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# Hypothalamic disease in craniopharyngioma patients.

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*Royal College of Surgeons in Ireland*

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# **Hypothalamic Disease in Craniopharyngioma**

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**Research conducted in the Academic Department of Endocrinology,  
Beaumont Hospital, Dublin 9, Ireland**

**Supervised by Prof. Christopher J Thompson**

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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 96188**

**Date**

## List Of Publications and Presentations Arising from Thesis

### Publications

Crowley RK, Hamnvik OP, O'Sullivan EP, Behan LA, Smith D, Agha A, Thompson CJ.

Morbidity and Mortality in Craniopharyngioma Patients after Surgery. *Clin Endocrinol (Oxf)*. 2010 73(4) 516-521

Crowley RK, Sherlock M, Agha A, Smith D, Thompson CJ.

Clinical insights into Adipsic Diabetes Insipidus; a large case series  
*Clin Endocrinol (Oxf)*. 2007 66(4):475-82

Crowley RK, Woods C, Fleming M, Rogers B, Behan LA, O'Sullivan EP, Kane T, Agha A, Smith D, Costello RW, Thompson CJ

Somnolence in Adult Craniopharyngioma Patients is a Common, Heterogeneous Condition That is Potentially Treatable  
*Clinical Endocrinology (Oxf)* 2011 74(6):750-5

## **Oral Presentations**

RK Crowley, D Ashley, OP Hamnvik, LA Behan, EP O'Sullivan, A Agha,  
D Smith, D O'Gorman, CJ Thompson.

Insulin resistance in craniopharyngioma patients compared to obese and  
overweight controls.

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RK Crowley, C Woods, M Fleming, B Rogers, RW Costello, CJ  
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High Prevalence of Sleep apnoea in patients after surgery for  
craniopharyngioma.

Irish Endocrine Society, Belfast, November 2007.

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9, OC5 S357

R Crowley, CJ Thompson, R Costello.

Sleep Disturbance following surgery for craniopharyngioma.

Irish Sleep Society, February 2007

## **Poster Presentations**

RK Crowley, P Barrett, C Woods, M Fleming, LA Behan, EP O'Sullivan, B  
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Hypothalamic resistance to signals of energy reserve, rather than excess appetite stimulation, may contribute to obesity in CP patients.

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Hypothalamic resistance to signals of energy reserve, rather than excess appetite stimulation, may contribute to obesity in CP patients.

Irish Endocrine Society, Cork, November 2009.

Published abstract: Irish Journal of Medical Science 2009 Vol 178 Suppl 10 P36

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Preservation of sexual dimorphism and diurnal variation in leptin levels of patients with craniopharyngioma.

British Endocrine Society, Harrowgate, April 2008.

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R Crowley, C Woods, M Fleming, B Rogers, R Costello, C Thompson.

High prevalence of sleep apnoea in craniopharyngioma patients after surgical intervention.

British Endocrine Society, Harrowgate, April 2008.

Published abstract: Endocrine Abstracts 2008 15 P206. \* Poster prize.

RK Crowley, O-P Hamnvik, E O'Sullivan, A Agha, CJ Thompson.  
High Prevalence of Obesity in Hypopituitarism Independent of Age at  
Diagnosis, Surgical Approach or Radiotherapy.  
American Endocrine Society, Toronto, June 2007

RK Crowley, RW Costello, CJ Thompson  
Sleep Disturbance Occurs Following Surgical Intervention for  
Craniopharyngioma and May Be Asymptomatic.  
American Endocrine Society, Toronto, June 2007

RK Crowley, OP Hamnvik, A Agha, CJ Thompson.  
Hypothalamic Obesity is Common in Patients undergoing Neurosurgical  
Procedures and is unrelated to Surgical Approach.  
European Congress on Obesity, Budapest, April 2007.  
Published abstract Int J Obesity 2007 31 Suppl 1: T2: PO.85

RK Crowley, RW Costello, CJ Thompson.  
Sleep Disorders in Craniopharyngioma Patients - a Preliminary Report.  
British Endocrine Society, Birmingham, March 2007.  
Published abstract Endocr Abstracts 2007 13 P249

Hamnvik OPR, Crowley RK, Agha A, Thompson CJ.  
Morbidity, Co-Morbidity and Mortality in an Irish Craniopharyngioma  
Cohort.  
Irish Endocrine Society, Galway 2006.

Published abstract Irish Journal of Medical Science 2006 175(4) Suppl 2:

13

Crowley RK, Smith D, Agha A, Sherlock M, Thompson CJ.

High Morbidity and Mortality in Adipsic Diabetes Insipidus.

American Endocrine Society, Boston, June 2006.

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High Morbidity and Mortality in Adipsic Diabetes Insipidus.

Irish Endocrine Society, Limerick, November 2005.

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Suppl 2 p12 (P43)

## Summary

Craniopharyngioma (CP) patients suffer from high rates of morbidity and mortality. The aim of this study was to describe hypothalamic morbidity and establish the standardised mortality ratio for CP patients attending the national neurosurgical unit in Ireland; to investigate abnormalities of thirst, sleep disorders and somnolence, glucose tolerance and abnormal hormonal responses to food intake, in CP patients.

The standardised mortality ratio for craniopharyngioma patients in this study was 8.75, with a predominance of deaths due to cardiovascular and respiratory disease. Over 80% of CP patients had panhypopituitarism, including diabetes insipidus. Four patients with diabetes insipidus had adipsia, which is associated in the published literature with increased morbidity and mortality. Tumour recurrence occurred in 46%, and hydrocephalus was diagnosed in 40% of CPs. Sixty-six percent of CPs were obese and 26% were overweight.

Seventy-one percent of CP patients suffered from somnolence and 46% were diagnosed with sleep apnoea; thus not all somnolence in CP was explained by sleep apnoea. Somnolence responded to treatment with continuous positive airway pressure therapy or modafinil.

Abnormal glucose tolerance was identified in 40% of CP patients, who were insulin resistant and obese or overweight. The CP patients with



abnormal glucose tolerance were older when diagnosed with CP than those with normal glucose tolerance.

CP patients had similar ghrelin levels to normal weight controls, which suggested that excess ghrelin was not the explanation for obesity in CP. However serum ghrelin levels in CP patients suppressed after food ingestion and were lower in patients with apnoea than in those without apnoea, which suggested that the hypothalamus remained sensitive to ghrelin signalling.

This study confirms the previous reports of high rates of morbidity and mortality in CP patients and identifies new targets for treatment of morbidity, such as dysnatraemia, somnolence and abnormal glucose tolerance.

## Chapter 1

### Introduction

#### 1.1 *Background*

Craniopharyngiomas (CP) are tumours of the sella and suprasellar region. The epidemiology of CP, management of tumour bulk and recurrence rates have been well described (1). The natural history of craniopharyngioma is inconsistent with that of other benign lesions; CP patients have increased mortality when compared to the background population (2-4), and also when compared to a hypopituitary cohort (3), which suggests that pituitary hormone deficiency alone does not explain excess mortality in CP. Improved management of these tumours has led to increased survival but CP continues to be associated with excess morbidity, low progression-free survival rate and reduced quality of life (4-8).

Our observations are that large tumour bulk and invasion into the hypothalamus distinguish CP patients from other hypopituitary groups; we postulate that hypothalamic damage contributes to the excess morbidity and mortality that many investigators report in patients with CP.

The hypothalamus is a collection of specialised nuclei and is a small structure vulnerable to damage from numerous insults including tumour bulk in CP and other suprasellar masses, surgery, trauma, infarct,

haemorrhage, infiltration and toxins (9-14). The hypothalamus integrates signals from the viscera, bloodstream, retina, and brainstem, and exerts control over the homeostasis of the body via hormonal and autonomic output (briefly summarised in Table 1.1). Hypothalamic morbidity such as deficiency in pituitary hormone releasing factors, hyperphagia and obesity, thirst disorders and abnormal temperature regulation can result from disruption to afferent and efferent signal pathways as well as to the nuclei themselves.

**Table 1.1.**

List of hypothalamic nuclei with known function, sources of input to nuclei and output.

AVP = arginine vasopressin, BP = blood pressure, HR = heart rate,  
HRFs = hypothalamic releasing factors, CVO = circumventricular organs  
(site of osmoreceptors; located anterior hypothalamus), DA = dopamine,  
NTS = nucleus of the solitary tract (vagal input; located in brainstem)

<u>Name</u>	<u>Function</u>	<u>Input from</u>	<u>Output to</u>
Supraoptic nucleus	Synthesis AVP + oxytocin	CVO, NTS	Neurohypophysis
Preoptic nucleus (medial, median, lateral components)	BP + HR, thirst, thermoregulation, sexual behaviour, HRFs	NTS, CVO, thermoreceptors (skin + mucous membranes)	Neurohypophysis, cerebral cortex
Suprachiasmatic nucleus	Circadian rhythm + sleep/wake	Retina	Pineal gland, other hypothalamic nuclei
Paraventricular nucleus	Synthesis AVP + oxytocin, HRFs, gastric reflexes, satiety	CVO, NTS,	Neurohypophysis, median eminence, brain stem, spinal cord
Anterior hypothalamus	Thermoregulation	Skin, spinothalamic tract	Autonomic NS, posterior hypothalamus
Ventromedial hypothalamus	Satiety, response to hypoglycaemia	Leptin, ghrelin, insulin	Vagal + SNS efferents, other nuclei
Mammillary nucleus	Memory	Amygdala, hippocampus	Thalamus
Dorsomedial nucleus	Feeding		
Arcuate nucleus	Synthesis DA, HRFs, appetite	Leptin, ghrelin, insulin	Median eminence, other nuclei, locus coeruleus
Lateral hypothalamus	Wakefulness, hunger, thirst	Glucose (?)	
Posterior hypothalamus	BP control, thermoregulation, histamine production for wakefulness and suppression of feeding	Pre-optic nucleus, skin	Autonomic NS, supra-chiasmatic nucleus

The sequelae of damage to the hypothalamus due to CP and other pathologies have been the subject of many case reports (15, 16).

However the prevalence of hypothalamic damage in CP and the clinical

manifestations of this damage are poorly understood and rarely investigated in a prospective study design. The lack of formal investigation into hypothalamic morbidity in CP compared to other pituitary lesions reflects the significant challenges that studying the CP population presents to the clinical researcher. CP is a rare disease with an incidence of 0.13 per 100,000 person years (17); most centres therefore have small numbers of CP patients. During the time needed to collect a case series, several different neurosurgeons may have shared responsibility for CP resections, who may have favoured different neurosurgical techniques and who may have had differing abilities and success rates. In addition, management guidelines for resection, radiotherapy or hormone replacement therapy may change several times during the collection of a series of patients substantial enough to allow statistical analysis (18). The age at diagnosis of CP is defined by a bimodal distribution with peaks at 5-14 years and 65-74 years (17); it may confound results to include patients with both CP of childhood-onset (CO) and adult-onset (AO) in studies, since there are reports of differences in presentation, quality of life and cardiovascular disease risk factors between CO and AO groups (10, 19). CP patients often present to neurosurgeons with visual loss or headache and may only be seen by an endocrinologist in the post-operative phase; thus the data on pre-operative hormone deficiencies are often incomplete and the effect of hypothalamic damage due to tumour bulk alone compared to that from surgical resection is difficult to establish (10, 20). The recruitment of appropriately-matched controls for studies of CP is also difficult because

CP patients are younger at onset of pituitary hormone deficiency than patients with other causes of tumoural hypopituitarism, such as non-functioning adenomas, and suffer from complications of treatment, such as obesity, at a younger age (21). All of these factors contribute to the heterogeneity of presentations and complications in CP patients; and in the absence of randomised controlled trials of treatment modalities, management decisions must be made on a case-by-case basis with the aim of optimisation of progression-free survival while preserving hypothalamic function and quality of life. The research, upon which this thesis is based, was undertaken in order to provide more information about hypothalamic disease in CP and to identify areas for intervention that may improve survival and quality of life.

The aims of the thesis and the data already known about hypothalamic pathology are the basis of this introduction.

## **1.2 *Aim of Thesis***

The aims of the thesis are described below:

1. To describe hypothalamic disease processes in our own CP cohort.
2. To establish the standardised mortality ratio for CP patients in Ireland.
3. To investigate abnormalities of thirst in CP patients and to identify associated morbidities.

4. To investigate sleep disorders and somnolence in CP patients and examine responses to treatment.
5. To establish the rate of abnormal glucose tolerance in CP patients and to analyse factors that impact on insulin sensitivity.
6. To analyse abnormal hormonal responses to food intake in CP, in order to assess the potential impact of abnormal appetite signalling on obesity in CP.

### **1.3 *Hypothalamic-Pituitary Dysfunction***

Anterior pituitary dysfunction is the most intensively studied and most commonly reported neuroendocrine complication of CP and its treatment, and can be a result of direct damage to the pituitary, interruption of hypothalamic releasing factor delivery by stalk damage or direct insult to the hypothalamic nuclei responsible for synthesis of releasing factors and dopamine (22). Intrasellar CPs are associated with more endocrine deficiencies at presentation than CP tumours of the third ventricle (23), which suggests that endocrine deficiency in CP is predominantly due to direct damage to the pituitary. Although physicians often identify endocrine dysfunction at presentation, it may not be the presenting complaint from the patient (24) and the incidence of endocrine dysfunction as the primary indication for referral varies with the age of the patient at diagnosis (1, 10, 25).

Endocrine symptoms at the time of presentation with CP vary according to factors such as the age of the patient and the location of the tumour itself. Retrospective chart reviews suggest that up to 93% of childhood CP cases have growth failure at presentation, up to 24% of adolescents have failure of sexual development or delayed puberty (24), and between 10 and 85% of adults present with hypogonadism (1). CO CP patients are more likely than AO to present with pressure symptoms, such as headache or cranial nerve palsies, than with endocrine dysfunction (10, 24, 26). Precocious puberty as a manifestation of hypothalamo-pituitary dysfunction has been reported in CP patients (1, 24, 26-28), but is rare, and was not identified in 36 patients with CP out of a series of 115 children with suprasellar tumours evaluated for signs of early puberty (29). The authors of the suprasellar tumour review postulated that location of tumour alone did not predispose to precocious puberty, since CP patients in the series were not affected, but that other tumours of the suprasellar region, such as germ cell tumours, were functional and may have secreted neurotransmitters that affected gonadotrophin-releasing hormone secretion (29). In those cases of precocious puberty that present in CP patients, the condition responds to surgical management (27); but precocious puberty has also been described in CP children as a complication of treatment modalities such as radiotherapy or intracystic bleomycin (30, 31).

There are less data on formal pre-operative assessment of endocrine deficiency in CP patients than for post-operative deficiencies, because



patients may present to a neurosurgeon with pressure symptoms and undergo resection before formal pituitary assessment can be performed. Comparison of rates of anterior pituitary hormone deficiencies between centres is difficult because testing protocols and diagnostic criteria are not standardised, but tumour bulk prior to surgical resection certainly affects adeno-hypophyseal function (32). Pre-operative assessment of growth hormone (GH) reserve revealed deficiency in up to 100% of children and 86% of adults, but these patients comprised only a third of children and a tenth of adult patients in the overall cohort who may have been pre-selected for pituitary testing because they had features of endocrine deficiency at pre-operative review (10). Gonadotrophin deficiency is present in 30-74% adults (4, 10, 33), the variation in reported rates is due to differences in diagnostic criteria between centres. Adrenocorticotrophin (ACTH) deficiency is more common in children than adults pre-operatively and occurs in 20-70% of patients (4, 10); hyponatraemia secondary to ACTH deficiency can be the presenting complaint in CP (34). TSH deficiency affects 25-46% of patients before surgery (4, 10).

When comparison was made between pre-operative pituitary function and data from the post-operative setting in a Dutch cohort of CP patients, there was no recovery of endocrine function after surgery for CP, and there was an increase in hypopituitarism from 60% pre-operatively to 89% after surgery (4). Higher rates of post-operative hypopituitarism in CP, compared to pituitary adenomas, was confirmed in a recent meta-

analysis (35). Rates of hypopituitarism increase in CP patients post-operatively but not in patients who undergo surgery for other suprasellar lesions such as Rathke's cleft cysts (36). A KIMS review revealed that CP patients suffered from higher rates of hypopituitarism post-operatively than patients with non-functioning adenoma (37). The difference in rates of post-operative hypopituitarism between lesions may be due to the ease with which a surgeon can dissect a cyst or adenoma from surrounding tissue; this is technically more demanding in CP, and is not always possible. CP tumour tissue invades surrounding tissue, which explains the high rates of post-operative damage to the hypothalamus when a surgeon attempts to excise CP with a clear margin, and also the high rates of recurrence in CP because of the difficulty in achieving gross total resection (38, 39). CP patients have a lower peak GH response to stimulation than patients with other pituitary tumours, which suggests more severe GH deficiency after CP resection; again this is suggestive of a more locally invasive tumour that requires more extensive resection than pituitary adenoma (40). In an assessment of other factors in CP that could impact upon post-operative pituitary function, Kendall-Taylor, in her analysis of the KIMS database, reported that there was no difference in post-operative rates of hormone deficiency between CP patients diagnosed in childhood compared to those diagnosed as adults (19). Panhypopituitarism is present post-operatively in over 85% of CP patients (4, 10, 19, 37).

The high frequency of hormone deficiencies in CP is clinically significant because endocrine deficiencies contribute to reduction in quality of life and to mortality in CP patients (3, 41, 42). Tomlinson highlighted that GH and sex steroids were under-replaced in hypopituitary patients enrolled in the West Midlands database, with replacement rates of 24% and 67% respectively in a population that included 118 CP patients (3). Failure to replace sex steroids adequately was associated with increased mortality in the West Midlands database (3). The decision not to replace a deficient hormone may be deliberate; in hypopituitary women of post-menopausal age, oestrogen therapy is contra-indicated because of the risk of breast cancer, and GH may not be replaced in paediatric CP, because some CP patients continue to grow despite GH deficiency (23, 26, 43). The phenomenon of “growth without growth hormone” in CP children is poorly understood, but is associated with suprasellar tumours (44) and delayed bone age (43), and is thought to be an effect of obesity and hyperinsulinaemia (23, 45, 46). Growth without growth hormone has also been reported in association with precocious puberty (47). There is no increased risk of CP recurrence with GH therapy (48-50) and GH-deficient CP patients are very sensitive to GH replacement; growth responds well to GH therapy in childhood (40). Discontinuation of GH replacement after childhood is associated with deterioration in lipid profile and quality of life (51). However, GH replacement is associated with weight gain and obesity in CP (20, 37); fat-free mass increases but fat mass and BMI do not decrease, unlike GH-replaced pituitary adenoma

patients (37). The mechanism for continued weight gain in CP patients in the face of appropriate GH replacement is unknown.

In summary, careful assessment of the CP patient by an endocrinologist both pre-and post-operatively allows for informed counselling for the patient and optimisation of replacement therapies with the aim of improved quality of life and survival.

#### **1.4 *Posterior Pituitary Dysfunction***

Central diabetes insipidus (CDI) can result from damage to the posterior pituitary, the stalk, the synthetic nuclei of the hypothalamus or to the osmoreceptors in the anterior hypothalamus that detect changes in plasma osmolality (52). In contrast to NFPA patients, who do not manifest diabetes insipidus prior to surgical intervention, polyuria secondary to CDI is a presenting feature in up to 30% of cases of CP (1), and is reported at presentation in paediatric CP at higher rates than in AO CP (10). The difference in prevalence of CDI at time of diagnosis between patients with CP and those with NFPA, illustrates the effect on hypothalamic function of differences in tumour bulk and location. Rates of CDI increase from 16-30% pre-operatively to over 95% after surgery (4, 32, 33, 36, 53). CDI remains more common post-operatively in CP patients than in those who are surgically treated for non-functioning pituitary adenoma (37).

Stalk disruption during surgery can also cause a transient syndrome of inappropriate antidiuresis (SIADH), as a result of dysregulated release of stored arginine vasopressin (AVP) (54). A triple-phase response of acute polyuria followed by transient SIADH followed by sustained polyuria was reported in 14 out of 117 CP patients after surgery in one series (54); 79 of the 117 patients had DI, and 76 of these had some degree of electrolyte imbalance post-operatively. Hyponatraemia is an independent risk factor for mortality (55) and difficulty in management of water balance in DI has been implicated in the high morbidity and mortality associated with CP (56), including a case of myelinolysis in a CP patient with other hypothalamic sequelae (57). The high rate of electrolyte imbalance post-operatively and the potential for fatal complications illustrates the importance of making the diagnosis of DI before surgery, to allow for careful management of water balance during surgery, and for the involvement of a specialist with an interest in water balance (58).

Polyuria secondary to CDI is managed with desmopressin (dDAVP) therapy. In one multivariate analysis, dDAVP replacement in CP patients was associated with weight gain (20). Weight gain on dDAVP therapy may be a marker for water retention and risk of hyponatraemia; such weight gain or serial admissions with dysnatraemia should prompt an assessment of thirst (see next section).

## 1.5 *Thirst*

Abnormalities of thirst are recognised sequelae of CP and may occur at rates as high as 20% after surgery (1, 53, 59, 60). Both excess thirst and reduced thirst have been reported after surgery for CP (53, 54, 59-61).

The osmoreceptors for thirst generation are located in close proximity to those for AVP release, in the anterior hypothalamus where they are vulnerable to damage by CP tumour mass or surgery (52). Smith and colleagues demonstrated that baroregulated release of AVP was normal in cases of adipsic diabetes insipidus (ADI) secondary to vascular or infiltrative disease but was absent in ADI secondary to CP, which suggested that the nuclei responsible for AVP synthesis as well as the osmoreceptor site were damaged by surgery or tumour bulk in CP (60). Hypodipsia is associated with treatment for large hypothalamic tumours (62), which explains why ADI is reported more frequently after CP surgery than after resection of pituitary adenoma (15, 63).

Thirst disorders in CP are intrinsically associated with diabetes insipidus, and the combination of these two abnormalities presents a significant management challenge for the physician (63). Desmopressin therapy for CDI is essential to control polyuria, but is hazardous in polydipsic patients because of the risk of dilutional hyponatraemia due to excess fluid intake that cannot be excreted in the presence of exogenous dDAVP (53).

Adipsic patients must drink a prescribed volume of fluid daily in order to maintain eunatraemic body weight, a surrogate marker of hydration status

(63). In order to prescribe fluid intake correctly, the patient must be weighed while eunatraemic, advised to drink 1.5-2 litres of fluid daily and weighed daily in order to replace any weight deficit with the corresponding volume of fluid (ie a weight 0.5kg below eunatraemic weight should prompt the patient to increase fluid intake by 0.5 litres) (63).

ADI is associated with recurrent dehydration and hypernatraemia (61). Other investigators have described myelinolysis and rhabdomyolysis during episodes of dysnatraemia (57, 61, 64). Failure to diagnose thirst disorders in CP can thus have serious clinical consequences; CP patients with recurrent dysnatraemia should be formally assessed with water deprivation tests paired with a visual analogue scale to assess thirst (65).

## **1.6 Obesity**

As a general principle it can be stated that obesity develops when there is an imbalance between energy intake, energy expenditure and energy storage (66, 67) and there is evidence in the literature to support disruption in all three elements of energy handling by CP patients (Table 1.2)(68). Under normal physiological conditions, energy intake is stimulated by ghrelin, which acts on the hypothalamus to release the appetite stimulants neuropeptide Y (NPY) and agouti-related peptide (69). Intake in normal individuals is balanced by the sensation of satiety and the signal to increase energy expenditure; both are generated by the

adipocytokine leptin in response to insulin, itself a satiety signal. Leptin signals energy sufficiency via its receptor in the hypothalamus, through the JAK-STAT pathway, which activates the sympathetic nervous system to increase energy expenditure (67, 70). The signal of energy sufficiency is absent in humans with genetic leptin deficiency or resistance, both rare syndromes that result in hyperphagia and obesity similar to that in the CP cohort (66, 67, 70). The frequency of development of obesity in CP patients has been attributed to disruption of these normal regulatory mechanisms for energy intake, expenditure or storage and has led to the description of an entity known as hypothalamic obesity.

**Table 1.2**

Mechanisms of weight gain and development of obesity in craniopharyngioma patients

<u>Excess energy intake</u>	<u>Reduced energy expenditure</u>	<u>Inappropriate energy storage</u>
Hyperphagia Leptin Resistance Insulin Resistance (Normal ghrelin)	Reduced vision Reduced mobility Somnolence / fatigue Reduced sympathetic tone Reduced thermogenesis	Hyperinsulinaemia

Hypothalamic obesity is defined as obesity that complicates diseases of the hypothalamus and their treatment; CP, and treatment of CP is the



most common single structural cause of this syndrome (66). Obesity occurs in 26-66% of CP patients treated with surgery and is associated with increased mortality in both the general and CP population (1, 71). A review of the KIMS database revealed that 47.1% of CP patients were obese and that the mean body mass index (BMI) in CP patients was higher than in non-functioning pituitary adenoma patients (37). Factors that have been implicated in the development of obesity in CP patients include tumour mass, tumour location, surgical damage, age of the patient at diagnosis and treatment modalities.

