched controls, without adversely affecting the secondary end point, quality of life.

## 3.2 Methods

### 3.2.1 Patients

Ten adult male hypopituitary patients secondary to organic pituitary disease, with known severe ACTH deficiency defined by a fasting morning total serum cortisol concentration <100nmol/l and a stimulated peak cortisol in response to insulin-induced hypoglycaemia of <400nmol/l were included.

Exclusion criteria: those aged less than 18 years, patients with acute medical or surgical illness, patients with advanced cardiac/pulmonary disease, patients with a terminal illness, patients on glucocorticoids for purposes other than ACTH deficiency and those on agents that interfere with corticosteroid metabolism. Female patients were excluded because of the unpredictable effects of oestrogen status on corticosteroid binding globulin (CBG) levels and thus total cortisol concentration and also free cortisol kinetics(126).

Growth hormone deficiency was defined as a peak GH response <3ng/ml to stimulation with insulin induced hypoglycaemia; TSH deficiency was defined as the presence of a low free T4 in association with a normal or low TSH. Gonadotropin deficiency was defined by a low morning serum testosterone on 2 separate occasions with a normal or low FSH/LH. Cranial Diabetes Insipidus was diagnosed on the basis of failure to achieve a peak urine osmolality >700mOsm/kg concentrate urine with a plasma osmolality >298pmol/l following a water deprivation test (WDT); however in some cases formal WDT was not carried out and CDI was diagnosed in the appropriate clinical setting (post neurosurgery) in conjunction with hypernatraemia (Na >145mmol/l), polyuria >3.5litres/24hours and an early morning fasting urine osmolality <300mOsm/kg.

The aetiology of hypopituitarism was as follows: 5 subjects had non-functioning pituitary adenomas, 2 had craniopharyngioma, 2 had macroprolactinoma and 1 had treated Cushing's disease (basal cortisol 87nmol/l and peak cortisol 113nmol/l), all had pituitary surgery and one patient had radiotherapy, with diagnosis and treatment taking place between 3 and 18 years prior to inclusion in the study. All but one subject had complete anterior pituitary failure, while the 10th was sufficient in gonadotropin activity. All patients were on appropriate hormone replacement, including GH, without alteration in dose for at least 3 months prior to and during the study. Hormone replacement therapy regimens were not adjusted during the study period, except for HC dose as per study protocol. All 10 patients had Diabetes Insipidus and each had normal electrolytes at each visit with no evidence of over or under-replacement with Desmopressin. The clinical characteristics of the patients are summarised in Table 3.1.

*Table 3.1 – Clinical characteristics of the patient group*

*Treated Cushing's disease, confirmed ACTH deficiency following therapy **and radiotherapy TSS – transphenoidal surgery, TCR – transcranial surgery, NFPA – non-functioning pituitary adenoma, ACTH adenoma – adrenocorticotropin producing adenoma, ADH – anti diuretic hormone, GH – growth hormone, TSH – thyroid stimulating hormone, r-GH – recombinant GH, T4 – thyroid hormone, dDAVP – Desmopressin, T- testosterone, BMI – body mass index.

| Patient | Age (years) | BMI (kg/m²) | Diagnosis | Pituitary Surgery | Other hormone deficiencies | Basal cortisol nmol/L | Peak stimulated cortisol nmol/L | Hormone replacement |
|---|---|---|---|---|---|---|---|---|
| 1 | 50 | 29.4 | NFPA | TSS | all | 30 | 30 | r-GH, T4,T, HC,dDAVP |
| 2 | 46 | 33.9 | ACTH adenoma* | TSS | all | 87 | 113 | r-GH, T4,T, HC,dDAVP |
| 3 | 56 | 36.2 | NFPA | TSS | all | 33 | 59 | r-GH, T4,T, HC,dDAVP |
| 4 | 71 | 27.1 | Macroprolactinoma | TCR | all | 29 | 29 | r-GH, T4,T, HC,dDAVP |
| 5 | 27 | 26.5 | Craniopharyngioma | TCR | all | 22 | 22 | r-GH, T4,T, HC,dDAVP |
| 6 | 55 | 39.6 | Craniopharyngioma | TCR | all | 30 | 31 | r-GH, T4,T, HC,dDAVP |
| 7 | 65 | 26.6 | NFPA** | TSS | all | 94 | 253 | r-GH, T4,T, HC,dDAVP |
| 8 | 33 | 30.7 | Macroprolactinoma | TSS | all | 32 | 70 | r-GH, T4,T, HC,dDAVP |
| 9 | 36 | 25.0 | NFPA | TSS | all | 30 | 68 | r-GH, T4,T, HC,dDAVP |
| 10 | 26 | 23.0 | NFPA | TCR | ACTH, GH, TSH, ADH | 63 | 213 | r-GH, T4, HC, dDAVP |

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### 3.2.2 Controls

10 healthy male controls, matched for age, body mass index (BMI) and waist circumference (WCM) underwent the same biochemical investigations. In addition to these 10 controls, we recruited 20 additional healthy matched controls, without a known chronic illness (total n=30) in regular employ, from similar ethnic and socio-economic background to our patient population to increase the control group solely for the purpose of quality of life (QoL) assessment.

### 3.2.3 Study Design

In this prospective clinical trial all 10 patients were randomised in a cross-over protocol to take 3 commonly prescribed doses of hydrocortisone, for 6 weeks of each dose regimen.

- **dose A** (20mg 0800hours and10mg 1600hours) or
- **dose B** (10mg 0800hours and 1600hours) or
- **dose C** (10mg 0800hours and 5mg 1600hours)

At the end of each treatment schedule patients were admitted at 0730 hours to our clinical research centre (CRC) for a 28 hour period. Following a physical examination that included recording pulse, blood pressure, BMI and WCM, a heparinised intravenous cannula was inserted into a peripheral vein. The morning dose of hydrocortisone was withheld until after baseline samples were taken at 0800 hours for cortisol, CBG, free T4, TSH, testosterone, sex-hormone binding globulin (SHBG), albumin, gonadotropins, prolactin and insulin like growth factor-I (IGF-I). Serum cortisol samples were taken hourly until midnight and 2 hourly from midnight until 0800hours the following morning

through the heparinised cannula into plastic tubes that facilitated clot formation. Samples were centrifuged at 3,000 rpm for 15 minutes and stored in 1ml aliquots at -20 °C until analysis. Subjects took the hydrocortisone dose at 0800 hours and 1600 hours as per study protocol. Meals were eaten at pre-defined times and lights were turned off at 2300 hours.

Subjects were administered the Short Form 36 (SF36) questionnaire(128) (Appendix 1) and the Nottingham Health Profile (NHP)(129) (Appendix 2) at 0900 hours on the first day of study admission, in order to assess quality of life (QoL) on each dose regimen. Detailed description of these questionnaires is provided in Chapter 2.

10 healthy matched controls underwent identical 24 hour serum cortisol profiling as the patient group. 30 healthy matched controls from similar socio-economic background to our patient population completed the QoL assessment

### 3.2.4 Analytical Methods

Serum total cortisol was measured using a chemiluminescent immunoassay with the Beckman Coulter Unicell DXI 800 with intra-assay coefficients of variation (CV) of 8.3%, 5% and 4.6% at serum cortisol concentrations of 76, 438 and 865nmol/l respectively. Serum CBG was measured using a radioimmunoassay, BioSource Europe S.A, Nivelles, Belgium. The interassay CV is 5.5% and 2.4% at serum CBG concentrations of 23.8 and 108 mg/l respectively while the intra-assay CV is 3.9% and 2.9% at 0.98 and 4.26mg/l respectively. Free cortisol was calculated using Coolens' equation(138) as shown in the equation below, where both CBG and total cortisol are expressed in μmol/L.

$$[(0.0167+0.182(CBG\text{-total cortisol}))^2+(0.0122 \times \text{total cortisol})]^{0.05} - [0.0167+0.182(CBG\text{-total cortisol})] \text{ (Coolens)}$$

Serum total testosterone, free T4, TSH, gonadotropins and prolactin were measured using fluoroimmunoassay (FIA), AutoDelfia (Perkin Elmer, Turku, Finland). IGF-I was measured using the chemiluminescent immunometric assay Immulite 2000 (Siemens Medical Solutions, Los Angeles, USA).

### 3.2.5 Statistical Methods

Analysis of variance (ANOVA) models were used to compare the integrated day curve of both 24hour serum total cortisol and 24hour calculated free cortisol profiles between the three dose regimens and also to that of the controls. ANOVA was also used to compare mean peak and trough post-absorption serum cortisol concentrations. Multiple comparison tests using the Bonferroni correction factor were applied to determine if results reached significance at the 5% level. Comparisons for age, BMI and WCM between patients and control group were made using the Student's t-test or the appropriate non-parametric test.

QoL questionnaires were coded for analysis by the investigators, in an effort to reduce the risk of bias during analysis of the results. Analysis of quality of life involved calculating age and gender specific mean and standard deviation (SD) score values from the normally distributed healthy control data to produce age and gender specific Z-scores for each patient on each dose regimen. The Z-score reveals how many units of the SD each subject is above or below the mean. The Z-score was calculated as follows: $Z=(x-\mu)/\sigma$, where x = individual QoL value, $\mu$ = mean QoL value of controls of equal

gender and age and σ = SD of QoL value of controls of equal gender and age. The Z-score was calculated for each domain in SF36 and NHP however because a higher score is associated with worse QoL in the NHP a positive Z score denotes worse QoL compared to healthy controls while in the SF36 a higher score indicates better QoL and therefore a negative Z score denotes decreased QoL compared to healthy controls.

## 3.3 Results

Patients and controls were appropriately matched for age, BMI and WCM as demonstrated in Table 3.2.

**Table 3.2** Patient and control baseline anthropometric and hormone status
Results expressed as mean ± standard deviation, BMI-body mass index, WCM-waist circumference

|  | Patients n=10 | Controls n=10 | p value |
|---|---|---|---|
| Age (years) | 46 ± 15 | 45 ± 15 | 0.9 |
| BMI (kg/m$^2$) | 29.8 ± 5.3 | 29.1 ± 4.6 | 0.5 |
| WCM (cm) | 105 ± 14 | 103 ± 11 | 0.53 |
| Basal Cortisol (nmol/L) | 76.8 ± 6.5 | 403.3 ± 122.4 | <0.0001 |
| Basal Free T4 (pmol/l) | 11.3 ± 2.1 | 10.9 ± 1.0 | 0.6 |
| Basal IGF-I (ug/L) | 163 ± 45 | 152 ± 32 | 0.5 |
| Basal Testosterone (pmol/L) | 14.2 ± 4.1 | 16.4 ± 7.7 | 0.4 |

## 3.3.1 Cortisol profile and corticosteroid binding globulin.

The median CBG was significantly lower in the hypopituitary subjects regardless of dose regimen at 20.67 (13.1-50.35mg/L) for dose A, 24.23 (12.5-63.3mg/L) dose B, 23.3 (15.6-55.23) dose C compared to controls 38.23 (24.3-69mg/L) ($p < 0.05$) but there was no significant difference in CBG levels between dose regimens.

The mean 24 hour total and calculated free cortisol profiles of control and the three dose regimens are shown in Figure 3.1 and 3.2 respectively. In view of the difference in CBG concentration between controls and subjects, the data presented from this point on in this chapter on refer to calculated free serum cortisol concentration.
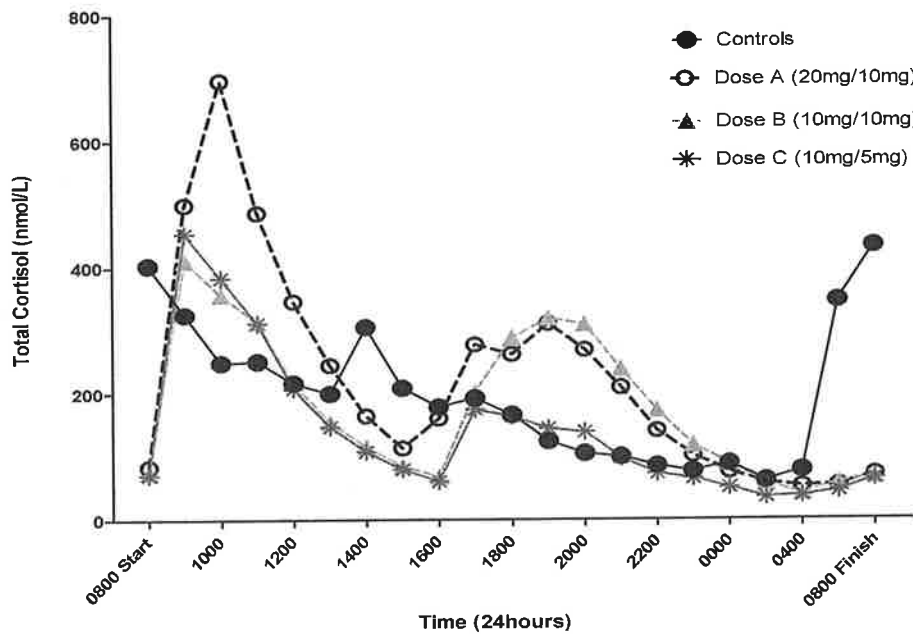


**Figure 3.1** Mean 24 hour serum total cortisol profile.
Hydrocortisone doses given at 0800hours and 1600hours

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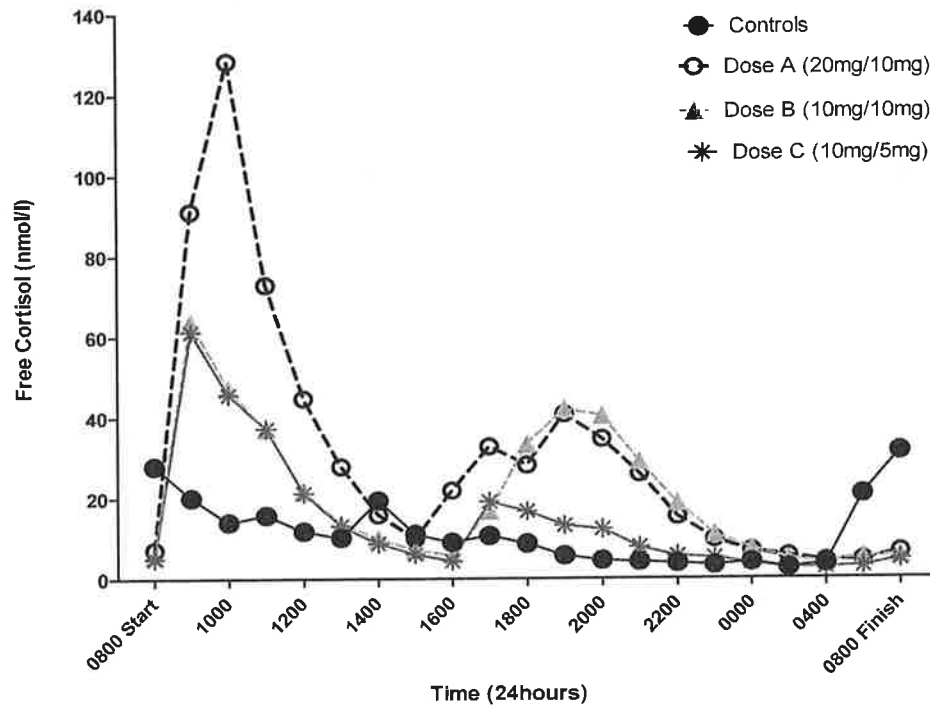
**Figure 3.2** Mean 24 hour calculated free cortisol profile.
Hydrocortisone dose given at 0800hours and 1600hours

In Figure 3.3 below the mean 24 hour free cortisol profiles (FCP) of each dose regimen are superimposed on the control FCP (Figure 3.2a-c). Both doses A and B produce supraphysiological values, while dose C mimics the FCP of the healthy controls more closely. If the timing of the second dose of regimen C was changed to 1400hours, an almost identical profile is produced except for the dawn peak in cortisol which no dose regimen currently available in clinical use could reproduce. (Figure 3.3d)

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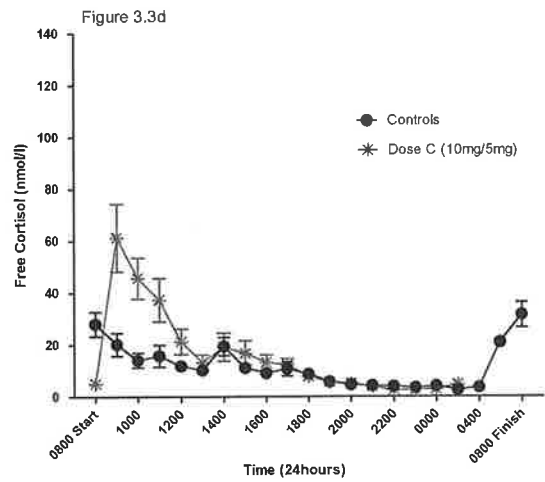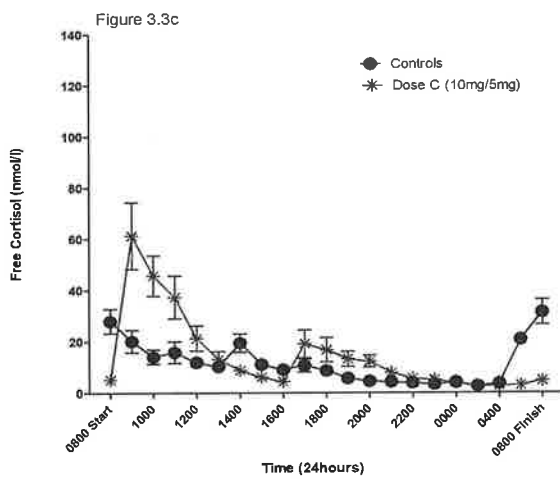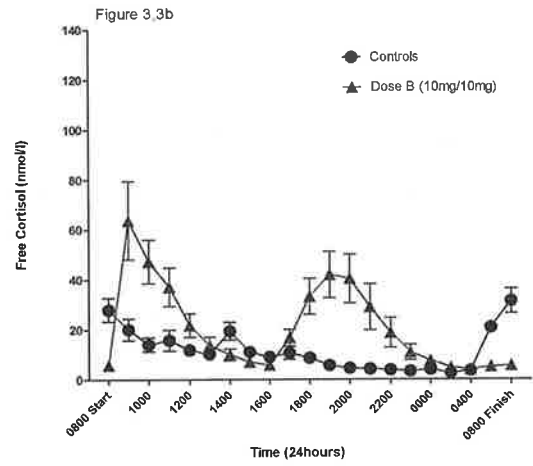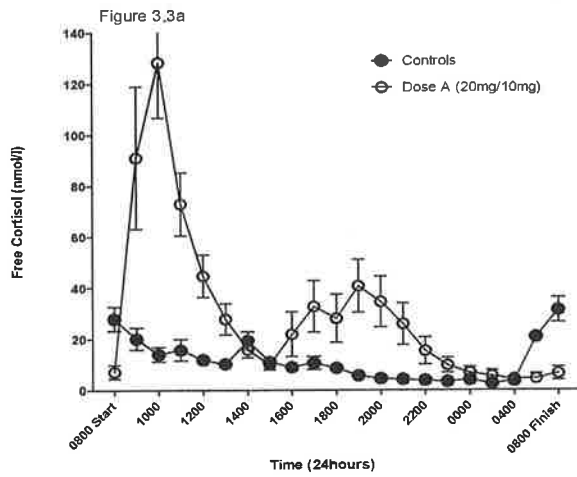
**Figure 3.3** 24 hour calculated free cortisol profiles in patients on each dose compared to controls

Hydrocortisone doses given at 0800hours and 1600hours, a) Controls and Dose A, b) Controls and Dose B, c) Controls and Dose C, d) Controls and Dose C with curve moved to left to demonstrate effect of a 1400hour dose.

### 3.3.2 Area under the curve, peak and trough free cortisol analysis

The area under the curve for dose A (AUC = 628.4nmol/24hr) was significantly higher than controls (213.7nmol/24hr p<0.000) while the AUC for dose B and C were not different from controls at 425.3nmol/24hr (p=0.17) and 294.6nmol/hr (p=1) respectively.

Peak post-absorption free cortisol following the morning dose ("day-time" peak) was significantly different for all dose regimens compared to controls (p<0.001 for dose A and B and p<0.05 for dose C), however following the afternoon dose, unlike dose A and B, peak post-absorption free cortisol ("night-time" peak) was not different in dose C compared to controls (p=0.06). Trough free cortisol values were not significantly different in any of the dose regimens when compared to controls (p=0.71). (Figure 3.4)
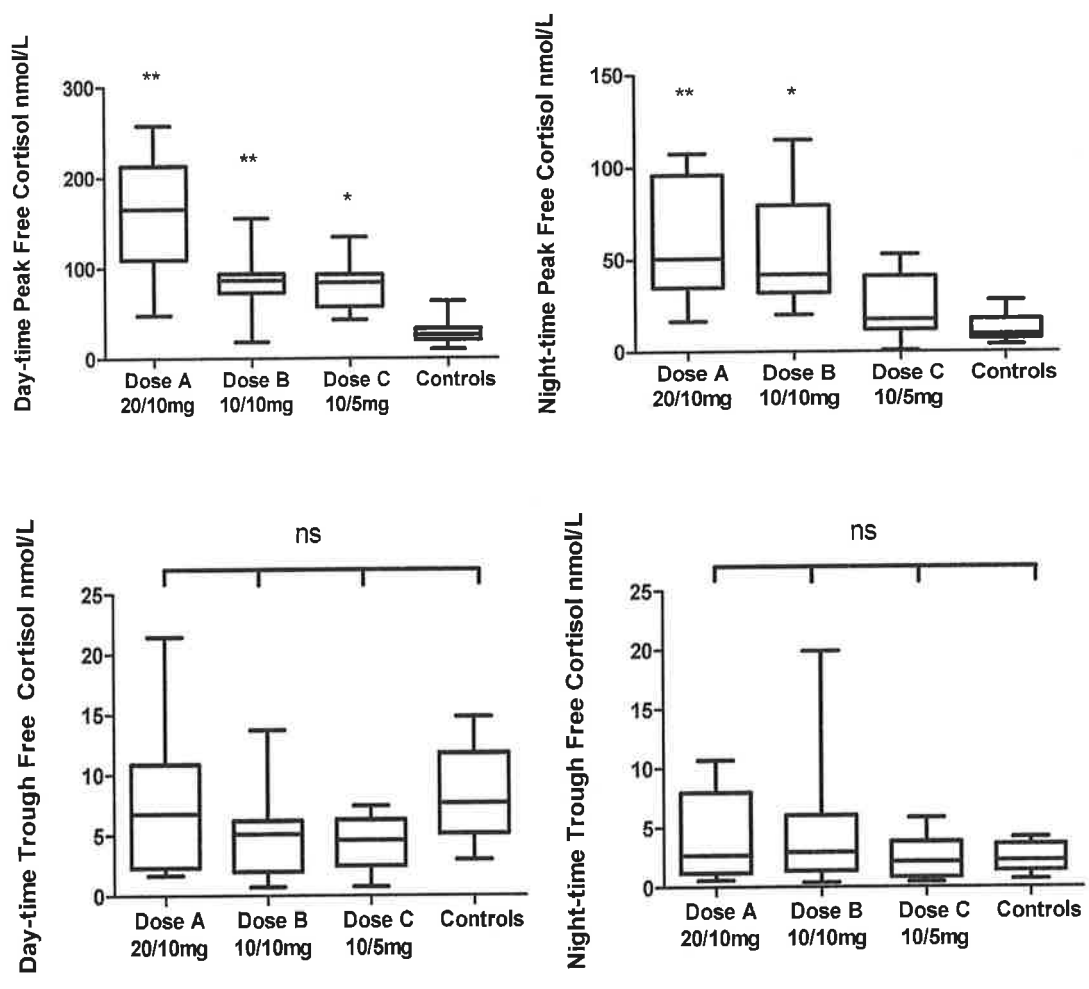
**Figure 3.4** Comparison of peak and trough free cortisol levels between dose regimens and to controls.

"Day-time" refers to the peak or trough following the 0800hours hydrocortisone dose, "night-time" refers to the peak or trough following the 1600hours hydrocortisone dose

** $p = <0.001$ compared to controls and dose C $p < 0.05$.

### 3.3.3 Quality of Life (QoL)

The overall QoL, as assessed by the SF36 and by the NHP was not significantly altered between dose regimens. However, the raw SF36 scores demonstrate a reduction in QoL in the role physical and role emotional domains in subjects on dose B and in role physical and vitality in those on dose C compared to controls. The NHP found impaired QoL with respect to energy level only in all three dose regimens compared to control subjects. (Table 3.3)

***Table 3.3* –** Raw quality of life scores between dose regimens and compared to controls
Data expressed as mean (SD), **p<0.001 compared to controls, *p<0.05 compared to controls,
no significant between dose differences. High scores indicate better quality of life for SF36 and
low scores indicate better quality of life for NHP.

| SF36 | Dose A 20/10mg | Dose B 10/10mg | Dose C 10/5mg | Controls |
|---|---|---|---|---|
| Physical Functioning | 88.5 (18.4) | 79.5 (24) | 80.5 (24.5) | 92.9 (13.4) |
| Role Physical | 77.5 (38) | 62.5 (35.8)** | 55 (45.3)** | 95.8 (12) |
| Bodily Pain | 85.1 (20) | 82.5 (23.8) | 76.5 (22.8) | 81 (21.2) |
| General Health | 62.8 (18.6) | 61.8 (13) | 59.8 (15) | 77 (12.7) |
| Vitality | 62.5 (24.9) | 47.5 (23.3) | 44 (24.5)* | 70 (13.3) |
| Social Functioning | 90 (17.4) | 92.5 (13.4) | 85 (18.4) | 91 (14.5) |
| Role Emotional | 83.3 (36) | 66.6 (41.5)* | 73.3 (40.9) | 91.6 (20.2) |
| Mental Health | 79.6 (17.8) | 80 (18.9) | 80 (17.4) | 78.8 (13.1) |

| NHP | Dose A 20/10mg | Dose B 10/10mg | Dose C 10/5mg | Controls |
|---|---|---|---|---|
| Energy Level | 36.3 (43.6)** | 35.1 (42.5)** | 41.3 (37.3)** | 3.2 (11.1) |
| Pain | 8.1 (22.2) | 7 (15.6) | 10.6 (21.6) | 2.1 (4.4) |
| Emotional Reaction | 8.5 (10.3) | 7.3 (15.5) | 8.6 (17.1) | 8.9 (13.4) |
| Sleep | 20.7 (23.2) | 15.3 (30.4) | 10.9 (17.7) | 11.2 (18.4) |
| Social Isolation | 8.5 (20.6) | 7.5 (13.3) | 0 (0) | 4.4 (13.6) |
| Physical Abilities | 8.9 (15.5) | 13.1 (21.8) | 14.4 (20.5) | 2.2 (5.6) |

## Quality of Life Standard deviation (Z) scores

The Z-scores for both the SF36 and the NHP confirmed that there was no difference in QoL between the three dose schedules however, subjects on doses B and C had impaired role physical in the SF36 with Z-scores of -2.768 (SD=2.97) and -3.39 (SD= 3.76) below the healthy mean ($p<0.01$ and $p<0.001$) respectively, while Dose A and C both demonstrated impaired energy level in the NHP with Z-scores of 2.98 (SD=3.94) and 3.44 (SD=3.38) respectively ($p < 0.05$) (Figure 3.5).

**SF36 Z Scores**

**NHP Z Scores**
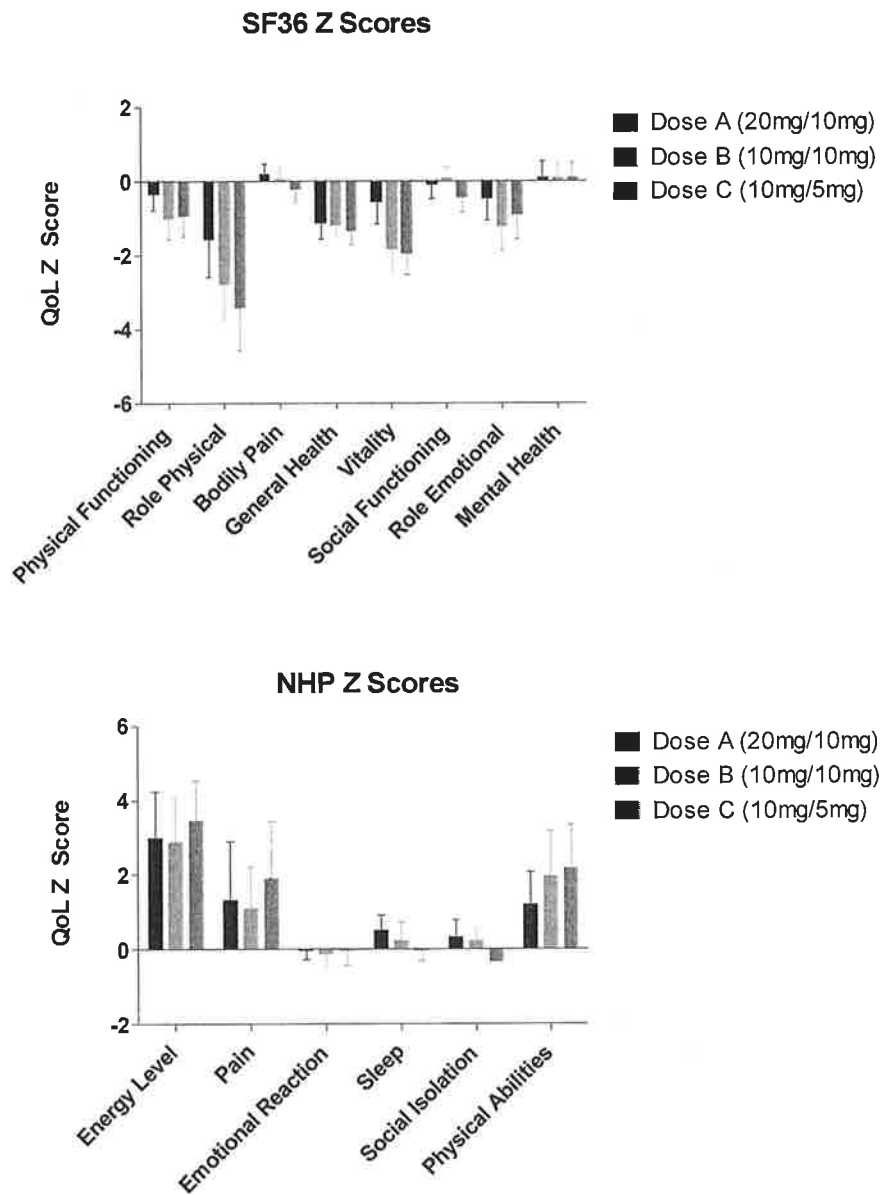
**Figure 3.5** Quality of life standard deviation scores for the SF36 and NHP questionnaires between dose regimens.

Qol – quality of life, SF36 – short form 36 QoL questionnaire, NHP – Nottingham health profile questionnaire, Z scores - standard deviation scores calculated using our control data.

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## 3.4 Discussion

This is the first prospective, randomised, controlled trial to assess three commonly used regimes of hydrocortisone replacement therapy in severe ACTH deficiency by comparing 24hour free cortisol profiles and quality of life with that of healthy matched controls. In this study it is demonstrated that the hydrocortisone dose regimen that most closely mimics normal physiology, both in overall cortisol exposure and in dynamics, is the lower dose, 10mg mane and 5mg tarde (dose C).

The mean peak serum total cortisol level in the morning in our healthy matched controls was 435 $\pm$ 139nmol/L, while the afternoon mean peak serum total cortisol was 313 $\pm$ 110nmol/L. This emphasises that previous recommendations by Drury and Besser to aim for a morning peak cortisol following GC replacement of between 700-1000nmol/L and a second peak of 420 to 500nmol/L(139), would result in grossly supraphysiological GC exposure. Previous recommendations to prescribe 30mg hydrocortisone in daily divided doses were based on an over-estimate of daily cortisol production of 12-15mg/m2/day(21, 22), however later studies using isotope dilution/mass spectrometry(24) and deconvolution analysis(25) showed daily cortisol production to be lower at approximately 5.7mg/m$^2$/day. Since those studies Peacey et al demonstrated that a reduction of hydrocortisone by 30% to 20mg daily lead to a 19% increase in osteocalcin, a marker of bone formation(36), yet doses of 10-20mg/m$^2$/day have been recommended to allow for presumed incomplete oral bioavailability and the short half-life of hydrocortisone(23).

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Although prescribing 30mg daily is considered outdated by many physicians it is clear that hypopituitary subjects are still being exposed to a wide range of glucocorticoid replacement doses in clinical practice as recent publications quoted use of glucocorticoid replacement equivalent to hydrocortisone doses of $\geq$ 30mg daily in 34%, 67% and 91% subjects(26, 28, 49) and $\geq$ 20mg daily in 80% of subjects in another study(29). Therefore, we chose to examine 30mg daily and 20mg daily which are commonly used doses in clinical practice, and to compare these doses to a lower hydrocortisone dose regimen of 15mgs daily in severely ACTH deficient hypopituitary subjects.

An important factor in our study is the consideration of serum cortisol dynamics. The 24hour cortisol profile we found in our control group is consistent with the expected diurnal rhythm of cortisol release(16, 17, 140) with an early morning peak that starts at approximately 0400 hours, a second smaller peak at approximately 1400 hours and low levels of cortisol from early evening to after midnight. Currently, no glucocorticoid replacement regimen available in clinical practice can precisely mimic this rhythm. Modified release hydrocortisone, when it becomes commercially available, may be able to overcome the difficulty in achieving the dawn cortisol rise(17, 141), however this may be more relevant to subjects with congenital adrenal hyperplasia where suppression of the early morning surge in ACTH is a desired outcome(142).

In our patient population who are severely ACTH deficient, taking hydrocortisone immediately upon waking is the closest approximation to physiology that we can currently achieve and prospective studies assessing metabolic outcome will be required

to assess the impact of modified release HC. While dose C is associated with a morning peak free cortisol that is higher than controls, controls it might be suggested that the morning dose should be reduced, however GC replacement is not only about dynamic but also overall cortisol exposure. Until it is possible to adequately mimic the early morning rise in cortisol, patients will be trying to catch up with their morning dose. A further reduction in dose at this time point would lead to a lower AUC that is less than that of controls and possibly under-replacement for that period of the day therefore some compensation for loss of the early morning rise in cortisol is reasonable. The afternoon dose peak mimics closely that of the controls and the overall AUC is the same as controls confirming that Dose C, 10mg mane and 5mg tarde is the most physiological replacement regimen although some adjustment by giving the afternoon 5mg dose near 1400hours seems appropriate as shown in figure 2d. Although we did not directly study the afternoon dose being administered at 1400hours and we have based some conclusions on "moving the curve to the left", we feel that this recommendation is valid. Plat et al examined the effects of administering 50mg Hydrocortisone at 0500hours compared to 1700hours in individuals whose endogenous cortisol production has been suppressed for the purposes of the study with metyrapone(99). While the mean cortisol levels were found to be the no different between the two time points (626 $\pm$ 44nmol/L v 650 $\pm$ 50nmol/L respectively), there was evidence of prolonged exposure to cortisol in the evening dose through evidence of a wider cortisol curve following that dose. It is unclear in our study whether giving the HC dose 2 hours earlier would make such a difference to the cortisol curve; however it can be inferred from the study by Plat et al that the peak cortisol level would be unchanged.

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There is no evidence available to suggest that hydrocortisone replacement doses ≤ 20mg are associated with increased frequency of adrenal crises and indeed higher replacement doses (for example 30 mgs daily) will still result in low early morning cortisol levels and do not avoid trough levels in the afternoon and evening. A recent audit of clinical practice by Druce et al on 99 patients with adrenal insufficiency of varying underlying pathologies demonstrated very low rates of hospital admission while also showing that there was no difference in the frequency of dose increases for inter-current illness between subgroups of patients on variable doses of hydrocortisone(15). It has been recommended to consider replacing glucocorticoid with the smallest dose that is compatible with safety and vitality while bearing in mind individual patient needs and potential medication interactions; adrenal crises can be avoided by detailed and repeated patient education rather than deliberate overtreatment(7). This policy seems to be sensible and it is particularly important to counsel the patients about increasing their glucocorticoid doses during periods of inter-current illness, a measure which may help to minimise the risk of an adrenal crisis.

It is our practice to prescribe twice daily hydrocortisone replacement based on the well-known diurnal variation described above. Thrice daily hydrocortisone replacement has been recommended by some authors as an appropriate replacement regimen based on the desire to avoid supraphysiological peaks and prolonged subphysiological trough levels(30, 31). However one of the main studies recommending thrice daily hydrocortisone compared subjects on a mean daily dose of 35mg hydrocortisone given at 0900, 1400 and 2000hours, with a twice daily regimen given at 0900 and 2000hours, eleven hours apart(30). Therefore, it is not surprising that by 2000hours the twice daily

group had much lower levels of hydrocortisone recorded than the thrice daily group. It is also worth noting that those in the thrice daily group had mean 1600hour cortisol levels of over 550nmol/l which is clearly supraphysiological for that time of day. As no cortisol measurements were provided after 2000hours in this study it is not clear if the cortisol levels thereafter were supraphysiological, or if low trough levels were successfully avoided in the thrice daily regimen. Although it was reported that patients 'feel better' on the thrice daily regimen in that study, this was based on a non-significant trend towards better well-being in the thrice daily group, compared to the twice daily group as assessed using a visual analogue scale that ranged from "on top of the world" to "wiped out".

In a retrospective, uncontrolled study Howlett et al also compared thrice and twice daily regimens, taking samples after the morning dose at 0900hours and prior to the 1230 and /or the 1730hours dose(31). The cut off for optimal replacement was defined arbitrarily. The criticism of the twice daily regimen in this paper was again based on prolonged trough levels in a dose regimen where the second dose was scheduled for the evening, thus unsurprisingly predisposing to non-physiological pre-dose trough levels. There were no cortisol measurements after the 1730hours dose, but it is likely the evening levels were supraphysiological, with no evidence that low early morning cortisol levels were avoided using the thrice or twice daily regimens in this study. In our study the trough free cortisol levels after the morning and evening doses were not significantly different between doses or compared to controls, suggesting that prescribing higher dose replacement to avoid low troughs is unproductive and more probably harmful.

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Mah et al performed pharmacokinetic study, evaluating the impact of a weight-based thrice daily regimen in subjects with cortisol deficiency, the majority of whom had primary adrenal insufficiency. They examined a 10mg "fixed dose" of HC in the morning in the fed and the fasting state, compared to a 0.12mg/kg dose also in the morning, without assessing the impact of the other two doses later in the day(32). Consuming HC in the fed state, prolonged elevated cortisol levels in the fixed dose group, while the weight-based regimen demonstrated less inter-individual variability compared to the fixed dose, although it did not result in lower peak cortisol levels compared to the other dose regimen. Weight-based prescribing is labour intensive for the patient as it involves cutting 10mg HC tablets into quarters in some cases, in order to modify the dose to the nearest mg, without any evidence of long term superiority in outcome. Another concern regarding weight based dosing is that replacement doses may reach over 25mg daily or higher in obese patients. It is conceivable that the combination of obesity and high total glucocorticoid doses may add to the already increased metabolic risk for those patients. In our study there was no evidence of adverse outcome in the short term on the low dose replacement for the patients with BMI >30kg/m$^2$.

Overall there is little evidence to suggest that a thrice daily regimen is superior and it is clear that the dynamics of a thrice daily regimen do not mimic diurnal rhythm, with the added important consideration of potentially reduced compliance with increasing frequency of dose administration. The main argument against twice daily regimens is concern regarding prolonged trough levels however the post-absorption trough level in our lowest dose regimen did not differ from the troughs in our controls, thus reducing this concern. If we recommend that patients take their hydrocortisone upon waking and

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again between lunchtime and 1400hours it may facilitate compliance while still providing adequate cortisol levels into the early evening, a time when the natural quiescent phase of cortisol dynamics begins in the healthy individual(16, 17, 140).

Our hypopituitary subjects on glucocorticoid replacement had lower serum CBG values than the matched controls thus making comparisons using the bio-available free cortisol rather than the total serum cortisol more reliable. No other study has reported the effect of glucocorticoid replacement on CBG levels and it is not clear whether this difference represents suppression of hepatic CBG production as a direct effect of exogenous glucocorticoids or whether it is related to the effects of growth hormone or other pituitary replacement on the liver. There is little evidence to this effect in the literature however Jansson et al demonstrated that while continuous GH infusion (1.4u/kg/day) increased serum CBG in hypophysectomised rats, intermittent 12 hourly subcutaneous injections either had no effect or suppressed serum CBG levels. Neither androgen nor oestrogen treatment altered CBG in these rat models(143). Three studies have evaluated the effect of initiating GH replacement on CBG levels and demonstrated a reduction from baseline CBG by 20-30% over 6 to 12 months(144-146), however no study compared baseline levels with healthy matched controls or evaluated the effect of glucocorticoid replacement. One of the studies that demonstrated a decrease of CBG, but stable SHBG and thyroid binding globulin (TBG) included 6 subjects with isolated GH deficiency and 16 with multipituitary hormone deficiency on a mean of 25mg HC daily, however there was no separate analysis of CBG levels between those two groups at baseline, so the effect of HC is not clear(146). Studies have suggested that CBG may have a more active role in localisation of cortisol action instead of solely regulating the

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amount of free hormone available(147). This may be relevant in a subject group with chronic over-exposure to cortisol. In our patients, all deficiencies including GH were appropriately replaced prior to inclusion and were not modified during the study.

While not as accurate as equilibrium dialysis in assessing free serum cortisol, Coolen's equation is an accepted surrogate measure of free cortisol and has been shown to correlate with measured serum free cortisol in a number of studies(148-153) While Barlow et al found Coolen's equation to underestimate measured free cortisol(151), Ho et al found close agreement between measured and calculated free cortisol(152). Discrepancies are amplified once CBG is saturated at cortisol levels of 650nmol/L(153). However that potential flaw in the use of calculated free cortisol is not as relevant to this study as only dose A was associated with total cortisol levels greater than 650nmol/L. Salivary cortisol has been suggested as an alternative method for measuring of free cortisol, however there are a number of practical challenges associated with use of this method, including contamination of levels by blood, food or even remnants of the oral dose of HC leading to significant variability from oral dosing of GC(146, 154); there is also a time delay that ranges between 15 minutes to 2hours from the consumption of GC and the appearance of the peak cortisol level in saliva(154) leading to challenges in interpretation of results. Measuring serum free cortisol is time consuming, labour intensive and expensive therefore the use of Coolen's equation in this setting was deemed appropriate and reliable.

We have also shown that it is possible to reduce hydrocortisone replacement to a more physiological dose without significantly compromising quality of life. Subjects with pituitary disease have been shown to have impaired QoL compared to the general population in a number of studies(47, 62-65) . This has been speculated to be due to several factors including radiotherapy(64) transcranial surgery(47) and hypopituitarism(64, 65). It has been shown that QoL is impaired in pituitary disease even in comparison to another chronic neurological disease (paraganglionoma) (65), suggesting that hypopituitarism and imperfect endocrine replacement may be contributing factors.  Benson et al demonstrated in a placebo-controlled randomised crossover protocol in secondary AI subjects that hydrocortisone 10mg in the morning and 5mg in the mid-afternoon was associated with better quality of life in some domains of the SF36, compared to 20mg divided in a thrice daily hydrocortisone schedule or to a prednisolone 5mg(70). The difference in QoL seen in that study was based on only 4 weeks of each dose regimen which corroborates the view that alterations in QoL can occur over a 4 week period. Our study population were all panhypopituitary, on all required pituitary hormone replacement therapy which was stabilised for 3 months prior to and for the duration of the study, thus minimising the potential confounding effect of other hormone replacement on the results with respect to the different cortisol regimens. We demonstrated that although QoL was impaired in some domains compared to controls, there were no differences in QoL between the three dose regimens, suggesting that the differences seen were not directly related to alterations of hydrocortisone replacement but the overall presence of pituitary disease and its general replacement. This provides reassurance that subjects can have their dose of hydrocortisone modified based on metabolic considerations without adversely impacting on quality of life. The underlying reasons for the reduced quality of life in some of the domains compared to

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controls are unclear and may be related to prior pituitary surgery, the other hormone replacement or the presence of panhypopituitarism itself.