The relative contributions to obesity of hypothalamic damage from CP tumour mass versus intervention have been widely discussed but not fully elucidated. Tumour mass and location, before surgical intervention, has some effect on weight gain in both adult and paediatric populations. Hyperphagia or excessive weight gain are complaints at presentation in 5% of children and 13% of adults with CP (10), and 17% of CP patients have excessive pre-operative weight gain associated with a hypothalamic lesion on imaging (72), whose accelerated weight gain pre-operatively is attributable to tumour effect alone (41). However, pre-operative weight gain could be an effect of unrecognised hypopituitarism as well as of tumour size and tumour invasion on surrounding hypothalamic tissue (10). Tumour location as a factor in obesity is confirmed in CO CP; the rate of obesity is higher in patients with a tumour that involves the hypothalamus (21, 44, 73, 74). The presence of hydrocephalus on imaging is predictive for obesity; this probably also reflects the large size

of the CP tumour (74). It is evident that tumour mass and location explain some of the obesity in CP reported by clinicians, but do not determine the complete picture.

Younger age of diagnosis of CP is associated with the development of obesity. Younger age at CP diagnosis in childhood is a risk factor for development of obesity in CO CP (21). Others suggest that AO CP carries a higher risk of obesity than CO CP (10); in the KIMS database patients with AO CP have an obesity rate of 51.8% compared with 39% in those diagnosed in childhood (19). The reason for the difference in obesity rates with age of onset is unclear. There are other differences between obesity in CO and AO CP; increased BMI correlates with poor quality of life in AO CP but not in CO CP, perhaps because adults have a greater awareness than children of deterioration in health and fitness after diagnosis with CP (19). Increased BMI in early childhood and at CP diagnosis correlate with weight gain after treatment for CO CP (73, 75, 76), but BMI at time of diagnosis in adults does not predict BMI at follow-up (20, 77). Adult studies lack the pre-morbid records of BMI that are readily available to paediatric investigators and without such records we cannot assess weight gain during the months prior to diagnosis of CP in adulthood.

Treatment for CP appears to have a greater effect on BMI than tumour mass alone. Obesity rates can double or treble after surgery (20, 41). Karavitaki calculated a 67% probability of development of obesity 20

years after treatment for CP (10); these rates were confirmed by De Vile in paediatric CP patients, whose BMI correlated with the degree of hypothalamic damage on post-operative MRI (72). Lustig identified tumoural characteristics and treatment factors associated with obesity in paediatric survivors of brain tumours. These include hypothalamic lesions, a histological diagnosis of CP and high-dose radiotherapy (21), but this study included relatively small numbers of CP patients. It is unclear whether radiotherapy is a marker for more severe disease or whether it is an independent risk factor for obesity and hypothalamic disease.

Thus it can be seen that tumour mass, location, surgery, the age of the patient and the use of radiotherapy can all contribute to hypothalamic damage and obesity, and there are three theories related to energy intake, energy expenditure and energy storage to explain the pathophysiology behind this damage.

### **1.6a *Hyperphagia***

Excess energy intake in the form of hyperphagia is a frequent explanation for post-operative weight gain in CP patients. Compulsive hyperphagia after CP treatment is a well-recognised phenomenon (10, 78-80).

Damage to the arcuate nucleus of the hypothalamus, conferring leptin resistance and increased insulin, is the putative cause of hyperphagia, the evidence for which was initially provided by animal studies (81). Post-

operative hyperleptinaemia is reported in paediatric patients with suprasellar CP, but not in sellar lesions, which suggests that hyperleptinaemia is a manifestation of hypothalamic damage (82). Leptin levels and insulin response to an oral glucose load are higher in children with hypothalamic obesity than in those with hypothalamic disease or simple obesity alone (83). Hypopituitarism itself is associated with high leptin because androgens suppress adipocyte production of leptin (70), but hypopituitarism alone cannot explain elevated leptin in a prepubertal group nor the difference between leptin levels in hypothalamic and sellar lesions.

Like leptin, insulin acts as a satiety signal on the arcuate nucleus to reduce food intake; insulin levels and systemic insulin resistance are increased in CP patients with hypothalamic involvement, which suggests that insulin levels rise to overcome hypothalamic insulin resistance (44, 69, 84). Insulin also acts as an efferent signal from the hypothalamus to the peripheral tissues via the autonomic nervous system (83), to suppress hepatic glucose production (85), and may be dysregulated in CP (83). The complex role of insulin as both an afferent and efferent signal in the hypothalamus makes interpretation of CP insulin levels and insulin sensitivity difficult. Although damage to the arcuate nucleus may reduce sensitivity to satiety signals, appetite signalling is not increased and it is interesting to note that arcuate nucleus damage does not cause ghrelin resistance and hyperghrelinaemia in CP (86). An elegant clinical experiment by Goldstone et al showed that ghrelin response to calorie

challenge in CP patients was comparable to that of BMI-matched controls, and CP ghrelin was lower than normal weight controls (86); these data suggested that obese CP patients reduced ghrelin and food intake appropriately in the context of excess energy stores.

Further clarification of the role of sensitivity to satiety signals in hypothalamic obesity may come from further investigation of those individuals who present with the converse syndrome - appetite loss in CP. Although hyperphagia and hyperinsulinaemia are frequently reported in CP, hypothalamic damage does not always result in weight gain and excess food intake. Reduced food intake has also been reported to occur in CP patients, but is rare compared to hyperphagia and is poorly understood. A small subset of CP patients presents with anorexia and the "diencephalic syndrome" which can be severe enough to be confused with anorexia nervosa (87, 88). Hypothalamic anorexia has been reported with other hypothalamic tumours and surgical removal of tumour was followed by excessive weight gain in one case series (89). However anorexia is an extremely unusual presenting complaint in CP and is difficult to study because of the rarity of presentation.

### **1.6b *Reduced energy expenditure***

Obesity in the CP population may result from reduced energy expenditure rather than hyperphagia (90); this was inadvertently demonstrated by a follow-up study to that which reported hyperleptinaemia (82), when the

same investigators assessed food intake in CP (91). CP patients in the study had lower caloric intake than normal-weight controls by a daily difference of 400 kilocalories, and CP patients had reduced daily physical activity counts (91). The investigators speculated that neurological or visual disability, daytime somnolence, inappropriate hormonal substitution or reduced sympathetic tone could cause reduced physical activity in CP patients (91). Visual deficits occur in 10-50% of survivors of CP (10, 33), and CP patients have a significant deterioration in physical function (92); both disabilities can lead to reduced mobility and explain reduced activity levels. Daytime somnolence and the effects of hormonal substitution are discussed in later sections.

A unifying theory to explain increased leptin and reduced activity in CP is that leptin resistance is implicated in sympathetic nervous system down-regulation (93); leptin administration to leptin-deficient mice promotes lipolysis, thermogenesis and increased movement secondary to sympathetic activation (67). Reduced sympathetic tone in CP patients is demonstrated by their lower adrenalin response to hypoglycaemia in an insulin tolerance test than controls (94, 95). Sympathetic activity as measured by urine sympathetic metabolites was lower in obese than normal-weight CPs and lower in those with hypothalamic lesions (96). Reduced sympathetic tone indicates damage to the ventromedial hypothalamus, impaired integration of peripheral signals by the arcuate nucleus or loss of hypothalamic outflow to the locus coeruleus (66, 67). Reduced sympathetic activity is an important diagnosis because it is

amenable to treatment with stimulants, such as dextroamphetamine which has been demonstrated to reduce weight gain in CP by increasing physical activity (97). Energy expenditure through physical activity is partially achieved through thermogenesis, which can be reduced as a complication of craniopharyngioma and other hypothalamic disease (14, 98). Disordered thermogenesis is discussed in a later section.

In summary, reduced energy expenditure may contribute to weight gain in CP patients and consideration should be given to management of physical disability or to treatment of reduced sympathetic tone with stimulants.

### **1.6c *Inappropriate energy storage***

An imbalance between energy intake and expenditure can occur when energy is stored excessively in the setting of excess insulin secretion. Hyperinsulinaemia in hypothalamic obesity could be the result of over-activity of the parasympathetic nervous system rather than a rise in insulin as a secondary response to obesity (99); lesions of the ventromedial hypothalamus in rats lead to hyperinsulinaemia and energy storage as adipose tissue (81), and excess insulin secretion is reduced by efferent vagotomy (100).

In humans it is difficult to confirm that hyperinsulinaemia is a direct response to hypothalamic damage because most human studies assess CP patients after stabilisation of post-operative weight gain, when they are already obese and insulin resistant (44, 86, 101). Hyperinsulinaemia promotes abnormal energy storage of fat in the liver in CP, causing non-alcoholic fatty liver disease in the acute phase post-surgery and after weight gain (80, 102-104).

If hyperinsulinaemia is truly a primary event in hypothalamic obesity, then it is an avenue for therapeutic intervention. Lustig hypothesised that octreotide therapy would reduce insulin secretion and weight, and performed a pilot trial of octreotide in patients with hypothalamic obesity (105). Unfortunately the only CP patient in the series did not tolerate octreotide, but the trial resulted in weight loss in 5 other patients and weight stabilisation in one. A placebo-controlled trial of thrice-daily octreotide in hypothalamic obesity reduced weight gain and insulin and was associated with improved quality of life (106).

It can be seen that the mechanisms regulating energy intake, output and storage are not independent, but identification of the dominant pathology can be used to tailor therapy to the individual patient. Gastric reduction surgery is an intervention that acts via multiple pathways, such as reduced food intake and insulin secretion, in hypothalamic obesity (107). Successful gastric bypass procedures in CP patients are now being described with greater frequency, resulting in weight loss and reduced



insulin levels; CP patients seem to be at risk of more complications because they have multiple co-morbidities at surgery (108-110). Other directed interventions to improve obesity include stimulants that impact on somnolence, which will be discussed in the next section.

## **1.7 *Abnormal Regulation of Sleep***

### **1.7a *Somnolence***

Normal physical activity in CP patients is important for quality of life and maintenance of normal weight; normal energy expenditure is difficult to maintain in the somnolent patient. Excessive daytime somnolence (EDS) is an abnormal tendency to fall asleep during the daytime and is reported prior to surgery for CP (10, 111). It can be difficult to differentiate clinically between somnolence and fatigue, which is reported more frequently by CP patients than by controls (92). EDS can be measured using the Epworth Sleepiness Score (ESS) and is diagnosed from scores of greater than 10 / 24 (112), or by the Multiple Sleep Latency Test (MSLT) which is a test of the time taken by the patient to fall asleep when given the opportunity to nap. Both the ESS and the MSLT has been used to confirm EDS in patients after hypothalamic surgery, including patients with CP (41, 113, 114).

Somnolence in CP may be related to obesity, which is associated with somnolence in itself, and is reported in obese CP patients (115-117).

The cause for EDS in CP patients is probably multi-factorial. EDS may

be due to conditions associated with CP, such as inadequate replacement therapy for pituitary hormone deficiencies, sedation secondary to anti-epileptic therapy, somnolence associated with obesity and untreated sleep-breathing disorders; or secondary to damage from tumour mass itself to cause unrecognised sleep disturbance and acquired narcolepsy.

### **1.7b *Somnolence and Hormone Replacement***

Over-replacement of GH and under-replacement of TSH could cause somnolence in CP. Acromegaly is associated with obstructive sleep apnoea (OSA) and somnolence (118); it is possible that GH over-replacement in GH-deficient CP patients causes EDS. GH excess does not explain somnolence at presentation with CP as reported by Karavitaki (10). Schneider et al studied the sleep of patients with GH deficiency before and after GH replacement and reported no change in sleep after treatment with GH (119), even though approximately half of the patients achieved IGF-1 levels in the supraphysiological range; this is suggestive of over-replacement with GH without any detrimental effect on sleep. A later study confirmed these findings of normal sleep architecture after 6 months of GH therapy (120); it is therefore unlikely that GH replacement is implicated in somnolence. Under-replacement with thyroxine is also an endocrine cause of somnolence, fatigue and OSA (121, 122). TSH deficiency is common in CP patients (1), but is usually promptly treated; its association with somnolence in CP is unknown.

### **1.7c *Somnolence and Seizure Disorders***

Seizures are common in the CP population and occur in 15-20% of patients (4, 41). Both untreated and treated epilepsy can impact on sleep (123). Untreated epilepsy is associated with sleep fragmentation (124), which predisposes patients to daytime somnolence, and treatment with anticonvulsants causes hypersomnia (125). In a non-CP epileptic population, vagal nerve stimulation (VNS) has been successfully used to reduce daytime sleepiness (126), but VNS has also been reported to cause a deterioration in OSA and would not be suitable for use in some CP patients.

### **1.7d *Somnolence and Obesity***

Obesity is associated with increased somnolence independent of sleep-breathing disorders (116, 127); the explanation for daytime somnolence associated with obesity alone is not known, but possible factors include depression (127), nocturnal sleep disturbance and circulating inflammatory cytokines (116). Obesity is a major risk factor for obstructive sleep apnoea (OSA). Although obesity is a well-recognised complication of CP, OSA in the CP population has not been studied prospectively.

OSA has been reported in 63% in a cohort with GH deficiency that included CP (120). Snow identified OSA in somnolent adolescents post-

hypothalamic surgery, in whom somnolence persisted despite use of continuous positive airway pressure (CPAP) therapy (113). Further investigation of somnolence in the CP population is indicated, since treatment is available (128) and untreated OSA is associated with increased morbidity and mortality and reduced quality of life (129-132).

### **1.7e Secondary Narcolepsy**

Not all the sleep abnormalities observed in CP patients can be attributed to obesity, however, and may be due to damage to the hypothalamic nuclei that control wakefulness. Two important wakefulness-promoting sites are located in the hypothalamus; the lateral hypothalamus, which secretes hypocretin, and the tuberomammillary nucleus, which produces histamine (133, 134); both areas are vulnerable to damage from tumour bulk or at tumour excision. Hypocretin is a wake-promoting neurotransmitter that is deficient in the CSF of idiopathic narcolepsy patients (135, 136). There are similarities between narcolepsy patients, who suffer from excessive sleepiness, cataplexy and other REM sleep phenomena, and those with somnolence associated with CP (137); patients with idiopathic narcolepsy, like CP patients, have reduced volume of hypothalamic grey matter on MR imaging (138). Acquired narcolepsy has been reported in patients with brain tumours; of 113 cases of acquired narcolepsy, Nishino reported that 29% were secondary to brain tumours, of which 70% were in the hypothalamus and 6 cases were CP patients (139). Evidence for a “secondary narcolepsy” in CP patients comes from a number of case reports of early REM sleep onset

in CP patients (114, 140, 141), and low hypocretin levels in the CSF of CP and hypothalamic tumour patients (16, 140, 142), which suggests damage to the lateral hypothalamus that mimics idiopathic narcolepsy. Interestingly, patients with idiopathic narcolepsy have other features in common with CP patients such as obesity and insulin resistance (135). It should be noted that cataplexy is not a feature of acquired narcolepsy and there is only one report of transient cataplexy after surgery for CP (143).

The clinical significance of a diagnosis of secondary narcolepsy is that such patients can benefit from a trial of stimulant therapy. Agents that combat EDS include stimulants such as methylphenidate, dexamphetamine and modafinil, all of which have been used in somnolence associated with craniopharyngioma (114, 141, 144). Methylphenidate and amphetamine exert their effects by increasing dopamine activity, but the action of modafinil is not clearly understood (133).

### **1.7f Other Sleep Abnormalities**

Multiple other poorly defined sleep abnormalities have been reported in CP patients. Sleep fragmentation occurs in young CP patients, who suffered from multiple awakenings after sleep onset (145); this is a relatively simple cause of daytime somnolence which is easy to assess with polysomnography during an overnight admission. Rehmann reported rapid sleep-wake cycles in a CP patient, whose sleep had

escaped normal regulation (111). A possible explanation for loss of normal sleep rhythm in CP is deficiency of melatonin, a hormone produced by the pineal gland with a role in sleep regulation. Muller found low melatonin concentrations in the saliva of obese CP patients (115), and reported reduced somnolence after a course of melatonin replacement (146).

The frequency of somnolence in CP patients and the wide variety of abnormalities of sleep reported in the CP literature illustrate the complexity of sleep regulation and its sensitivity to disturbance after hypothalamic damage. No single hypothalamic lesion can explain somnolence and sleep disorder in CP patients; this is partly due to the complexity of input to, and output from the hypothalamic nuclei. In summary, sleep problems in CP are common and are multifactorial but are amenable to treatment.

### **1.8 *Thermoregulation***

Poikilothermia is failure of body temperature regulation and is a rare disorder. The main thermoregulation centre is the hypothalamus, which effects its action both via autonomic outflow from the anterior hypothalamus to control sweating and vasodilation for heat loss, and vasoconstriction and shivering for heat generation; and via behavioural changes co-ordinated from the posterior hypothalamus (147). The core temperature of a healthy individual varies by as little as 0.2°C from target

(36.5-37.5°C), but can vary by as much as 6°C in patients with poikilothermia (147). The most common cause of poikilothermia is anaesthesia, which blocks behavioural responses to temperature change and alters the threshold temperatures at which autonomic regulation occurs (148).

A diagnosis of impaired temperature control is a rare but important diagnosis to make in CP patients – such a diagnosis would obviate the need for excessive investigation of episodes of hypo- or hyperthermia for sources of sepsis, would raise safety concerns in patients with hypothalamic obesity undertaking exercise programmes and would affect fluid management in those with co-existing diabetes insipidus or thirst disorders (14, 53). Impaired thermoregulation leads to reduced energy expenditure and is implicated in hypothalamic obesity (66); it is unsafe for patients who cannot regulate body temperature to engage in exercise for weight maintenance, because they cannot initiate sweating to maintain normal temperature and fluid balance.

Abnormal temperature regulation has not been prospectively studied in CP, but has been reported in both paediatric and adult cases (64, 149-151). Poikilothermia in CP is a post-operative complication (10, 152), but poikilothermia was reported at presentation in a woman with a large pituitary adenoma, which implied that tumour bulk itself could be implicated in poikilothermia (153). Poikilothermia has also been described after hypothalamic damage from toxins (14), neural tube

defects (154), multiple sclerosis (147) and pituitary adenoma (153) as well as craniopharyngioma. Hyperthermia secondary to hypothalamic damage responds to chlorpromazine (149, 150).

Poikilothermia in CP patients is complicated by other metabolic derangements such as dysnatraemia (57, 64), pancreatitis (64) and pancytopenia (64, 151), all of which are associated with hypothermia independent of a diagnosis of CP. Complications of hypothermia resolve with appropriate fluid management and careful re-warming.

The diagnosis of poikilothermia is difficult to make and requires management by a specialist in intensive care. Lack of temperature regulation is confirmed by controlled warming to a higher threshold than normal for onset of sweating, and cooling to a lower threshold than normal for vasoconstriction and shivering (147). Controlled temperature testing in CP should be considered in CP patients with unexplained hypo- or hyperthermia, particularly if considering participation in an exercise programme, and should be performed when the patient is eunatraemic.

## **1.9 Summary**

Craniopharyngioma patients are at risk of hypothalamic disease both pre- and post-operatively. Hypothalamic disease in CP is associated with increased morbidity and mortality; diagnosis of disorders of thirst and thermoregulation in particular may be difficult to confirm. Clinicians who



manage CP should have a high index for suspicion of disorders of thirst, somnolence, temperature regulation and energy handling which could predispose CP patients to weight gain.

Once the diagnosis of hypothalamic disease is made, careful consideration should be given to the possibility of treatment of individual disorders. Recent reports suggest that bariatric surgery is a viable option for CP patients with established obesity. Somnolence is treatable with CPAP in the setting of sleep apnoea, and modafinil or melatonin in acquired narcolepsy and idiopathic hypersomnolence.

In order to optimise quality of life and survival in CP, these patients should be managed in centres with experience of hypothalamic disease management where there is access to a multi-disciplinary, individualised approach to each patient.

## **Chapter 2**

### **Study Design and Methods**

#### **2.1 *Methodology***

This chapter contains a description of the methods used for the studies performed. Elaboration of each method is made in the individual chapters.

#### **2.2 *Identification and Profiling of Study subjects***

Patients with craniopharyngioma were identified from the hospital pituitary database and a chart review was performed. Details of demographic data, tumour characteristics, treatment modalities, complications and co-morbidities were recorded on a datasheet (Windows Excel). Patients who had died were identified from the medical records and from follow-up telephone calls to their general practitioners. Cause of death was recorded from medical records, post-mortem examination reports and from death certificates obtained with permission from the Office of the Registrar General. Those patients who were alive at commencement of the study were invited by letter to attend a specialist outpatient clinic. Patients who attended the outpatient appointment received an explanation of the study and a written patient information leaflet, and were encouraged to discuss participation in the study with their own family doctor. All patients who attended the specialist clinic received a

follow-up telephone call at least one week after the clinic, at which any further questions were answered and the patient was invited to participate in the study. All patients who had undergone surgical resection of a craniopharyngioma in Beaumont Hospital or attended the Endocrine service for medical follow-up were considered eligible for inclusion in the study. Exclusion criteria included age less than 16 years at the time of outpatient visit and inability to give informed consent. The Beaumont Hospital Ethics Committee approved the studies and information leaflets in advance.

### **2.3    *Outpatient Visit***

#### **2.3a   *Clinical data***

All patients enrolled in the study attended the outpatient clinic. A proforma was used to record date of diagnosis, presenting symptoms, intervention used (surgery  $\pm$  radiotherapy), pituitary hormone deficiencies post-intervention, hormone replacement therapies, post-operative complications, hypothalamic complications, Epworth Sleepiness score, family history of diabetes and other medications. Blood pressure was measured using a manual sphygmomanometer and was repeated at least twice if abnormal. Height and weight were measured for calculation of Body Mass Index (BMI, kg/m<sup>2</sup>). A BMI of greater than 30 kg/m<sup>2</sup> was considered representative of obesity. Waist and hip measurements were taken for calculation of waist-hip ratio. The most recent fasting blood glucose and lipid profile measurements were recorded. All details were entered into a spreadsheet (Microsoft Excel).

### **2.3b Sleepiness**

The Epworth Sleepiness Score (ESS) is an 8-part questionnaire that is used to give a subjective measure of daytime somnolence (112). Each question asks the patient what they perceive is their risk of falling asleep in 8 different everyday situations. The patient is asked to rate their likelihood of falling asleep from 1 (low risk) to 3 (high risk). Daytime somnolence is defined as an ESS greater than 10/24. The Epworth score has been validated against the Median Sleep Latency Test, the most objective measure of propensity to fall asleep (155). The ESS is reproducible in normal adults, is high in patients with apnoea and drops after treatment with CPAP therapy. The ESS has been used to assess somnolence in previous studies of CP, non-functioning adenoma and acromegaly patients (118, 156, 157). A copy of the ESS questionnaire is reproduced in Figure 2.1.

## Epworth Sleepiness Score

Situation	Chance of dozing
	0 = no chance of dozing 1 = slight chance of dozing 2 = moderate chance of dozing 3 = high chance of dozing
Sitting and reading	
Watching television	
Sitting inactive in a public place (e.g. a cinema or meeting)	
As passenger in a car for > 1 hour	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to a companion	
Sitting quietly after an alcohol-free lunch	
In a car, while stopped briefly in heavy traffic	
<b>Total Epworth Sleepiness Score</b>	

**Figure 2.1.**

## **2.4 Polysomnography**

### **2.4a Day of admission**

Patients were admitted at 5pm on the day of the polysomnography study. The patient was advised to take all medications, including hydrocortisone tablets and growth hormone injections, at their usual times, such that the sleep study represented a normal night for the patient. All leads were connected to a master headbox, which in turn was attached to the polysomnography monitor by two cables. The headbox design allowed

detachment of the two cables during the night, to allow temporary discontinuation of the study if required. The study was terminated between 7am and 8am on the following morning, according to the wake time of the patient. Removal of adhesive materials was performed with Remove wipes (Smith and Nephew Inc, Hull, England).

#### **2.4b Lead placement**

The electrode leads were cleaned before each patient's use using Stericcept. The patient's skin was prepared for lead placement with Nuprep abrasive skin preparation gel (DO Weaver, Aurora, USA), in order to reduce impedance. The copper heads of the EEG leads were covered in conductance gel (Ten20 conductive EEG paste, Biosense Medical, Aurora, USA) and applied to the skin, and human adhesive (Collodion Adhesive, SLE Diagnostics, Croydon, UK) was used to secure the leads.

Electroencephalography (EEG) leads were placed in the frontal, central, occipital and reference sites according to the 10/20 system of EEG lead placement (158). In total 6 recording electrodes plus ground and reference electrodes were used. The 10/20 system referred to the position of an electrode in relation to the underlying cortex, with each electrode placed 10% or 20% along one of the major axes of the skull. Right hemisphere leads were numbered with even numbers and left hemisphere electrodes with odd numbers. The EEG measured post-synaptic potentials fired in a co-ordinated manner from large groups of

neurons during sleep, which allowed identification of distinct rhythm types by their wave frequencies in order to diagnose sleep stage. The EEG was represented as a bipolar montage; each channel was represented as a waveform of the difference between two recording electrodes.

Leads for leg EMG were attached to the skin over the medial malleoli of both lower limbs; two leads were attached to each leg for leg movement analysis. Two leads for chin EMG analysis were placed below the chin. Leads for electro-oculography (EOG) were placed at the outer edges of the orbits bilaterally; EOG allowed differentiation between REM and Non-REM sleep. Skin preparation and lead attachment was identical for EEG, EMG and EOG leads. Electrocardiography (ECG) was performed with three stickers (Skintact ECG electrodes, Leonhard Lang GmbH, Innsbruck, Austria) placed as per standard lead cardiac monitoring; one on the skin over the right acromion process, one in the same position on the left and the third on the left flank. ECG was used to record R-R interval and variation throughout the sleep study.