The importance of appropriate glucocorticoid replacement is increasingly recognised. In their retrospective observational study Filipson et al demonstrated that subjects on hydrocortisone equivalent doses <20mg/day did not differ in metabolic endpoints compared to those who were ACTH sufficient, while increasing hydrocortisone doses were associated with increased BMI and an adverse metabolic profile(29). Recently, in a study on patients with multiple pituitary hormone deficiencies who were not on growth hormone replacement, a reduction in hydrocortisone dose from a mean dose of 26.3mg daily to 13.1mg daily demonstrated that after 6-12 months subjects had lost a mean of 7.1kg total body fat and 4.1kg abdominal fat and an improvement in QoL assessed by the adult growth hormone deficiency questionnaire, however there were no changes in lean body mass, bone mineral content and insulin resistance as assessed by HOMA-IR(69). The recent observational study that found increased mortality with increasing HC dose in hypopituitary patients for treated non-functioning pituitary adenoma supports the case for prescribing the lowest dose of hydrocortisone tolerated(48).

There are a few limitations to this arm of my study. Firstly, it may not be possible to extrapolate the results to all severely ACTH deficient hypopituitary subjects as only male Caucasian population were studied. This study may also have been underpowered to reliably assess the between dose differences in quality of life, however the main aim of the study was to demonstrate safety and tolerability of low dose HC replacement and

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this has been shown. In this study, the second dose of hydrocortisone was given at 1600hours, however giving the dose at 1400hours seems more appropriate from figure 2d. Although as a general rule, 15 mgs of hydrocortisone in two divided doses may be appropriate for most patients based on our findings, it is important to tailor replacement to the individual bearing in mind the effect of other medications that alter the metabolism of hydrocortisone, including the initiation of growth hormone replacement or anti-epileptic medications and also different life style demands such as night shift work, as those subjects may require altered prescribing. A key feature leading to the successful and safe reduction in hydrocortisone replacement dosing is detailed and repeated patient education regarding the management of inter-current illness thus avoiding adrenal crisis through appropriate management rather than through over replacement of hydrocortisone.

In conclusion, in this part of the study comparing 24 hour free cortisol profile of hypopituitary patients with severe ACTH deficiency to that of healthy matched controls we have demonstrated that a low dose regimen of 10mg mane and 5mg tarde of HC most closely mimics healthy control cortisol dynamics without significant compromise in their quality of life compared with higher doses. In the next chapters I will present further studies that were performed to assess the impact of these regimens on tissue cortisol exposure through assessment of urinary cortisol metabolites, bone health and cardiometabolic risks.

## Chapter Four: The impact of 3 hydrocortisone dose regimens, and the resulting cortisol dynamics, on markers of bone turnover

## 4.1 Introduction

Optimising glucocorticoid (GC) replacement in hypoadrenal subjects in clinical practice remains challenging and GC excess has long been associated with osteoporosis(87, 155). However the adverse effects of glucocorticoid on bone health have largely been demonstrated in studies using supraphysiological doses of GC for anti-inflammatory therapeutic effect(109-111), yet an increased relative risk of 1.55 (95% CI 1.2-2.01) for vertebral fractures has also been seen with long term use of lower doses of prednisolone <2.5mg/day(112).

Although GC replacement therapy for primary and secondary adrenal insufficiency aims to use physiological dose regimens, there is conflicting evidence that GC replacement is also associated with deleterious effects on bone. The majority of available studies are in subjects with primary adrenal failure, with some studies finding a reduced bone mineral density (BMD) in men with increasing hydrocortisone (HC) equivalent doses(113, 114), and lower femoral neck BMD that correlated with weight adjusted GC dose(115), while others found no difference in BMD compared to the control population(20, 28), except in subjects on prednisolone who had a significant decrease in BMD(116).

There is a paucity of data on the effect of glucocorticoid replacement on bone metabolism in patients with ACTH deficiency. Peacey et al demonstrated in a cross-sectional and prospective cohort of 32 patients, 20 of whom had secondary adrenal insufficiency, that a reduction in GC dose by 30%, to 20 mg daily, was associated with a 19% increase in OC[1-49], a marker of bone formation, and a weak but significant negative correlation between absolute BMD and dose of hydrocortisone (HC) replacement(36). This finding was replicated in an observational study by Chikada et al in a group of primary (n=10) and secondary (n=5) hypoadrenal patients who demonstrated a negative correlation between HC dose and BMD and also cumulative HC dose and BMD(122). Wichers et al prospectively randomised 9 patients to 3 different HC dose regimens in a double-blind study and also demonstrated a significant increase in OC[1-49] as the dose of hydrocortisone decreased from 30mg to 15mg, but there was no control group and no comment on the replacement status of the other pituitary hormones, which can have significant effects on bone turnover(68).

The aim of this arm of my study was to determine in a prospective, randomised controlled trial, the effect of three commonly used HC replacement regimens on markers of bone turnover in a group of male hypopituitary patients, fully replaced with all other pituitary hormone replacement, including growth hormone.

## 4.2 Methods

### 4.2.1 Patients and Controls

As described previously, ten adult hypopituitary men with known severe ACTH deficiency, defined by a fasting morning total serum cortisol concentration <100nmol/l and a stimulated peak cortisol of <400nmol/l in response to insulin-induced hypoglycaemia (glucose <2.2mmol/l) were included.

All patients were on appropriate hormone replacement including growth hormone (GH), without alteration in dose for at least 3 months prior to and during the study. All 10 patients had diabetes insipidus and were on Desmopressin. No patient had serum sodium abnormalities to suggest under or over replacement with Desmopressin. Anterior pituitary hormone replacement therapy regimens were not adjusted during the study period, except for HC dose as per study protocol. No patient was on calcium or vitamin D supplementation.

Exclusion criteria specific to this arm of the study were conditions associated with altered bone turnover such as Paget's disease of bone, known osteoporosis or fracture within the previous 1 year. We excluded patients on glucocorticoids for purposes other than ACTH deficiency and those on agents that interfere with corticosteroid metabolism or bone metabolism such as anti-epileptic medications(120). As stated previously, female patients were excluded because of the variable effects of oestrogen status on bone turnover marker measurement(102), and also on corticosteroid binding globulin (CBG) levels, thus affecting total cortisol concentrations and cortisol kinetics(126). Healthy male controls (n=10), matched for age, BMI and WCM, were enrolled to undergo the same biochemical investigations and clinical examination as the patient group.

## 4.2.2 Study Design

10 patients were randomised in an open cross-over protocol to take 3 commonly prescribed doses of hydrocortisone, dose A (20 mg 0800 hours, 10 mg 1600 hours), dose B (10 mg 0800 hours and 1600 hours) or dose C (10 mg 0800 hours and 5mg 1600 hours). All 10 patients completed 6 weeks of each schedule and underwent assessment thereafter. In view of the short half-life of HC the patients took each dose regimen for a full 6 weeks to allow adequate time for a "washout" of the previous dose. At the end of each 6 week treatment schedule patients were admitted to our clinical research centre overnight. Following a physical examination, blood samples were drawn as described in Chapter 2 through a heparinised IV cannula hourly for 24 hours for cortisol measurement. Basal free thyroxine, thyrotropin, testosterone, sex hormone binding globulin, albumin, gonadotropins, prolactin and insulin like growth factor-I (IGF-I) parathyroid hormone (PTH), 25-hydroxyvitamin D (25OHD), calcium, albumin and renal function were sampled upon commencement of each 24 hour admission. In order to control for circadian variation and the effect of food intake on bone turnover markers(156, 157), subjects fasted from midnight during the admission and the morning dose of hydrocortisone was withheld until after venous sampling for bone turnover markers, which was completed between 0730 hours and 0800 hours (see below). Samples were centrifuged at 3,000 rpm for 15 minutes and stored in 1ml aliquots at -80 °C until analysis. 10 healthy matched controls underwent identical biochemical profiling as the patient group.

## 4.2.3 Laboratory Methods

### Bone Formation Markers

OC[1-49] and PINP were measured using an electrochemiluminescence immunoassay on the Elecsys 2010 analyser (Roche Diagnostics, Mannheim, Germany) as described in detail in Chapter 2. Normal reference ranges are age and sex dependent. Bone ALP, a marker of both bone mineralisation and maturation was measured by an immunoenzymatic assay (Immunodiagnostic Systems Ltd, Boldon, UK) on an automated enzyme linked immunosorbent assay platform as described in Chapter 2.

### Bone Resorption Markers

CTX-I was measured using an electrochemiluminescent immunoassay on the Elecsys 2010 analyser (Roche Diagnostics, Mannheim, Germany). TRACP5b was measured by ELISA (Immunodiagnostic Systems Ltd, Boldon, UK).

### Bone remodelling indices

As the normal reference range of bone turnover markers is dependent on age and sex, we calculated each result in standard deviation (SD) units, the Z-score, using measured data from our healthy matched control group. The Z-score was calculated as follows: $Z=(x-\mu)/\sigma$, where x = individual bone marker value, $\mu$ = mean bone marker value of controls of equal gender and age and $\sigma$ = SD of bone marker value of controls of equal gender and age. Using the Z-scores we were then able to calculate the bone remodelling balance index as follows: (formation (PINP) Z score – resorption (CTX-I) Z

score)(158, 159). We also calculated PINP:CTX-I ratio as an approximation of bone remodelling balance(160, 161).

Other biochemical indices

Serum 25OHD, PTH, cortisol and all other pituitary hormone measurements were measured as described in Chapter 2. Peak morning and afternoon cortisol levels were based on a single maximum cortisol level post HC administration for each patient. "Day-time" trough levels were based on a minimum single level prior to the next dose at 4pm afternoon, while "night-time" trough levels were based on the single minimum cortisol level after the afternoon dose, but before 2am in order to account for the lack of physiological morning cortisol rise.

## 4.2.4 Statistical Methods

Results are reported as mean (SD) or median (interquartile range, IQR) as appropriate. Bone turnover markers and other biochemical indices were analysed for normality using the D'Agostino-Pearson normality test. Between group differences were assessed using ANOVA, or repeated measures ANOVA or the non-parametric equivalent, followed by application of a multiple comparison test. Correlations were analysed using the Spearman or Pearson correlation co-efficient, as appropriate based on normality tests. Significance was defined for p-values <0.05.

## 4.3 Results

Detailed patient characteristics have already been presented in Chapter 3, Table 3.1 Patients were appropriately age, sex, BMI and WCM matched with controls. There were no differences in measured thyroid hormone, testosterone, IGF-I, 25OHD, or PTH between the patients on stable pituitary replacement therapy and healthy control measured hormone status (Table 4.1).

**Table 4.1** Patient and control baseline data
Results expressed as mean ± standard deviation (SD), BMI-body mass index, WCM-waist circumference, eGFR – estimated glomerular filtration rate

|  | Patients n=10 | Controls n=10 | p value |
|---|---|---|---|
| Age (years) | 46 ± 15 | 45 ± 15 | 0.9 |
| BMI (kg/m$^2$) | 29.8 ± 5.3 | 29.1 ± 4.6 | 0.5 |
| WCM (cm) | 105 ± 14 | 103 ± 11 | 0.53 |
| Basal cortisol (nmol/L) | 76.8 ± 6.5 | 403.3 ± 122.4 | <0.001* |
| Free T4 (pmol/l) | 11.3 ± 2.1 | 10.9 ± 1.0 | 0.6 |
| IGF-I (ug/L) | 163 ± 45 | 152 ± 32 | 0.5 |
| Testosterone (pmol/L) | 14.2 ± 4.1 | 16.4 ± 7.7 | 0.4 |
| Creatinine (µmol/L) | 100 ± 5 | 77 ± 3 | 0.001* |
| eGFR (mL/min/1.73m$^2$) | 73 ± 5 | 97.6 ± 5 | 0.004* |
| Serum calcium (mmol/L) | 2.32 ± 0.01 | 2.30 ± 0.02 | 0.47 |
| Albumin (g/L) | 40 ± 0.8 | 39 ± 2 | 0.66 |
| PTH (ng/L) | 41 ± 3 | 32 ± 2 | 0.06 |
| 25OHD (nmol/L) | 49 ± 3.9 | 56 ± 3 | 0.17 |

Although the control group had lower mean creatinine compared to patients (Table 4.1), only one patient had a eGFR <60ml/min/1.73m2 and there was no difference in renal function between the three hydrocortisone dose regimens, (p=0.8, Table 4.2). Serum electrolytes were recorded at each visit and there were no abnormalities suggestive of over or under replacement with Desmopressin, nor was there any difference in 24hour urine volume between dose regimens or compared to controls (p=0.18 and p=0.7 respectively). There was no correlation between 25OHD and PTH in either the group as a whole (r= – 0.04, p=0.8) or when analysed for patients only (r= 0.02, p=0.9) or for controls only (r= 0.08, p=0.8). There was also no correlation between renal function or 25OHD (r= 0.2, p=0.2) or PTH (r= -0.15, p=0.4) in the whole group or when analysed for patients and controls separately. No patient or control had PTH above the normal reference range of 65ng/L.

**Table 4.2** Bone and renal indices between dose regimens and compared to controls

Data are expressed as Mean ± SD or Median (Interquartile Range) *difference between dose A and Dose C,
**difference between A and C and B and C, ^difference between controls and all doses,
^^difference between controls and dose C, v- compared to

| | Dose A 20 mg/10 mg n=10 | Dose B 10 mg/10 mg n=10 | Dose C 10 mg/5mg n=10 | p value v dose regimens | Controls n=10 | p value dose v controls |
|---|---|---|---|---|---|---|
| PTH (ng/L) | 42.9 ± 10 | 38.3 ± 13 | 41.9 ± 12.9 | 0.34 | 32.3 ± 8.2 | 0.17 |
| 25 OHD (nmol/L) | 54.1 ± 18.8 | 48.4 ± 13 | 44.6 ± 13.5 | 0.18 | 56.2 ± 9.7 | 0.25 |
| Creatinine (µmol/L) | 99.2 ± 20.5 | 103 ± 17 | 99.1 ± 17.5 | 0.82 | 77 ± 3 | 0.001^ |
| GFR (ml/min/1.73m2) | 75 ± 21 | 70.2 ± 14 | 75 ± 18 | 0.80 | 97.6 ± 5 | 0.006^ |
| Calcium (mmol/L) | 2.32 ± 0.07 | 2.33 ± 0.07 | 2.32 ± 0.07 | 0.88 | 2.30 ± 0.02 | 0.84 |
| PINP (µg/L) | 54.9 (36.4-139.5) | 71.6 (42.9-126.7) | 102.4 (54.9-166.1) | 0.045* | 45.65 (37.8-30.2) | 0.14 |
| OC[1-49] (µg/L) | 21.9 (19.1-42.3) | 26.3 (20.5-39.3) | 34.2 (23.7-43.4) | 0.006** | 18.6 (15.7-30.2) | 0.06 |
| bone ALP (µg/L) | 16.5 ± 10.6 | 15.5 ± 7.9 | 14.4 ± 7.6 | 0.4 | 12.1 ± 3.5 | 0.95 |
| CTX-I (µg/L) | 0.51 (0.29-0.96) | 0.47 (0.26-0.64) | 0.58 (0.29-0.84) | 0.43 | 0.32 (0.27-0.56) | 0.65 |
| TRACP5b (IU/L) | 2.92 (2.27-3.76) | 2.69 (2.27-3.53) | 2.76 (2.07-3.09) | 0.97 | 2.79 (2.64-2.97) | 0.92 |
| PINP:CTX-I Ratio | 137 ± 43.5 | 181 ± 79.3 | 208 ± 56 | 0.015** | 136 ± 49.2 | 0.02^^ |
| Formation Z Score | 0.035 (-0.66 - 3.21) | 0.66 (-0.42 - 2.71) | 1.81 (0.03 - 4.19) | 0.045* | - | - |
| Resorption Z Score | 0.39 (-0.56 - 2.38) | 0.19 (-0.75 - 0.98) | 0.72 (-0.6 - 1.85) | 0.43 | - | - |

### 4.3.1 Bone Turnover Markers

Bone formation markers, PINP was significantly higher for dose C compared to A and OC[1-49] was higher in dose C compared to both dose A and dose B. In fact PINP was 86% higher and OC was 56% higher in dose C compared to dose A . There was no difference in bone ALP, or in the two markers of bone resorption, CTX-I and TRACP5b. (Table 4.2)

There was no correlation between the mean 24 hour cortisol concentration and any bone turnover marker for dose A or for controls; however mean 24 hour cortisol concentrations for dose B demonstrated a moderate, non-significant negative correlation for OC[1-49], PINP and bone ALP, and a strong, significant negative correlation with CTX-I and TRACP5b (Figure 4.1). The lowest dose regimen, dose C has moderate to strong negative correlations for mean 24 hour cortisol concentrations and each bone turnover marker, however only OC[1-49] and CTX-I are statistically significant (Figure 4.1).
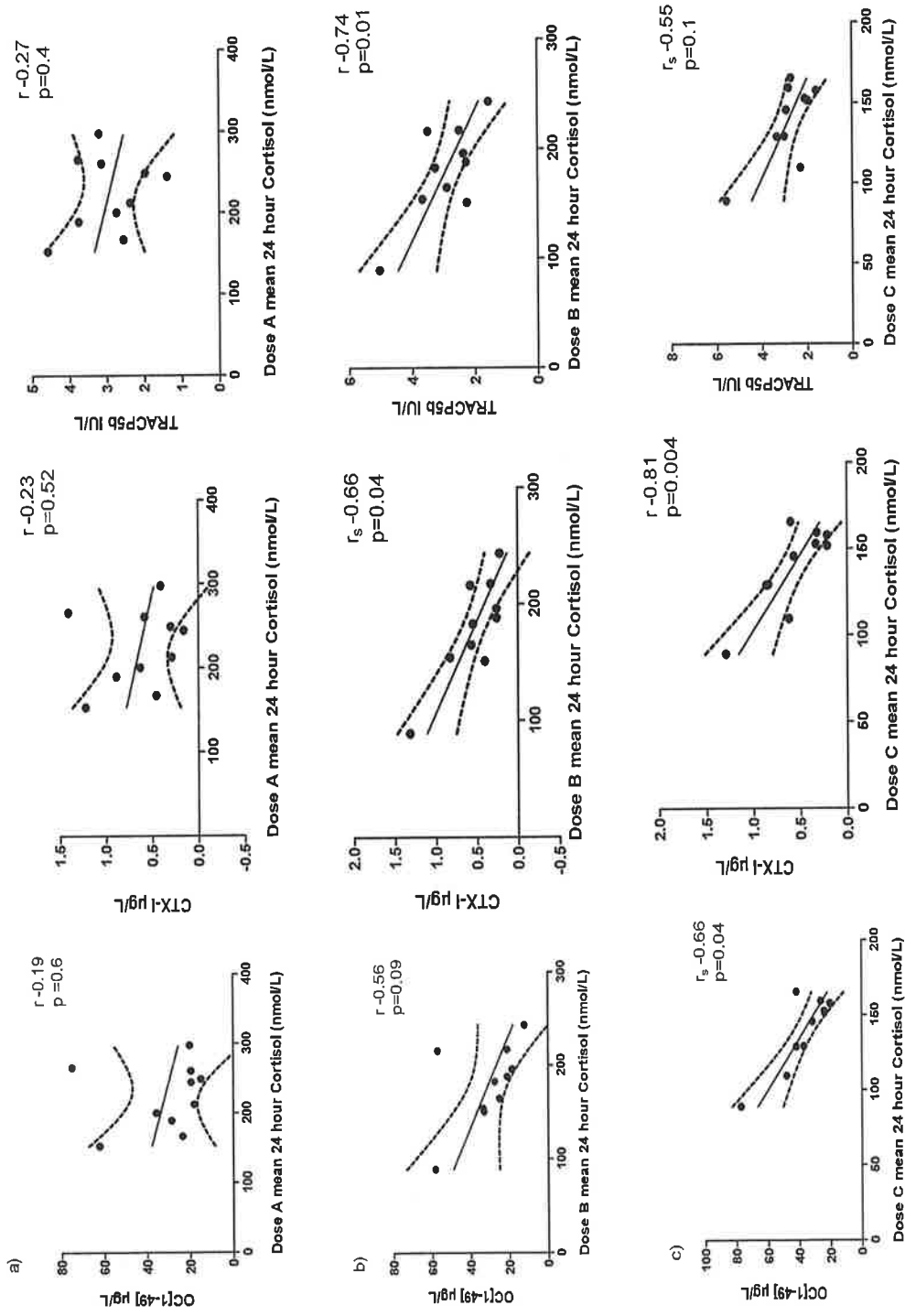
**Figure 4.1-** Mean 24 hour total cortisol concentration correlations with selected bone turnover markers for dose regimens a) Dose A correlations, b) dose B correlations, c) dose C correlations