I set up each polysomnography study after initial tuition from a representative from Respironics and from a technician from the EEG laboratory; and was also instructed in interpretation of polysomnography studies by Prof Richard Costello, who performed the final interpretation for the studies as an investigator blinded to the underlying diagnosis.

#### **2.4c *Measurement of respiratory effort***

Respiratory effort was measured by the placement of two elastic straps around the patient's torso. The thoracic effort was recorded by a strap placed around the chest at the level of the manubriosternal angle and the abdominal effort by a strap at the level of the umbilicus. The straps were adjusted to fit smoothly around the patient without discomfort.

#### **2.4d *Measurement of airflow***

Airflow during sleep was measured using an airflow sensor (Adult Thermistor Airflow Sensor, Healthdyne for Respirationics, France) secured to the upper lip with an adhesive strip.

#### **2.4e *Measurement of oxygen saturation***

Blood oxygen saturation was measured continuously during the study by means of pulse oximetry. The oximeter probe was secured to the index finger of the patient using adhesive tape.

#### **2.4f *Record of body position***

The body position of the patient during sleep, and any changes therein, were recorded by a body position sensor, which was secured to the anterior aspect of the thoracic effort strap using Velcro.



#### **2.4g *Record of snoring***

Snoring was recorded using a small microphone. This was placed over the larynx of the patient and secured by a strap around the neck.

#### **2.4h *Analysis of sleep record***

Sleep analysis was performed using the Alice 4 (Respironics, France). Each sleep study was recorded onto a PC card and downloaded onto a personal computer for analysis by the Alice software. Sleep reports were generated using Microsoft Word. A respiratory physician, who was blinded to the BMI and ESS of the patient, validated the study reports.

The following temporal data was recorded from the sleep assessment: Total recording time, Time in Bed, Total Sleep Time, Time from Sleep Onset to last Sleep, Time to Sleep Onset, Wake time during sleep, REM time, NREM time (sleep stages 1-4) and Slow wave sleep time (Sleep stages 3 and 4). The number of events per hour of sleep was calculated from the above data.

The technical criteria for the polysomnography study were as follows:

#### **2.4h i *Neurological parameters(159):***

Alpha rhythms: 8-13 Hz frequency

Beta rhythms: Frequency > 13 Hz

Theta rhythms: Frequency 4-7 Hz, varying morphology

Delta rhythms: All frequencies up to 4 Hz. High amplitude, low frequency

Stage 1 sleep was defined as the presence of vertex waves – synchronous symmetric waves with attenuation of alpha rhythm and increased beta activity.

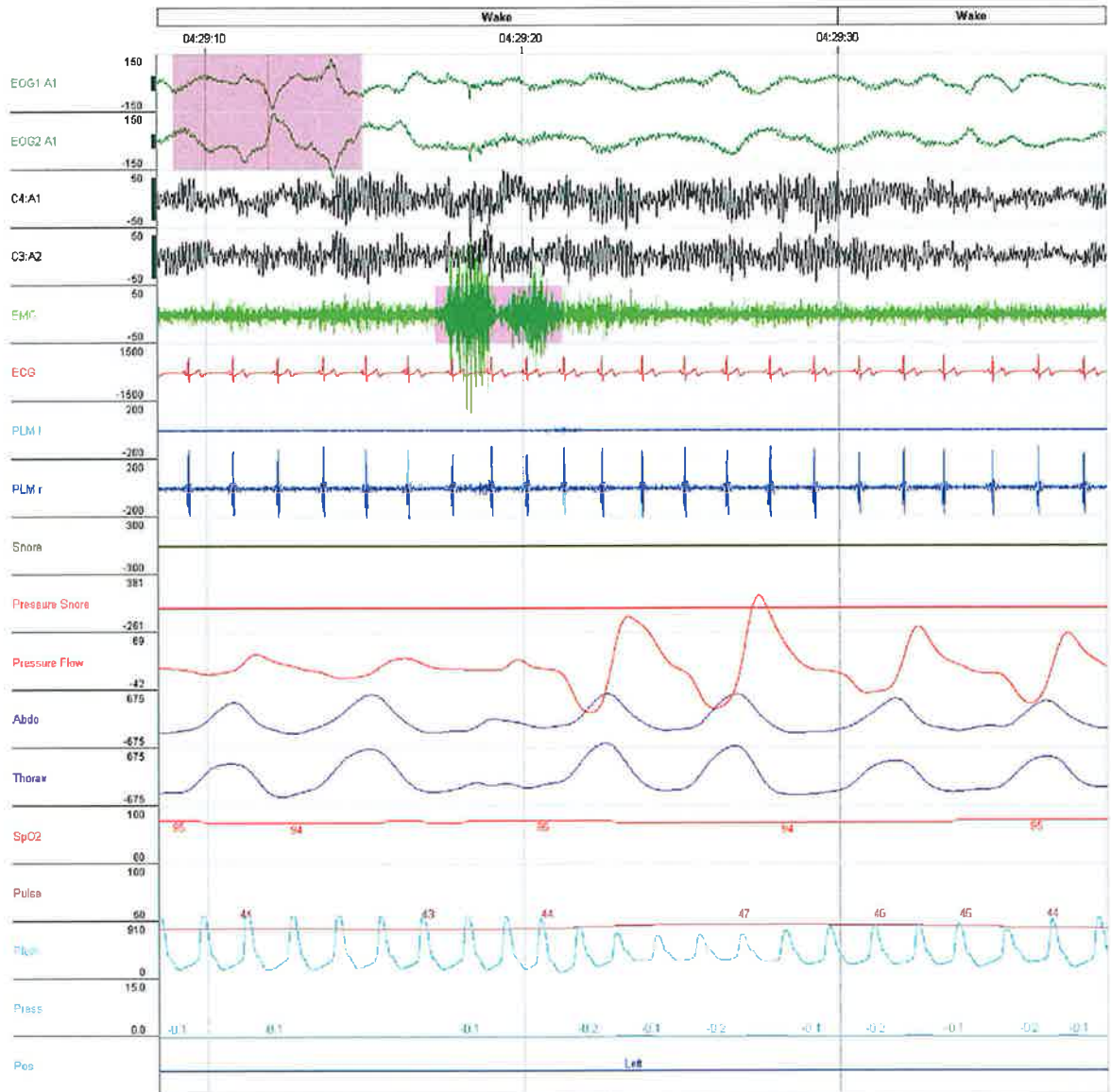
Stage 2 sleep was defined by the presence of sleep spindles and K complexes (Figure 2.3). Sleep spindles were defined as sinusoidal waves with a frequency of 12-14 Hz. A K complex was defined as a high amplitude diphasic wave with an initial sharp transient followed by a high amplitude slow wave.

Stage 3 sleep was defined as sleep with 20-50% delta wave activity.

Stage 4 sleep was defined as sleep with greater than 50% delta wave activity.

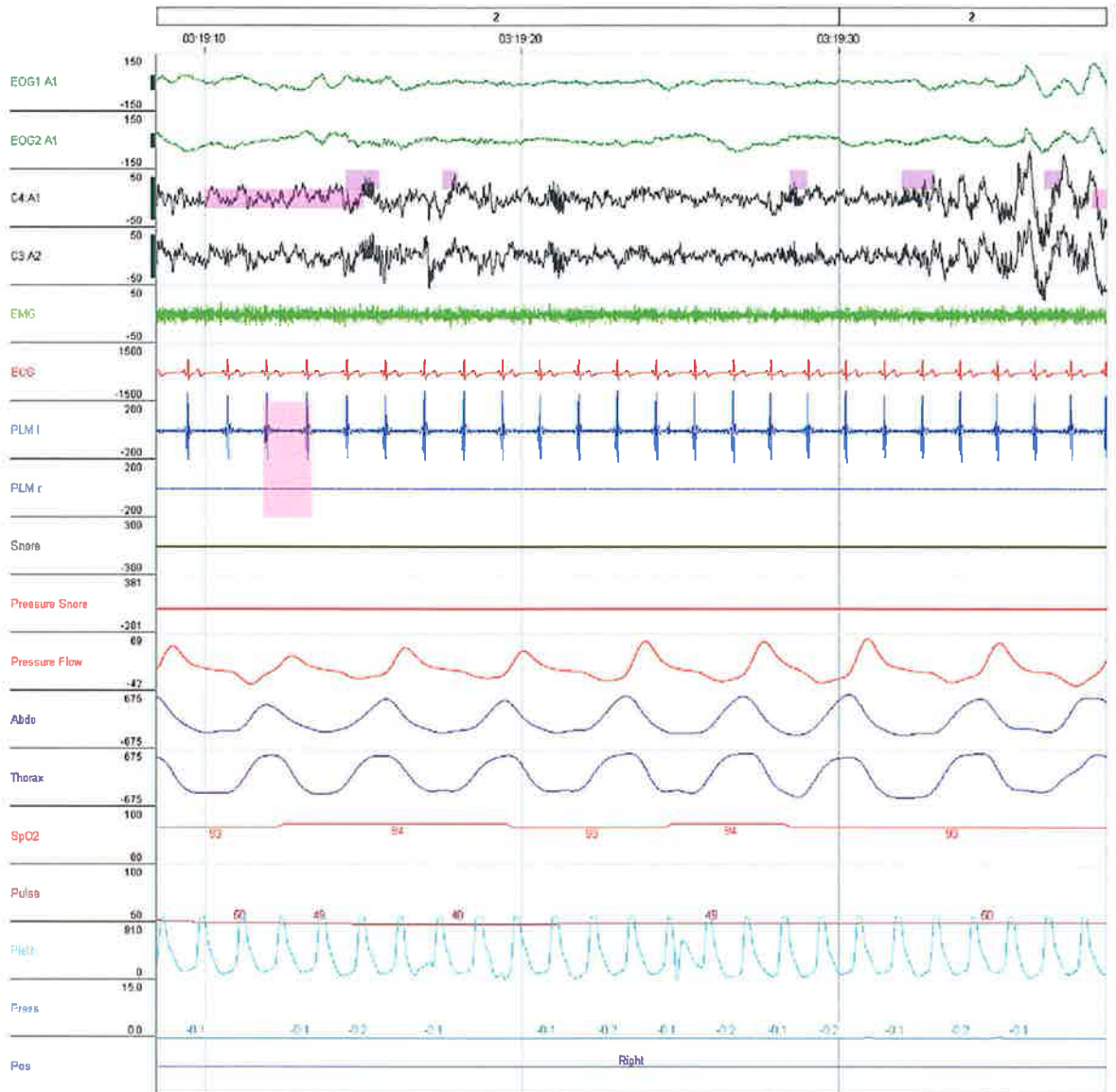
Slow wave sleep was defined as sleep stages 3 and 4.

Rapid Eye Movement (REM) sleep was defined as sleep with rapid eye movements, loss of muscle tone and sawtooth EEG waves (Figure 2.4).



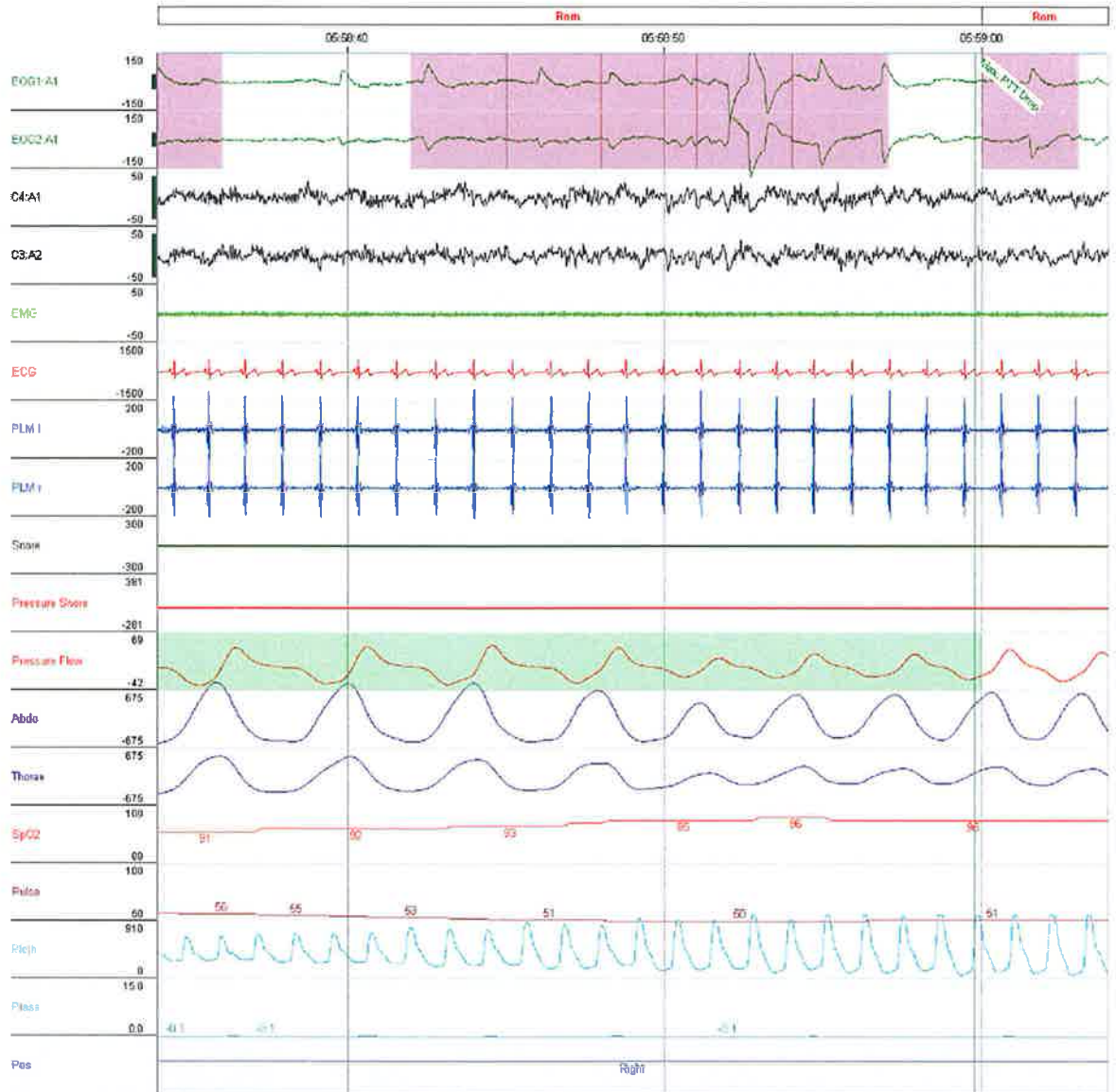
**Figure 2.2** Waking polysomnography

See activity highlighted in electro-oculogram (EOG) and electro-myogram (EMG) leads.



**Figure 2.3** Stage 2 sleep

Note K complexes highlighted in purple in EEG leads



**Figure 2.4: REM sleep**

Note eye movements in electro-oculogram (EOG) leads and flat trace in electro-myogram (EMG) lead, consistent with loss of muscle tone.

## **2.4h ii *Respiratory parameters***

An episode of central apnoea was defined as cessation of airflow without concurrent respiratory effort.

An episode of obstructive apnoea was defined as cessation of airflow with concurrent respiratory effort.

Hypopnoea was defined as 50% drop in airflow from baseline.

10 seconds was the minimum duration acceptable for diagnosis of central, obstructive and mixed apnoeas or hypopnoeas.

An oxygen saturation drop of 4% from baseline and / or heart rate drop of 2.5% from baseline was the minimum acceptable change following an apnoeic event.

Obstructive Sleep Apnoea Syndrome was defined as polysomnographic finding of more than five obstructive apnoeas, greater than 10 seconds in duration, per hour of sleep; and one / more of:

Frequent arousals from sleep associated with the apnoeas

Bradycardia

Arterial oxygen desaturation associated with the apnoeas.

For a diagnosis of OSAS, standard clinical criteria were also required, ie the patient reported excessive sleepiness (which may have been reported by someone other than the patient) and associated features such as snoring, morning headache or dry mouth upon awakening (137).

## **2.5 *Early morning arterial blood gas measurement***

All patients underwent sampling of arterial blood upon waking from the sleep study, in order to diagnose early morning hypoxia and to exclude daytime hypercarbia. The samples were taken from the radial artery using aseptic technique, with a 25g needle (BD Microlance, Drogheda, Ireland), into a prepared syringe that contained 7u of lyophilised heparin (Gaslyte syringe, Marquest, Vital Signs, Colorado, USA). Hypoxia was defined as morning arterial oxygen of less than 10 kPa and hypercarbia defined as morning arterial carbon dioxide of greater than 6kPa.

## **2.6 *Pulmonary Function Tests***

All patients underwent spirometry and lung volume measurement to exclude obesity hypoventilation syndrome or other respiratory disease.

## **2.7 *Appetite Study***

Patients who were admitted for polysomnography also underwent evaluation of the hormones governing appetite and satiety. In order to mimic a normal daily eating pattern, patients were advised to eat their midday meal as normal and then to avoid snacking or consumption of any fluid other than water prior to admission at 5pm. An 18 gauge cannula (Optiva 2, Medex Medical Ltd, Hastingsdon, England) was placed in the antecubital fossa using aseptic technique. 22.5 ml of venous blood was

withdrawn from the cannula at time 0, for measurement of leptin, ghrelin, insulin and adiponectin. Patients drank 333ml of Fortisip, which is equivalent to 500 kCal, in order to assess hormonal response to caloric intake. A further 15ml of venous blood was drawn at 15, 30, 60, 90, 120, 150 and 180 minutes post-administration of Fortisip. The patency of the cannula was maintained by a flush of normal (0.9%) saline after sample withdrawal, and the first 5ml of blood was discarded before sampling, in order to avoid sample dilution by the saline flush. Samples were centrifuged at 3000 rpm for 15 minutes, separated into aliquots and stored in a  $-80^{\circ}\text{C}$  fridge prior to assay. Sampling was repeated in the morning after the sleep study. This protocol is a modification of that used by Goldstone et al to assess appetite (86).

## **2.8 Assessment of glucose metabolism**

Patients attended the Diabetes Day Centre for assessment of glucose tolerance. Patients were advised to fast from 10pm the night before and to take their normal medications with a sip of water, in order to assess glucose tolerance under normal conditions. An 18g cannula (Optiva 2, Medex Medical Ltd, Hastingdon, England) was placed in the antecubital fossa under aseptic technique. 20ml of blood was withdrawn: 2 serum samples for estimation of insulin and C peptide and a citrated sample for estimation of glucose. The sample bottle for C peptide estimation was chilled on ice prior to sampling and transported immediately on ice to the laboratory for centrifugation. 75g of glucose was dissolved in 300ml of water and given to the patient for consumption as an oral glucose



challenge. The patient was advised to remain seated until repeat sampling was performed at 120 and 180 minutes.

### **2.9 Assessment of Osmoregulatory function and thirst**

A subgroup of craniopharyngioma patients with adipsic or polydipsic diabetes insipidus underwent assessment of thirst as part of a larger study of morbidity and mortality in a cohort of patients with adipsic diabetes insipidus previously identified at Beaumont Hospital. The data on thirst, morbidity and mortality in the CP patients alone were included in the CP study. Patients were diagnosed with DI and had confirmation of thirst disorders by measurement of the AVP and thirst response to osmotic stimulation with hypertonic sodium chloride infusion.

Desmopressin was withheld for 24 hours prior to study and patients were encouraged to drink two litres in excess of their urine output, in order to maintain normal plasma osmolality before commencing the study.

Patients were admitted on the morning of study, rested recumbent and intravenous cannulae were inserted in the antecubital veins, one for infusion and the other for venesection. Hypertonic (855 mmol/l) sodium chloride solution was then infused intravenously at 0.05 ml/kg/min for two hours. Blood was withdrawn at 30-minute intervals during the infusion and centrifuged immediately at -4°C for 15 minutes at 2000G. The plasma supernatant was removed and divided into aliquots. One aliquot was used for immediate measurement of plasma osmolality by the depression of freezing point method. The other was frozen at -70°C for later measurement of AVP. Thirst was measured at blood sampling times

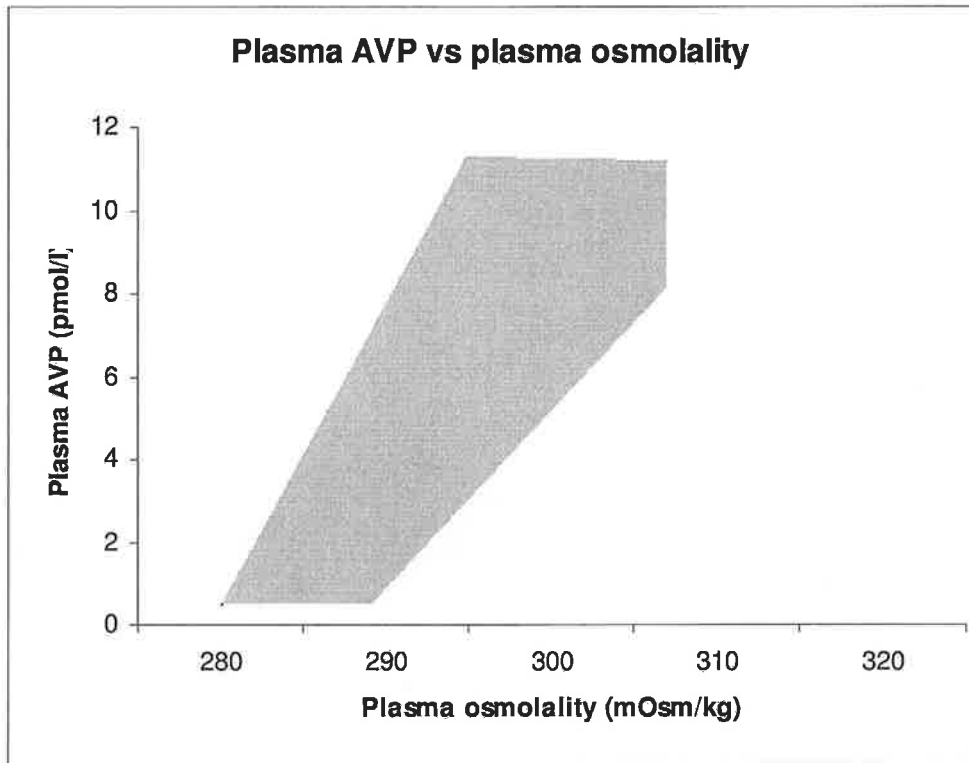
using a 10cm long visual analogue scale (160, 161). As an objective corollary of thirst ratings, the volume of tap water drunk in 30 minutes after cessation of infusion was observed and recorded.

Adipsic DI was defined as follows:

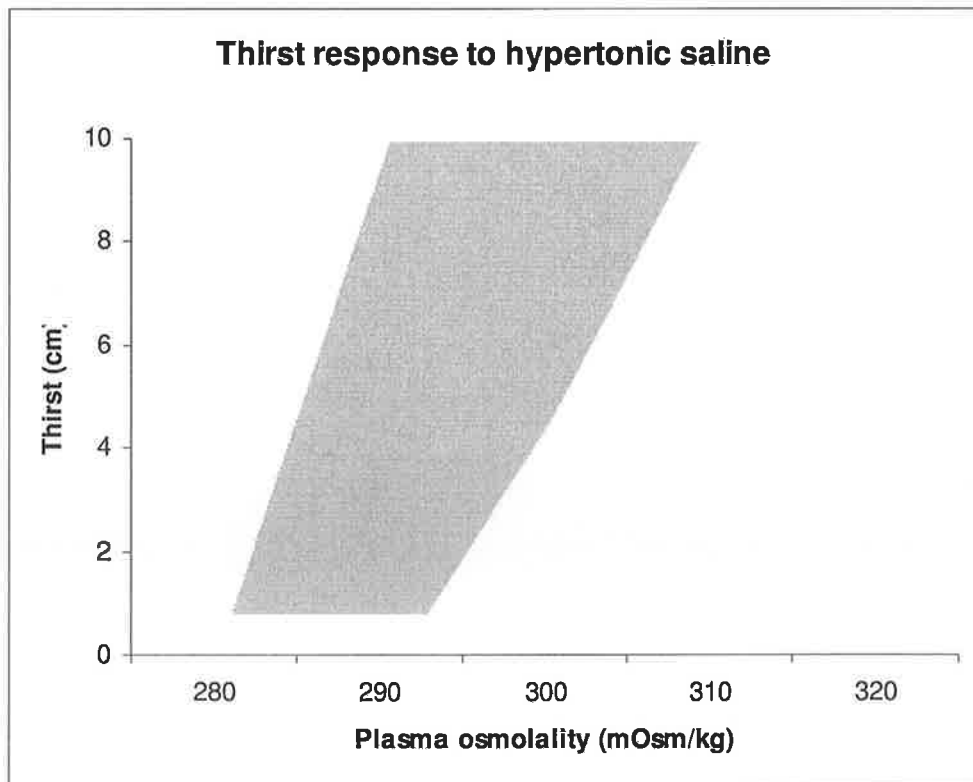
1. hypotonic polyuria (> 4 litres / 24h) with subnormal AVP responses to osmotic stimulation
2. subnormal thirst responses to hypertonic saline, and water intake less than half of the lower limit of normal in healthy controls (<500ml)

Polydipsia was defined as water intake greater than 1,900 ml (normal intake + 2 standard deviations above the upper limit for a craniopharyngioma patient with DI)(53).