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When the mean 24 hour cortisol concentrations for patient data, regardless of dose regimen, were pooled there was a moderate significant negative correlation for OC [1-49] and PINP and a near significant negative correlation for CTX-I (Figure 4.2). Non-significant data for TRACb5 and Bone ALP are not shown.
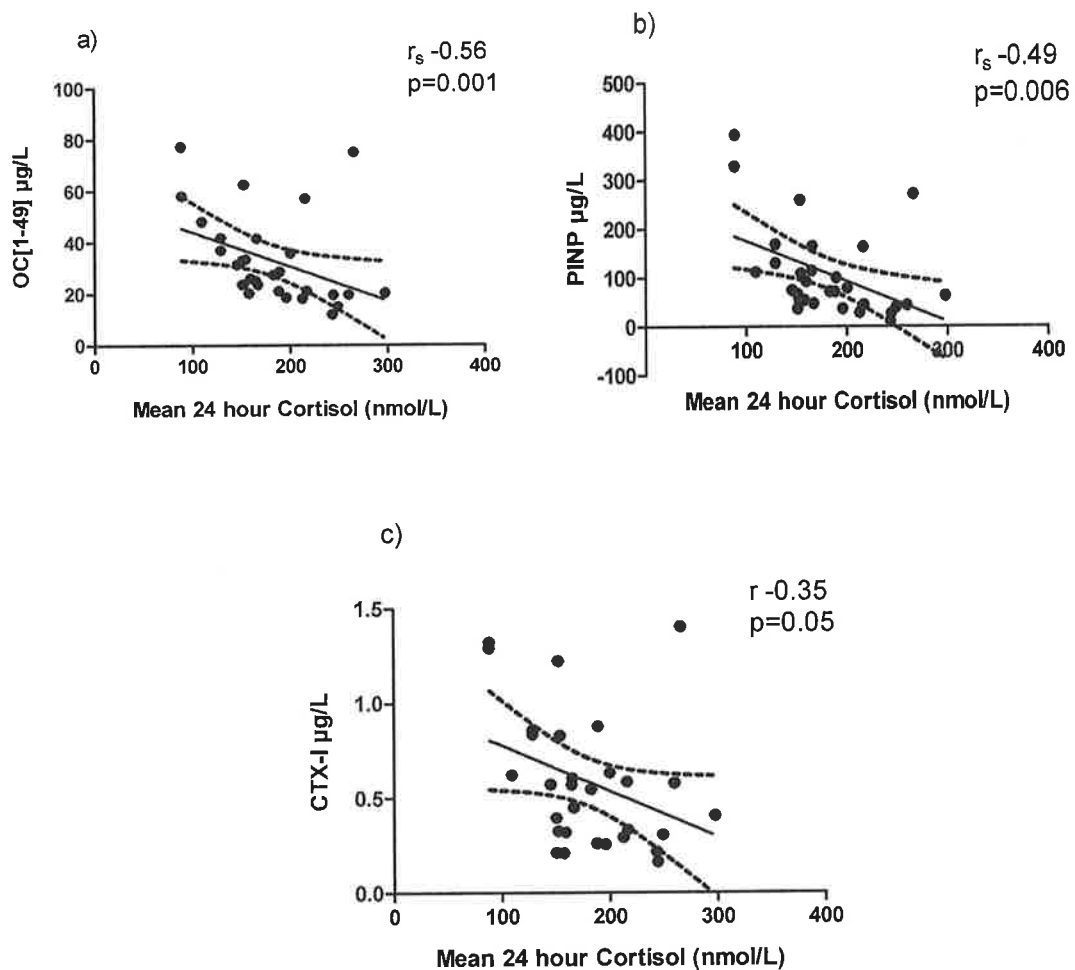


**Figure 4.2** Mean 24 hour total cortisol concentration correlations with selected bone turnover markers for all dose regimens pooled
a) OC[1-49], b) PINP, c) CTX-1, r –pearson correlation, rs –spearman correlation.

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There was no correlation in any dose regimen between peak total serum cortisol concentration after either the morning or afternoon dose of hydrocortisone and any bone turnover marker (Table 4.3). For dose A and controls, there was no correlation between day-time or night-time trough levels and any bone marker. Day-time and night-time trough levels for dose B correlated moderately with bone turnover but were only significant for CTX-I in the night-time trough afternoon, r= -0.65, p=0.04, (Table 4.3). Dose C day-time trough levels demonstrated strong significant negative correlations for all bone turnover markers as shown in Figure 4.3, while there were moderate negative correlations between the night-time trough and bone turnover markers that were non-significant except OC[1-49] r= -0.64, p=0.05 (Table 4.3).

**Table 4.3** Peak and trough total cortisol correlations with bone markers for patients and controls
*Significant correlation, ^near significant correlation

|  |  | Dose A | Dose B | Dose C | Controls |
|---|---|---|---|---|---|
| **Trough Cortisol Day-time** | OC[1-49]ug/L | r 0.15 p=0.68 | r -0.39 p=0.26 | **r -0.89*** **p=0.0008** | r 0.16 p=0.6 |
|  | PINP ug/L | r 0.14 p=0.7 | r -0.43 p=0.22 | **r -0.83*** **p=0.005** | r 0.22 p=0.54 |
|  | Bone ALP ug/L | r 0.11 p=0.76 | r -0.5 p=0.14 | **r -0.74*** **p=0.02** | r 0.13 p=0.71 |
|  | CTX-I ug/L | r -0.04 p=0.9 | r -0.61^ p=0.06 | **r -0.77*** **p=0.009** | r 0.07 p=0.84 |
|  | TRACP5b IU/L | r -0.34 p=0.33 | r -0.61^ p=0.06 | **r -0.64*** **p=0.05** | r -0.16 p=0.66 |
| **Trough Cortisol Night-time** | OC[1-49]ug/L | r -0.36 p=0.31 | r -0.53 p=0.11 | **r -0.64*** **p=0.049** | r -0.49 p=0.15 |
|  | PINP ug/L | r -0.37 p=0.3 | r -0.49 p=0.15 | r -0.43 p=0.2 | r -0.20 p=0.58 |
|  | Bone ALP ug/L | r -0.17 p=0.63 | **r -0.58^** **p=0.08** | r -0.33 p=0.34 | r -0.01 p=0.9 |
|  | CTX-I ug/L | r -0.49 p=0.14 | **r -0.65*** **p=0.04** | r -0.46 p=0.18 | r -0.52 p=0.12 |
|  | TRACP5b IU/L | r -0.41 p=0.24 | r -0.48 p=0.16 | r -0.29 p=0.18 | r -0.29 p=0.42 |
| **Peak Cortisol Day-time** | OC[1-49]ug/L | r 0.15 p=0.75 | r -0.17 p=0.49 | r 0.46 p=0.18 | r -0.16 p=0.65 |
|  | PINP ug/L | r 0.2 p=0.56 | r 0.26 p=0.47 | r 0.23 p=0.53 | r 0.16 p=0.66 |
|  | Bone ALP ug/L | r 0.37 p=0.28 | r -0.53 p=0.1 | r 0.41 p=0.23 | r -0.13 p=0.70 |
|  | CTX-I ug/L | r 0.13 p=0.71 | r 0.23 p=0.51 | r 0.26 p=0.47 | r -0.38 p=0.28 |
|  | TRACP5b IU/L | r 0.31 p=0.4 | r -0.05 p=0.87 | r 0.15 p=0.67 | r -0.23 p=0.51 |
| **Peak Cortisol Night-time** | OC[1-49]ug/L | r 0.11 p=0.75 | r -0.16 p=0.64 | r -0.36 p=0.31 | r -0.14 p=0.68 |
|  | PINP ug/L | r 0.21 p=0.56 | r -0.21 p=0.56 | r -0.13 p=0.73 | r -0.18 p=0.6 |
|  | Bone ALP ug/L | r 0.37 p=0.27 | r -0.54 p=0.11 | r -0.55 p=0.1 | r -0.37 p=0.28 |
|  | CTX-I ug/L | r 0.14 p=0.71 | r -0.19 p=0.58 | r -0.3 p=0.39 | r -0.44 p=0.2 |
|  | TRACP5b IU/L | r 0.31 p=0.39 | r -0.05 p=0.87 | r -0.14 p=0.7 | **r -0.7*** p=0.03 |

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## 4.3.2 Bone remodelling balance and bone marker indices

There was a significant increase in the formation Z-score to a median (IQR) of +1.81(0.03-4.19) when subjects were on dose C (Table 4.2), whereas there was no significant difference in resorption Z-scores between dose regimens. The bone remodelling balance index showed a significantly positive remodelling balance for dose C compared to the other dose regimens (p=0.03) (Figure 4.3).
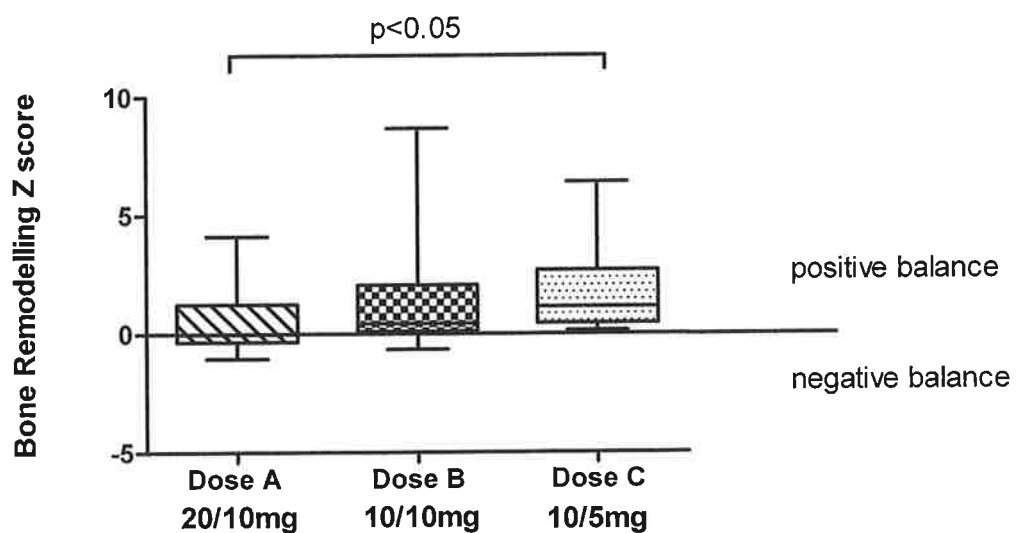


**Figure 4.3** Bone remodelling balance between dose regimens
z-score – standard deviation score,
calculated as (formation (PINP) Z score – resorption (CTX-I) Z score)

The PINP:CTX-I ratio was significantly higher for dose C (208.7±56.5) compared to the other dose regimens (A: 137.5 ± 43.5; B: 181.7±79.3)(p=0.015) (Figure 4.4), consistent with increased formation on that dose. The ratio was also higher in dose C compared to controls (136.3 ± 49.2) (p=0.02).

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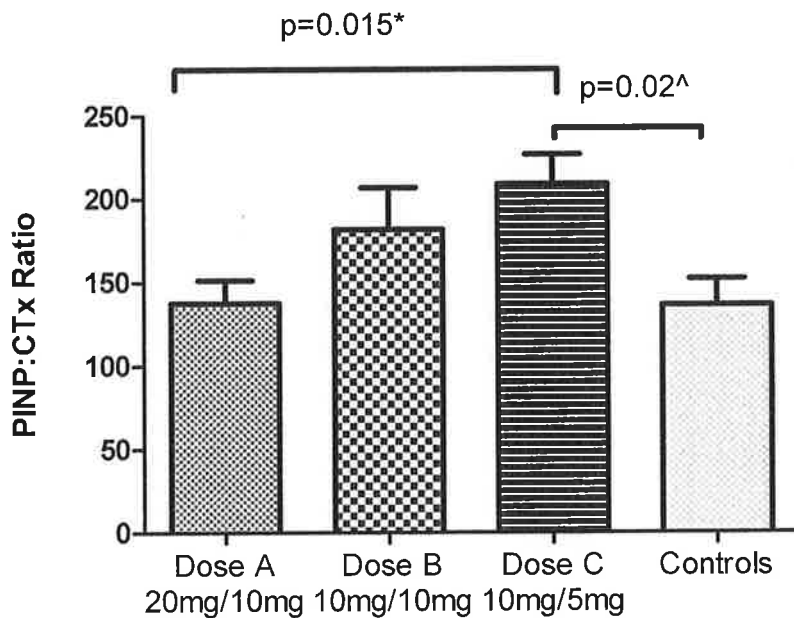
**Figure 4.4** PINP:CTX-I ratio between dose regimens and compared to controls.
*repeated measures ANOVA between doses, ^standard ANOVA compared to controls
PINP – pro collagen Type 1 telopeptide, CTX-I – C Terminal cross-linking telopeptide

### 4.3.3 Renal indices and bone markers

There was no correlation between renal function or serum PTH and any of the bone turnover markers when analysed for the whole group, patients alone or controls alone (data not shown). However, there was a negative correlation in the whole group between 25OHD and PINP (r= -0.39, p=0.01), OC[1-49] (r= -0.39, p=0.01 ), CTX-I (r= -0.32, p=0.04) and bone ALP (r= -0.43, p=0.006), but not TRACP5b (r= -0.24, p=0.12). This correlation was lost when patients and controls were analysed separately.

## 4.4 Discussion

This study has demonstrated in a prospective, randomised-controlled crossover trial on the effects of 3 commonly used HC replacement regimens in panhypopituitary subjects on full pituitary replacement that using a lower dose HC leads to increased bone formation markers, without a significant change in resorption markers. We have also demonstrated a relationship between 24 hour serum cortisol profile and bone formation markers, particularly between trough serum cortisol levels and both bone formation and resorption markers. Our results indicate that lower cortisol exposure in hypopituitary patients, receiving what would be regarded as "physiological" hydrocortisone replacement, leads to a positive bone remodelling balance with increased bone formation, both of which are associated with a more favourable bone effect.(158)

Glucocorticoids (GC) induce a negative calcium balance as evidenced by reduced intestinal calcium absorption and increased renal calcium excretion with reduced bone formation through suppression of osteoblast activity(106). There are conflicting data regarding GC effects on resorption, but it has been shown that GC administration promotes osteoclastogenesis(107) which, in combination with the changes described above, could lead to increased bone fragility with consequent increased fracture risk(106, 108).

Bone turnover markers are a rapid and accessible way to assess bone metabolism, yet they are associated with a number of pre-analytical factors that may impact on their measurement. In order to increase the reliability and reproducibility of our results, we controlled for a number of these variables including circadian variation, food intake and

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physical activity(162) by taking the samples on each of the three occasions at the same time in the morning, fasting, and in a standardised setting. It is known that endocrine status effects bone turnover(162), and our patients were on the full, appropriate and stable modern pituitary replacement therapy including GH with no differences in circulating peripheral hormones concentrations compared to the healthy matched controls. We are, therefore, confident that the observed alterations in bone turnover seen in our cohort are due to the effect of the alteration in glucocorticoid dose during the study protocol.

We demonstrated a 19-56% increase in OC[1-49], similar to the findings of Peacey et al, who showed that a 30% dose reduction in 19 patients with either primary or secondary hypoadrenalism was associated with a 19% increase in OC[1-49], and they demonstrated a weak negative correlation between absolute BMD and the dose of HC prior to dose adjustment(36). Wichers demonstrated in 9 hypopituitary patients with severe ACTH deficiency in a cross-over double blind study an increase in OC[1-49] with a reduction in glucocorticoid dose, significant for reduction from 30 mg to 20 mg (p<0.05) and from 30 mg to 15 mg (p<0.01) after only two weeks on each treatment schedule (68). They were unable to demonstrate any alterations in other bone turnover markers, including markers of bone resorption. In that study however there was no control group, patients were GH deficient but not receiving GH replacement and there was no comment on other pituitary replacement. Suliman et al examined the effect of 3 glucocorticoid replacement schedules on bone turnover markers in 9 subjects (1 ACTH deficient): HC 10 mg morning, 5 mg evening; HC 10 mg morning, 5mg at 1600 hours and 5 mg at evening; dexamethasone at 0.1mg/15kg body weight daily with comparison of findings

to unmatched, younger, healthy controls(95). They demonstrated lower ionised calcium and higher 25OHD across all replacement schedules compared to controls with no difference in PTH between doses or compared to controls. Except for a reduced resorption marker, urinary free deoxypyridinoline, in those receiving dexamethasone compared to hydrocortisone, there were no other differences in markers of bone formation or resorption between schedules.

In our study, the changes in bone formation correlated negatively with trough, rather than peak cortisol concentrations, indicating that glucocorticoid clearance may have a significant influence on bone health. Cortisol dynamics are likely to be as important as the overall prescribed dose since studies on bone turnover and mineral density have demonstrated more pronounced adverse effects in patients on synthetic long-acting glucocorticoids (prednisolone and dexamethasone) compared to those on hydrocortisone or cortisone acetate(20, 95, 115, 116). Trials assessing metabolic outcome using extended or modified release hydrocortisone will be of interest.

Changes in the "uncoupling index" or "bone remodelling balance" have been shown to correlate with later changes in BMD(158, 159) and a positive bone remodelling balance with associated increased bone turnover is associated with increased bone formation and mineralisation. However, very high bone turnover is associated with an increased fraction of newly mineralised bone which may be associated with suboptimal resistance(163). Observational studies have shown that there is an increase in bone resorption, without a concurrent increase in bone formation in men after the age of 60

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years and this imbalance is thought to be responsible for age related bone loss in healthy men(164, 165). We have demonstrated a more positive bone remodelling balance, and increased PINP:CTX-I ratio reflecting increased bone formation on low dose hydrocortisone. Our control subjects are likely to have quiescent bone milieu and since hypopituitary patients are at higher risk of bone loss than healthy men increased bone formation suggests a favourable bone remodelling balance leading to net bone gain.

Whether the differences we have demonstrated in bone formation following dose reduction are likely to be maintained in the long term is unclear. Hermus et al demonstrated, in 9 premenopausal women with Cushing's disease, that surgical remission resulted in a 400% increase in OC[1-49]. Increased bone formation was persistent at 2 years with an ongoing 100% increase in OC[1-49] from pre-operative levels(166). Van Staa et al found, in a population of patients prescribed glucocorticoid for anti-inflammatory purposes, that the relative rate of vertebral fracture decreased from 2.4 in the first year after discontinuation of glucocorticoid to 1.8 and subsequently returned to healthy control values thereafter(112). These findings indicate that beneficial effects of glucocorticoid dose reduction on bone may be prolonged.

There are a few limitations with respect to this part of the study. There was no true "wash-out" period in this study and it is conceivable that this may confound the analysis. Our patient group had reduced renal function compared to controls and renal impairment is known to affect bone turnover and bone marker measurement although this is usually

only significant for GFR <50mls/min/1.73m$^2$, in particular for OC and CTX-I(162). The relatively reduced renal function in our cohort is unlikely to have significantly impacted on our results as only 1 patient had a GFR <50mls/min/1.73m$^2$ (45mls/min/1.73m$^2$); that patients results did not differ significantly from the group and sub-analysis excluding those results did not alter the findings of this arm of the study. There was no difference in renal function between dose regimens allowing accurate between group comparison of hydrocortisone effect on bone turnover markers which is the primary aim of this study. Although 2 patients had controlled hypertension and were on angiotensin converting enzyme inhibitors, no patient in the whole group had evidence of overt secondary hyperparathyroidism and there was no correlation between renal function, PTH or 25OHD. We did demonstrate a weak negative correlation for the whole group between 25OHD and bone turnover markers, except TRACP5b. This finding may possibly reflect the effect of hypovitaminosis D on bone turnover markers but is unlikely to account for between-dose differences as the correlation was lost when analysed for patients alone or between-dose regimens.

In conclusion, this study has demonstrated the role of cortisol dynamics in bone turnover in HC replaced hypopituitary subjects, along with a beneficial metabolic end organ effect of aiming to prescribe lower replacement glucocorticoid doses through increased bone formation and a positive bone remodelling balance.

## Chapter Five: Tissue Cortisol Exposure

## 5.1 Introduction

Hypopituitary patients exhibit abnormalities of protein, fat and carbohydrate metabolism, which lead to metabolic and body composition alterations that are likely both secondary to the pituitary hormone deficiency and to clinical attempts to replace those hormones. There is a propensity to central obesity as visceral fat deposition is significantly increased compared to control subjects with similar BMI(167). In the general population visceral adiposity is associated with the metabolic syndrome, insulin resistance/diabetes mellitus, hypercholesterolaemia and hypertension(168, 169). Although the role of GH deficiency and replacement in metabolic outcomes in hypopituitary subjects has been extensively investigated(170), the contribution of ACTH deficiency and glucocorticoid replacement on morbidity and mortality in this patient group is under increasing scrutiny. Importantly, even patients who have only mild increases in serum cortisol levels are susceptible to changes in adipose tissue distribution(171), that when seen in Cushing's syndrome are associated with adverse metabolic outcome.

Although physicians have aimed to prescribe lower glucocorticoid replacement regimens in recent years, it is possible that subtle increased chronic glucocorticoid exposure in patients on GC replacement therapy might contribute to increased morbidity. In a study on GH deficient adults on GH replacement, Dullaart et al demonstrated an adverse metabolic profile in 117 ACTH deficient subjects on GC replacement compared to 48

ACTH sufficient, with higher triglycerides (p=0.001) and increased prevalence of hyperglycaemia or known DM (p=0.03)(26). The differences between the groups persisted after adjustment for age, sex and smoking status. This data is consistent with the study by Fillipson et al on a cohort of GH deficient hypopituitary patients wherein patients on hydrocortisone replacement had increased total cholesterol, triglycerides, waist circumference and HbA1c compared to the ACTH sufficient patients(29). Patients on hydrocortisone equivalent doses of <20mg/day did not differ in metabolic endpoints compared to the ACTH sufficient patients, while those patients on hydrocortisone equivalent doses ≥ 20mg/day had an adverse metabolic profile. In that study all new cases of stroke and MI occurred in the ACTH deficient GC replaced group. These findings may reflect the fact that patients with both GHD and ACTHD may have more severe pituitary disease and are likely to be on other pituitary hormone replacement that may have impact on metabolic outcome, but the results demonstrate the need to reassess GC replacement practices in order to identify the pathophysiology behind the increased morbidity and mortality, along with investigating the impact of dose reduction.