Normative data was derived from a data bank comprised of responses of 40 healthy controls. Normal AVP response to increased serum osmolality is shown in Figure 2.5 and thirst response is shown in Figure 2.6 below.



**Figure 2.5:** Serum AVP response to increased serum osmolality in normal controls (shaded area)

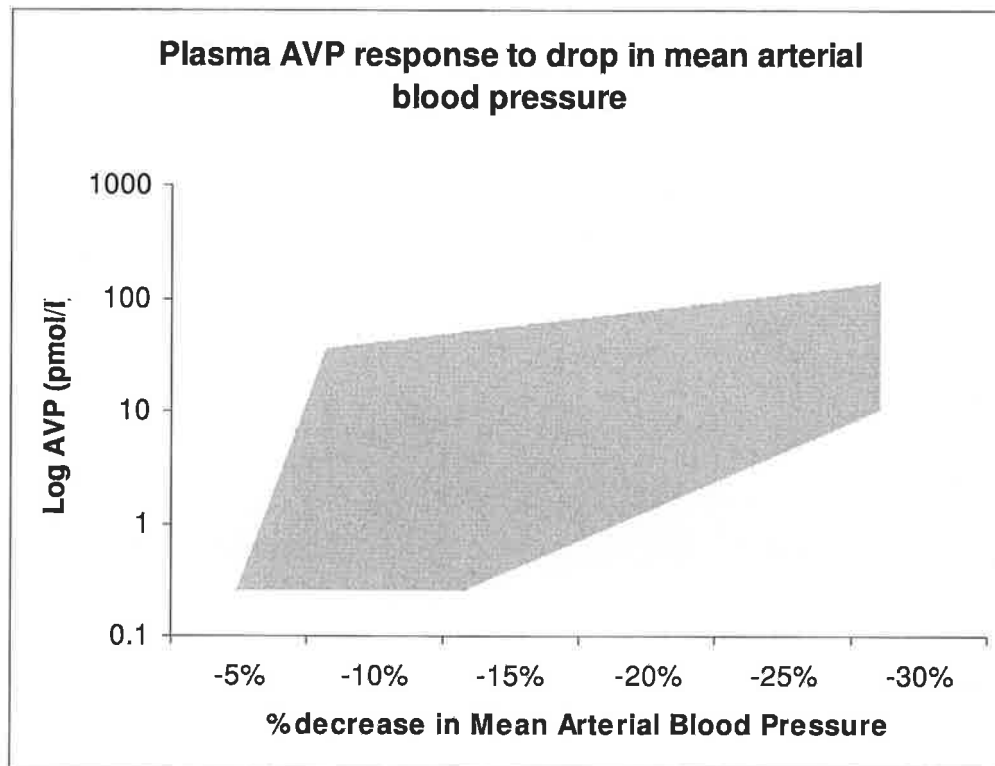


**Figure 2.6:** Thirst response to increased serum osmolality in normal controls (shaded area)

## **2.10 Assessment of Baroregulated Release of Arginine**

### ***Vasopressin (AVP)***

Desmopressin was withheld for 24 hours prior to study and patients were encouraged to drink two litres in excess of their urine output, in order to maintain normal plasma osmolality. Patients were admitted on the morning of the study. Blood pressure was measured three times at baseline and the mean arterial pressure (MAP) was calculated for each reading, using the calculation  $[(2 \times \text{diastolic}) + \text{systolic}] / 3$ . The average of three readings was used as the baseline MAP, and the target MAP was calculated as 70% of baseline. Intravenous cannulae were inserted, one for infusion and the other for venesection. Trimetaphan camsylate solution was infused at 0.5 mg/min and blood pressure was measured every 5 minutes using a mercury sphygmomanometer. The rate of infusion was doubled every 15 minutes until the mean arterial blood pressure had fallen by 30% from baseline, at which point the infusion was discontinued, and the foot of the bed was elevated. Blood was withdrawn at 5-minute intervals during the infusion. Repeat blood pressure measurements were checked until the patient had returned to baseline. Normal AVP response to reduced MAP is shown in Figure 2.7. The assessment of thirst and baroregulation of AVP in CP patients was an extension of work begun by Dr Diarmuid Smith in the area of ADI associated with hypothalamic diseases in patients treated in the unit (53, 60).



**Figure 2.7:** Serum AVP response to reduced mean arterial pressure in normal controls (shaded area)

## 2.11 *Laboratory techniques*

### 2.11a *Estimation of leptin*

The assay for leptin was not available in the endocrine laboratory, so I set up and validated the assay using a commercial kit for radioimmunoassay (Mediagnost, Reutlingen, Germany). The manufacturer calibrated the kit against the WHO International Standard NIBSC Code 97/594. Samples were removed from storage at  $-80^{\circ}\text{C}$  and allowed to thaw at room temperature, mixed by repeated inversion and centrifuged at 3000 rpm for 20 minutes prior to assay. Kit components were brought to room temperature before use. The assay was performed in duplicate.

Streptavidin-coated tubes were provided in the kit. Tubes were labelled as follows:

Tubes 1+2: total counts (TC)

Tubes 3+4: zero standard ( $B_0$ )

Tubes 5-18: standards 1-7

Tubes 19, 20, 61, 62, 103 + 104: medium control

Tubes 21-102, excluding controls: patient samples

Standards of recombinant leptin were provided ready-prepared and ranged from 1-64ng/ml leptin.

Specific antibody from anti-human leptin antibody was reconstituted with 7ml of the assay buffer provided.

Capture antibody from anti-rabbit IgG was reconstituted with 7ml of the assay buffer provided.

The tracer was Iodine  $^{125}$  at < 60 kBq, reconstituted in 13ml assay buffer.

Control material lyophilised from human serum (concentration ca 7ng/ml) was provided with the kit and was reconstituted with 750 $\mu$ l assay buffer.

All reagents were mixed on a roller-mixer for at least 30 minutes before use.

25 $\mu$ l of buffer was added to tubes  $B_0$ , in order to provide a zero standard.

25 $\mu$ l of standards 1-7 was added to tubes 5-18. 25 $\mu$ l of commercial control was added to tubes 19+20, 61+62 and 103+104 to assess intra-assay consistency. 25 $\mu$ l of the sample material for leptin estimation was

added to tubes 21-102. A reverse-pipetting technique was employed to improve pipette accuracy. 50µl of capture antibody, followed by 50µl of specific antibody were added to all tubes beginning with tube 3. 100µl of tracer was added to all tubes. The tubes were shaken overnight on a shaking device at room temperature for a minimum of 15 hours. Tubes 1+2 (total counts) were removed and the liquid in the remaining tubes was drained by careful decantation. 500 µl of assay buffer was added to all tubes except 1+2 and decanted.

The radioactivity of all tubes was measured using a gamma counter. Standard curve and result calculation was performed with a commercial computer data reduction programme (Gamma Wizard), as follows:

Binding value B was calculated from the average counts of each pair of standards

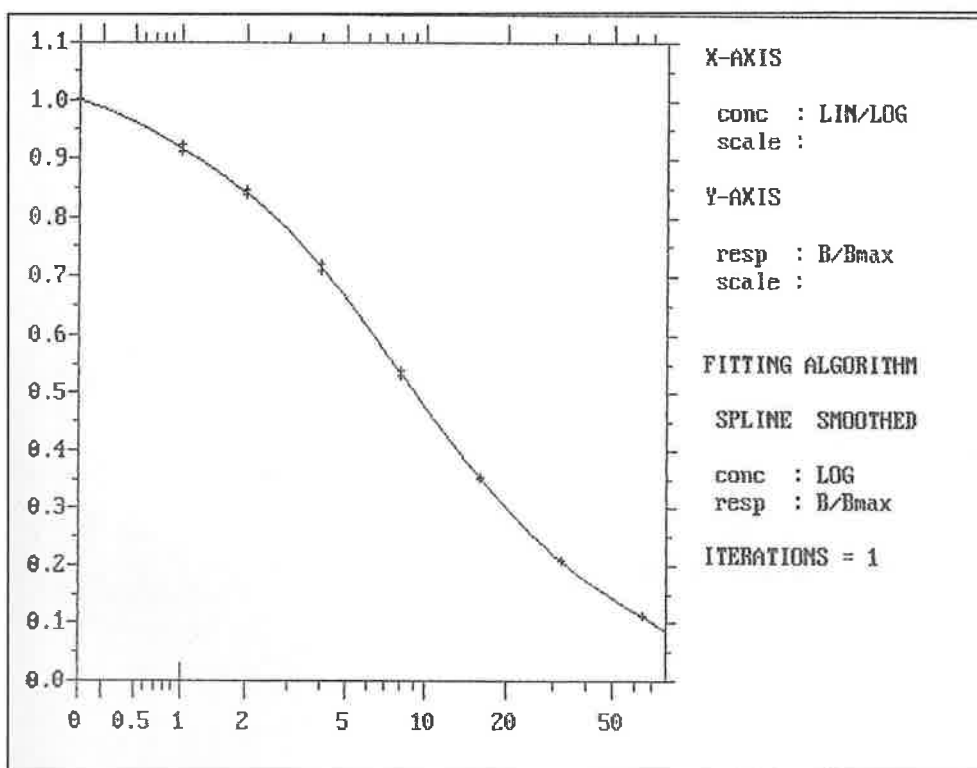
$B_0$  was the average counts of tubes 3+4

Percent bound =  $B/B_0 \times 100$

The percent bound versus the concentration of the standards was plotted on a semi-logarithmic scale

Percentage bound of the zero standard =  $B_0/\text{total counts} \times 100$  (should be > 20%)

Curve generated by Gamma counter shown in Figure 2.8



**Figure 2.8:** Standard curve for leptin RIA, generated by Gamma Wizard.

X-axis shows the concentration of standards 1-7, Y-axis shows the percentage bound for each standard.

In-house validation of this assay was performed using local control material. Serum from a female patient with anorexia nervosa, amenorrhoea and a BMI of  $14.6 \text{ kg/m}^2$  (sample 1) was used to represent low levels of leptin. Serum samples from a female adult with a BMI of  $22.9 \text{ kg/m}^2$  (sample 2) and from a male adult with a BMI of  $23.5 \text{ kg/m}^2$  (sample 3) were used to represent normal levels of leptin for women and men respectively. Serum from a patient with morbid obesity (BMI  $61 \text{ kg/m}^2$ ) (sample 4) was used to represent a high leptin level. These four samples were assayed from sample 1 to 4, ten times, to assess reproducibility. Sample 1 was consistently estimated to be lower than the



lowest standard provided (1ng/ml), therefore the leptin from the anorexic patient was considered to be below the limit of detection of this assay. All 4 samples were used in later assays as independent control material.

Calculation of co-efficient of variation (CV) was performed as follows:

$CV = (\text{standard deviation} / \text{mean}) \times 100,$

where standard deviation =  $\sqrt{(1/n-1) \cdot \Sigma (x_i - \text{mean})^2}.$

Samples 1-4 were used for calculation of intra-assay CV for leptin. Each sample was assessed 10 times in one assay run, and a mean and standard deviation was calculated at each leptin level. The formula for calculation of CV was applied. This yielded an intra-assay CV of 34.5% at leptin level 0.2 ng/ml, 1.5% at 8.5 ng/ml, 2.4% at 18 ng/ml and 4.6% at 70 ng/ml.

Samples 1-4 were assessed twice in all leptin assay runs for calculation of inter-assay CV for leptin. The formula for calculation of CV was applied. This yielded an inter-assay CV of 36% at 0.2 ng/ml, 9.3% at 8.5 ng/ml, 2.7% at 18 ng/ml and 6.5% at 70 ng/ml.

### **2.11.b Estimation of ghrelin**

The assay for ghrelin was not available in the endocrine laboratory, so I set up and validated the assay using a commercial kit for radioimmunoassay (Mediagnost, Reutlingen, Germany). The kit was calibrated by the manufacturer against the internal test of Prof Brabant at the Hannover Medical School. Samples were removed from storage at  $-80^{\circ}\text{C}$  and allowed to thaw at room temperature, mixed by repeated

inversion and centrifuged at 3000 rpm for 20 minutes prior to assay. The assay was performed in duplicate.

Tubes were labelled as follows:

Tubes 1+2: total counts (TC)

Tubes 3+4: non-specific binding (NSB)

Tubes 5+6: zero standard ( $B_0$ )

Tubes 7-18: standards 1-6

Tubes 19, 20, 61, 62, 103 + 104: medium control

Tubes 21-102, excluding controls: patient samples

Standards of recombinant human ghrelin were provided in lyophilised form and were reconstituted with 750  $\mu$ l of the assay buffer provided; levels ranged from 200-6400 pg/ml ghrelin.

The non-specific binding was lyophilised from rabbit IgG and reconstituted with 1 ml of assay buffer.

The first antibody was reconstituted with 10.5 ml of the assay buffer provided.

The second antibody from anti-rabbit IgG was reconstituted with 1 ml of the assay buffer provided; then the dissolved material was immediately decanted into 55ml of precipitation reagent cooled to  $< 8^\circ\text{C}$ .

The tracer was Iodine  $^{125}$  at  $< 55$  kBq, reconstituted in 10.5 ml assay buffer.

Control material lyophilised from human serum was provided with the kit and was reconstituted with 750 $\mu$ l assay buffer.

All reagents (except second antibody-precipitation reagent mix) were mixed on a roller-mixer for at least 30 minutes before use.

Day 1: 100µl of buffer was added to tubes B<sub>0</sub>, in order to provide a zero standard, and NSB. 100µl of standards 1-6 was added to tubes 7-18. 100µl of commercial control was added to tubes 19+20, 61+62 and 103+104 to assess intra-assay consistency. 100µl of the sample material for ghrelin estimation was added to tubes 21-102. 100 µl of NSB was added to tubes 3+4. A reverse-pipetting technique was employed to improve pipette accuracy. 100µl of the first antibody was added to all tubes beginning with tube 5. The tubes were mixed with a vortex-mixer and incubated at 2-8°C overnight (between 20 and 24 hours).

Day 2: 100µl of tracer was added to all tubes. Tubes 1+2 were stoppered and removed. The tubes were mixed with a vortex-mixer and incubated at 2-8°C overnight (between 16 and 20 hours).

Day 3: The second antibody was added to the precipitation reagent and mixed with a vortex-mixer immediately prior to use. 500 µl of the second antibody-precipitation reagent mix was added to each tube, beginning with tube 3, while the tubes were immersed in a bath of iced water, to maintain cooled temperature. The tubes were mixed with a vortex-mixer and incubated for precipitation at 2-8°C for 1 hour. An extended washing procedure was used to increase precision, as recommended by the manufacturer. After incubation, the tubes were centrifuged at 3500 rpm

for 20 minutes at a temperature of 4°C. All tubes except 1+2 were drained, by careful decanting. 1ml of ice-cold distilled water was added to each tube and all were centrifuged again at 3500 rpm for 20 minutes at a temperature of 4°C. The liquid was decanted and the radioactivity of the tubes was counted using a gamma-counter.

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

Corrected binding value B was calculated from the average counts of each pair of standards (and controls and samples) minus the average of NSB

$B_0$  was the average counts of tubes 3+4, corrected for NSB

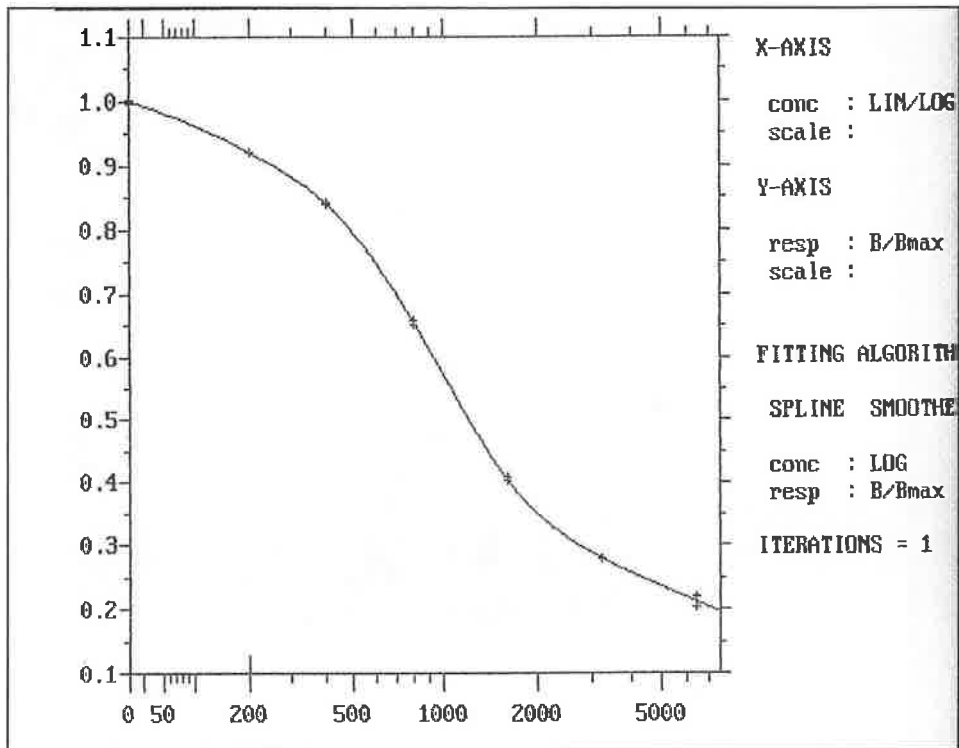
Percent bound =  $B/B_0 \times 100$

The percent bound versus the concentration of the standards was plotted on a semi-logarithmic scale

The NSB / TC % was calculated for quality control, this should be < 5%

The Percentage bound of the zero standard (=  $B_0/TC$  %) should be > 20%

The curve generated by the Gamma counter is shown in Figure 2.9:



**Figure 2.9:** Standard curve for ghrelin RIA, generated by Gamma Wizard.

X-axis shows the concentration of standards 1-6, Y-axis shows the percentage bound (calculated by average counts of radioactivity corrected for non-specific binding) for each standard.

In-house validation of this assay was performed using local control material.

A healthy female volunteer underwent the morning appetite study and ingested 500 kcal of Fortisip. Ghrelin levels were sampled as per the standard protocol for the appetite study. Ghrelin samples at the 8 sample timepoints were processed 5 times in succession to assess the reproducibility of the assay results. The extended washing procedure and the use of ice bath to chill samples were found to improve reproducibility.

Calculation of co-efficient of variation (CV) was performed as follows:

$CV = (\text{standard deviation} / \text{mean}) \times 100,$

where standard deviation =  $\sqrt{(1/n-1) \cdot \sum (x_i - \text{mean})^2}.$

It was not possible to obtain low, normal and high range ghrelin samples locally, therefore repeated samples from a healthy female volunteer were used for calculation of intra-assay CV and the medium control material provided by the manufacturer was used for calculation of inter-assay CV.

Intra-assay CV was 5% +/- 1.55% at ghrelin 1028 pg/ml

Inter-assay CV was 6.4% in the range ghrelin 896-1160 pg/ml.

### **2.11c Estimation of adiponectin**

I performed the adiponectin ELISA in the Dublin City University laboratory under the supervision of Dr David Ashley, who had set up the assay and validated it for his studies of insulin resistance in the community.

Laboratory estimation of adiponectin was performed using a commercial kit for enzyme-linked immunosorbent assay (ELISA) for total adiponectin (Quantikine Acrp30 Immunoassay, R&D Systems, Abingdon, UK). The manufacturer calibrated the kit against a highly-purified recombinant human adiponectin produced at R&D Systems. Samples were removed from storage at  $-80^{\circ}\text{C}$  and allowed to thaw at room temperature, mixed by repeated inversion and centrifuged at 3000 rpm for 20 minutes prior to assay. Samples were then diluted 100-fold by the addition of 10 $\mu\text{l}$  of serum to 990 $\mu\text{l}$  of calibrator diluent (buffered protein base, provided by kit).

Prior to assay, 500ml of wash buffer was prepared by dilution of 20 ml of wash buffer concentrate in distilled water. Colour Reagent A (hydrogen peroxide) and B (tetramethylbenzidine) were mixed in equal volumes 10 minutes prior to their use as the substrate solution. The lyophilised Adiponectin standard (500ng) was reconstituted with calibrator diluent to produce a stock solution of 250 ng/ml and mixed with a roller mixer. The stock solution was diluted serially with 200 µl of calibrator diluent to produce adiponectin standards of 0 ng/ml (calibrator diluent alone), 3.9, 7.8, 15.6, 31.2, 62.5, 125 and 250 ng/ml (stock solution) respectively.

The adiponectin microplate was a 96-well polystyrene plate coated with a mouse monoclonal antibody against the adiponectin globular domain. 100 µl of assay diluent (buffered protein) was added to each well of the microplate. The adiponectin standards were added to the first 8 wells and diluted patient serum was added to the remaining wells. All samples were added in duplicate. The microplate was covered in an adhesive strip and was incubated for 2 hours at room temperature. After incubation, the wells were aspirated and washed 4 times using an autowasher. Excess liquid was removed by inversion and blotting of the microplate. 200µl of adiponectin conjugate (mouse monoclonal antibody against adiponectin globular domain, conjugated to peroxidase) was added to each well and the microplate was incubated for a further 2 hours. After incubation, the aspiration and washing procedure was repeated. 200 ml of substrate solution was added to each well and the microplate was protected from light for a 30-minute incubation at room

temperature. 50 ml of Stop Solution (sulphuric acid) was added to each well.

The optical density of each well was determined using a microplate reader set to 450 nm, at a correction of 540 nm and then repeated at a correction of 570 nm.

#### **2.11d *Estimation of glucose***

Where serum samples were taken for glucose measurement, samples were centrifuged and removed from red cells to minimise glycolysis.

None of the samples were icteric or lipaemic. Glucose was estimated using the hexokinase method, an enzymatic UV test, on an automated Olympus analyser. The principle of the hexokinase method is based on the generation of NADPH via the following reactions:

Glucose + ATP (in the presence of hexokinase and  $Mg^{2+}$ ) generates  
glucose-6-phosphate + ADP

G-6-P +  $NAD^+$  (in the presence of G6P dehydrogenase) generates  
gluconate-6-P + NADH +  $H^+$

NADH generated by these reactions is absorbed at 340nm (UV region) in a manner proportional to the concentration of glucose in the sample.

The analyser was calibrated to the Olympus System Calibrator Cat. no. 66300, which is traceable to the National Institute of Standards and Technology Standard Reference Material 965.

The glucose assay was performed in the Clinical Biochemistry department of Beaumont Hospital.



### **2.11e *Estimation of insulin***

Serum samples were taken, allowed to clot 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 using a chemiluminescent immunoassay on the Beckman Coulter™ Access® Immunoassay System. This was a simultaneous one-step immunoenzymatic “sandwich” assay. The samples were added to a reaction vessel along with mouse monoclonal anti-insulin alkaline phosphatase conjugate and paramagnetic particles coated with mouse monoclonal anti-insulin antibody. Serum insulin was bound to the solid-phase antibody and the conjugate reacted with a different antigenic site on the insulin molecule. Material not bound to the solid phase was removed after incubation by separation in a magnetic field and washing. Lumi-Phos 530, a chemiluminescent substrate was added to the reaction vessel and light generated by the reaction was measured with a luminometer. Light production was directly proportional to the concentration of insulin in the sample.

The insulin assay was performed in the Clinical Endocrinology Department of Beaumont Hospital.

### **2.11f *Estimation of arginine vasopressin (AVP)***

(as per Standard Operating Procedure for measurement of Arginine Vasopressin, with thanks to Prof Steve Ball)

Samples for AVP measurement were taken using lithium heparin bottles and were immediately placed on ice, stored at – 70 degrees Celsius, and sent to the Department of Clinical Biochemistry at the Newcastle upon Tyne Hospitals NHS Trust, where they performed estimation of serum AVP (162). The assay was locally validated in Newcastle and calibrated against a local standard. A 1ml sample was extracted with C18 resin and the extract was pre-incubated overnight with antibody. <sup>125</sup>I-labelled AVP was added before a further overnight incubation and the assay was completed by addition of a second antibody on Day 3. Activity counts were measured using a Cobra Gamma Counter. The limit of detection of the assay was 0.3 pmol/l, with intra- and inter-assay co-efficients of variation of 9.7% and 15.3% (162).

#### **2.11g Estimation of plasma osmolality**

Plasma osmolality was measured in the Department of Clinical Biochemistry in Beaumont Hospital and estimated using a supercooling method on an osmometer from Advanced Instruments. The inter-assay % CV for the osmometer used in this study was 0.5% at a plasma osmolality of 290.5 mOsm, with an intra-assay CV of 1.4%.

#### **2.12 Statistical Analysis**

Statistical analysis was performed using JMP software (SAS California). Elaboration of each test statistics used is given in the appropriate chapter. Cumulative mortality data were generated from a formula written

in Excel by Ms Gill O'Connor and used to calculate Standardised Mortality Ratio (Chapter 3).

## Chapter 3

### Profile of Morbidity and Mortality of Craniopharyngioma Patients attending Beaumont Hospital

#### 3.1 *Introduction*

Craniopharyngioma (CP) has been associated with increased morbidity and mortality in comparison to other causes of hypopituitarism (2, 3, 92, 163). The increased mortality has been attributed to both cardiovascular and respiratory disease, but the reported susceptibility to cardio respiratory disease is poorly understood. The reported morbidity associated with craniopharyngioma includes high rates of hypopituitarism, hypothalamic complications and impaired quality of life (41).

The aim of this part of the study was to establish mortality rates in CP patients treated at our institution and to calculate the Standardised Mortality Ratio (SMR). We also wished to document the spectrum of morbidity, and the potential contribution of co-morbidities, such as obesity, hypertension, dyslipidaemia and diabetes mellitus, to the increased mortality in CP. We compared the prevalence of obesity in our CP population to that of another patient cohort with tumoural hypopituitarism (non-functioning pituitary adenoma) and to that of a hypopituitary group of similar age (post-traumatic hypopituitarism), who were managed in the same centre.

### **3.2 Methods**

Patients were identified from the hospital pituitary database, which contained details from all patients treated between 1980 and 2008. 70 patients (39 male) with craniopharyngioma were identified from the database; 21 patients had died at the time of analysis.

#### **3.2a Mortality**

Patients who died while under follow-up in our hospital had cause of death identified from hospital casenotes and local post-mortem information. For patients who were lost to follow-up, or who were followed up in other institutions, the family practitioner was contacted in order to identify patients who had died.

Cause of death was established from death certificates from the Office of the Registrar General and from post mortem data. The Central Statistics Office of Ireland provided records of mortality rates for the Irish population by age and gender. The background Irish mortality rate per 1000 population for each age group and gender was expressed as a decimal and converted into a cumulative mortality by summation of previous yearly mortality.

The expected mortality by age and gender of each craniopharyngioma patient was calculated:

Background cumulative mortality for year of death - Background cumulative mortality for the year of diagnosis with craniopharyngioma

The craniopharyngioma Standardised Mortality Ratio (SMR) was defined as:

Number of deaths observed / number of expected deaths in an age- and gender-matched population

Irish data were available from the years 1950-2004; thus the background mortality in 2005 to 2008 was assumed to equal that of 2004. Poisson probability distribution was used to calculate the 95% confidence interval (CI) for the SMR. The difference between SMR for men and women was estimated using the equation described by Altman and Bland (164).

### **3.2b Morbidity**

Case note review was performed for collection of morbidity data and details were recorded in a spreadsheet (Windows Excel). Biographical details included gender, date of birth and age at diagnosis and death.

Case management details included; surgery and radiotherapy management, surgical approach, recurrence of tumour, pituitary hormone replacement therapy and dosage and prescription of statin, anti-hypertensive and anti-epileptic therapy. Prevalence of anterior pituitary hormone deficiency and diabetes insipidus was recorded.

The most recent measurement of fasting blood glucose and lipid profile, and blood pressure, height, weight and BMI measurements from the most recent outpatient office measurement were noted. Historical diagnoses

of diabetes mellitus, hypertension or dyslipidaemia were also recorded. The diagnosis of diabetes mellitus was based on standard WHO criteria of positive OGTT results, two fasting blood glucose concentrations > 7.0 mmol/l or a random blood glucose of > 11.1 mmol/l when accompanied by osmotic symptoms. Hypertension was diagnosed by three office readings of > 140 / 90mmHg, or use of an anti-hypertensive to achieve normal blood pressure. Dyslipidaemia was defined as total cholesterol > 5mmol/l or LDL cholesterol > 2.6 mmol/l, or use of a statin.

GH deficiency (GHD) was defined as a peak GH response of less than 3 µg/l in response to insulin tolerance test (ITT) or glucagon stimulation test (GST), as defined by locally derived normative data (165). ACTH deficiency was defined as a peak cortisol less than 500 nmol/l on ITT or 450 nmol/l on GST, as previously defined (165). Gonadotrophin deficiency in pre-menopausal women was defined as amenorrhoea for more than 6 months in the presence of low oestradiol and normal or low gonadotrophins. In post-menopausal women gonadotrophin deficiency was defined as gonadotrophins of pre-menopausal range. In men gonadotrophin deficiency was defined as symptomatic low testosterone, and inappropriately low gonadotrophins. TSH deficiency was defined as low T4 in the presence of low or normal TSH. Diabetes insipidus was diagnosed in the post-operative period by the presence of polyuria and hypernatraemia, according to the criteria of Seckl and Dunger (166) and confirmed with water deprivation test or hypertonic saline infusion six weeks after discharge. Adipsia or polydipsia was diagnosed by use of an

infusion of hypertonic saline (855 mmol/l) with measurement of thirst as described in detail in Chapter 2 (53). Prevalence of other morbidity such as obesity, defined as body mass index (BMI) greater than 30 kg/m<sup>2</sup>, hydrocephalus, seizures, visual loss and venous thrombosis was recorded from the case notes.

Body mass index (BMI) data were compared to that of other hypopituitary groups; 89 patients with hypopituitarism after surgery for non-functioning pituitary adenoma (NFPA) and 29 patients with hypopituitarism after traumatic brain injury (TBI), also identified from the pituitary database.

### **3.2b Statistics**

Mean and standard deviation were used to describe data that were normally distributed; otherwise the median and range was reported. BMI data were compared with Wilcoxon's test and sub analysed with Tukey's HSD. Significance of sub analysis findings was determined by post hoc t test with correction for multiple comparisons. JMP 8 (SAS Institute, California, USA) was used for statistical analysis. The local Ethics Committee approved the study.

### **3.3 Results**

Median follow-up of craniopharyngioma patients from time of diagnosis was 8 years (range 1-50 years). Twenty-four patients (34%) were diagnosed with CP in childhood.



### 3.3a Mortality

Twenty-one CP patient deaths were observed between 1988 and 2007.

The expected mortality in this population was 2.4; therefore SMR was 8.75 (95% CI 5.4-13.3). SMR for women (10.51, 95% CI 5.04-19.3) and for men (7.55, 95% CI 3.77-13.52), but the difference between mortality for men and women did not achieve statistical significance (RR 1.388, CI 0.55-3.51) (164). Causes of death are reported in Table 1.

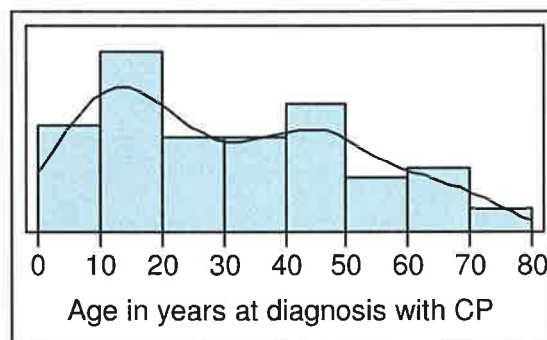
**Table 3.1.** Mortality data for 21 craniopharyngioma patients.

F = female, M = male, CP = craniopharyngioma. Cause of death reported as stated on death certificate (not located for patient 5).

<u>Patient</u>	<u>Gender</u>	<u>Age at Diagnosis CP</u>	<u>Age at death</u>	<u>Cause of Death</u>
1	F	64	71	Myocardial infarction
2	F	48	51	CP
3	F	74	81	Respiratory arrest
4	M	47	49	Respiratory arrest
5	M	63	68	Not reported
6	M	26	61	Epilepsy
7	M	34	38	Metastatic Lung Carcinoma
8	F	52	58	Small cell lung carcinoma
9	F	30	32	Pulmonary Embolus
10	F	66	68	Respiratory Tract Infection
11	M	70	82	Cerebral Thrombus
12	M	36	45	Aspiration Pneumonia
13	M	43	43	Cerebral Infarct
14	F	44	46	CP
15	F	39	40	CP
16	M	3	8	Pneumonia
17	F	16	23	Increased Intracranial pressure
18	F	13	13	Pulmonary Embolus
19	M	7	19	Glioma
20	M	48	53	Left ventricular failure
21	M	11	20	Head trauma secondary to seizure

### 3.3b Morbidity

Age at diagnosis was distributed in a bimodal pattern (Figure 3.1). Sixty-eight patients underwent surgical intervention; 2 patients were diagnosed with CP on radiological appearance alone and did not undergo surgery because of preservation of visual fields. Eight patients underwent surgery by a trans-sphenoidal approach, 5 of which required a later craniotomy. On average, 60 pituitary resections were performed in the centre each year; there were eight neurosurgeons in the centre, 1 of whom was a specialist pituitary surgeon. Three successive pituitary surgeons were responsible for the service over the time period studied.



**Figure 3.1.** Distribution of CP patients by age in years at diagnosis.

Line demonstrates bimodal pattern of distribution

Radiotherapy details were not available for 6 patients (4 deceased, 1 attended follow-up elsewhere, 1 failed to follow up). Of the remaining 64 patients, 27 (42%) underwent radiotherapy; 16 after recurrence, 1 as prophylaxis with early recurrence and 10 of these patients received prophylactic radiotherapy without recurrence. Records were incomplete

in 3 deceased patients; of the remaining 67, 31 patients (46.2%) suffered from tumour recurrence.

Growth hormone deficiency (GHD) was diagnosed in 53 of 58 patients (91%) for whom the GH axis was tested; of the remaining 12 patients, 8 died prior to 1997 when assessment for adult GHD became routine in our unit and 4 did not follow-up with our service. Of those 5 patients considered to have normal GH, 2 did not undergo surgery and the remaining 3 were considered to be GH replete by an endocrinologist because of a normal IGF1, before dynamic testing became routine in adults. Of those identified with GHD, 62% received GH replacement. GH was not replaced because of tumour mass or recurrence in 8% of GHD patients, discontinued after perceived lack of benefit in 16%, discontinued after the development of diabetes in 5.4%, or not commenced because of patient age (over 70 years) in 5.4%. GH replacement therapy was monitored by serial measurement of IGF1 and titrated to maintain IGF1 in the upper half of the normal reference range. Median GH dose was 0.35 mg with a range of 0.2 – 1.8 mg. Analysis of mortality in GHD patients, who received replacement (7/41) compared with those who did not (4/12), was not significant ( $\chi^2$ ,  $p = 0.2$ ).

ACTH status was not documented in 4 patients; 3 were deceased and 1 attended another institution. Of those for whom data was available, 61 patients (92%) had ACTH deficiency. 2 of the patients with intact ACTH secretion had not undergone surgery, 2 failed to follow-up with our

service and 1 was managed elsewhere. All patients diagnosed with ACTH deficiency received replacement therapy with hydrocortisone or prednisolone. Median daily dose of steroid was equivalent to 20 mg of hydrocortisone, range 10 – 30mg, where 5mg prednisolone was considered equivalent to 20mg of hydrocortisone.

Gonadotrophin status was not assessed in 1 paediatric patient, not documented for 5 deceased patients and unknown in 2 who did not follow-up in our institution. Of those for whom data was available, 58 patients (93.5%) were deficient. Gonadotrophin deficiency was treated with sex hormone replacement in all men and in women who were younger than 51 years of age at the time of diagnosis.

Thyrotrophin (TSH) status was not documented for 1 patient who attended another institution and for 4 deceased patients. Of the remaining patients, 56 (86%) were TSH deficient. All patients with TSH deficiency received replacement therapy with thyroxine. Median daily dose was 112 µg of thyroxine, range 50 – 250 µg.

Diabetes insipidus (DI) was documented in 56 patients (81%), who all received replacement desmopressin (dDAVP) therapy. Median dDAVP dosage frequency was twice per day, with a range of nil to 6 doses per day. Patients were advised to miss one dose at the weekend in order to avoid fluid overload. Four patients (7.1%) had adipic diabetes insipidus.

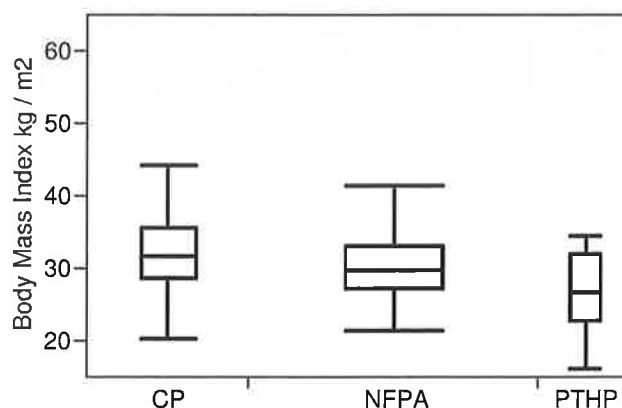
Lipid profile and blood pressure were recorded after appropriate hormone replacement therapy. Median total cholesterol was 5.0 mmol/l, with a range of 3.3 – 8.2 mmol/l (target range <5 mmol/l). Median LDL cholesterol was 3.2 mmol/l, with a range of 1.1 – 5.2 mmol/l (target < 2.6 mmol/l); HDL was 1.1 mmol/l (range 0.6 – 2.8 mmol/l, target > 1mmol/l); and triglyceride was 2.2 mmol/l (range 0.6 – 6.7 mmol/l, target <1.9 mmol/l). Twenty-three patients of the 49 survivors (46.9%) had total cholesterol of more than 5 mmol/l, of whom 11 were known to be on a statin. There was no difference in any lipid parameter between CP patients who died and those who continued to attend the outpatients.

Five of the surviving 49 patients had a blood pressure greater than 140/90 mmHg; of these, only 2 were treated with anti-hypertensives. One other patient had achieved normal blood pressure with an ACE inhibitor. Ten patients of historically normal blood pressure were followed up elsewhere. Data therefore suggested that 6/39 (15.3%) had hypertension.

Six patients out of 52 for whom results were available had diabetes mellitus (11.5%); 1 was diagnosed with Type 1 in childhood prior to the diagnosis of CP. Median fasting glucose was 4.8 mmol/l, (range 2.3 – 11 mmol/l).

Median BMI in CP patients was 31.8 kg/m<sup>2</sup>, (range 20.3 – 61.2 kg/m<sup>2</sup>). 66% of CP patients had a BMI in the obese range (greater than 30 kg/m<sup>2</sup>)

and 26% were overweight (BMI 25 – 29.9 kg/m<sup>2</sup>). The CP patients were compared to two other hypopituitary cohorts identified from the local database: non-functioning adenoma patients (NFPA; median BMI 29.8, 47% obese) and those with post-traumatic hypopituitarism (PTHP; median BMI 26.6, 31% obese). Patients with post-traumatic hypopituitarism were of similar age to the CP patients and were included in the study in an attempt to control for the effect of age in 2007 the patients with non-functioning older (Table 3.2). There was a significant difference in BMI across the three groups (Figure 3.2 and Table 3.2,  $p < 0.001$ , Wilcoxon). Sub analysis revealed that there was no difference between the BMI of CP and NFPA patients (Tukey HSD) but there was a difference between both CP and NFPA patients compared to PTHP patients. Student t test with correction for multiple comparisons revealed no significant difference between CP and NFPA BMI, but a significant difference between CP and PTHP ( $p < 0.001$ ) and between NFPA and PTHP ( $p < 0.05$ ). There was also a significant difference in age between the groups (Table 2).



**Figure 3.2.**

Comparison of BMI (kg/m<sup>2</sup>) between hypopituitary patients with different underlying causes of hypopituitarism.

CP = craniopharyngioma (n = 70), NFPA = non-functioning adenoma (89), PTHP = post-traumatic hypopituitarism (n = 29) (p < 0.001 for comparison across all 3 diagnoses).

**Table 3.2.**

Comparison of CP patients to NFPA and PTHP groups.

Age at diagnosis is different for NFPA group compared to both CP and PTHP group. Difference between CP and PTHP group is borderline non-significant (p = 0.052). BMI is different in PTHP group from both CP and NFPA. P values in the table represent the difference across all 3 groups.

Diagnosis	CP (n = 70)	NFPA (n = 89)	PTHP (n = 29)	p value
Median Age (years)	27.5	52	41	< 0.001
Median BMI (kg/m <sup>2</sup> )	31.7	29.8	26.6	< 0.001
% BMI > 30	66	47	31	< 0.01

Hydrocephalus was documented radiologically in 40.3% of patients, and was more common in patients who were deceased (12 of 14 for whom hydrocephalus documented) at the time of analysis than in live patients (13 of 48 for whom documented)(chi square,  $p < 0.0001$ ). Seizures affected 49% of patients and were associated with hydrocephalus (chi square,  $p < 0.0001$ ). Seventy-seven percent of patients had documented visual field loss.

Seven patients had a history of development of either a deep venous thrombus (DVT) or a pulmonary embolus (PE). Post-mortem studies revealed that PE was the cause of death in 2 / 21 deceased patients (Table 3.1).

### **3.4 Discussion**

Craniopharyngioma (CP) is a tumour associated with a higher mortality than pituitary adenomata that is not explained by endocrine morbidity alone. Our management of craniopharyngioma is comparable to that in other tertiary centres with a policy of gross total resection of tumour, but our findings may be less applicable to those centres with a more conservative surgical approach to CP. The elevated SMR for CP in our centre (8.75) is comparable to that of published international studies. In an analysis of the West Midlands UK database reported by Tomlinson et al, the SMR was 9.28 in craniopharyngioma patients (3), an excess



mortality that was attributed to respiratory and cardiovascular disease. A single-centre Swedish study, which analysed mortality in 60 CP patients, and which documented an elevated SMR of 5.5 (2), reported that 7 of 27 deaths were related to pneumonia or PE. Our single-centre study also documents an excess of deaths due to respiratory disease. There is a consistent reporting of excess respiratory and vascular disease in the available literature. Knowledge of the mechanism of death in our patients, such as respiratory failure, would have been of greater clinical value in assessment of mortality in CP than the cause of death as reported in Table 3.1. Because this was a retrospective study, we depended on the accuracy of death certificate reports, the majority of which were completed in hospitals other than our own; in Ireland, physicians are legally required to report cause rather than mechanism of death.

The authors of both the UK and the Swedish studies suggested that untreated GH deficiency contributed to the excess mortality of hypopituitary patients. There are confounding factors in their studies, for consideration when analysing whether their data fully substantiates this suggestion. Both series included patients diagnosed prior to the use of dynamic testing for GHD in adults, which questions the accuracy of diagnosis of GH deficiency in some patients. We have attempted to correct for likely GHD in our study. The 3 patients in our study who were assessed before the adoption in 1997 of formal dynamic testing for GH deficiency, and who were considered to have adequate GH secretion on

the basis of a normal IGF1 result, would probably have failed GH stimulation testing, given that they had evidence of deficiency of all other pituitary hormones (167). The true rate of GHD in our CP cohort is therefore more likely to be 96%. We have a policy of offering GH replacement to all eligible GHD adults with craniopharyngioma, which differs slightly from historical practice in the West Midlands centre; our data did not show any survival benefit of GH treatment compared with those who were not treated.

Female patients with hypopituitarism due to any cause have a higher mortality than their male counterparts (2-4). Our data suggested a trend in this direction, though the relative risk of death for women compared to men did not reach statistical significance; this reflects the small absolute numbers of deaths involved in our series. It has been speculated that sub-optimal sex steroid replacement in women may contribute to this reversal of gender-associated mortality when compared to the background population, and the Swedish group have suggested that the pre-diagnosis clinical history of oestrogen deficiency should be included in the total time of oestrogen deficiency in order to give some indication of increased cardiovascular risk (4, 168). We would observe, however, that one of our patients, who subsequently died of respiratory failure, developed multiple pulmonary emboli on oestrogen replacement, during an episode of hypernatraemic dehydration due to adipsic diabetes insipidus. Hormonal treatment is not always straightforward, therefore, in this group of patients.

One patient died from an anaplastic astrocytoma 12 years after radiotherapy for CP. Glioma post-radiotherapy for CP is a recognised but rare complication of treatment, with 15 cases previously reported in the literature (169-171). Our patient satisfied Cahán's criteria for a diagnosis of a radiation-induced tumour (172) – the glioma originated in the previously irradiated region; it was diagnosed over 10 years after irradiation; it was of different histology from the original tumour; and the patient did not suffer from a phakomatosis that would have predisposed him to tumour development. In one previous case report, the time elapsed between irradiation of the craniopharyngioma and the development of astrocytoma was only 4 years and the authors queried whether this could be related to the combined effect of GH therapy and radiotherapy (173). Our patient had received GH therapy, but this is generally considered to be safe in CP patients (48). Although post-radiotherapy glioma is a rare complication of treatment, it should be considered when planning a treatment strategy to minimise hypothalamic morbidity in CP.

The excess mortality in CP patients may be related to hypothalamic damage and its complications, such as adipsic DI and obesity (3, 4, 20, 72). A recent, single centre study in Holland reported an elevated SMR of 2.88 in 54 CP patients, with higher rates of obesity and diabetes than the background Dutch population (4). Obesity is a recognised complication of CP and also of the effects of treatment of CP, with

surgery and radiotherapy (10, 37, 66). We report a very high prevalence of CP obesity (66%) in our CP cohort compared to the prevalence in Irish people of 18% (174). We expected a high rate of obesity in our CP cohort, but were surprised by the high incidence of obesity in our NFPA cohort, which was not statistically different to that in CP. The NFPA group was significantly older than the CP cohort, and as age is an independent associate of obesity, we compared the rates of obesity in CP to age-matched patients with hypopituitarism secondary to brain trauma; our data showed a considerable excess of obesity in the groups with tumoural hypopituitarism. Patients with PTHP appear to suffer from significantly less obesity than patients with tumoural hypopituitarism, but they have a much shorter duration of follow-up because PTHP has been recognised relatively recently. The excess of obesity in the younger CP group is clinically very relevant, as obesity in a younger population predicts a higher risk of future morbidity and mortality. Obesity has been suggested, for instance, to contribute to cardiovascular mortality in CP (4).

Neurosurgical intervention in CP was often performed before full pre-operative endocrine assessment (2); thus we could not assess the relative effects of pre-operative CP tumour bulk and surgical intervention on pituitary function. GH, gonadotrophin and TSH deficiencies in our cohort were comparable to international reports (1, 4). ACTH deficiency has been reported to range from 55 to 88% in a review of the literature by Karavitaki, but our prevalence figures were a little higher at 92%.

Craniotomy is a frequently used surgical approach for management of craniopharyngioma, particularly in cases of large tumour size and suprasellar location (1, 19, 37). Our use of the craniotomy approach was at the higher end of international practice and might have contributed to the high rates of hypopituitarism and hypothalamic complications reported in our cohort. In spite of our local practice to attempt gross total resection, recurrence rate (46%) was comparable to previous studies (1). Gross total resection is rarely achieved in CP patients, and where surgical clearance is attempted, imaging studies can reveal a remnant in 18 – 26% of cases (1).

Craniopharyngioma patients are at increased cardiac and cerebrovascular risk. Our cardiovascular risk assessment policy identified dyslipidaemia. The low rate of statin prescription probably reflects the reluctance of medical practitioners to prescribe lipid-lowering agents to a young population like the CP cohort. A recent analysis of Irish cardiovascular mortality since the 1980s attributed 30% of improvement in survival to a 4.6% decrease in the population total cholesterol (175), which suggests that our Irish CP cohort could benefit from more aggressive management of dyslipidaemia, although there are no data for benefit of treatment of dyslipidaemia in this group. Hypertension does not appear to be as prevalent a cardiovascular risk factor in the CP group, although those who had hypertension were undertreated. The rate of diabetes (11.5%) is much higher than that of

the background Irish population (4.8%) (176). The majority of measurements of fasting glucose were recorded at baseline of pituitary stimulation testing, prior to hormone replacement. The true rate of diabetes mellitus may be higher because GH and glucocorticoids are known to affect glucose metabolism (177, 178), hence measurement of fasting glucose after hormone replacement may be a more appropriate evaluation. Given the spectrum of cardiorespiratory disease in CP, it may be valuable to adopt a formal screening programme for vascular risk factors such as diabetes mellitus, hypertension and hyperlipidaemia, and a more aggressive treatment schedule, particularly considering the relatively small numbers of patients involved and the consequent low cost of implementing cardiovascular screening.

Frequent visual field loss and hydrocephalus reflected the large size of the tumours. The prevalence of seizures in our patient cohort was closely correlated with hydrocephalus and was higher than the rate of seizure in Oxford CP patients, which reported a 26% probability of seizure over 20 years of follow-up (10). Hydrocephalus was more prevalent in patients who subsequently died, but not in patients diagnosed in childhood; this data from our study is a novel finding (1).

In conclusion, we have shown that mortality is increased in an Irish CP population and that women are more affected than male patients. In our cohort increased mortality was not related to GH deficiency, or replacement, but was associated with a diagnosis of hydrocephalus, a

probable marker of tumour size. There was a high rate of respiratory mortality, comparable to that previously published. We did not find evidence for inappropriate hormone replacement therapy as a cause of morbidity or mortality in this study. Dyslipidaemia was common; both dyslipidaemia and hypertension were rarely treated in this cohort.

Our study results confirm the high morbidity and mortality associated with a diagnosis of craniopharyngioma.

## Chapter 4

### Posterior Pituitary Function; Morbidity and Mortality associated with Thirst Disorders in Craniopharyngioma

#### 4.1 *Introduction*

In Chapter 3 I reported that 56 patients in our CP cohort (85%) were diagnosed with diabetes insipidus (DI) and that 4 of these patients were diagnosed with lack of thirst (adipsia). In this chapter I describe the diagnosis of thirst abnormalities and the morbidity and mortality associated with adipsic DI in craniopharyngioma patients.