At the tissue level glucocorticoid action is modulated by isozymes of 11 beta-hydroxysteroid dehydrogenase (11β-HSD), type 1 and 2. Although 11β-HSD1 is a bidirectional enzyme, in vivo it primarily acts in the liver and in adipose tissue to regenerate active cortisol from inactive cortisone and is abundantly expressed in those tissues(133). 11β-HSD1 itself is modulated by many factors, including GH/IGF-I (172), thyroid hormone, insulin, cytokines, glucocorticoids and sex steroids(133). In view of such interactions, patients with hypopituitarism on various hormone replacements may have alterations in tissue specific exposure to glucocorticoid, independent of circulating

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values. In vitro, glucocorticoids themselves positively regulate 11β-HSD1 mRNA and activity and expression and activity of 11β-HSD1 has been implicated in many of the features of metabolic syndrome, notably hepatic glucose output, the accumulation of visceral adipose tissue and muscle insulin resistance(133).

In view of the in vitro evidence suggesting up regulation of the enzyme pathway by glucocorticoids it was our hypothesis is that 11β-HSD1 is up-regulated in hypopituitarism due to un-physiological glucocorticoid replacement therapy and the ensuing increase in tissue specific cortisol generation contributes to the metabolic changes reported in hypopituitarism. Previous studies in the hypopituitary population have concentrated on the effect of GH deficiency and replacement on corticosteroid metabolism, while none to our knowledge have examined the effect glucocorticoid dose on tissue exposure to cortisol in this group.

We aimed to prospectively examine the effect of 3 commonly prescribed HC replacement regimens on 11β-HSD1 by assessing the urinary corticosteroid metabolite profile within a cohort of panhypopituitary, GHD males, replaced with GH and all other pituitary hormones, compared to healthy matched controls in a randomised crossover protocol.

## 5.2 Methods

### 5.2.1 Patients and Controls

Ten adult male hypopituitary patients with known severe ACTH deficiency were included in a randomised-controlled, crossover study of three different hydrocortisone replacement regimens. Patients had been diagnosed and treated for pituitary tumours between 3 to 18 years prior to inclusion in the study. All patients were on stable appropriate pituitary hormone replacement, including GH, without alteration in dose for at least 3 months prior to and during the study. Hormone replacement therapy regimens were not adjusted during the study period, except for HC dose, as per study protocol. Hormone deficiencies were defined based on criteria previously explained in Chapter 2.

Subjects were randomised to a cross-over protocol of 3 commonly prescribed doses of HC for 6 weeks of each regimen as previously described. At the end of each 6 week treatment schedule patients underwent a physical examination that included recording body mass index (BMI), waist circumference (WCM), baseline pituitary blood tests, biochemical profiling as previously described in previous chapter 2 and a 24 hour urine collection for measurement of urinary corticosteroid metabolites. First void urine on the day of admission was discarded and the urine collection commenced thereafter for 24 hours as per standard clinical practice. Quantitative data on excretion of individual steroids requires accurate 24 hour sampling and 1ml of a 24-hour collection for analysis.

10 healthy male controls, matched for age, BMI and WCM underwent the same biochemical investigations and clinical examination.


### 5.2.2 Laboratory Methods

The major route of cortisol metabolism comprises the interconversion of cortisol (Kendall's compound F) to cortisone (Kendall's compound E) through the activity of 11 β-HSD isozymes or reduction of the C4-5 bond by either 5α-reductase or 5β-reductase to yield 5α-THF (allo THF) and tetrahydrocortisol (THF) respectively[133] THF, allo-THF and tetrahydrocortisone (THE) are rapidly conjugated with glucuronic acid and excreted in the urine[133] . (Figure 5.1)
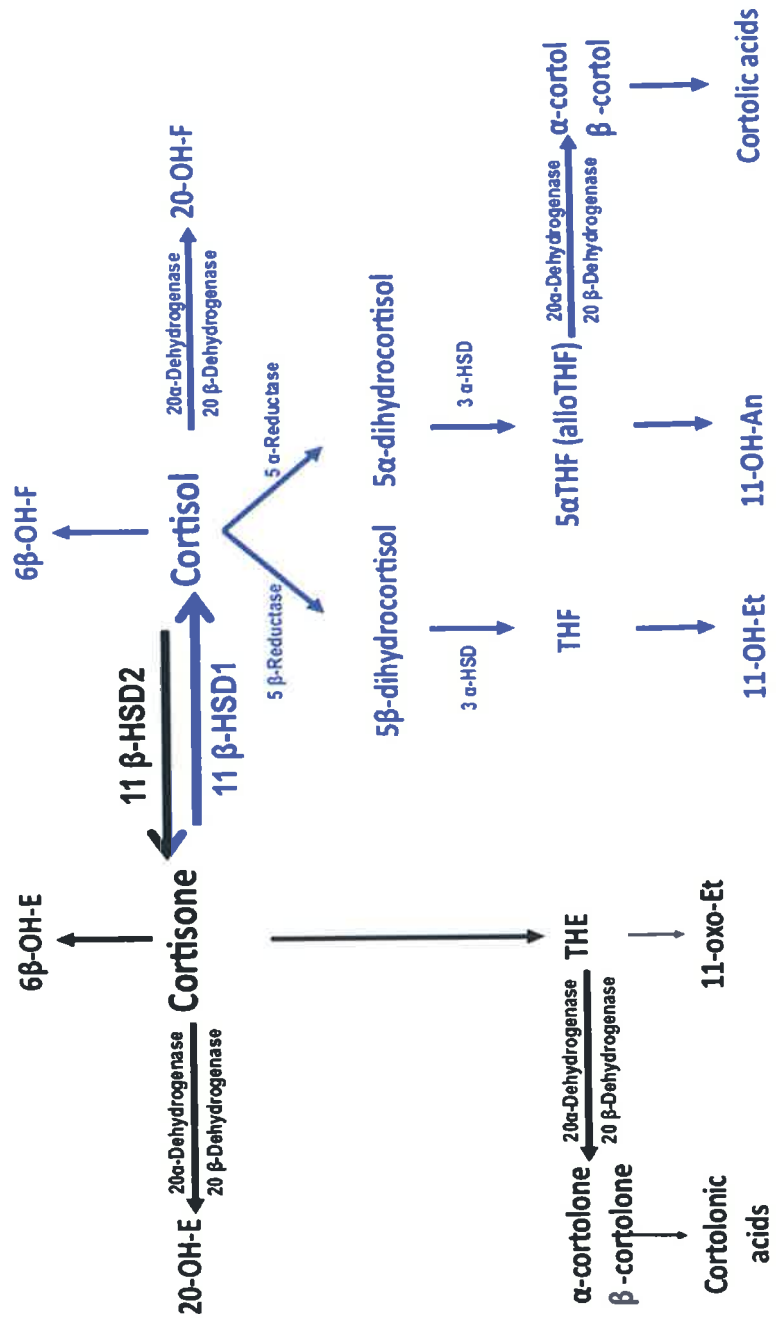
**Figure 5.1** The major pathways involved in cortisol metabolism
(E= cortisone, F= cortisol, Et = etiocholanolone, An = androsterone, 11βHSD – 11 β hydroxysteroid dehydrogenase).

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## Measurement of 11βHSD activity:

Gas Chromatography/Mass Spectrometry (GC/MS) urinary steroid analysis was carried out at the Institute for Biomedical Research, Centre for Endocrinology, Diabetes and Metabolism (CEDAM), University of Birmingham and was based on the method described by Palermo et al(130). At the CEDAM approximately 40 steroids are targeted for selected-ion-monitoring analysis, which cover all disorders of steroid synthesis and metabolism. The pathway has been divided to reflect steroid hormone production into metabolites of androgens, mineralocorticoids, progesterone and glucocorticoids (Figure 5.2).
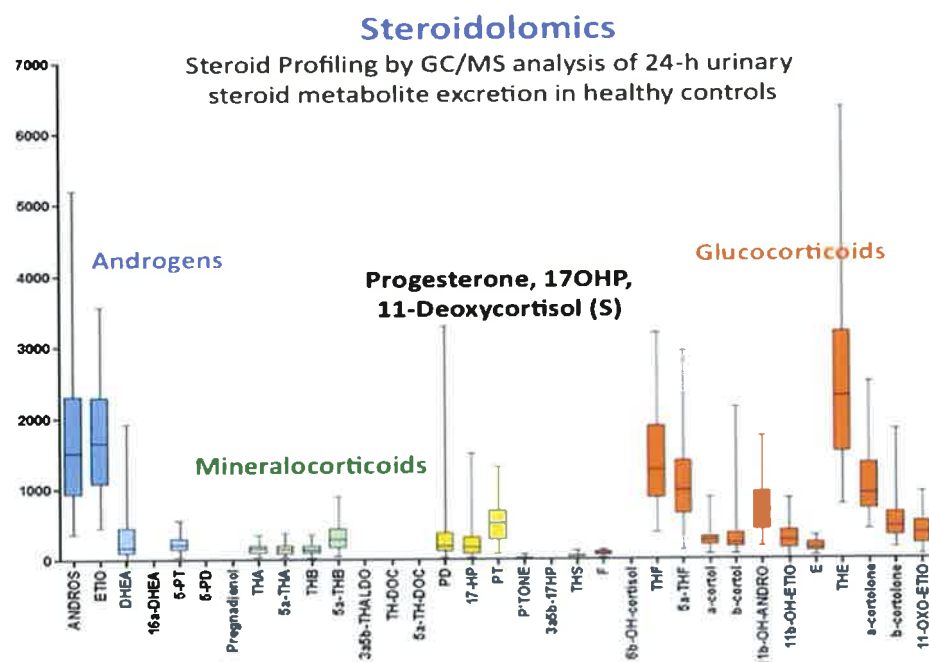


**Figure 5.2** Representative graph of steroid metabolite excretion (μg/24 hours) assessed by 24 hour urinary GC/MS in healthy adults
Results divided into metabolites of androgens, mineralocorticoids and progesterone (17-OHP and 11-deoxycortisol) and glucocorticoids. Box and whisker plots represent mean and 5th and 95th percentile. Courtesy of CEDAM

Most hormonal imbalances caused by enzyme deficiencies result in depletion of a steroid product and build-up of the upstream precursors. Thus, a ratio of metabolites of the substrate to metabolites of the product should indicate if there is such a deficiency. In this paper ratios of glucocorticoid metabolites are used to determine the relative activity of 11 β-HSD1 and 11 β-HSD2.

### 5.2.3 Laboratory Analysis at CEDAM

The following isotope labelled internal standards were used; $(9,11,12,12-^2H)$ cortisol and $(9,12,12-^2H)$ cortisone. The standards were calibrated by high performance liquid chromatography (HPLC) analysis of solubilised, non-labelled standard on known weight. Free steroid was extracted using Sep-pak C18 cartridges 104. Labelled steroid $d_4$-cortisol (0.18μg), and $d_3$-cortisone (0.12μg), as well as internal standards (stigmasterol and cholesteryl butyrate), 200μg were then added. The samples were then derived using 100μl of 2% methoxyamine hydrochloride in pyridine and 50μl of trimethylsilylimidzole. Lipidex chromatography was then used to purify the steroid derivative.

GC/MS was carried out using a Hewlett Packard 5970 mass spectrometer and 15m fused-silica capillary column, 0.25mmID, 0.25μm film thickness (J&B Scientific, Folsom CA, USA) using 2μl of sample. Steroids were quantified by comparing individual peak area to the peak area of the internal standards, for cortisol fragment 605m/z compared to 609 m/z and for cortisone fragment 531 m/z compared to 534 m/z. The relative peak area was calculated and the metabolite concentration expressed as μg/24hr. A quality

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control (QC) was analysed with each batch.  The intra and inter-assay co-efficient of variance was <10%.

Pituitary hormone analysis and pituitary hormone deficiencies are as described in Chapter 2.

### 5.2.4 Statistical methods

Continuous data were summarised using means and standard deviations (or standard error of mean) if parametrically distributed or medians and inter-quartile ranges if non-parametrically distributed.  Parametric data was compared using a paired t-test and non-parametric data was analysed using a Mann-Whitney test.  Multiple comparisons were assessed using one-way analysis of variance (ANOVA), or Kruskal-Wallis for non-parametric data.  The level for statistical significance was taken at $p<0.05$.

## 5.3 Results

### 5.3.1 Patient Characteristics

The aetiology of hypopituitarism was as follows: 5 subjects had non-functioning pituitary adenomas, 2 had craniopharyngioma, 2 had macroprolactinoma and 1 had treated Cushing's disease (basal cortisol 87nmol/l and peak cortisol 113nmol/l), all had pituitary surgery and one patient had radiotherapy. All but one subject had complete anterior pituitary failure, with the 10th patient being sufficient in gonadotropins. All 10 patients

had Diabetes Insipidus and all had normal electrolytes at each visit and no evidence of over or under-replacement with Desmopressin (Table 3.1, Chapter 3). There was no difference in urinary volume between dose regimens (p=0.18) or compared to controls (p=0.7). The patient group had reduced renal function compared to controls (GFR 73 $\pm$ 5ml/min/1.73m$^2$ v 97.6ml/min/1.73m$^2$, p=0.004), there was no difference in renal function between dose regimens (p=0.8). Patients were appropriately replaced with pituitary hormone replacement, including GH, and were matched for age, BMI and WCM with control subjects (Table 5.1).

**Table 5.1** Patient and control baseline characteristics
Results expressed as mean $\pm$ standard deviation, BMI-body mass index, WCM-waist circumference

|  | Patients n=10 | Controls n=10 | p value |
|---|---|---|---|
| Age (years) | 46 $\pm$ 15 | 45 $\pm$ 15 | 0.9 |
| BMI (kg/m$^2$) | 29.8 $\pm$ 5.3 | 29.1 $\pm$ 4.6 | 0.5 |
| WCM (cm) | 105 $\pm$ 14 | 103 $\pm$ 11 | 0.53 |
| Basal Cortisol (nmol/L) | 76.8 $\pm$ 6.5 | 403.3 $\pm$ 122.4 | <0.0001 |
| Basal Free T4 (pmol/l) | 11.3 $\pm$ 2.1 | 10.9 $\pm$ 1.0 | 0.6 |
| Basal IGF-I (ug/L) | 163 $\pm$ 45 | 152 $\pm$ 32 | 0.5 |
| Basal Testosterone (pmol/L) | 14.2 $\pm$ 4.1 | 16.4 $\pm$ 7.7 | 0.4 |

## 5.3.2 Urinary steroid metabolites

The urinary free cortisol excretion was significantly increased in the highest replacement dose A compared to both lower doses and to controls as shown in Figure 5.3. There was no difference between dose B and C or compared to controls.
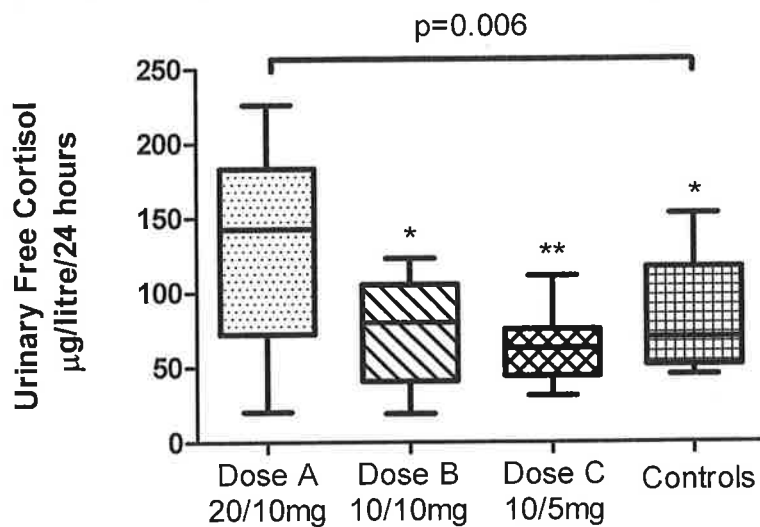


**Figure 5.3** Urinary free cortisol µg/L/24hours between dose regimens and controls. *p<0.05, **p<0.01 compared to dose A

Dose A (20mg/10mg) was associated with significantly higher total cortisol metabolites compared to the other dose regimens (p<0.05 v dose B, p<0.001 v dose C) and compared to healthy controls (p<0.01), while there was no difference between Dose B (10mg/10mg), Dose C (10mg/5mg) and control subjects. (Figure 5.4)

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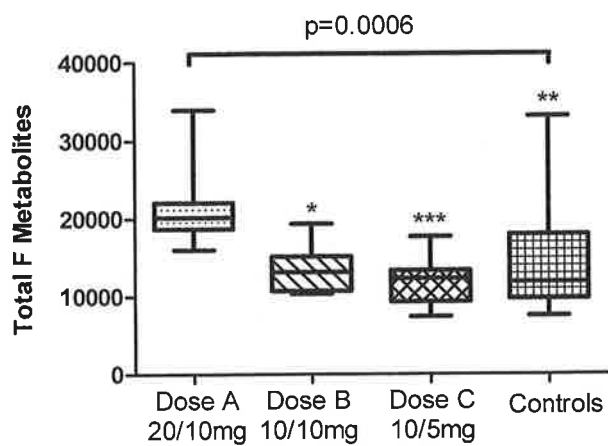
**Figure 5.4** Total cortisol metabolites/24 hours between doses and controls.
*p<0.05, **p<0.01 and ***p<0.001 v dose A

UFF/UFE is a measure of 11 β-HSD 2 activity, inactivating cortisol to cortisone. The ratio

is considered normal between 0.5-0.8 (Palermo), it was elevated above the normal

range in dose A only and significantly different to controls, while the lower doses were

not different to healthy controls (Figure 5.5).
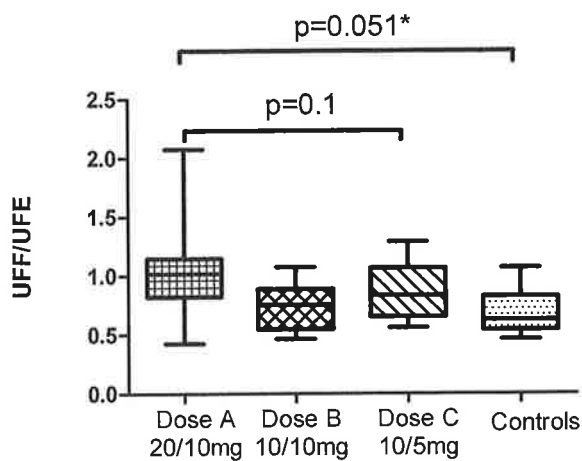


**Figure 5.5** Urinary free cortisol (UFF)/urinary free cortisone (UFE) ratio between doses
and controls
*difference between dose A and controls (p=0.02)

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Although THF+allo THF/THE ratio was increased across all dose regimens compared to healthy controls, it was highest in dose A. (Figure 5.6)
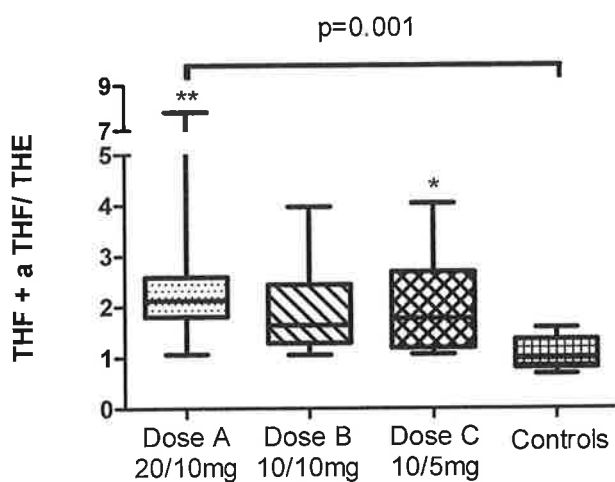


Figure 5.6 THF+alloTHF/THE ratios between doses and compared to controls
*p<0.05 and **p<0.001 compared to controls.

5 α reductase activity was increased for all dose regimens compared to controls, although this was not significant (p=0.055) it reflects increased liver enzyme activity and therefore is a manifestation of increased tissue exposure to glucocorticoids. (Figure 5.7)
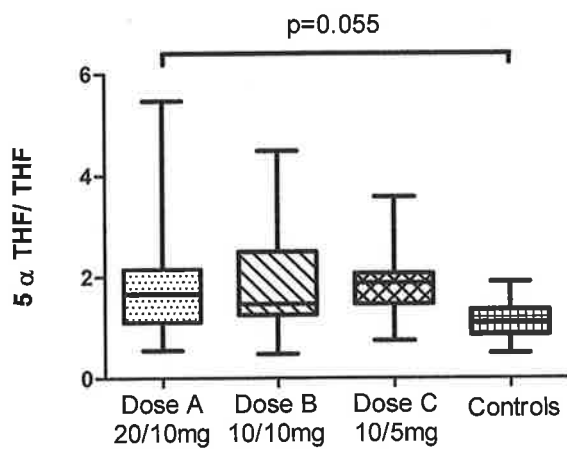
**Figure 5.7** 5 α reductase activity between doses and compared to controls

### 5.3.3 Interaction of Body Mass Index and Waist Circumference on Urinary Cortisol Metabolites

There was no correlation between BMI or WCM and any cortisol metabolite, or metabolite ratio, on any dose regimen, as shown below in Figure 5.8 using THF +allo THF/THE ratio as a sample.
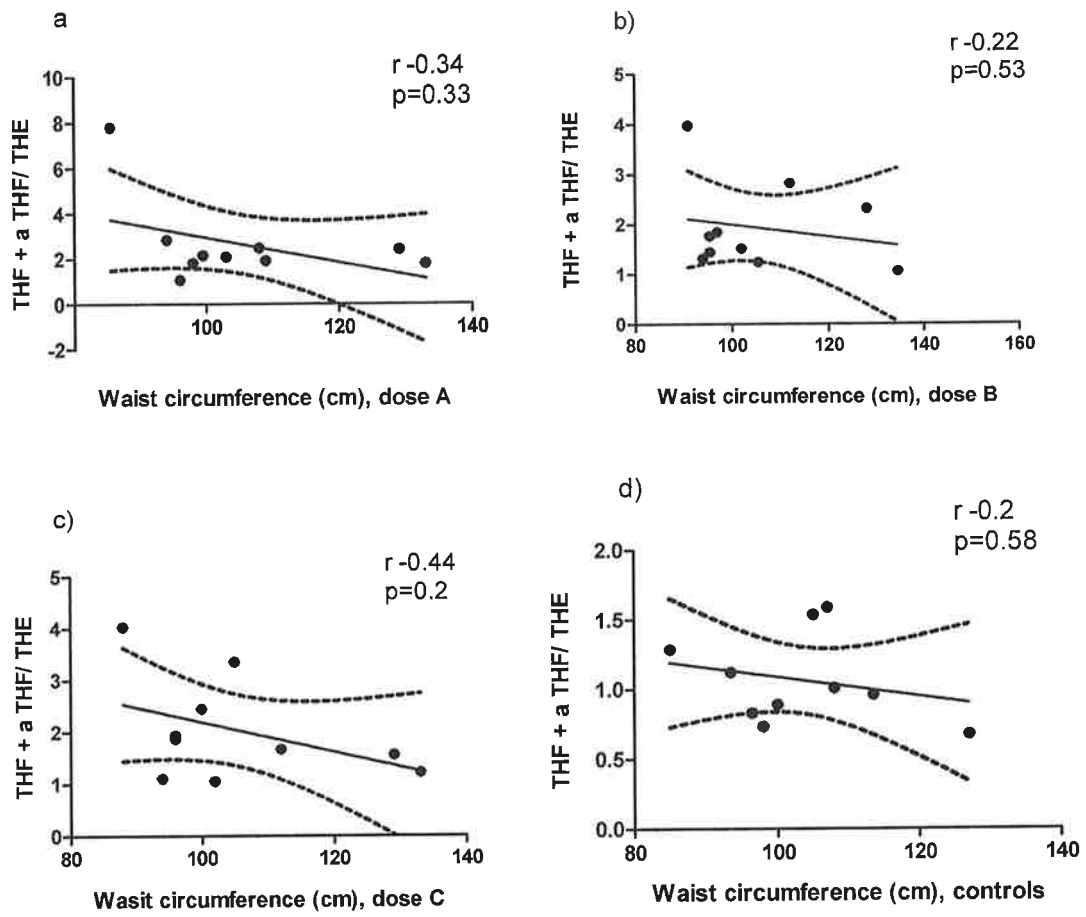
**Figure 5.8** Correlation between waist circumference and 11βHSD1 activity for each dose regimen and controls
r- pearson correlation

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## 5.4 Discussion

In this prospective, randomised, controlled study of male hypopituitary patients on full pituitary hormone replacement, including GH, we demonstrated that hypopituitary patients who receive hydrocortisone therapy have significant alterations in corticosteroid metabolism. Although urinary free cortisol levels were increased in all dose regimens compared to controls, the findings of significantly elevated total cortisol metabolites in high dose hydrocortisone replacement reflect tissue level over-exposure to cortisol. We demonstrated increased 11 β-HSD1 activity and 5 α-reductase activity across all dose regimens; with the highest activity in the higher dose regimen. These results provide further evidence that excess exogenous glucocorticoid is not simply excreted by the renal system but undergoes intracellular metabolism that leads to increased tissue exposure. This may contribute to the metabolic and phenotypic patterns we see in association with hypopituitarism through the interplay of GHD, GH and cortisol replacement therapies.


Hypopituitarism is associated with increased morbidity and mortality(14) with increased levels of obesity, dyslipidaemia and hyperglycaemia(26, 60, 173, 174). Recent observational studies have shown that in patients with acromegaly(49) that ACTH deficiency is an independent predictor for mortality and daily doses of hydrocortisone of greater than or equal to 25mgs per day were associated with increased mortality, predominantly due to cardiovascular disease, findings which have been replicated in studies on patients diagnosed with and treated for non-functioning pituitary adenomas(48).  There has been increasing awareness that the replacement doses of

hydrocortisone previously prescribed were greater than the cortisol production rate in healthy controls(24, 25) and dose reduction studies have been undertaken. The majority of studies assessing the tissue cortisol exposure have done so to investigate the impact of GHD and GH replacement(144, 172, 175, 176), rather than examining the impact of GC replacement per se and none have specifically considered the severity of ACTH deficiency. Hypopituitary patients with partial ACTH deficiency have shown similar cortisol day curves to healthy controls suggesting that these patients may be over-treated by conventional steroid replacement therapy (125).

Visceral adiposity and decreased lean mass is reported in patients with GHD and this improves following treatment with GH(60, 167, 177, 178). Untreated GHD is associated with THF+alloTHF/THE ratios that are increased by about 50% from baseline, while subjects with acromegaly have reduced urinary THF+alloTHF/THE ratios(172). Studies in GHD hypopituitary patients commencing GH replacement show a reduction in the THF+alloTHF/THE ratio, without in alteration in the UFF/UFE ratio, suggesting a decrease in 11β-HSD1 reductase activity(175). Importantly, patients in these previous studies of GH replacement may also have been ACTH deficient and may or may not have received glucocorticoid replacement therapy(172, 175, 176). It could be speculated that many of the changes in body composition, both increased fat mass and decreased muscle mass, reported with GH therapy in patients with hypopituitarism may be due to inhibition of corticosteroid metabolism (in particular 11 β-HSD1) by GH treatment. In our study however, patients were fully replaced with GH and the dose was unchanged for 3 months prior to involvement in the study, yet the ratios of THF+alloTHF/THE were elevated across all dose regimens compared to healthy controls suggesting that the

elevated 11βHSD 1 activity was primarily related to glucocorticoid exposure, rather than GH status. Glucocorticoids themselves have been reported previously to increase 11β-HSD1 activity and expression(179, 180) which may lead to a "forward feedback regulation" of 11β-HSD1 by cortisol, resulting in a further amplification of tissue specific glucocorticoid action. Expression and activity of 11β-HSD1 has been implicated in many of the features of metabolic syndrome, notably hepatic glucose output, the accumulation of visceral adipose tissue and muscle insulin resistance(133).

Visceral adiposity is known in the general population to be associated with insulin resistance and/or diabetes mellitus, hypercholesterolaemia and hypertension(168, 169). Bujalska et al. first proposed that excessive activity of 11 β-HSD1 enzyme within visceral adipose tissue could lead to increased adipose tissue levels of glucococorticoids and 'Cushing's disease of the omentum' (181). The increase in both THF+alloTHF/THE ratios and total cortisol metabolites in our cohort of patients taking hydrocortisone therapy could lead to significant changes in adipose tissue biology and ultimately contribute to increased metabolic risk. We were unable to demonstrate any correlation between waist circumference and any cortisol metabolite in our study that might indicate altered visceral adiposity, although that was not a primary end point of this work as it would not be expected to change significantly in 6 weeks. Studies are ongoing in CEDAM to assess the impact of GC replacement on visceral adiposity in conjunction with analysis of urinary cortisol metabolites.

Recommendations to use urinary free cortisol (UFC) measurement as a method to assess patients for over or under-replacement of cortisol have been made by some groups(31, 38). This requires diligent collection of urine for a 24 hour period with reference to an established normative range when interpreting results and has been shown to be associated with considerable inter-individual variability(10). Assessment of UFC reflects only the total 24 hour dose and does not account for over exposure at certain time points or under exposure to cortisol at other time points nor does it quantify overall tissue exposure to cortisol. Although UFC was highest in the highest dose regimen there was considerable overlap between dose regimens and this emphasises the impracticality of using UFC to titrate HC dose clinically. It is possible that future research may lead to greater understanding of the role of corticosteroid metabolites and that measurement of 24 hour urine metabolites could be used to tailor individual therapy in the future.

There are some flaws to this arm of the study. We included male Caucasian patients only and as such it may not be possible to extrapolate the results to the general population. The UFF/UFE ratio, indicative of 11βHSD 2 activity was abnormal in patients on the highest dose regimen which may cloud the interpretation of the THF + allo THF/THE ratios, although it suggests increased overall tissue exposure to cortisol and its metabolites. We did not measure changes in visceral adiposity as we did not expect a change over a 6 week period and this is an area for future research.

In conclusion, this study demonstrates significant abnormalities in corticosteroid metabolism in patients with ACTH deficiency treated with conventional doses of hydrocortisone therapy in keeping with increased $11\beta$-HSD1 activity. Induction of $11\beta$-HSD1 is associated with central adiposity, which confers an increased metabolic risk. Higher doses of glucocorticoid replacement therapy can directly, and indirectly via enhanced $11\beta$-HSD1 activity, contribute to a deleterious metabolic phenotype. Understanding of the increased tissue exposure across all HC dose regimens, most significantly in the highest dose regimen may lead to alteration in prescribing practices.

## Chapter Six: The effect of 3 hydrocortisone regimens on blood pressure indices and glucose metabolism as markers of metabolic risk

## 6.1 Introduction

The primary causes of morbidity and mortality in hypopituitarism are cardiovascular or cerebrovascular in origin and in most(41, 44, 46), but not all studies(43, 47), vascular and cardiovascular mortality is increased. Patients with glucocorticoid excess (Cushing's syndrome) have significant morbidity and increased mortality; hypertension is present in approximately 80% of patients with endogenous Cushing's syndrome(87, 155), while hypercortisolism also leads to hyperglycaemia through insulin resistance, hepatic gluconeogenesis and glycogenolysis. Although the increase in mortality in hypopituitarism is likely multifactorial, recent studies in subjects with treated acromegaly and non-functioning pituitary adenomas have suggested that inappropriate glucocorticoid replacement is an independent risk factor for increased mortality(48, 49). Therefore, it is feasible that inappropriate glucocorticoid replacement may result in subtle chronic over-exposure to cortisol and lead to increased morbidity in this group.

Despite this evidence for increased vascular mortality in hypopituitary patients the cause remains unclear. There is conflicting data regarding the role of hypertension in morbidity and mortality in hypopituitarism. Some studies have shown no difference between patient or controls in absolute systolic or diastolic BP(51, 52), but increased prevalence of treated hypertension in hypopituitary patients that remained significant after controlling

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for BMI(50), while a study by Dunne et al demonstrated lower 24hour ambulatory BP in 13 GH deficient hypopituitary subjects compared to matched controls(53). Such conflicting data may suggest there is no relationship between the increased cardiovascular mortality seen in this group and hypertension, however excepting Dunne et al, these studies used clinic BP recordings rather than 24 ambulatory measurements, few studies reported data regarding concurrent anti-hypertensive medication use and subjects were not uniformly replaced with pituitary hormones.

Impaired glucose tolerance (IGT) is an established risk factor for cardiovascular disease and insulin resistance has been shown to be associated with increased mortality(78). A small number of studies have prospectively examined the effect of different glucocorticoid regimens and doses in hypopituitary patients on glucose metabolism compared to matched controls and failed to demonstrate any difference in fasting glucose or insulin levels(53, 69, 95). It must be noted that in those studies subjects were GH deficient and were not replaced with GH. Even when blood glucose and plasma insulin levels are similar to those seen in controls, GHD patients treated for pituitary disease have been shown to be insulin resistant(182).

In view of the discrepancies in the literature regarding the effect of glucocorticoid replacement in hypopituitary subjects on blood pressure and glucose metabolism, we aimed to examine, in a prospective randomised-controlled manner, the effect of three commonly prescribed regimens of hydrocortisone replacement on markers of cardiometabolic outcome in a group of panhypopituitary adults with severe ACTH

deficiency, fully replaced with all pituitary hormones including growth hormone and compared to age, sex and BMI matched controls.

## 6.2 Methods

### 6.2.1 Patients and Controls

Ten adult hypopituitary men with known severe ACTH deficiency, defined by a fasting morning total serum cortisol concentration <100nmol/l and a stimulated peak cortisol of <400nmol/l in response to insulin-induced hypoglycaemia (glucose <2.2mmol/l) were included.

All 10 subjects had been diagnosed and treated for sellar tumours between 3 to 18 years prior to inclusion in the study. 5 patients had been treated for non-functioning pituitary adenoma, 2 for macroprolactinoma, 2 for craniopharyngioma and the 10th had treated, cured, Cushing's disease with panhypopituitarism and was 8 years post definitive treatment, still requiring HC replacement, with a morning pre hydrocortisone cortisol level <100nmol/L. 9 patients had complete anterior pituitary failure, 1 patient was deficient in all anterior pituitary hormones, except LH and FSH; all patients were on appropriate hormone replacement including growth hormone (GH), without alteration in dose for at least 3 months prior to and during the study. All 10 patients had diabetes insipidus and were on Desmopressin and no patient had serum sodium abnormalities to suggest under or over replacement with that medication. Anterior pituitary hormone replacement therapy regimens were not adjusted during the study period, except for HC

dose as per study protocol. No patient had known diabetes, 2 patients had controlled hypertension and were on stable doses of angiotensin-converting enzyme inhibitor medication that was not changed during the study. Exclusion criteria were as documented in previous chapters and with particular relevance to this study, patients with uncontrolled hypertension or a known diagnosis of diabetes mellitus were excluded.

Healthy male controls (n=10), matched for age, body mass index (BMI) and waist circumference (WCM) with no known pre-existing diabetes or uncontrolled hypertension, were enrolled to undergo the same biochemical investigations and clinical examination as the patient group.

## 6.2.2 Study Design

10 patients were prospectively randomised in an open cross-over protocol to each experience 3 dose regimens of commonly prescribed doses of hydrocortisone, dose A (20 mg 0800 hours, 10 mg 1600 hours), dose B (10 mg 0800 hours and 1600 hours) or dose C (10 mg 0800 hours and 5mg 1600 hours). In view of the short half-life of HC the patients took each dose regimen for a full 6 weeks to allow adequate time for a "washout" of the previous dose.

### 6.2.3 Study Procedures.

At the end of each 6 week treatment schedule patients were admitted to our clinical research centre overnight for metabolic investigations as previously described in Chapter 2. On each admission between 0730hours and 0800hours patients were fitted with validated oscillometric devices to record 24 hour ambulatory blood pressure, (SpaceLabs 90202 or 90207) and underwent oral glucose tolerance challenge with serum sampling for insulin and glucose following an overnight fast. Subjects took the pre-designated hydrocortisone dose at 0800hours, 1600hours and at 0800hours the following morning. 10 healthy matched controls underwent identical biochemical profiling as the patient group.

### 6.2.3.1 Ambulatory Blood Pressure Measurement

We used validated oscillometric recording devices, (SpaceLabs 90202 or 90207 ref), programmed to obtain blood pressure readings at 30-minute intervals for 24 hours throughout each 26 hour admission period(183). Failed recordings automatically triggered a repeat recording within a 2 minute interval. All of the recorded clinical data were transferred into the dabl Cardiovascular software package (dabl Ltd) in order to produce a report for each event.

### 6.2.3.2 Insulin sensitivity assessment

In order to reproduce "everyday" conditions, the oral glucose tolerance test (OGTT) was conducted under standard clinical conditions of full hormone replacement, since both

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glucocorticoid and growth hormone deficiency and replacement therapy have effects on glucose metabolism(184-186). Subjects fasted from 2300hours the previous night and took the designated hydrocortisone dose at 0800hours with a small sip of water, as per study protocol. The OGTT was performed 1 hour later in order to standardise the effect of hydrocortisone on the results. 75g of glucose was dissolved in 300mls of water to be consumed as an oral glucose challenge. Paired samples for insulin and glucose were taken at Time 0, before consumption of the glucose load and at 30, 60, 90 and 120 minutes (Time 30, Time 60, Time 90 and Time 120) post oral consumption of glucose.

## 6.2.4 Laboratory methods

Glucose was analysed using the hexokinase method on an automated Beckman Coulter AU5400 analyser and serum insulin levels were estimated used a chemiluminescent immunoassay on the Unicel DxI Beckman Coulter Immunoassay System, as previously described in Methods, Chapter 2.

## 6.2.5 Data Analysis

### 6.2.5.1 Ambulatory Arterial Stiffness Index

Vascular disease is associated with increasing stiffness of the vascular tree(187) therefore we chose to assess arterial stiffness using the non-invasive method of Ambulatory Arterial Stiffness Index (AASI)(188, 189). Arterial stiffness varies nonlinearly with distending pressure: as mean arterial pressure increases, stiffness increases exponentially. In subjects with less compliant arteries, increases in the distending

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pressure, above a certain threshold, are associated with a greater increase in systolic pressure than diastolic pressure. In those with very stiff vessels, although systolic pressure sharply rises with each increase in mean arterial pressure, diastolic pressure may even decline. The AASI is a validated predictor of vascular mortality(188, 190-192) and is calculated from the half-hourly readings within the 24-hour ABPM recordings. Using the data from the 24 hour ABP machines, we computed, for each participant the regression slope of diastolic on systolic blood pressure. We did not force the regression line through the origin (intercept 0),8,19 Therefore AASI is defined as: 1 minus the regression slope of DBP/SBP. The stiffer the arterial tree, the closer the regression slope and AASI are to 0 and 1, respectively(189).

### 6.2.5.2 Glucose and insulin homeostasis

Normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) were diagnosed according to standard World Health Organisation criteria (WHO)(193). Insulin resistance was calculated with the homeostatic model assessment of insulin resistance (HOMA-IR) using fasting insulin (mU/l) x glucose(mmol/l)/22.5(194) and we also used the computer model HOMA2-IR, as recommended by the authors who initially described HOMA, in order to correct for the effects of hyperglycaemia on hepatic and peripheral glucose resistance(195). Insulin measurements in ng/ml were multiplied by a factor of 175 to convert measurements to pmol, in keeping with international recommendations, for the purposes of HOMA2 calculation (196). Dynamic estimation of insulin sensitivity was measured using the Oral Glucose Insulin Sensitivity (OGIS) method(197). The OGIS is

an index of insulin sensitivity calculated from OGTT glucose and insulin measurements, which has been validated against the hyperinsulinaemic-euglycaemic clamp(197).

### 6.2.6 Statistical Analysis

Results are reported as mean (SD) or median (interquartile range, IQR) as appropriate. Between group differences were assessed using ANOVA, or repeated measures ANOVA or the non-parametric equivalent, followed by application of a multiple comparison test. Correlations were analysed using the Spearman or Pearson correlation co-efficient, as appropriate based on normality tests. Significance was defined for p-values <0.05. Statistical Analysis was performed using GraphPad Prism Windows version 5.0 (GraphPad Software, La Jolla, California, USA).

## 6.3 Results

Patients and controls were appropriately matched for age, BMI and WCM as shown in previous chapters. There were no differences in measured pituitary hormones, excluding cortisol, between patients and controls suggesting appropriate pituitary hormone replacement.