Adipsic diabetes insipidus (ADI) is a rare disorder, characterised by hypotonic polyuria due to vasopressin (AVP) deficiency, and failure to generate the sensation of thirst in response to hypernatraemia. Thirst is almost invariably preserved in hypothalamic DI (53, 65). The sensation of thirst and compulsion to drink is the key homeostatic mechanism that prevents hypernatraemic dehydration in patients with untreated hypothalamic DI. Loss of thirst leads to failure to respond to aquaresis with appropriate fluid intake, and predisposes to the development of significant hypernatraemia. Adipsic DI has been described in association with craniopharyngioma (53), clipping of anterior communicating aneurysms following subarachnoid haemorrhage (9, 60, 63, 179), surgery for suprasellar pituitary adenoma (15), head injury (65), toluene exposure



(14) and developmental disorders (180-184) but the rarity of the condition dictates that clinical information is derived mainly from case reports.

Therefore, the incidence of co-morbidities and associated conditions is not well described. We describe adipsic DI in 4 craniopharyngioma patients studied as part of a larger case series of 13, in order to document the morbidity and mortality in CP patients of this rare, but complex condition.

Many of the conditions associated with ADI are treatable, such as sleep apnoea, or preventable with active patient management, such as dysnatraemic seizure or the development of obesity. We provide some recommendations for assessment and treatment of these patients, based on our extensive clinical experience of this rare condition.

## **4.2 *Methods***

### **4.2a *Patients***

We studied 4 patients with ADI (1 male, age 16 – 56 years) secondary to craniopharyngioma. Three of the four patients were identified as part of a previous study of thirst abnormalities in CP conducted by Dr Diarmuid Smith (53). The fourth patient had recurrent hypernatraemia after surgery for CP, in spite of dDAVP therapy for DI and free access to water; the diagnosis of adipsia was suspected and confirmed with hypertonic saline infusion. Clinical details are shown in Table 4.1. AVP and thirst response to hypertonic saline infusion, and AVP response to hypotension

were compared to responses of nine healthy control subjects (3 female), chosen from a local database of subjects with normal AVP responses to hypertonic saline and matched for age to CP patients ( $33.0 \pm 10.5$  years compared to CP ( $33.3 \pm 19.7$  years,  $p = 0.8$ ).

**Table 4.1**

Clinical details of patients with adipsic DI secondary to CP.

F = female, M = male, PHP = panhypopituitarism.

<u>Patient</u>	<u>Gender</u>	<u>Anterior pituitary deficiencies</u>	<u>Age of disease onset (years)</u>	<u>Age of hypothalamic assessment (years)</u>
1	F	PHP	15	16
2	F	PHP	16	22
3	M	PHP	36	56
4	F	PHP	41	41

#### **4.2b Osmoregulatory function**

All patients had the diagnosis of adipsic DI confirmed by measuring the AVP and thirst response to osmotic stimulation with hypertonic sodium chloride infusion, as described in Chapter 2. The limit of detection of the AVP assay was 0.3 pmol/l (162).

Adipsic DI was defined as follows:

hypotonic polyuria (> 4 litres / 24h) with subnormal AVP responses to osmotic stimulation

subnormal thirst responses to hypertonic saline, and water intake less than half of the lower limit of normal in healthy controls (<500ml)

Normative data was derived from a data bank comprised of responses of 40 healthy controls.

#### **4.2c *Baroregulated AVP responses***

All patients underwent assessment of Baroregulated AVP release as described in Chapter 2. Documentation of baroregulated AVP response is standard practice in our unit when a patient has been diagnosed with ADI.

#### **4.2d *Anterior pituitary function***

Adrenocorticotrophin (ACTH) and growth hormone (GH) secretion were tested by the measurement of cortisol and GH in response to stimulation with the insulin tolerance test (ITT) in the patient who had no history of seizure. In the 3 patients for whom the ITT was contraindicated due to seizures, the cortisol and growth hormone response to intramuscular glucagon was measured. Normal cortisol and GH responses were defined as described in Chapter 3. TSH and gonadotrophin deficiency were defined as described in Chapter 3.

#### **4.2e *Other Morbidities***

Details of other co-morbidities were derived from retrospective case-note analysis of the patients. Those patients who gave a clinical history of snoring, somnolence, sleep disturbance or apnoeic periods underwent analysis with overnight monitoring of oxygen saturation (Stardust, Respironics, France, 2 patients), or polysomnography (Alice 4, Respironics, France, 2 patients). Full polysomnography included EEG, EMG, EOG and ECG recordings. Thoracic and abdominal straps

recorded respiratory effort. A nasal cannula was used to record airflow, a microphone to record snoring and a digital probe to record oxygen saturation. Sleep was scored from the downloaded data as described fully in Chapter 2. All patients who underwent polysomnography had an Epworth Sleepiness score (112) recorded, a morning arterial blood gas sample and pulmonary function testing including spirometry, lung volumes and diffusion lung capacity (Vmax22, SensorMedics, Ca, USA) as described in Chapter 2.

#### **4.2f Ethics**

All studies had local area ethical approval. All subjects, or where applicable the next of kin, gave informed written consent.

#### **4.2g Statistics**

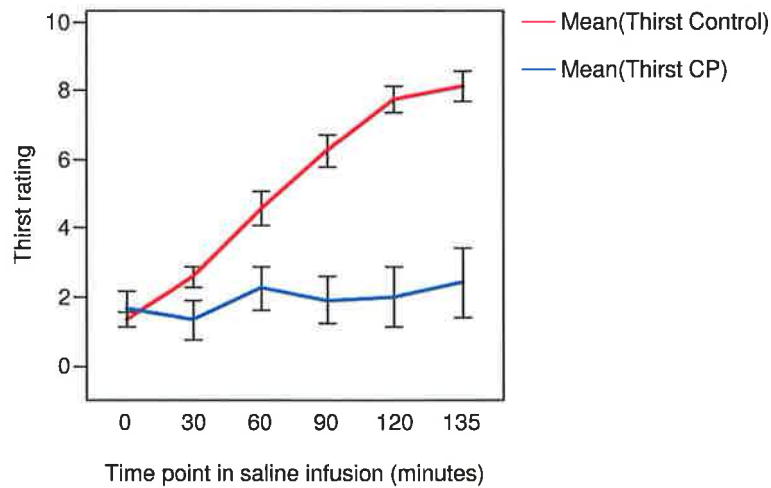
Changes in plasma osmolality, plasma AVP and thirst with hypertonic saline infusion were analysed using ANOVA for repeated measures. Mean arterial pressures and plasma AVP pre-and post-trimetaphan were compared using the Wilcoxon Signed Rank test. The volumes drunk post-infusion were compared using the t-test. The software used was JMP 8 (SAS, California). Statistical significance was identified by  $p < 0.05$ .

### **4.3 Results**

#### **4.3a Osmoregulatory studies**

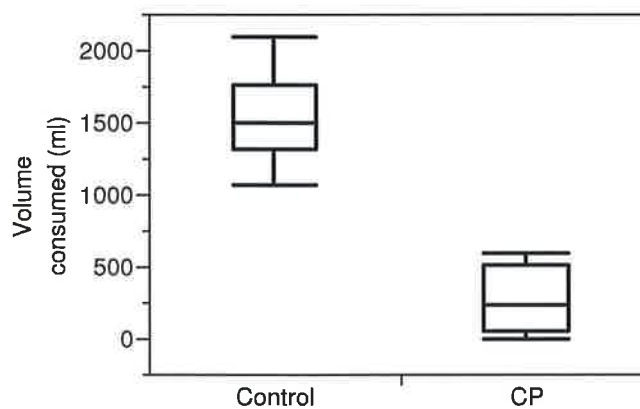
During hypertonic saline infusion, plasma osmolality in the 4 CP patients rose from  $293 \pm 8$  to  $314 \pm 13$  mOsm/kg (mean  $\pm$  SD,  $p = 0.01$ ), which

was significantly higher than controls ( $p < 0.05$ ), whose plasma osmolality rose from  $288 \pm 2$  to  $305 \pm 2$  mOsm/kg ( $p < 0.01$ )(Figure 4.1). The rise in plasma osmolality in CP patients failed to stimulate a rise in plasma AVP concentrations ( $0.43 \pm 0.18$  to  $0.43 \pm 0.12$  pmol/l,  $p = 0.5$ )(Figure 4.2). All 4 patients were classified as having hypothalamic diabetes insipidus on the basis of these results. Thirst ratings remained unchanged during hypertonic saline infusion ( $1.7 \pm 1.6$  to  $2.0 \pm 2.3$  cm,  $p = 0.59$ ) and were significantly less than healthy controls ( $p < 0.05$ ), in which thirst ratings increased from  $1.2 \pm 1.7$  to  $7.7 \pm 1.7$  ( $p < 0.0001$ ) (Figure 4.3). The mean volume of water drunk in each subject was  $265\text{ml} \pm 250$ , significantly less than in healthy controls ( $1544\text{ml} \pm 306$ ,  $p < 0.001$ )(Figure 4.4). These data are in contrast to the brisk rise in thirst responses to hypertonic saline reported in controls (160), patients with DI (65) and CP patients with DI (53) and confirmed that each patient was hypodipsic, in addition to having AVP deficiency.



**Figure 4.3**

Thirst response to hypertonic saline infusion in CP patients (shown in blue) and in controls (shown in red)( $p < 0.01$ ). Error bars show standard error of the mean. Time point extended to 135 minutes to show time period where fluid consumption was permitted



**Figure 4.4**

Volume of fluid (ml) consumed in 30 minutes post-termination of hypertonic saline infusion ( $p < 0.001$ ).

#### 4.3b Baroregulatory studies

The intravenous infusion of trimetaphan produced a fall in mean arterial blood pressure from  $93 \pm 7$  to  $64 \pm 2$  mmHg ( $p < 0.01$ ). All four CP patients lacked AVP response to hypotension (mean change in AVP 0.17

$\pm 0.17$  pmol/l,  $p = 0.4$ ). No patient experienced a severe hypotensive episode during infusion of trimetaphan.

#### **4.3c Anterior pituitary dysfunction**

All of the patients had panhypopituitarism, and were on hormone replacement therapy, where appropriate.

#### **4.3d Other co-morbidities**

The plasma sodium concentration at diagnosis is shown for each patient in Table 4.2. Three patients had hospital admissions due to hypernatraemia after the diagnosis of adipsic DI had been confirmed and treatment schedules commenced. In one of these patients, admissions were repeated.

**Table 4.2.** Plasma concentration at diagnosis of adipsic DI and number of hospital admissions with hypernatraemia or hyponatraemia

<u>Patient</u>	<u>Gender</u>	<u>Plasma sodium at diagnosis (mmol/l)</u>	<u>Hospital admissions with hypernatraemia</u>	<u>Hospital admissions with hyponatraemia</u>
1	F	153	4	None
2	F	160	1	None
3	M	157	None	None
4	F	162	1	None

Three patients had seizures at some point during their clinical course (Table 4.3).

**Table 4.3**

Other morbidities associated with diagnosis of Adipsic DI.

DVT = deep venous thrombosis, BMI = body mass index.

<u>Pt</u>	<u>Sleep apnoea</u>	<u>DVT</u>	<u>BMI</u> kg/m <sup>2</sup>	<u>Other morbidity</u>	<u>Age at Death</u> (years)
1	No	No	61.1	Hypercholesterolaemia, diabetes mellitus	
2	Yes	Yes	43.0	Seizures, hydrocephalus	24
3	No	No	34.7	Seizures	45
4	Yes	No	28.2	Hypernatraemic seizure	

One patient developed deep venous thrombosis and multiple pulmonary emboli, requiring anti-coagulation with warfarin and discontinuation of oestrogen replacement therapy.

Two patients had sleep apnoea identified on overnight oximetry and / or polysomnography; one of these patients was clinically obese (BMI > 30 kg/m<sup>2</sup>) and the other was overweight at the time of the study but developed obesity within a year after diagnosis with ADI (BMI 32.2 kg/m<sup>2</sup>). The sleep apnoea was clinically significant and in spite of treatment with continuous positive airway pressure therapy one of these patients eventually died of hypercapnoeic respiratory failure. Three of the patients were found to be obese (BMI > 30 kg/m<sup>2</sup>) and the fourth developed obesity in the months after the study.

Two patients died, one of respiratory failure and one of aspiration pneumonia. Although the second patient was known to suffer from



seizures there was no reference in his death certificate to a seizure prior to aspiration.

#### **4.4 Discussion**

In this carefully documented series of CP patients with the rare condition of adipsic diabetes insipidus, we have shown that the morbidity and mortality of this difficult disease extend beyond abnormalities of water balance in a small group of patients. In particular, we have demonstrated mortality at a young age. We have reported a clinically significant thrombotic event, serious obesity, and seizure disorders.

All patients in the series were meticulously proven to have DI. They all had documented polyuria due to absent osmoregulated AVP secretion in response to hypertonic saline infusion and good clinical response to desmopressin therapy. Adipsia was proven in all cases by the demonstration of attenuated thirst and water intake during osmotic stimulation.

The AVP response to hypotension was absent in craniopharyngioma patients (60). Many cases of adipsic DI in the literature, including all of those due to surgical clipping of anterior communicating artery aneurysms, have been demonstrated to have intact baroregulated AVP secretion (60). Most patients with a history of surgery to an anterior communicating artery aneurysm have brisk AVP responses to hypotension, indicating intact supraoptic and paraventricular nuclei, and

normal functioning posterior pituitary glands. The finding of intact synthetic nuclei localises the lesion in these patients to the osmoreceptor unit in the anterior hypothalamus which is the site for the sensory component of thirst, thought to be situated in the circumventricular organs (185), and which has neural connections to the cerebral cortex or “effector” site for thirst (186). In contrast, all four patients with craniopharyngioma described in this sub-study had absent baroregulated AVP responses. All 4 patients had developed adipsic DI after having undergone extensive trans-cranial surgery for large, aggressive tumours. It is likely in these patients that extensive surgical damage created lesions in the supraoptic and paraventricular nuclei, neurohypophyseal unit and the osmoreceptors in the subfornical organ and the organum vasculosum laminae terminalis (185).

In one patient in this series, deep venous thrombosis occurred as a complication of acute hypernatraemic dehydration. This is a serious complication in adipsic DI. In this young woman, multiple pulmonary emboli with respiratory failure occurred which required long term anticoagulation and necessitated withdrawal of oestrogen replacement therapy (187). Venous thrombosis has been reported previously in animal case reports of hypernatraemia (188) and in paediatric patients with adipsic DI (189), and we have previously reported it in other cases of ADI with hypernatraemic dehydration.

Our experience with this CP patient and with those suffering from ADI secondary to other pathologies has prompted us to alter our practice to include the routine prescription of prophylactic subcutaneous heparin during episodes of hypernatraemic dehydration. Although obesity, reduced mobility and, in females, supraphysiological sex steroid replacement are all risk factors for venous thrombosis in this cohort, we believe that thrombosis occurs in adipic DI as a complication of plasma volume contraction and consequent plasma hyperviscosity. We have not documented thrombosis during hypernatraemic dehydration where prophylactic heparinisation has been employed. The administration of low molecular weight heparin should be considered carefully in those patients with adipic DI who have recently undergone neurosurgery, and in the post-operative period strict fluid balance management to prevent hypernatraemic dehydration is essential.

Previous case reports of ADI described sleep apnoea (14). Formal sleep studies in tandem with pulmonary function testing confirmed obstructive sleep apnoea and excluded obesity-related hypoventilation syndrome in these patients. One patient in this study underwent sleep studies twice, the second time after gamma knife radiotherapy for craniopharyngioma recurrence, which is recognised to increase the incidence of hypothalamic morbidity; after this procedure her apnoea-hypopnoea index increased and CPAP therapy was initiated. The other patient with apnoea, who required nocturnal CPAP, also had severe respiratory compromise; such that continuous oxygen therapy was required,

respiratory failure was progressive and death resulted. Excessive daytime somnolence has been reported in children with craniopharyngioma although sleep apnoea has not consistently been reported. We believe that the surgical insult to the hypothalamus in patients with craniopharyngioma predisposes these patients to weight gain and apnoea, and that respiratory stress from apnoea may explain the excess respiratory mortality previously reported in the craniopharyngioma group (3). As sleep apnoea is common in adipic DI and has the potential to reduce quality of life and contribute to early mortality, we recommend formal assessment of somnolence and sleep in patients with ADI. Symptoms of daytime somnolence might otherwise be overlooked in patients receiving anticonvulsants or in patients with limited exercise capacity secondary to neurological deficits.

There was a very high incidence of obesity in the group. Obesity has been reported in association with hypothalamic disorders, including childhood craniopharyngioma (21, 190, 191) and is most probably multifactorial in origin. It has been speculated that damage to the arcuate nucleus of the hypothalamus affecting the satiety centre may lead to overeating and failure to curb appetite in response to hormones such as leptin (66, 192). Elevated leptin levels disproportionate to BMI have been described in children with craniopharyngioma, suggesting acquired leptin resistance (due to damaged hypothalamic nuclei) (82). The spouse of one of these four patients observed that his wife was hyperphagic, but this patient also suffered from short-term memory loss – loss of satiety

and lack of recall for food eaten may have contributed to rapid weight gain in this patient. However, research in craniopharyngioma patients has also suggested that diminished physical activity may also contribute to obesity (91) and one of these patients was wheelchair-bound and had low energy expenditure.

Other abnormalities were also documented during case note analysis. Seizure disorders were common and in all affected patients, developed after neurosurgical intervention. Poikilothermia has been reported previously in hypothalamic dysfunction (193) and with adipsia (154) and has been observed in other patients with ADI in our institution, but not in the 4 with craniopharyngioma.

#### **4.5 *Recommendations for management***

The management of water balance in adipsic DI has been comprehensively reviewed previously (63). Our recommendations for management of the complications of water balance are in agreement with those previously published in *Clinical Endocrinology* by Ball et al, and are summarised in Table 4.4. However, even with close monitoring, it is difficult to reproduce the close control of plasma sodium concentrations that characterises physiological osmoregulation. Patients had hospital admissions due to hypernatraemic dehydration and one developed venous thrombosis while hypernatraemic. Usually dehydration was secondary to intercurrent illness, such as gastroenteritis, with inadequate

fluid intake. The swings in plasma sodium concentration are not trivial; seizures complicated severe hypernatraemia. Daily weighing can give a clue to rapid alterations in body water, but regular review with frequent measurement of plasma sodium or plasma osmolality is essential for these patients.

**Table 4.4**

Recommendations for management of water balance for patients with adipsic diabetes insipidus. AVP = arginine vasopressin, dDAVP = desmopressin

Replace AVP deficit with desmopressin
Monitor fluid intake as inpatient and titrate fluid / dDAVP to achieve eunatraemia
Weigh patient while eunatraemic
Advise 1.5-2 litre fluid intake per day
Weigh daily
If < eunatraemic weight, replace with equivalent volume of fluid eg if 0.5kg deficit, drink 0.5 litres fluid
Advise to increase fluid intake in times of increased exercise or increased ambient temperature
Regular measurement of plasma sodium

In addition to the guidelines for managing water balance in this condition, we recommend the administration of low molecular weight heparin to patients with adipsic DI during periods of volume contraction and

hypernatraemia. We recommend screening for sleep apnoea with an Epworth sleepiness score and a high index of suspicion, based on snoring history, daytime somnolence and early morning headache, even when the Epworth score is normal. We believe that ADI should be regarded as a marker for obesity risk, and that these patients may benefit from early referral to a dietician, or entry into a formal exercise programme if clinically appropriate. Advice about portion control may help counteract excess calorie intake as a result of hyperphagia. Prevention of obesity should be the management focus, as treatment is difficult. Obesity contributes to the vulnerability to sleep apnoea we have described in these patients, which we believe is an important predictor of mortality in this group.

In summary, adipsic diabetes insipidus is a rare complication of craniopharyngioma, which is associated with increased morbidity and mortality. We regard ADI as a marker for extensive hypothalamic damage. It is now policy in our department to actively test for other hypothalamic disorders such as sleep disorders, and to counsel patients with ADI about the likelihood of developing obesity. In the acute phase of hypernatraemia associated with cranial diabetes insipidus, we recommend prophylactic anticoagulation with low molecular weight heparin, a policy that would not have previously been implemented in those patients who are post-neurosurgical intervention.

hypothesised that if sleep apnoea were prevalent in the craniopharyngioma population, it would be a potential explanation for the excess respiratory mortality reported in CP (3, 130).

This study was therefore designed to test the hypothesis that sleep apnoea is prevalent in the craniopharyngioma population and that it contributes to the somnolence and fatigue so characteristic of this patient population.

## **5.2 *Methods***

### **5.2a *Clinical subjects***

Subjects with craniopharyngioma were identified from the hospital pituitary database. Control subjects who were overweight or obese were consecutive patients recruited from referrals to the respiratory outpatients for exclusion of sleep apnoea as a cause of hypertension. Twenty-eight craniopharyngioma and twenty-three obese or overweight people were evaluated. Subject details including diagnosis, age, gender, body mass index (BMI) and pituitary hormone deficiencies were recorded. Seven (25%) of the craniopharyngioma subjects were diagnosed before the age of 18 and were defined as "childhood-onset" CP (CO). All CP patients with ACTH deficiency received hydrocortisone replacement and those with TSH deficiency received thyroxine. All men and all women of premenopausal age (less than 51 years) with gonadotrophin deficiency received gonadal steroid replacement. Patients with growth hormone deficiency who were less than 70 years of age received growth hormone



replacement. Growth hormone was discontinued in two of the craniopharyngioma patients, in one patient following tumour recurrence and in one patient after the development of diabetes mellitus. One craniopharyngioma patient refused growth hormone therapy in adulthood and was not receiving growth hormone at the time of assessment. Exclusion criteria for entry into the study included age less than 18 years at the time of outpatient visit and inability to give informed consent. The local hospital ethics committee gave approval for the study.

### **5.2b *Somnolence and Fatigue***

The Epworth Sleepiness Score was used to assess somnolence, as described in Chapter 2 and shown in Figure 2.1. Daytime somnolence is defined as an ESS greater than 10 / 24. Independent reports of somnolence by patients' families or physicians were also used for assessment of somnolence, in cases where this could be confirmed with objective evidence such as falling asleep during consultations or change of employment secondary to somnolence.

### **5.2c *Sleep apnoea***

All subjects were assessed for one night and were admitted for polysomnography at 5pm on the day of the study, as described in Chapter 2. All patients with hypopituitarism were advised to take hormone replacement therapy as prescribed. Polysomnography was performed using the Alice 4 (Respironics, France). Sleep stage was

diagnosed using the well validated criteria of Rechtschaffen and Kales (158).

An episode of central apnoea was defined as cessation of airflow for at least 10 seconds without respiratory effort. An episode of obstructive apnoea was defined as cessation of airflow for at least 10 seconds with respiratory effort. Hypopnoea was defined as a 50% drop in airflow from baseline. An oxygen saturation drop of 4% from baseline and / or heart rate drop of 2.5% from baseline was the minimum acceptable change following an apnoeic event. Sleep stage assessment (as described in Chapter 2, Figures 2.2-2.4) and respiratory event diagnosis, were performed by a sleep disorders physician who was blinded to the diagnosis and clinical features of the subjects. The apnoea – hypopnoea index (AHI) was calculated by the number of apnoeic and hypopnoeic events per hour of sleep. An AHI of greater than 5 events per hour was considered to be diagnostic of sleep apnoea, as per the criteria of the American Sleep Disorders Association (137). Spirometry, lung volumes and in selected patients carbon monoxide diffusion capacity, were performed to exclude the diagnosis of obesity hypoventilation syndrome, as per the criteria of the American Sleep Disorders Association (137).

#### **5.2d Patient Management**

All subjects who were diagnosed with sleep apnoea were offered a trial of continuous positive air pressure (CPAP) therapy. Any patient who failed to tolerate CPAP, or had no improvement in measures of somnolence

with CPAP was offered a trial of modafinil, a non-amphetamine wakefulness-promoting agent licensed to treat excessive daytime somnolence associated with apnoea (198). Those patients who did not have a diagnosis of sleep apnoea, but had measurable somnolence, were also offered a trial of modafinil therapy. This was not a formal clinical trial of therapy for somnolence, and the decision to offer therapy was made by a respiratory physician to whom all patients with sleep apnoea or somnolence were referred, as agreed with the local Ethics Committee.

### **5.2e Statistics**

Non-parametric data were described using the median and interquartile range, or total range where considered by the investigators to be clinically appropriate. Continuous variables were compared using Wilcoxon's test and were sub analysed using t test with Bonferroni correction where appropriate. Categorical variables were assessed by Chi Square. Correlation was assessed with Spearman's Rho and logistic regression was used for multivariate analysis. Statistical significance was accepted where the p value was less than 0.05. Statistical analysis was performed using JMP 8 software (SAS Institute, California, USA).