### 6.3.1 Blood Pressure Analysis

There were no differences in systolic BP or diastolic blood BP, either between dose regimens or compared to controls. (Table 6.1)

**Table 6.1** 24hour ambulatory blood pressure levels between dose regimens and compared to controls
Blood pressure (BP) expressed in mmHg, data expressed as mean ± SD

| BP mmHg | Dose A 20mg/10mg | Dose B 10mg/10mg | Dose C 10mg/5mg | Control | p value |
|---|---|---|---|---|---|
| 24 Hour Systolic | 115 ± 12 | 117 ± 12 | 115 ± 13 | 121 ± 10 | 0.67 |
| 24 Hour Diastolic | 70 ± 8 | 68 ± 8 | 68 ± 7 | 73 ± 8 | 0.60 |
| Day-time Systolic | 119 ± 10 | 120 ± 12 | 122 ± 13 | 128 ± 9 | 0.38 |
| Day-time Diastolic | 74 ± 7 | 71 ± 8 | 74 ± 8 | 79 ± 8 | 0.16 |
| Night-time Systolic | 105 ± 16 | 109 ± 16 | 103 ± 13 | 110 ± 13 | 0.70 |
| Night-time Diastolic | 60 ± 10 | 62 ± 12 | 57 ± 7 | 63 ± 10 | 0.54 |

A nocturnal systolic dip <10% is considered abnormal and 3 subjects on Dose A were in this category, compared to 5 on Dose B, 2 on Dose C and 1 control subject; 1 patient on dose A, 3 on Dose B, none on dose C and no controls had a nocturnal diastolic dip <10% . Overall the physiologic nocturnal dip in systolic and diastolic blood pressure was blunted in subjects on higher dose HC replacement; however this was significant only for dose B (10mg/10mg) compared to dose C (10mg/5mg). (Figure 6.1)
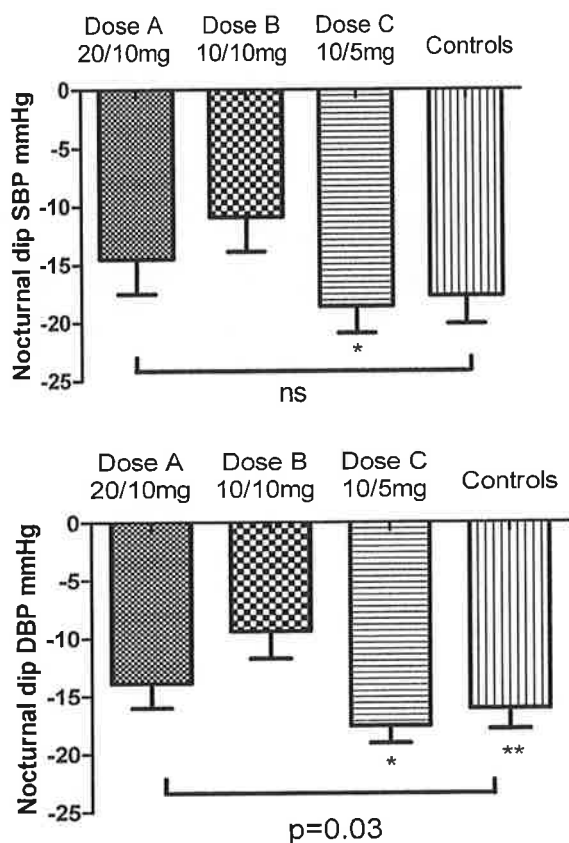
**Figure 6.1** Mean Nocturnal systolic and diastolic BP dip for patients and controls.
* - difference between dose C and dose B, ** difference between controls and dose B, BP- blood pressure, SBP – systolic BP, DBP- diastolic BP

Ambulatory Arterial Stiffness Index (AASI) was calculated as described previously. The closer the AASI is to 1, the stiffer the arterial tree. Low dose HC replacement demonstrated the lowest AASI compared to the other two dose regimens. (Figure 6.2)
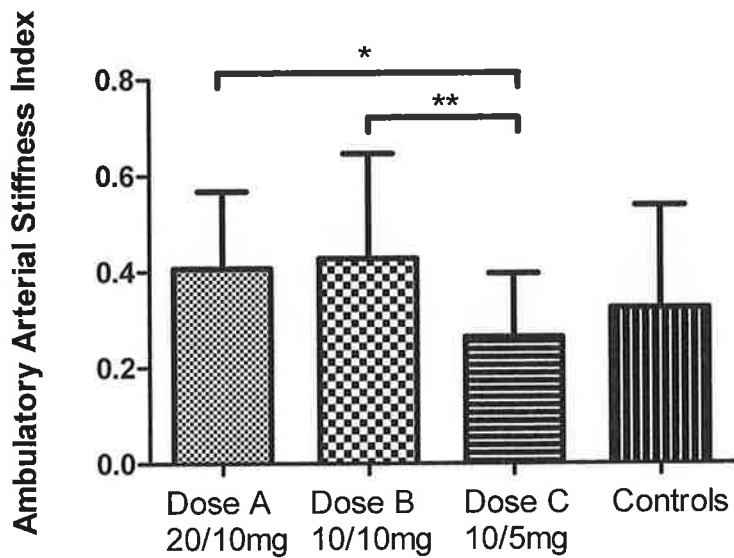
**Figure 6.2** Ambulatory arterial stiffness Index between dose regimens and controls
AASI closer to 1 indicates a stiffer arterial tree * - p=0.04 for dose A compared to dose C, ** - p=0.02 for dose B compared to dose C

## 6.3.2 Glucose Metabolism analysis

3 patients had abnormal glucose levels at some point during the study. This would not have been diagnosed in the out-patient setting by examining fasting glucose only. 2 control subjects had abnormal glucose levels. (Table 6.2)

*Table 6.2 Abnormal glucose metabolism in the patients and controls*

BMI – body mass index, WCM – waist circumference, NFPA – non-functioning pituitary adenoma, IGT – impaired glucose tolerance, IFG – impaired fasting glucose, DM – diabetes mellitus

| Subject | Age years | BMI kg/m2 | WCM cm | Pathology | Fasting Plasma Glucose mmol/l | 2 hour Plasma Glucose mmol/l | Glucose Abnormality |
|---|---|---|---|---|---|---|---|
| Patient 1 | 50 | 29.4 | 105 | NFPA | Dose A - 5.1 | Dose A - 5.3 | Dose A – normal |
| | | | | | Dose B - 5.1 | Dose B - 8.1 | Dose B – IGT |
| | | | | | Dose C - 5.1 | Dose C - 3.7 | Dose C - normal |
| Patient 3 | 56 | 36.2 | 128 | NFPA | Dose A - 4.7 | Dose A - 9.1 | Dose A – IGT |
| | | | | | Dose B - 4.5 | Dose B - 11.2 | Dose B – Type 2 DM |
| | | | | | Dose C - 5.2 | Dose C - 10.9 | Dose C – IGT |
| Patient 7 | 65 | 26.6 | 103 | NFPA | Dose A - 5.0 | Dose A - 7.9 | Dose A – IGT |
| | | | | | Dose B - 5.2 | Dose B - 6.8 | Dose B – normal |
| | | | | | Dose C - 5.2 | Dose C - 8.9 | Dose C – IGT |
| Control 7 | 33 | 38.5 | 127 | - | 6.4 | 8.9 | IFG and IGT |
| Control 10 | 66 | 30.7 | 108 | - | 5.9 | 8.5 | IGT |

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## 6.3.3 Analysis of glucose and insulin levels

Fasting plasma glucose levels were significantly higher in matched controls compared to the patients on all three doses of hydrocortisone replacement (Figure 6.3a), while there was no difference in fasting serum insulin levels or 2 hour glucose and insulin levels following the oral glucose load either between dose regimens or compared to controls. (Figure 6.3b)
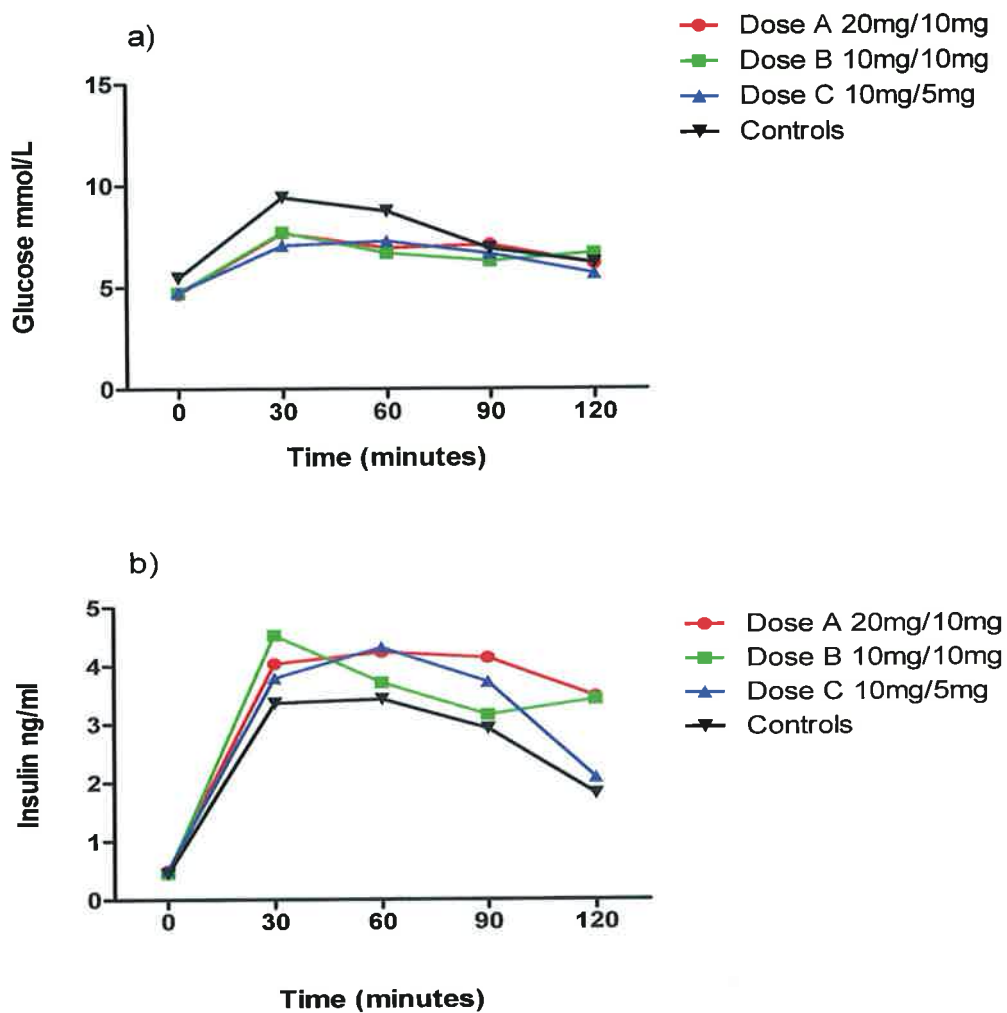


Figure 6.3 Mean glucose (a) and insulin (b) levels at each time point during the75g oral glucose challenge test.

There was no difference in area under the curve (AUC) for glucose, for each dose compared to controls (p=0.36), or between doses (p=0.31) or for insulin compared to controls (p=0.98) or between doses (p=0.60). Post hoc test for linear trend for insulin was non-significant. (Figure 6.4)
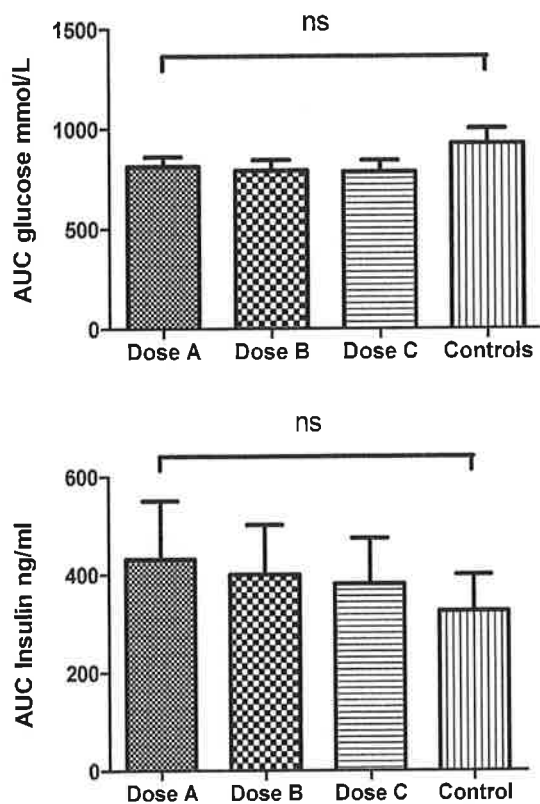


**Figure 6.4** Area under the curve for glucose and insulin following oral glucose tolerance testing
ns – non significant, AUC – area under the curve

There was no difference in insulin sensitivity between dose regimens (p=0.68) or compared to controls (p=0.9). (Figure 6.5)
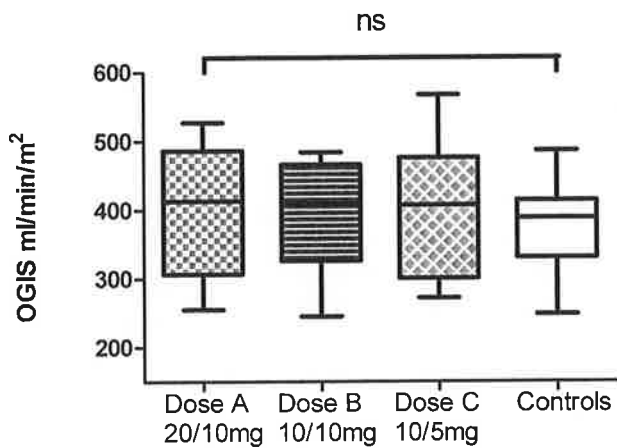
**Figure 6.5** Oral glucose insulin sensitivity (OGIS) index between doses and controls
ns- non significant

There was no difference in insulin resistance based on HOMA-IR, p=0.81. Data was not normally distributed therefore it was log transformed and results remained unchanged, p=0.9.
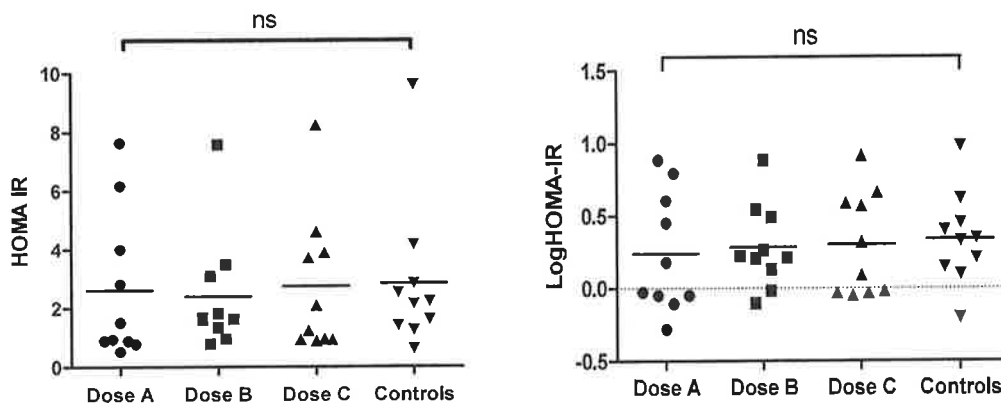


**Figure 6.6** HOMA-IR and LogHOMA-IR between dose regimens and compared to controls.
ns – no significant difference, HOMA – homeostatic model assessment, IR- insulin resistance

## 6.4 Discussion

In this randomised, controlled, cross-over study of panhypopituitary subjects on complete pituitary hormone replacement, we have demonstrated that lower dose HC replacement is associated with a lower arterial stiffness index and a more physiological nocturnal blood pressure profile, although that was only significant for dose B compared to dose C. We have also demonstrated that although there is no difference in insulin and glucose metabolism between these commonly prescribed dose regimens, 30% of the patient cohort had abnormal glucose metabolism at some point in the study compared to 20% matched controls. GH has impacts on insulin sensitivity and cardiovascular status, body composition, lean body mass, cortisol metabolism through the 11 Beta hydroxysteroid dehydrogenase pathway(60, 167-169, 182) and also endothelial function, therefore on stable GH replacement, with IGF-I in the target range it is reasonable to assume that any alterations in metabolic endpoints in this cohort are truly the effects of changes in cortisol dose regimens.

Rosen and Bengtsson analysed 333 hypopituitary patients and demonstrated an increased SMR in the whole group at 1.95, which was higher in women (2.7) compared to men (1.7)(41). Although most studies suggest that the increased morbidity and mortality is primarily vascular in origin, the cause for adverse vascular outcome is not clear. Sherlock et al demonstrated in a cohort of patients with acromegaly that ACTH deficiency is a predictor for mortality and daily doses of hydrocortisone of greater than or equal to 25mgs per day were associated with increased mortality, independent of age, sex, calendar period and radiotherapy and over 30% of deaths were from cardiovascular causes(49). These findings were corroborated by Zueger et al who retrospectively

examined 105 patients with non-functioning pituitary adenomas, mean follow up of 12.7 ± 9 years, and demonstrated increased hazard ratios for mortality with both increasing weight adjusted glucocorticoid dose and with increasing total daily glucocorticoid replacement doses, HR for mortality increased from 1 at 5-19mg daily, to 2.03 at 20-29mg daily and 4 at ≥ 30mg daily (p=0.029 for trend)(48). While the majority of studies evaluating cardiometabolic risk in hypopituitarism have focussed on the effects of GH deficiency, and in some cases GH replacement, Filipsson et al demonstrated an adverse metabolic profile including dylipidaemia, an elevated HbA1c and waist circumference in GHD subjects who were treated for ACTH deficiency compared to GHD, ACTH sufficient patients. Interestingly ACTH deficient patients on HC equivalent doses <20mg daily did not differ in metabolic outcomes compared to their ACTH sufficient counterparts, while the adverse metabolic profile was associated with HC equivalent doses >20mg and was more pronounced in those on doses >25mg daily(29).


It is generally accepted that ABP has greater predictive value than casual out-patient clinic BP readings(183). We demonstrated no difference in 24 ABPM between dose regimens or compared to controls in our group of GH-replaced hypopituitary subjects. This is consistent with data from the only other prospective study in hypopituitary subjects to use 24 hour ABP, in which 13 hypopituitary GHD subjects had a dose reduction from 30mg daily to 15 mg daily and repeated 24hour ABPM 3 months following the dose reduction demonstrated no difference, however they did note that the patient cohort had lower BP regardless of dose compared to controls(53).

It is known that blunting of the nocturnal dip in blood pressure is a predictor of increased cardiovascular risk independent of absolute 24 hour BP(198) and is associated with increased arterial stiffness(199). Loss of the nocturnal systolic and/or diastolic blood pressure dip is considered pathological and has been identified in Cushing's syndrome(200) and with use of exogenous high dose glucocorticoids prescribed for anti-inflammatory purposes(201). There is little data regarding the effects of currently prescribed GC replacement regimens on the circadian rhythm of blood pressure. A nocturnal dip of 10% or less is considered abnormal (202) and we have demonstrated a blunted nocturnal dip in subjects on the higher dose replacement regimens, although this was only statistically significant for dose B compared to dose C and control subjects. Such alteration in circadian blood pressure may reflect abnormal cortisol dynamics in subjects on exogenous GC replacement. This has not previously been evaluated in the hypopituitary group on full replacement with respect to GC dose comparison.

Matsumura et al studied the effect of exogenous glucocorticoids on blood pressure in 5 subjects with secondary adrenal insufficiency(203). They examined three glucocorticoid regimens: once daily HC, dose ranging between 15-25mg; twice daily HC at the same total dose given at 0800hours and 2000hours and lastly prednisolone 3.75-5mg daily. 24 hour BP analysis was performed before GC replacement and afterwards, essentially comparing a GC deficient state with a GC-replaced state. Unsurprisingly, mean BP increased post-GC replacement in all replacement regimens, while once daily HC replacement was associated with a more significant nocturnal drop than twice daily. The only other study to use 24 hour BP to evaluate the effect of different dose regimens in the hypopituitary patient population did not report whether there was any alteration in circadian BP rhythms(53). In view of these results and the mixed evidence regarding the

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contribution of hypertension to the morbidity and mortality in hypopituitary subjects, it is very likely that loss of circadian variation in BP is an important marker of vascular risk in this patient group.

Vascular disease is associated with increasing stiffness of the arterial tree and a number of studies in hypopituitary patients have demonstrated reduced large vessel reactivity(59) or reduced endothelium derived dilation as a reflection of abnormal vascular function(55, 57). Arterial stiffness as measured by pulse wave velocity is a predictor of cardiovascular events, independent of pulse pressure(204, 205). We have demonstrated that low dose HC replacement is associated with reduced Ambulatory Arterial Stiffness Index. AASI has been shown to correlate with pulse wave velocity and with both central and peripheral augmentation indices; AASI appears to be able to identify arterial dysfunction at a younger age than pulse pressure(188). In a study of 11,291 adults not on antihypertensive medication at the time of ABPM recording, AASI has been shown to predict death from stroke, and was found in normotensive subjects to be more predictive of stroke and cardiovascular mortality than pulse pressure(192). Our findings regarding the AASI are particularly relevant in hypopituitarism where cerebrovascular mortality is increased anywhere from a SMR of 1.7 to 4.9(14) and notably in those diagnosed at a young age(45). Further long term prospective studies are required to truly assess the utility of the AASI in predicting vascular mortality in the hypopituitary patient population.

Reduced AASI in our cohort on low dose HC replacement is unlikely to represent under-replacement with glucocorticoid, as no patient reported postural symptoms, a finding that

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is also consistent with reported data from Dunne et al who reduced HC from 30mg to 15mg daily without any onset of postural symptoms. The same group also demonstrated higher forearm blood flow on the lower dose HC regimen(15mg daily), compared to the higher dose, indicative of improved vascular reactiveness(53). Our findings with respect to the nocturnal dip and the AASI suggest controlled HC dose reduction may be beneficial for long term health of the vascular tree.

Reports of increased prevalence of IGT and DM in hypopituitary patients compared to controls has been reported to varying degrees, ranging from 16-18% IGT and 2-13% overt DM, with the differences likely explained by varying degrees of hypopituitarism and hormone replacement(92, 93). Interestingly a retrospective study examining the impact of GC replacement on metabolic outcomes in GH replaced hypopituitary subjects in the KIMS database identified 9.4% of ACTH deficient subjects to have hyperglcaemia (fasting glucose > 6.1nmol/l) or known type 2 diabetes mellitus (T2DM), compared to none of the ACTH sufficient patients p=0.03. This difference remained significant after adjusting for age, sex and smoking status (p=0.04). The mean HC equivalent replacement dose in that study was 20mg, however 34.2% were on 30mg or more daily. GH dose was not different between the ACTH sufficient and the ACTH deficient groups (26).

Using the WHO criteria (193), 30% of our patient cohort had abnormal glucose metabolism at some point in the study, without a clear pattern in relation to hydrocortisone replacement regimen. Only 1 patient had abnormal glucose tolerance on all three dose regimens, while only 20% had abnormal glucose metabolism on the two

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lower dose regimens which is more consistent with previous reported data. Notably no patient had impaired fasting glucose, indicating that measurement of fasting glucose in the outpatient setting would not be an adequate means of screening this patient population.

Danilowicz et al examined 11 panhypopituitary subjects with a mean BMI 31.5kg/m2, not replaced with GH and reduced their mean HC dose from 26mg daily to 13mg daily. They demonstrated no change in HOMA-IR after 6 months on the lower dose regimen(69). Dunne et al also studied 13 hypopituitary subjects, however their mean BMI was lower at 24kg/m$^2$ and they also were not on GH replacement(53). A reduction in dose from 30mg daily to 15 mg daily resulted in no change in fasting glucose or HbA1c after 3 months on the new regimen. It is important to note that in each of those studies only fasting levels of glucose and insulin were assessed; we would not have identified any glucose abnormalities in our cohort if we had relied on fasting levels only. In our cohort of panhypopituitary patients, fully replaced on GH we were unable to demonstrate any difference in markers of insulin sensitivity or resistance between dose regimens. Our control group had higher fasting glucose levels, and 20% had impaired glucose tolerance. This may reflect the fact that in order to match for BMI and WCM we recruited obese and overweight subjects, who are at higher risk of abnormal glucose metabolism than the overall general population Those with abnormal glucose tolerance in the patient group had a mean BMI of 30.7kg/m2 ($\pm$4.9), while the controls with abnormal glucose tolerance had a mean BMI of 34.6kg/m2 ($\pm$5.5). It is possible that hypopituitary subjects have altered physiology and develop IGT at lower BMI than healthy controls, however in this study the numbers are too small to analyse a subpopulation. Studies of insulin and glucose physiology in relation to glucocorticoid use have primarily used high dose GC

and therefore it is difficult to extrapolate the results to our study. Plat et al demonstrated an immediate suppressive effect of 100mg HC on insulin secretion, without a change in glucose concentration, while there was also a delayed effect with the appearance of a state of relative insulin resistance that occurred 4-6 hours after administration of the bolus dose(206). The harmful effects of hydrocortisone on insulin sensitivity were delayed and occurred 3 hours after the cortisol peak; however the mean peak cortisol level in that study was 850nmol/l, which is significantly higher than we have previously demonstrated with low dose hydrocortisone replacement in Chapter 3(207).