## **5.3 Results**

### **5.3a Clinical Subjects**

The characteristics of the subjects are shown in Table 5.1. The obese or overweight subjects, who were matched for gender and weight, were

older than the CP patients ( $p < 0.01$ ). Spirometry and other respiratory function tests were normal (Table 5.1) in the craniopharyngioma patients. Two CP patients did not undergo surgical resection because the tumour was intrasellar; the other 26 patients underwent surgery for suprasellar extension of the mass, of whom 1 had a trans-sphenoidal resection followed by later craniotomy and 25 underwent craniotomy. With regard to the two patients who did not have a histological diagnosis; the first patient had a large suprasellar tumour with a stable appearance on gadolinium-enhanced MRI over 7 years of follow-up; the second patient initially had a CT that showed calcification and then an MRI that showed a small mass lesion located just above the diaphragma sella. The infundibulum of the pituitary was displaced to the right by the lesion. On T1 weighted images the lesion showed mixed signal and was considered to be most consistent with a craniopharyngioma. Neither patient had visual loss or pressure symptoms, thus surgery was not indicated. Ten CP patients (36%) had received radiotherapy. The majority of CP patients were panhypopituitary, which comprised GH, gonadotrophin, ACTH and TSH deficiency, and cranial diabetes insipidus (DI).

**Table 5.1.** Sleep study Patient Characteristics compared to Controls.

Values expressed as median (total range). CP = craniopharyngioma, Ob / OW = obese / overweight control. M:F = male : female ratio. BMI = body mass index. FVC = forced vital capacity as percentage of predicted based on gender, age and BMI. FEV1 = forced expiratory volume in 1 second. NS = non-significant result,  $p$  value  $> 0.05$

Parameter	CP (n = 28)	Ob / OW (n = 23)	p value
Age Years	40.5 (19-67)	53 (34-73)	< 0.01
Gender M:F	17:11	13:10	NS
BMI kg/m <sup>2</sup>	33.6 (26.1-61.1)	33.7 (28-63.8)	NS
FVC % pred	89 (57-130)	99 (80-118)	NS
FEV1 / FVC	77 (60-86)	75 (68-85)	NS
GH deficiency	26 (92%)	Not applicable	
Gonadotrophin deficiency	26 (92%)	Not applicable	
ACTH deficiency	26 (92%)	Not applicable	
TSH deficiency	26 (92%)	Not applicable	
AVP deficiency	22 (79%)	Not applicable	

### 5.3b Somnolence

Somnolence was reported by 20 of 28 (71%) craniopharyngioma patients, and by 4 of 23 obese subjects (17%,  $p < 0.001$ ), Table 5.2. The ESS was higher in the craniopharyngioma patients (median 7.5, IQR 6, 10.7) than in the obese controls, (4.0, IQR 4, 8,  $p < 0.01$ ) shown in Figure 5.1.

Excessive somnolence was diagnosed clinically in 13 patients with ESS of 10 or less. 4 of these patients had disruption of education secondary to falling asleep in classes, 1 patient fell asleep during a consultation, 1 took early retirement from employment secondary to somnolence, 2 former housewives fell asleep repeatedly during family activities and were unable to perform their household tasks as previously and 5 patients

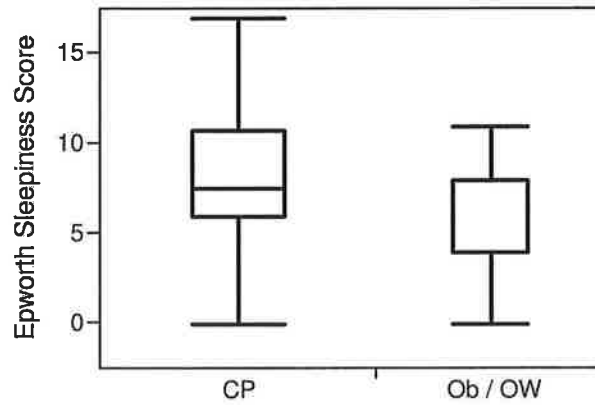
temporarily took leave of absence from employment or changed job secondary to somnolence. There was no difference in the prevalence of somnolence between the childhood and adult onset CP groups ( $p = 0.6$ ). There was no difference in the proportion of patients who were somnolent between the patients who had undergone radiotherapy and those who had not ( $p = 0.5$ ). There was no difference in current age ( $p = 0.2$ , figure 5.2) or in age at diagnosis ( $p = 0.25$ , figure 5.3), between somnolent and non-somnolent CP patients. There was no difference in T4 levels between CP subjects who were somnolent (10.7, IQR 8.7,15.3 pmol/l) and those who did not report sleepiness (8.8, IQR 7.8,9.1 pmol/l,  $p = 0.06$ , figure 5.4). Similarly, there was no difference in IGF1 standard deviation score between CP subjects who were somnolent ( $-0.4 \pm 2$ ) and those who did not report sleepiness ( $0.4 \pm 2$ ,  $p = 0.4$ ).

**Table 5.2.**

Somnolence and diagnosis of apnoea

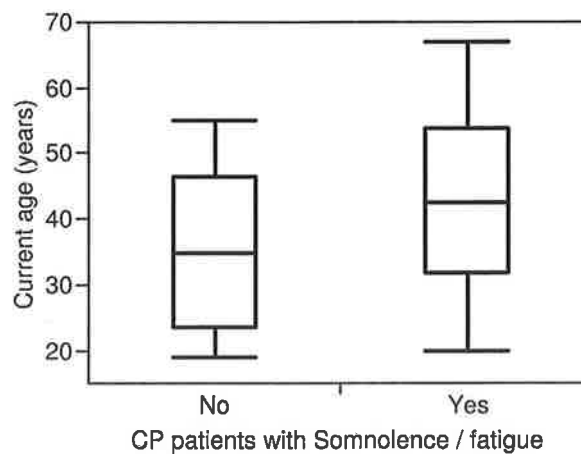
Clinical Somnolence or fatigue includes all patients who reported sleepiness, or those in whom sleepiness or tiredness was objectively reported by a third party. ESS = Epworth Sleepiness Score, daytime somnolence defined by score greater than or equal to 11. AHI = apnoea hypopnoea index, sleep apnoea defined by score greater than or equal to 5. Ob / OW = obese / overweight control group. NS non-significant

	CP (n = 28)	Ob / OW (n = 23)	p value
Number of subjects with Clinical Somnolence or fatigue	20 (71.4%)	4 (17.3%)	< 0.001
No. subjects with ESS $\geq$ 11	7 (25%)	1 (4.3%)	<0.05
Median ESS	7.5	4	< 0.01
No. subjects with Apnoea (AHI > 5)	13 (46%)	14 (60.8%)	NS
Median AHI per hour	4.5 (2-49.2)	5 (0-35)	NS



**Figure 5.1.**

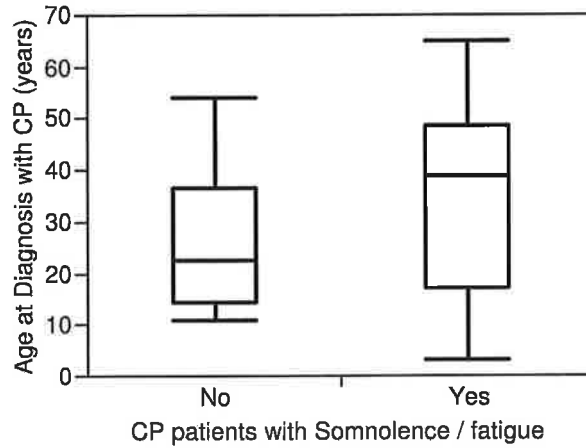
Epworth Sleepiness Score in CP patients compared to obese / overweight (Ob / OW) controls ( $p < 0.01$ )



**Figure 5.2.**

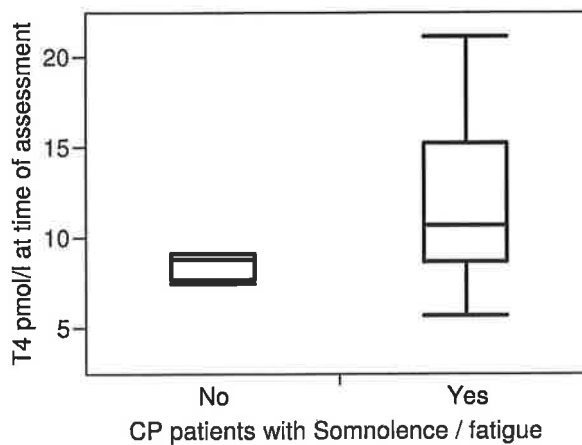
Comparison of current age between CP patients who suffer from somnolence / fatigue (Yes) and those who do not (No,  $p = 0.2$ )





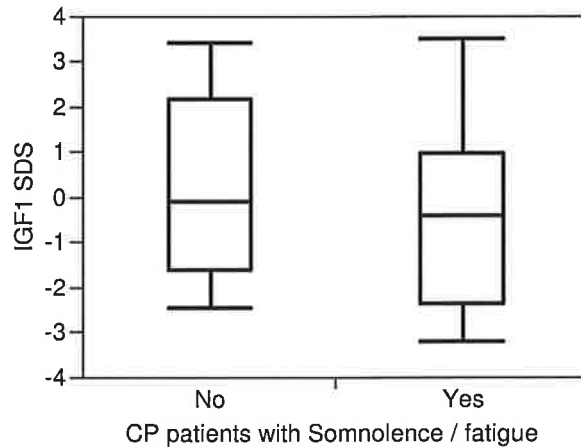
**Figure 5.3.**

Comparison of age at diagnosis with CP between CP patients who suffer from somnolence / fatigue (Yes) and those who do not (No,  $p = 0.25$ )



**Figure 5.4.**

Comparison of T4 level at time of sleep assessment between CP patients who suffer from somnolence / fatigue (Yes) and those who do not (No,  $p = 0.06$ )



**Figure 5.5**

Comparison of IGF-1 standard deviation score (SDS) at time of sleep assessment between CP patients who suffer from somnolence / fatigue (Yes) and those who do not (No,  $p = 0.4$ )

### 5.3c *Diagnosis of sleep apnoea*

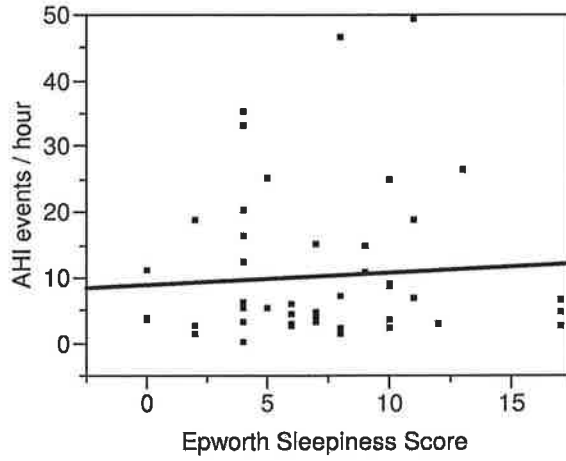
There was no difference in the rate of diagnosis of obstructive sleep apnoea in craniopharyngioma patients (13 of 28) and obese or overweight controls (14 of 23,  $p = 0.2$ ). The details of the sleep studies and respiratory sleep events are shown in Table 5.3. Sleep efficiency and total sleep time was higher in the craniopharyngioma group (Table 5.3), which reflects the somnolence observed in the CP population. No patient was diagnosed with sleep-onset REM. ESS did not correlate with AHI within CP patients, nor in the overall study ( $r = 0.08$ ,  $p = 0.6$ , Figure 5.6). Body mass index (BMI) did not correlate with AHI within CP patients, nor in the overall study ( $r = 0.25$ ,  $p = 0.08$ , Figure 5.7). There was no difference in median serum T4 concentrations between subjects

who were diagnosed with sleep apnoea (9.7 IQR 7.8,13.8 pmol/l) and those who had normal sleep breathing (10.3 IQR 8.7,14.6,  $p = 0.5$ , Figure 5.8). Similarly, there was no difference in IGF1 standard deviation score between subjects who were diagnosed with sleep apnoea ( $0.5 \pm 2$ ) and those who had normal sleep breathing ( $- 0.7 \pm 2$ ,  $p=0.07$ , Figure 5.9).

**Table 5.3.** Sleep Parameters

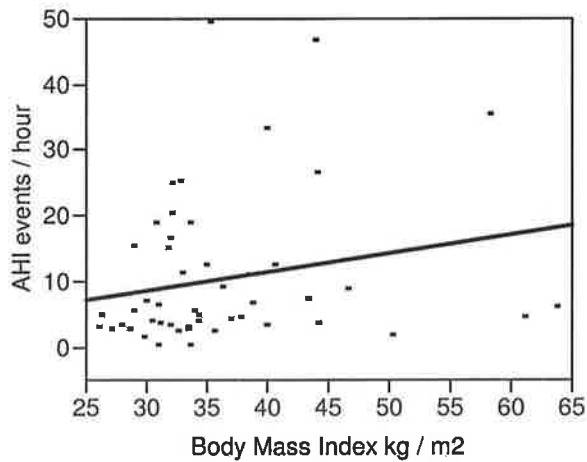
Median and Total ranges shown. SL: sleep latency in minutes, TST: total sleep time in hours, REM %: percentage sleep time spent in Rapid Eye Movement sleep, Min SaO<sub>2</sub>: minimum oxygen saturation, AHI: apnoea hypopnoea index, Ob / OW: obese / overweight controls, NS non-significant  $p > 0.05$

Sleep Data	CP	Ob / OW	p
SL Minutes	29 4-56	38 9-64	NS
TST Hours	7.03 5.6-10.1	6.18 4.1-6.5	0.02
Sleep efficiency %	85 77-95	77 55-88	0.02
Sleep Stage 1 %	21 10-26	14 13-15	NS
Sleep Stage 2 %	45 43-47	49 45-53	NS
Slow-wave sleep %	14 7-23	11 5-23	NS
REM %	23 13-26	16 7-24	0.14
Min SaO <sub>2</sub> %	78 75-86	88 80-93	0.04
AHI Events / hour	4.5 2-49	5 0-35	NS



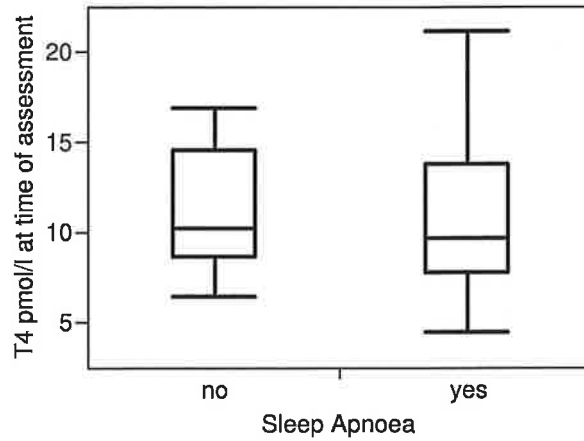
**Figure 5.6**

Correlation of Apnoea Hypopnoea Index (AHI) with Epworth Sleepiness Score (ESS) showing line of best fit,  $r = 0.08$ ,  $p = 0.6$



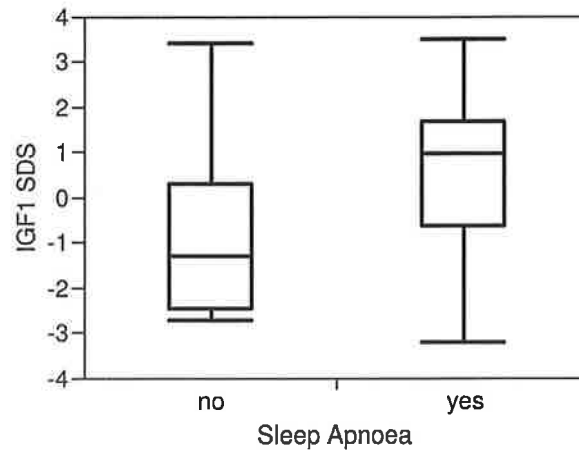
**Figure 5.7**

Correlation of Apnoea Hypopnoea Index (AHI) with Body Mass Index (BMI) showing line of best fit,  $r = 0.25$ ,  $p = 0.08$



**Figure 5.8**

Comparison of T4 level at time of sleep assessment between CP patients without sleep apnoea (no) and those with sleep apnoea (yes) ( $p = 0.5$ )



**Figure 5.9**

Comparison of IGF1 standard deviation score (SDS) at time of sleep assessment between CP patients without sleep apnoea (no) and those with sleep apnoea (yes) ( $p = 0.07$ )

### **5.3d Patient Management**

All patients who were diagnosed with apnoea were offered a trial of continuous positive airway pressure (CPAP) therapy. CPAP treatment reduced ESS from 10 (IQR 8,13) to 5, (IQR 3,7),  $p = 0.01$  (Table 5.4). Two patients refused CPAP therapy; one of these was a male patient who had no complaints of somnolence or fatigue and who continued to work full-time (AHI 11, ESS 0). The other patient (AHI 6.7, ESS 11) refused CPAP therapy on the grounds of claustrophobia. Nine craniopharyngioma patients complained of somnolence but did not have apnoea on polysomnography, in these patients CPAP would not have provided clinical benefit. Five somnolent CP patients were offered modafinil therapy, of whom 4 reported clinical improvement with an overall reduction in ESS from a median of 8 (IQR 5,9.5) to 1 (0,2)  $p = 0.01$ . A further 3 somnolent CP patients who did not have apnoea were not compliant with anterior pituitary hormone replacement therapy and non-adherence was considered the likely contributory factor to their somnolence. One patient died before any treatment could be offered.

**Table 5.4.**

Management outcomes for CP patients who were diagnosed with apnoea

M = male, F = female. BMI = body mass index. AHI = apnoea hypopnoea

index, events per hour. ESS = Epworth Sleepiness Score. CPAP =

continuous positive airway pressure therapy

<u>Patient</u>	<u>Gender</u>	<u>Age</u> years	<u>BMI</u> kg/m <sup>2</sup>	<u>AHI</u> Events per hour	<u>ESS</u>	<u>Outcome</u>
1	M	60	38.5	10.5	9	Did not tolerate CPAP, benefit from modafinil
2	F	60	30	6.7	11	Refused CPAP
3	F	34	43.3	6.9	8	CPAP + modafinil, ESS drop to 2
4	F	67	32.8	24.8	5	Did not tolerate CPAP or modafinil
5	M	31	28.97	14.9	7	Benefit from CPAP
6	M	53	44	46.4	8	Benefit from CPAP – weight loss 8 kg, ESS drop to 4
7	M	31	38.9	6.3	17	Lost 20kg weight on CPAP
8	M	55	33	11	0	Refused CPAP
9	M	47	32.1	24.5	10	Benefit from CPAP
10	M	65	35.4	49.2	11	Benefit from CPAP, ESS drop to 7
11	F	36	44.1	26	13	Benefit from CPAP, ESS drop to 7
12	M	43	36.37	8.8	10	Trial of modafinil, in view of low AHI (fatigue disproportionate to degree of apnoea)
13	F	42	32.1	20	4	Trial of CPAP; patient did not tolerate - ESS drop to 0 with modafinil

## 5.4 *Discussion*

The data from this study confirms the widespread clinical observation that somnolence is very common in CP patients. In addition, our observation that the craniopharyngioma subjects were more somnolent than a weight-matched control group of subjects indicates that obesity is not the sole determinant of the presence of somnolence in CP. This was emphasised by the lack of correlation between BMI and AHI. Sleep apnoea was the most common cause of sleepiness in the craniopharyngioma group and those patients who were diagnosed with sleep apnoea responded well to treatment with CPAP. In those patients with somnolence but in whom apnoea was excluded, symptoms improved in response to modafinil therapy.

We assessed somnolence with the well-validated Epworth Sleepiness Score, but we also recorded patient and relatives' reports of fatigue and somnolence. Our clinical observations on the patient cohort in our study caused us to question whether the ESS was accurate for use in the CP patient group. Although the rate of somnolence, defined by an ESS greater than 10, in our CP cohort was similar (35.7%) to that reported in a Dutch study (33.3%, (156)), we noted that some craniopharyngioma subjects who had objective evidence of somnolence, such as falling asleep before lights out or during phlebotomy, reported relatively normal Epworth Sleepiness Scores. We therefore suspect that the high rate of somnolence in our group may still be an underestimate of the true rate of



somnolence in this group. Although the Epworth Score may be the gold standard screening tool for somnolence, a separate, disease-specific screening tool may be needed in this cohort in order to accurately define somnolence. In addition, as the ESS included questions about driving, reading and watching television that were inappropriate for patients with seizure disorders and visual loss, we may have underestimated somnolence as assessed by ESS alone. In our study protocol, patient-reported, family reported or health care reported incidents of somnolence, such as falling asleep during phlebotomy, medical consultations and during time at work, were utilised in order to define what we thought was the true incidence of somnolence (Table 5.2).

We had hypothesised that as craniopharyngioma patients suffer from high rates of obesity (1, 21, 37), which is a major risk factor for the development of sleep apnoea (195), our cohort would have a high incidence of sleep apnoea. Our data confirmed a high prevalence of sleep apnoea in craniopharyngioma subjects, which was similar to the rate in obese subjects. However our data showed that there was not a close relationship between the rate of apnoeic events and body mass index in the CP group. This would indicate that obesity is not the sole determinant of the development of sleep apnoea in CP patients, and would further suggest that screening for sleep apnoea in CP should not be confined to obese patients. There are a number of possible mechanisms for sleep apnoea in addition to weight change. Dysfunction of the upper airway dilator muscles from abnormal levels of hormone

replacement such as GH and thyroxine is possible, though hormonal data argues for appropriate hormone replacement and makes this unlikely. Hypothalamic damage from tumour and/or surgery may also lead to abnormal sleep cycles or changes in local neurotransmitters such as hypocretin that affect the sleep/wakefulness cycle. We did not perform invasive nerve conduction studies nor sample hypocretin and thus can only speculate about the possible contribution of the above factors. The relationship between destructive surgery and hypothalamic complications that is reported in CP makes this a strong contender for causality; however this hypothesis would need a prospective study to document sleep quality before and after surgery to prove causality.

Although we studied a small cohort of patients, the subjects were recruited in an unselected, consecutive manner from the outpatient department and not on the grounds of somnolence or the clinicians' assessment of the subjects' apnoea risk. It is the largest study of which we were aware, to use polysomnography in the craniopharyngioma population, and although the study was open-label in nature, the physician who assessed the polysomnography recordings was blinded to the diagnosis and characteristics of each subject. We therefore feel that the high rate of somnolence and sleep apnoea are true reflections of the clinical scenario in craniopharyngioma patients and are therefore applicable to craniopharyngioma patients in attendance at any standard outpatient service.

The severity of obesity in the craniopharyngioma population made it difficult to match controls for both BMI and age, with the result that the obese controls were significantly older. Increasing age is a risk factor for sleep apnoea (199), however, so the high incidence of sleep apnoea in the younger CP group assumes even greater significance. In a recent Canadian study of sleep-disordered breathing (SDB) in adolescent patients with CP, there was a higher incidence of SDB in CP patients than obese adolescents (196). We were unable to replicate this observation in this adult study, possibly due to the older age profile in our obese group. Seven of the 15 paediatric CP patients in the Canadian study were not prescribed GH replacement at the time of polysomnography because they had already achieved target height; in our cohort all adult patients with GH deficiency were offered replacement unless there was a contra-indication to GH therapy. Untreated growth hormone deficiency may have contributed to the high incidence of sleep disturbance in the paediatric study, although the effect of GH replacement on sleep disturbance remains unclear (119, 120, 200, 201). It is also noteworthy that 6 of 13 subjects in the paediatric study had an abnormal AHI but only 2 were offered CPAP therapy. A much higher proportion of adult CP patients in our study were offered treatment with CPAP or modafinil in a manner appropriate for individual AHI scores (128), and demonstrated benefit.

In this study we observed multiple potential explanations for somnolence in craniopharyngioma subjects, such as sleep apnoea (155), non-

adherence with drug therapy (121) and a further group with unexplained hypersomnolence. This group of unexplained hypersomnolent patients may have a “secondary narcolepsy” due to damage of the hypothalamic nuclei that secrete hypocretin, although this remains hypothetical without the measurement of cerebrospinal fluid hypocretin concentrations and is unlikely in the absence of sleep-onset REM in the polysomnography studies (113, 135, 202). Melatonin deficiency secondary to hypothalamic damage has also been described in somnolent CP patients (115, 203) and melatonin supplementation has also been used to treat somnolence in CP patients in previous case series (146).

Although somnolence in craniopharyngioma is more heterogeneous in aetiology than we had hypothesised, the high rate of sleep apnoea and the response of somnolent patients with and without sleep apnoea to therapy suggest a new approach to targeted therapy in craniopharyngioma patients. Patients who were able to tolerate CPAP were able to show significant reductions in ESS and two patients (patients 6 & 7, Table 5.4) were able to lose significant weight as their exercise levels increased with CPAP treatment. Somnolent CP patients without sleep apnoea responded well to modafinil therapy with improvements in daytime sleepiness. Patients with narcolepsy respond to modafinil (133); the good response of the majority of our subjects with unexplained hypersomnolence to modafinil suggests that secondary narcolepsy may be a cause of somnolence in this group. The number of patients treated with either CPAP or modafinil in this study was small, but

the responses that we report are sufficiently encouraging to suggest that a larger prospective study to evaluate the role of these treatments would be a useful contribution to our treatment of this difficult condition. Until the results of such a trial are published, we would recommend that clinicians should consider that sleep apnoea and unexplained somnolence are potentially treatable complications of craniopharyngioma.