There are a few potential limitations to this study, it is conceivable that lack of difference in 24 hour ABP parameters between doses and compared to controls is a Type 2 statistical error, however previous studies with similar small numbers demonstrated differences in BP in a similar patient population size and similar baseline BP readings, so it is unlikely that this is the case. 2 of our patients were on anti-hypertensive medication and while this may have altered the comparison to controls, it is unlikely to have altered the between dose comparison as the dose of antihypertensive was not altered during the study. There has been debate previously regarding the use of a surrogate marker of arterial stiffness such as the AASI; AASI may also be affected by the mechanical properties of the small vessels, which may be the reason it is a useful predictor of stroke in the normotensive population. In fact this may be a strength for the AASI as a test in the hypopituitary cohort, in whom hypertension does not appear to be a prominent feature. It is a quick and easy calculation that can be taken from any ABPM and may be of use in the clinical setting to identify at risk patients. We did not include HbA1c as part of our assessment as we would not expect a significant change in a 6 week period(208). In view of the extensive literature regarding the effect of GH and GC

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replacement on lipid metabolism we also opted not to assess lipid metabolism during this study.

In summary this is the first study to prospectively evaluate 3 commonly prescribed HC dose regimens in panhypopituitary subjects on full pituitary hormone replacement with respect to the circadian rhythm of blood pressure, arterial stiffness index and the oral glucose tolerance test as a marker of glucose and insulin metabolism. Currently prescribed doses of hydrocortisone replacement do not result in significant differences in absolute BP levels or improvements in insulin sensitivity, yet lower doses are associated with a less stiff arterial tree, a more physiological nocturnal BP dip and therefore most likely confer reduced vascular risk. Physicians should aim for safe dose reductions of hydrocortisone in order to reduce the adverse impact of excess replacement. 30% of our cohort had IGT at some point in the study and therefore it is important to consider OGTT as part of the routine assessment of hypopituitary patients. Although no differences between doses were identified for 24 hour BP, 2 patients were on anti-hypertensive medication and in a population at increased risk of vascular morbidity it is also worth considering performing a 24 hour ABP in order to identify those at highest metabolic risk.

# Chapter Seven: Summary Discussion and Recommendations

## 7.1 Summary Discussion

In this prospective study of panhypopituitary males, fully replaced on all pituitary hormones, including GH, I have assessed the impact of randomised hydrocortisone dose alterations on a wide range of metabolic endpoints, with a view to identifying the dose regimen that results in the most physiological replacement in this cohort. This is the first study to prospectively evaluate these clinical endpoints in a cohort on full pituitary replacement with comparison to matched controls. This work contributes valuable knowledge and understanding of glucocorticoid replacement in hypopituitarism. In the absence of a reliable clinical or biochemical marker for adequacy of GC replacement, these data may assist in clinical decision making regarding hydrocortisone replacement in hypopituitary patients with severe ACTH deficiency.

Following the confirmation that daily cortisol production rates had been overestimated(24, 25), attempts have been made to reduce prescribed doses of glucocorticoid replacement in secondary adrenal insufficiency(36, 69, 95). Despite this clinical practice varies significantly and concern for theoretical risk of increased adrenal crises has resulted in continued use of higher doses of HC than may be necessary or beneficial. In the first arm of this study I have demonstrated that 24 hour serum cortisol profiles on low dose HC replacement closely mimic those of the control population, while acknowledging the absent early morning cortisol rise. In secondary adrenal insufficiency it is possible that this early morning rise is less important than in primary adrenal

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insufficiency; there is no requirement to suppress the concurrent rise in ACTH as there would be in primary AI or in congenital adrenal hyperplasia. The mean trough levels following each dose were not different between regimens or compared to controls, refuting the suggestion that higher dose replacement protects patients from prolonged exposure to trough levels. Importantly the dose reduction in this study was achieved without adverse impact on overall quality of life. Finally, an interesting finding was that of reduced cortisol binding globulin levels across all dose regimens compared to controls. While this may be secondary to exogenous GH administration, the effect of exogenous GC replacement at low doses on CBG warrants further investigation.

It is generally accepted that high doses of glucocorticoid are associated with adverse effects on bone health, primarily leading to osteoporosis. The majority of studies leading to this conclusion studied high dose GC used for anti-inflammatory purposes and recent studies examining the effect of lower doses of GC replacement on bone health have been less consistent in their findings. Consistent with published data(36), a randomised dose reduction in my study resulted in a 56% increase in osteocalcin and an 86% increase in PINP, both markers of bone formation, compared to the highest dose regimen. In conjunction with little change in markers of bone resorption these results suggest a positive bone remodelling balance, alterations in which have been shown in other studies to correlate with future changes in BMD. Interestingly, I demonstrated that cortisol dynamics are likely to be as important as overall dose in relation to overall bone health. Mean 24 hour cortisol concentrations correlated negatively for the lower dose regimens in some markers of bone formation and resorption, again suggesting that as cortisol increases, bone turnover is suppressed. Trough cortisol levels after the morning and afternoon dose also correlated negatively with bone markers and these correlations

were most significant in the lowest dose regimen indicating that low trough levels of cortisol at physiological time-points may be required to sustain bone health. This is the first study to demonstrate the impact of cortisol replacement dose and dynamics in hypopituitarism. The findings are consistent with suggestions that even at low doses, long-acting synthetic glucocorticoids such as prednisolone are associated with increased risk of osteoporosis(112).

In general, published data in the literature regarding cortisol metabolism in hypopituitarism is focussed on the interaction of GH deficiency and GH replacement on various metabolic endpoints, without significant reference to the effect of GC replacement dose or dynamics on tissue cortisol exposure. I demonstrated in this population that although urinary free cortisol is significantly increased in dose A compared to the other does regimens and controls, this finding dose not simply reflect benign excretion into the urine as tissue cortisol exposure was increased across all dose regimens, with the highest 11βHSD1 and 5αReductase enzyme activity evidenced in the highest HC dose regimen. These elevations in tissue cortisol exposure may contribute to the adverse metabolic phenotype associated with hypopituitarism and pituitary hormone replacement. Further studies are required to elucidate the effect of different GC dose alterations in the setting of GH deficiency and GH replacement as well as the impact these replacements have on metabolic outcomes.

There is increased vascular mortality and morbidity in hypopituitarism(14), and although the causes are likely multifactorial, the contribution of hypertension has been debated, with some studies finding no difference in blood pressure, or lower BP compared to

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controls, while others have demonstrated increased frequency of treated hypertension. In this study I have demonstrated that hypopituitary males on higher dose hydrocortisone replacement have a blunted nocturnal BP dip, and an increased arterial stiffness index, both of which are associated with increased risk of vascular mortality. This is the first study to demonstrate in hypopituitary subjects that, despite normal absolute BP levels, increased vascular risk may be present and may be related to elevated doses of glucocorticoid replacement. Impaired glucose tolerance in the general population is also associated with increased mortality(75, 77) and although there was no difference in markers of insulin sensitivity or resistance between the three HC dose regimens or compared to controls, 30% of patients involved in this study had abnormal glucose tolerance at some point in the study, compared to 20% of the matched controls. Not only do these findings confirm an adverse metabolic outcome on these subjects, but it suggests future increased disease burden for these patients and the health care services.

## 7.2 Areas for future research

Although this work contributes to the knowledge regarding optimisation of glucocorticoid replacement there are many areas in which future research would be beneficial. The impact of dose reduction on long term bone health and of the different dose regimens on the 24hour circadian rhythm of bone turnover markers is an area that may present some interesting findings. It is not clear why cortisol binding globulin is reduced in the hypopituitary cohort and studies assessing the role of GH and GC replacement in modulating CBG may reveal valuable information with respect to tissue cortisol exposure and delivery. Prospective comparison studies of short acting hydrocortisone with

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modified release hydrocortisone with respect to metabolic and quality of life outcomes are required to prove superiority of the latter. Lastly a study that assesses the impact of GHD and GH replacement in conjunction with assessing the impact of different GC replacement regimens may contribute more information regarding tissue cortisol exposure and the adverse metabolic phenotype associated with hypopituitarism.

## 7.3 Recommendations for Clinical Practice

1.  It is reasonable to recommend that physicians aim to prescribe lower dose glucocorticoid replacement in hypopituitary patients with severe ACTH deficiency based on the findings of previous research and this work. Dose reduction should be undertaken in a controlled manner, bearing in mind individual patient requirements and concurrent use of medications that may alter cortisol metabolism.

2.  Evidence to suggest that weight-based dosing or thrice daily regimens are superior to twice daily replacement in severe ACTH deficiency is scant and to facilitate patient compliance and dose reduction twice daily HC replacement is preferable. Our current practice is to recommend that patients take their morning dose immediately upon waking and their second dose between 12 and 2pm in the afternoon, assuming the patients are not night-shift workers.

3.  There is no evidence that HC doses <20mg are associated with increased risk of adrenal crisis and we have demonstrated that higher dose replacement does not avoid low trough levels. Adrenal crises may be avoided by careful and repeated patient education regarding dose adjustments during inter-current illness or

periods of prolonged physiological stress, rather than by routine replacement with higher doses of hydrocortisone.

4. If available, short acting glucocorticoid replacement is preferable for glucocorticoid replacement in secondary adrenal insufficiency, as long acting synthetic glucocorticoids such as dexamethasone and prednisolone appear to be associated with adverse metabolic effects due to exposure to GC at inappropriate time points. This advice may not apply to modified release hydrocortisone as these preparations are aiming for a more dynamic cortisol profile, however metabolic outcome studies using these preparations will be very informative.

5. It is likely that these recommendations are unchanged for the GHD subject not on GH replacement as the impact of GH replacement at most is to slightly reduce bioavailable cortisol through reduction of 11βHSD1 activity, yet the clinical impact of this is still debated (walker). This work was performed in patients on GH replacement and the lowest dose was adequate and physiological replacement.

6. As discussed in the introduction, monitoring hypopituitary patients for over or under-replacement is challenging and there is no single biochemical or clinical marker to facilitate this. This study demonstrates that clinic blood pressure readings and fasting glucose measurements may not be adequate to identify hypopituitary patients with an adverse metabolic profile and as such the use of oral glucose tolerance testing and 24 hour blood pressure assessment should be considered during the long term follow up of these patients. Individual measurement of bone turnover markers or urinary corticosteroid measurements have not been validated for routine clinical assessment to date and I recommend instead a baseline bone density scan followed by interval testing during long term follow up of bone health. Urinary free cortisol does not appear to be helpful in

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monitoring cortisol replacement as there is considerable overlap between dose regimens and studies have demonstrated significant inter-individual variability.

These recommendations are based on my findings in a group of hypopituitary male subjects and therefore may be applicable for this group only.

Hypopituitarism is a rare condition that may be becoming more prevalent due to increasing awareness of post-traumatic and post cranial irradiation therapy hypopituitarism. It is associated with considerable morbidity and mortality and it is vital to reduce the adverse impact that inappropriate hormone replacement may have in this setting in order to decrease the disease burden patients and health care services experience.

# Appendix 1

## The SF-36 questionnaire

This survey asks for your views about your health. This information will help you keep track of how you feel and how well you are able to do your usual activities.

Answer every question by selecting the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

| 1 | In general, would you say your health is: | | | | |
|---|---|---|---|---|---|
| | Excellent | Very good | Good | Fair | Poor |
| | O | O | O | O | O |

| 2 | Compared to one year ago, how would you rate your health in general now? | | | | |
|---|---|---|---|---|---|
| | Much better than one year ago | Somewhat better now than one year ago | About the same as one year ago | Somewhat worse now than one year ago | Much worse now that one year ago |
| | O | O | O | O | O |

3   The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

| | | Yes, limited a lot | Yes, limited a little | No, not limited at all |
|---|---|---|---|---|
| A | Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports | O | O | O |
| B | Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf | O | O | O |
| C | Lifting or carrying groceries | O | O | O |
| D | Climbing several flights of stairs | O | O | O |
| E | Climbing one flight of stairs | O | O | O |
| F | Bending, kneeling, or stooping | O | O | O |
| G | Walking more than a mile | O | O | O |
| H | Walking several blocks | O | O | O |
| I | Walking one block | O | O | O |
| J | Bathing or dressing yourself | O | O | O |

4   During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

| | | Yes | No |
|---|---|---|---|
| A | Cut down on the amount of time you spent on work or other activities | O | O |
| B | Accomplished less that you would like | O | O |
| C | Were limited in the kind of work or other activities | O | O |
| D | Had difficulty performing the work or other activities (for example, it took extra effort) | O | O |

5   During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

| | | Yes | No |
|---|---|---|---|
| A | Cut down on the amount of time you spent on work or other activities | O | O |
| B | Accomplished less than you would like | O | O |
| C | Did work or other activities less carefully than usual | O | O |

Subject Initials:............... Subject No:...... Visit No:............

| 6 | During the <u>past 4 weeks</u>, to what extent have your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups? | | | | |
|---|---|---|---|---|---|
| | Not at all | Slightly | Moderately | Quite a bit | Extremely |
| | O | O | O | O | O |

| 7 | How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>? | | | | |
|---|---|---|---|---|---|
| | None | Very mild | Mild | Moderate | Severe | Very severe |
| | O | O | O | O | O | O |

| 8 | During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and housework)? | | | | |
|---|---|---|---|---|---|
| | Not at all | Slightly | Moderately | Quite a bit | Extremely |
| | O | O | O | O | O |

9 These questions are about how you feel and how things have been with you during the <u>past 4 weeks</u>. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the <u>past 4 weeks</u>...

| | | All of the time | Most of the time | A good bit of the time | Some of the time | A little of the time | None of the time |
|---|---|---|---|---|---|---|---|
| A | Did you feel full of pep? | O | O | O | O | O | O |
| B | Have you been a very nervous person? | O | O | O | O | O | O |
| C | Have you felt so down in the dumps that nothing could cheer you up? | O | O | O | O | O | O |
| D | Have you felt calm and peaceful? | O | O | O | O | O | O |
| E | Did you have a lot of energy? | O | O | O | O | O | O |
| F | Have you felt downhearted and blue? | O | O | O | O | O | O |
| G | Did you feel worn out? | O | O | O | O | O | O |
| H | Have you been a happy person? | O | O | O | O | O | O |
| I | Did you feel tired? | O | O | O | O | O | O |

| 10 | During the <u>past 4 weeks</u>, how much of the time have your <u>physical health or emotional problems</u> interfered with your social activities (like visiting friends, relatives, etc)? | | | | |
|---|---|---|---|---|---|
| | All of the time | Most of the time | Some of the time | A little of the time | None of the time |
| | O | O | O | O | O |

| 11 | How TRUE or FALSE is <u>each</u> of the following statements for you? | | | | |
|---|---|---|---|---|---|
| | | Definitely true | Mostly true | Don't know | Mostly false | Definitely false |
| A | I seem to get sick a little easier than other people | O | O | O | O | O |
| B | I am as healthy as anybody I know | O | O | O | O | O |
| C | I expect my health to get worse | O | O | O | O | O |
| D | My health is excellent | O | O | O | O | O |

*Thank you for completing these questions!*

Subject Name (Please Print) :..............................Subject Signature:...........................Date:..........
*Subject to complete details above*

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# Appendix 2

### Nottingham Health Profile

Overview:

The Nottingham Health Profile is intended for primary health care, to provide a brief indication of a patient's perceived emotional, social and physical health problems.

Breakdown of questionaire

(1) Part I: 38 questions in 6 subareas, with each question assigned a weighted value: the sum of all weighted values in a given subarea adds up to 100

• energy level (EL): 3

• pain (P): 8

• emotional reaction (ER): 9

• sleep (S): 5

• social isolation (SI): 5

• physical abilities (PA): 8

(2) Part II: 7 life areas affected

Completing questionaire

• each question answered "Yes" or "No"

• important that all questions are answered

• if the patient is not sure whether to say "yes" or "no" to a problem, s/he are instructed to answer the one more true at that time.

Part I

| Question | Yes | No | Section | Weight |
|---|---|---|---|---|
| I'm tired all the time. | | | EL | 39.20 |
| I have pain at night. | | | P | 12.91 |
| Things are getting me down. | | | ER | 10.47 |
| I have unbearable pain. | | | P | 19.74 |
| I take pills to help me sleep. | | | S | 22.37 |
| I've forgotten what it's like to enjoy myself. | | | ER | 9.31 |
| I'm feeling on edge. | | | ER | 7.22 |
| I find it painful to change position. | | | P | 9.99 |
| I feel lonely. | | | SI | 22.01 |

| | | | Section | Weight |
|---|---|---|---|---|
| I can walk about only indoors. | | | PA | 11.54 |
| I find it hard to bend. | | | PA | 10.57 |
| Everything is an effort. | | | EL | 36.80 |
| I'm waking up in the early hours of the morning. | | | S | 12.57 |
| I'm unable to walk at all. | | | PA | 21.30 |
| I'm finding it hard to make contact with people. | | | SI | 19.36 |

| Question | Yes | No | Section | Weight |
|---|---|---|---|---|
| The days seem to drag. | | | ER | 7.08 |
| I have trouble getting up and down stairs and steps. | | | PA | 10.79 |
| I find it hard to reach for things. | | | PA | 9.30 |
| I'm in pain when I walk. | | | P | 11.22 |
| I lose my temper easily these days. | | | ER | 9.76 |
| I feel there is nobody that I am close to. | | | SI | 20.13 |
| I lie awake for most of the night. | | | S | 27.26 |
| I feel as if I'm losing control. | | | ER | 13.99 |
| I'm in pain when I'm standing. | | | P | 8.96 |
| I find it hard to get dressed by myself. | | | PA | 12.61 |
| I soon run out of energy. | | | EL | 24.00 |
| I find it hard to stand for long (e.g., at the kitchen sink, waiting in a line). | | | PA | 11.20 |
| I'm in constant pain | | | P | 20.86 |
| It takes me a long time to get to sleep. | | | S | 16.10 |
| I feel I am a burden to people. | | | SI | 22.53 |
| Worry is keeping me awake at night. | | | ER | 13.95 |
| I feel that life is not worth living. | | | ER | 16.21 |

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| I sleep badly at night. | | | S | 21.70 |
|---|---|---|---|---|
| I'm finding it hard to get along with people. | | | SI | 15.97 |
| I need help to walk about outside (e.g., a walking aid or someone to support me). | | | PA | 12.69 |
| I'm in pain when going up or down stairs. | | | P | 5.83 |
| I wake up feeling depressed. | | | ER | 12.01 |
| I'm in pain when I'm sitting. | | | P | 10.49 |

Part II

| Is your present state of health causing problems with your: | Yes | No |
|---|---|---|
| Work? <br><br> (that is, paid employment) | | |
| Looking after the home? <br><br> (cleaning & cooking, repairs, odd jobs around the home, etc.) | | |
| Social life? <br><br> (going out, seeing friends, going to the movies, etc.) | | |
| Home life? <br><br> (that is, relationships with other people in your home) | | |
| Sex life? | | |
| Interests and hobbies? <br><br> (sports, arts and crafts, do-it-yourself, etc.) | | |
| Vacations? <br><br> (summer or winter vacations, weekends away, etc.) | | |

Interpretation

• number of questions in each section affected

   • relative level affected, in which the sum of the relative weights are subtracted from 100%, giving values between 0 and 1, with 0 indicating poor and 1 good health

# Appendix 3

## ACTH Study Proforma

Admission Date_____          Admission dose regimen_____

Patient Study ID_____          Issues while on dose_____

**Study Procedures:**

1. Admission 0700hours to 0800hours _____
   a. Clinical Examination
      i. BP_____ Weight_____height_____WCM_____
      ii. General examination_____
   b. Confirmation of dose schedule_____ no alterations in dose_____
      i. This morning's dose not yet taken_____
   c. 24hour BP monitor attached     □     Time initiated_____
   d. IV cannula inserted _____
   e. Baseline samples taken for
      i. Cortisol 0800 "A" □  CBG □ TSH, FT4, PRL, FSH/LH, Testo, IGF-I □ PTH,
         □ 25OHD □ calcium, albumin, renal indices □ Cannula flushed _____
   f. 24hour urine collection commenced_____
2. **Hydrocortisone dose taken at 0800hours_____**
3. Serum cortisol sample taken at
   **0900** □   **1000** □   **1100** □   **1200** □   **1300** □   **1400** □   **1500** □
   Explain any delay or missed sample_____
4. Quality of life questionnaires completed between 0900 and 1000hours □
5. **Hydrocortisone dose taken at 1600hours_____**
6. Serum cortisol sample taken at
   **1600** □   **1700** □   **1800** □   **1900** □   **2000** □   **2100** □   **2200** □   **2300** □   **0000** □
   *****Fasting from 2300hours******0200** □   **0400** □   **0600** □   **0800"B"** □
   Explain any delay or missed sample_____
7. Collection of the following samples between 7am and 8am while fasting and before HC -
   Bone turnover markers _____ (centrifuge immediately)
8. **Hydrocortisone dose taken at 0800hours_____**
9. 24 hour BP monitor detached_____time_____
10. 24 hour urine collection completed_____time_____ total volume_____
11. 0900 hours_____ 75g oral glucose challenge consumed
    a. Time 0 □ Time 30□ Time 60 □ Time 90 □ Time 120□
12. Pre-discharge examination completed_____ patient well □   cannula removed □
13. Next dose schedule or completion of study confirmed_____

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