## Chapter 6

### **Abnormal glucose tolerance is common in craniopharyngioma patients and is associated with older age at diagnosis with craniopharyngioma**

#### ***6.1 Introduction***

Craniopharyngioma (CP) is a benign tumour of the sella and suprasellar region that I have demonstrated in Chapters 3-5 to be associated with increased morbidity and mortality. The increased mortality in CP patients is secondary to cardiovascular disease and to respiratory pathology (2, 3). CP patients suffer from high rates of obesity, which is a risk factor for cardiovascular disease (21, 37). Obesity and dyslipidaemia have been reported in CP patients, but other cardiovascular risk factors have not been well described (19, 204). We hypothesise that diabetes and abnormal glucose metabolism may be more prevalent in the CP population than is described in the literature, and that the presence of abnormal glucose metabolism may be a contributing factor in the high mortality rate from cardiovascular disease that has been reported in the obese CP population.

In this study we aimed to establish the prevalence of abnormal glucose tolerance and to assess insulin sensitivity, in an adult CP cohort.

## **6.2 Methods**

Twenty subjects with craniopharyngioma (11 male) were identified from the hospital pituitary database; none of these patients were known to have diabetes. Five of the craniopharyngioma subjects were diagnosed before the age of 18 and were defined as “childhood-onset” CP (CO). Two CP patients did not undergo surgical resection because the tumour was intrasellar; the other 18 patients underwent surgery for suprasellar extension of the mass. Of the two patients who lacked a histological diagnosis; the first patient had a suprasellar tumour with a stable appearance on gadolinium-enhanced MRI over 7 years of follow-up; the second patient initially had a CT that showed calcification and then an MRI that showed a small mass lesion of mixed signal located just above the diaphragma sellae. Neither patient had visual loss or pressure symptoms, thus surgery was not indicated. Eight CP patients (40%) had received radiotherapy. The majority of CP patients were panhypopituitary, which comprised GH, gonadotrophin, ACTH and TSH deficiency, and cranial diabetes insipidus (DI). All patients with hypopituitarism received hormone replacement therapy as described in Chapter 3. Growth hormone was discontinued in one of the craniopharyngioma patients following tumour recurrence. One craniopharyngioma patient refused growth hormone therapy in adulthood and was not receiving growth hormone at the time of assessment. Twenty obese or overweight controls (13 male) were recruited from a study of metabolic parameters in healthy individuals conducted at Dublin City University. Subjects were advised to fast from 10pm the night before

and CP patients were advised to take their normal steroid replacement and thyroxine replacement with a sip of water, in order to assess glucose tolerance under normal clinical conditions. Glucose tolerance testing was conducted under normal clinical conditions of full hormone replacement in order to assess glucose metabolism under everyday conditions, since both steroid and GH deficiency states and replacement therapy have effects on glucose metabolism (177, 205, 206). An 18g cannula was placed in the antecubital fossa under aseptic technique. 20ml of blood was withdrawn: a serum sample for estimation of insulin and adiponectin, and a citrated sample for estimation of glucose. 75g of anhydrous glucose was dissolved in 300ml of water and given to the subjects for consumption as an oral glucose challenge. The subjects were advised to remain seated until repeat sampling was performed at 120 and 180 minutes.

Glucose was estimated using the hexokinase method, on the Beckman Coulter (formerly Olympus) AU 5400 chemistry analyser, and insulin with the Unicel Dxl 800 Beckman Coulter chemiluminescent immunoassay system. Laboratory estimation of adiponectin was performed using a commercial kit for enzyme-linked immunosorbent assay (ELISA) for total adiponectin (Quantikine Acrp30 Immunoassay, R&D Systems, Abingdon, UK).

Normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type 2 diabetes were diagnosed



according to standard American Diabetes Association criteria (207). For the purpose of this study, Abnormal Glucose Tolerance (AGT) was defined as the presence of either IFG or IGT or type 2 diabetes. 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 (208) 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 (209). Insulin measurements in units were multiplied by a factor of six to convert measurements to pmol, in keeping with international recommendations, for the purposes of HOMA2 calculation (210). Dynamic estimation of insulin sensitivity was measured using the Oral Glucose Insulin Sensitivity (OGIS) method (211). The OGIS is an index of insulin sensitivity calculated from OGTT glucose and insulin measurements, which has been validated against the hyperinsulinaemic-euglycaemic clamp (211) and has been used to assess insulin sensitivity in previous studies of Irish patients (212, 213). OGIS is reliable in cases where glucose absorption is normal; none of our patients had a history of malabsorption, or symptoms to suggest that this was the case.

Exclusion criteria for entry into the study included age less than 18 years at the time of outpatient visit and inability to give informed consent.

Control subjects were excluded if they were known to have diabetes or pre-diabetes before entry into the study. Beaumont Hospital and Dublin City University ethics committees gave approval for the study.

Normally distributed data were described using mean and standard deviation and compared using t test. Non-parametric data were described using median and 95% confidence interval and were compared using Wilcoxon's test. Correlation analysis was performed using Spearman's Rho test. Multiple regression analysis was used to assess the impact of continuous variables in a model. Statistical significance was accepted at  $p < 0.05$ .

### **6.3 Results**

Comparison of CP and control group characteristics is shown in Table 1. One CP patient and 1 control were diagnosed with diabetes. Fasting glucose in CP patients was  $5.2 \pm 0.8$  mmol/l and in controls was  $5.3 \pm 0.6$  mmol/l,  $p = 0.2$  (Figure 6.1). Fasting insulin was higher in CP patients ( $62.8 \pm 42$  pmol/l) compared to controls ( $34.9 \pm 29$ ,  $p = 0.01$ , Figure 6.2).

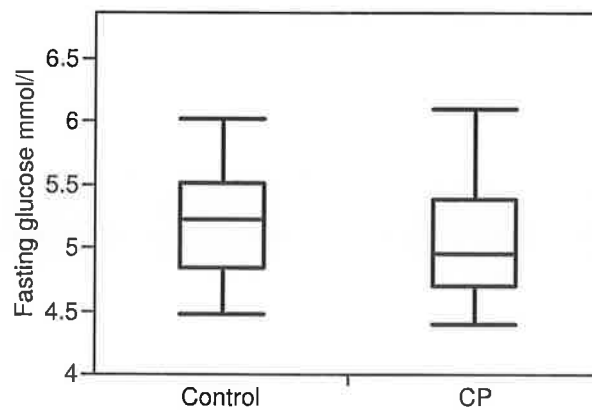
**Table 6.1.**

Comparison of CP and control groups in glucose tolerance study

CP = craniopharyngioma, BMI = body mass index, M:F = male:female,

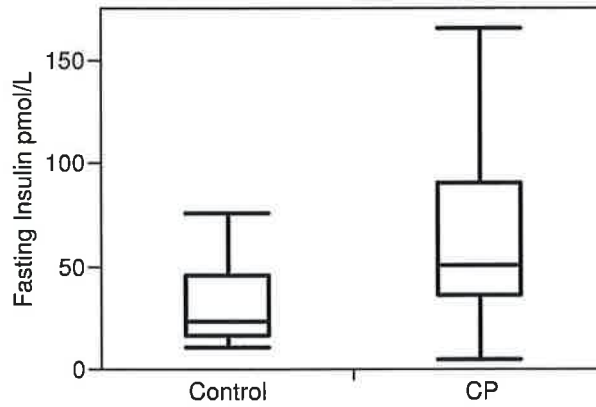
AGT = abnormal glucose tolerance

Parameter	CP (n = 20)	Controls (n = 20)	p value
BMI (kg/m <sup>2</sup> )	35.3 ± 7.3	32.0 ± 1.6	0.06
Age (Years)	42 ± 14	51 ± 14	0.04
M:F	11:9	13:7	0.7
Fasting adiponectin (ng/ml)	6.6 ± 4.8	7.4 ± 3.5	0.2
HOMA	2.47 ± 1.9	1.34 ± 1.1	0.05
HOMA2 IR	1.2 ± 0.2	0.7 ± 0.1	0.02
AGT	8	4	0.4



**Figure 6.1.**

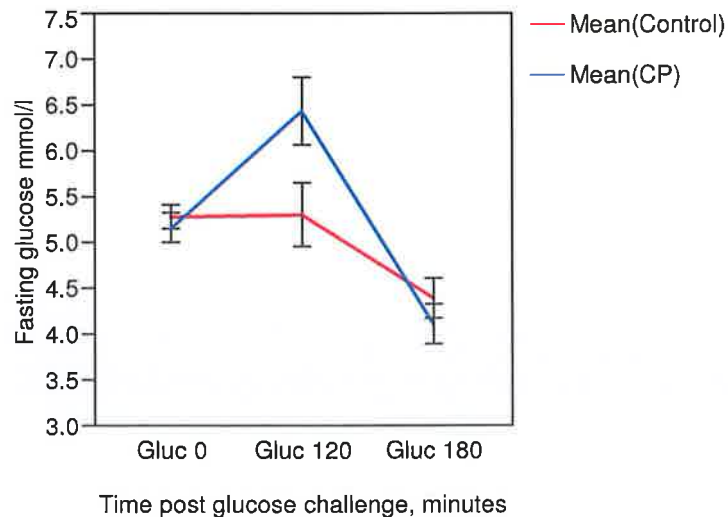
Comparison of fasting glucose between controls and CP patients (p = 0.2)



**Figure 6.2.**

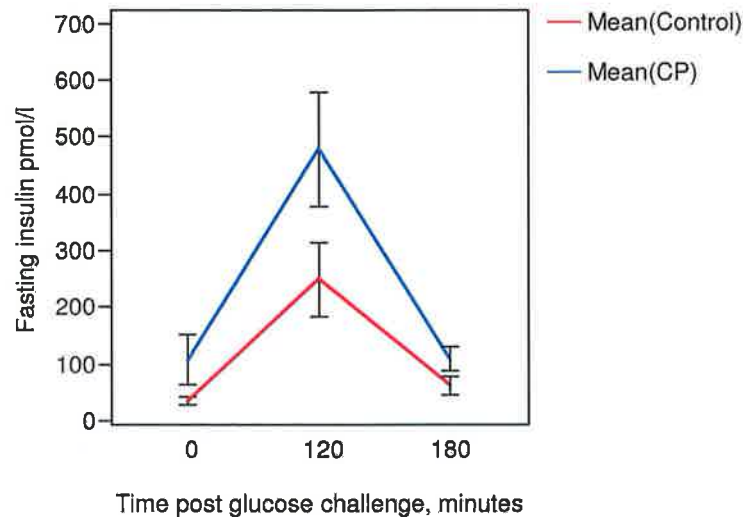
Comparison of fasting insulin between controls and CP patients ( $p = 0.01$ )

There was no difference between CP and controls in glucose response post OGTT (ANOVA,  $p = 0.4$ , Figure 6.3) or in insulin response (ANOVA,  $p = 0.06$ , Figure 6.4). OGIS was higher in controls ( $480 \pm 97$  ml/min/m<sup>2</sup>) than in CP patients ( $415 \pm 70$  ml/min/m<sup>2</sup>,  $p < 0.05$ ).



**Figure 6.3.**

Comparison of glucose response post-oral glucose challenge in CP patients compared to controls, error bars demonstrate standard error of the mean,  $p = 0.4$



**Figure 6.4.** Comparison of insulin response post-oral glucose challenge in CP patients compared to controls, error bars demonstrate standard error of the mean,  $p = 0.06$

Within CP patients, there was no difference between CO and adult-onset (AO) CP in any glucose or insulin measurement, including OGIS and HOMA ( $p > 0.05$ ). The proportion of patients with abnormal glucose tolerance was not higher in AO CPs ( $p = 0.6$ ). CP patients with NGT are compared to those with AGT in Table 6.2.

**Table 6.2**

Comparison of CP patients with and without abnormal glucose tolerance

NGT = normal glucose tolerance, AGT = abnormal glucose tolerance,

BMI = body mass index, T4 = serum T4 level, IGF-1 SDS = Insulin-like

growth factor standard deviation score, steroid dose (mg) =

hydrocortisone or hydrocortisone-equivalent prednisone dose

Parameter	NGT (n=12)	AGT (n=8)	p
BMI (kg/m <sup>2</sup> )	34.3 ± 5.6	36.9 ± 9.5	0.4
Current age (years)	38 ± 14	49 ± 12	0.06
Age at diagnosis (years)	27 ± 18	43 ± 13	0.04
Years since diagnosis	11 ± 12	6 ± 4	0.2
T4	9.4 ± 2.7	13.2 ± 4.8	0.03
IGF-1 SDS	-0.5 ± 2	0.9 ± 2	0.17
Steroid dose (mg)	21 ± 5.7	21 ± 4.5	0.9

IGF-1 SDS did not correlate with HOMA ( $p > 0.1$ ) but did correlate negatively with OGIS ( $r = -0.57$ ,  $p = 0.02$ ). Serum T4 level did not correlate with HOMA or OGIS.

Age at diagnosis with CP correlated with HOMA ( $r = 0.66$ ,  $P < 0.01$ ), which did not correlate with current age ( $r = 0.38$ ,  $p = 0.1$ ) or BMI ( $r = 0.25$ ,  $p = 0.3$ ) in the CP patients. None of these factors remained significant in a multiple regression analysis model for factors which influenced HOMA. There was a strong negative correlation between OGIS and age at diagnosis with CP (Spearman's rho,  $r = -0.78$ ,  $p < 0.001$ ). There was also a negative correlation between OGIS and current age ( $r = -0.65$ ,  $p < 0.01$ ), and OGIS and BMI in CP ( $r = -0.55$ ,  $p < 0.05$ ). A multiple regression analysis model for OGIS revealed that 78% of variance in OGIS is accounted for by current age, age at diagnosis and BMI; age at diagnosis ( $p < 0.01$ ) and BMI ( $p < 0.01$ ) remained significant in the model. A model that included IGF1 SDS accounted for 82% of the variation in OGIS but IGF1 SDS itself was not significant when included in the model ( $p = 0.1$ ).

#### **6.4 Discussion**

This study demonstrates that abnormal glucose tolerance is common in adult CP patients (40%); the rate of AGT in CP failed to achieve statistical significance when compared with that of a BMI-matched obese population (20%,  $p = 0.3$ ), which may reflect the small size of the study population. The rate of 40% is comparable to that found by one other study conducted in Canada (214); in the Canadian study the difference between the CP group ( $n = 15$ ) and controls did achieve statistical

significance, because there was no AGT identified in the Canadian control group.

The control subjects in our study were significantly older than the CP patients. Within the CP cohort, current age was not a significant factor in models of factors such as BMI, which reasonably could be expected to impact on fasting (HOMA) or dynamic (OGIS) measures of insulin resistance. Nonetheless, increasing age is a major risk factor for insulin resistance and pre-diabetes (207). Although CP patients are younger, in this small study they appear to have the same rate of AGT as the controls; it is clinically significant that CP patients are exposed to the negative impact of hyperglycaemia upon cardiovascular risk profile from a younger age. Measurement of glucose and insulin at more time intervals, such as at 30 and 60 minutes, may have yielded more information about subtle differences between glucose handling in the CP compared to the control populations, but would not change the overall findings of prevalence of AGT in the 2 groups.

The prevalence of AGT in the CP population has not been established from earlier publications, partly because GH deficiency was not always treated in patients included in other studies; untreated GH deficiency is associated with insulin resistance and would be expected to have an impact upon glucose tolerance (206). A study of GH-deficient adolescents who did not receive GH replacement reported AGT in 2 of 12 patients, which was a lower incidence of AGT than that shown in our



adult study, perhaps because of the youth of the adolescent subjects (104); and another Canadian study already mentioned in this discussion, in which half of the adolescents with CP received GH replacement, revealed AGT in 40% of patients, the same rate as that shown in our study (214). A recent study of cardiovascular risk in CP did include adults on GH replacement; although fasting glucose results in the diabetes range were quoted in the article, the proportion of CP patients diagnosed with AGT was not reported (194). Differences in growth hormone replacement rates may have had an impact on the variation in rates of AGT reported in these studies.

We used OGIS as well as HOMA to assess glucose metabolism because our cohort included patients with abnormal glucose tolerance and with diabetes, in whom HOMA does not correlate with hyperinsulinaemic euglycaemic clamp findings (211). A German study found greater insulin resistance (as measured by a median HOMA of 6.0) than we measured (median HOMA 1.9), in a study of adolescent obese CP patients. The German group included younger patients than those in our cohort, thus they would be expected to be less insulin resistant than our patients; a third of the patients in that study did not receive GH replacement and thus would be expected to be more insulin resistant than our patients, and rates of AGT were not reported so the validity of HOMA results in the German study cannot be assessed (101). For these reasons it is difficult to directly compare the German study to the results we have documented in our cohort. A French study of adolescents with CP demonstrated that

hypothalamic involvement and greater insulin resistance prior to surgery (measured by a HOMA of 3.65) was associated with increased weight gain after surgery, but again the study was done in GH-deficient patients who had not received replacement and would be expected to have insulin resistance, and the prevalence of AGT was not reported (84).

With regard to hormone replacement in these patients it is interesting to note that serum T4 levels were significantly higher in CP patients with AGT than in those with NGT. The local reference range for T4 is 7-16 pmol/l, and the unit policy is titration of thyroxine replacement doses in hypopituitary patients to achieve a T4 level in the upper half of the normal reference range (11.5 – 16 pmol/l), which was not achieved in the group with NGT. It is difficult to assess the clinical significance of this finding, since serum T4 level did not correlate with either of the measurements of insulin sensitivity / resistance used in this study. There are three possible explanations for the difference in free T4 levels between the 2 groups. The first explanation is that thyroxine is diabetogenic and is directly responsible for the higher rate of AGT in the group with higher T4 levels. Free T4 levels were higher in AGT patients than in controls in a recent Greek study in which fT4 correlated with insulin resistance (HOMA), and the authors postulated that thyroid hormones could be implicated in the pathogenesis of diabetes (215). Their findings have not been replicated in other studies of thyroid hormone and insulin resistance (216) , and such studies have not been done in hypopituitary groups. The second possible explanation is that free T4 levels are higher in the AGT group

because insulin resistance causes a drop in thyroid hormone binding protein and a consequent increase in circulating free T4, analogous to the effect of insulin resistance to lower sex hormone binding globulin and increase circulating free testosterone in polycystic ovary syndrome. It is not routine practice in our laboratory to measure total T4, thus we cannot assess the impact on fT4 of changes in levels of thyroid binding globulin levels in AGT. This explanation is unlikely given the reliability of fT4 measurement in patients on oestrogen. The third possibility is that clinicians are more aggressive in titration of thyroxine therapy in patients with clinical evidence of insulin resistance such as increased BMI, or in patients who report difficulty in losing weight, and thus the higher T4 levels are really a marker of the clinicians' perception of the patient; this is a very subjective clinical judgement and would be almost impossible to measure in the clinic. There is little in the literature regarding T4 and AGT in hypopituitary patients and thus these 3 possibilities remain highly speculative.

Unlike serum T4 measurements, there was no difference in IGF-1 SDS between the AGT and NGT CP groups, and in these the local therapeutic target of a normal age- and gender-adjusted IGF-1 was achieved with recombinant GH therapy. Both groups received the same dose of steroid replacement therapy.

Abnormal glucose tolerance is an important diagnosis because diabetes in CP has been reported to be associated with significant morbidity such

as diabetic ketoacidosis and non-alcoholic fatty liver disease (79, 104). The impact of a diagnosis of diabetes on cardiovascular mortality in CP remains unclear because the prevalence of diabetes and cardio- or cerebrovascular disease in this population has not been established to date.

In summary, abnormal glucose tolerance is common in CP patients who are more insulin resistant than BMI-matched controls. CP patients with abnormal glucose tolerance are older when diagnosed with CP than those with normal glucose tolerance. CP patients are exposed to a negative glucose metabolism profile from a young age.

## Chapter 7

### **Ghrelin in craniopharyngioma patients is similar to that of obese controls and is lower in patients with sleep apnoea**

#### **7.1 Introduction**

Craniopharyngioma (CP) is a benign tumour that is particularly associated with obesity (21). The cause of obesity in CP is multifactorial. Pathophysiological changes which have suggested to contribute to the development of obesity in CP include increased calorie intake secondary to hyperphagia (78), increased energy storage secondary to hyperinsulinaemia (21, 106) and reduced energy expenditure, secondary to reduced physical activity and basal metabolic rate (90, 91, 93). In my study of CP patients I have already identified a high rate of hyperinsulinaemia (Chapter 6) and also of somnolence, which is a potentially important cause of reduced energy expenditure (Chapter 5). Both of these factors could be contributing to the development of obesity in our CP cohort. Sleep disturbance is implicated in abnormal eating patterns and obesity in the background population (217, 218), and sleep-disordered breathing is reported in obese CP patients (113), which we have confirmed in our own cohort (Chapter 5). In this chapter I propose to assess abnormal appetite signalling, which may lead to hyperphagia, as a potential contributor to obesity in CP. I will analyse the signals of appetite and energy repletion in CP patients and assess whether there is any link between appetite signalling and the abnormal sleep I have already described in this population.

The appetite signal measured in this study was ghrelin, the first enteric signal demonstrated to stimulate appetite, via its action on the hypothalamus. Ghrelin is an acyl-peptide produced by the stomach, with peak plasma concentrations reported prior to food ingestion; plasma concentrations are reduced in obesity (70). Reduced ghrelin levels after gastric bypass have been implicated in the sustained weight loss seen in patients post bariatric surgery (69). Ghrelin is also a GH-secretagogue and a weak stimulant of prolactin secretion; and is implicated in the pathophysiology of seizure disorders, disorders of sleep and memory dysfunction (219, 220), all of which I have reported in CP patients in our cohort. Measurement of the adipocytokine leptin was used to assess the signal of satiety and energy store repletion to the hypothalamus. Plasma concentrations have been reported to be increased in obese individuals and in CP patients, and it has been argued therefore that CP patients may be leptin-resistant (70, 82, 221).

In this study I aimed to measure fasting ghrelin and leptin levels in CP patients and compare these to normal controls. Increased ghrelin has been reported in seizure disorders (222), which are common in CP (1), thus I also compared the plasma concentrations of ghrelin in CP patients with and without seizures. Ghrelin is reported to have an impact on sleep and sleep stage; thus ghrelin levels of CP patients with and without sleep apnoea were compared. I also assessed the impact of a diagnosis of

sleep apnoea on the ghrelin and insulin response to an appetite challenge in CP patients.

## **7.2 Methods**

Twenty patients with craniopharyngioma (9 male) were identified from the hospital pituitary database. Subject details including diagnosis, age, gender, body mass index (BMI) and pituitary hormone deficiencies were recorded. All CP patients with pituitary hormone deficiencies received hormone replacement therapy as described in Chapter 3. Growth hormone was discontinued in two of the craniopharyngioma patients, in one patient following tumour recurrence and in one patient after the development of diabetes mellitus. One craniopharyngioma patient refused growth hormone therapy in adulthood and was not receiving growth hormone at the time of assessment. Exclusion criteria for entry into the study included age less than 18 years at the time of outpatient visit and inability to give informed consent. The local hospital ethics committee gave approval for the study.

### **7.2.a Fasting hormone profile**

CP patients attended for phlebotomy at 8am and underwent sampling of fasting leptin and ghrelin. Hypopituitary patients were advised to take hormone replacement therapy in accordance with their normal replacement regimen. Twenty two healthy controls (9 male), none of whom were diagnosed with a seizure disorder or sleep disorder, were recruited, had measurement of height and weight to calculate BMI and

underwent fasting hormone sampling for comparison with CP samples. Controls were divided into those of normal BMI and those who were overweight or obese for the purposes of data analysis.

### **7.2.b Appetite challenge and sleep study in CP patients**

CP patients were admitted at 1600 hours and underwent insertion of an intravenous cannula in the antebrachial fossa. Blood was withdrawn for measurement of ghrelin, leptin and insulin. The patients were given 500 kcal as a mixed liquid meal (330ml Fortisip) and repeat sampling for ghrelin and insulin was performed at 15, 30, 60, 90, 120, 150 and 180 minutes post-calorie ingestion, in a modification of the protocol previously described by Goldstone et al (86). Following the appetite challenge, patients underwent full polysomnography to assess for sleep apnoea. The appetite challenge was repeated in the morning after polysomnography.

Polysomnography was performed using the Alice 4 (Respironics, France). Sleep stage was diagnosed using the criteria of Rechtschaffen and Kales (158). Apnoea and hypopnoea were defined as described in Chapter 2. Sleep stage and respiratory event diagnoses were performed by a sleep disorders physician who was blinded to the diagnosis of the subjects. The apnoea – hypopnoea index (AHI) was calculated by the number of apnoeic and hypopnoeic events per hour of sleep. An AHI of greater than 5 events per hour was diagnostic of sleep apnoea, as per the criteria of the American Sleep Disorders Association (137).



### **7.2.c Assays**

All samples were stored at -80° C. Laboratory estimation of leptin and ghrelin were performed using a commercial kit for radioimmunoassay (Mediagnost, Reutlingen, Germany) as described in Chapter 2. Insulin levels were estimated with a chemiluminescent immunoassay on the Unicel Dxl 800 Beckman Coulter Access Immunoassay System. Glucose was estimated using the hexokinase method, on the Beckman Coulter (formerly Olympus) AU 5400 chemistry analyser. 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 (208).

### **7.2.d Statistics**

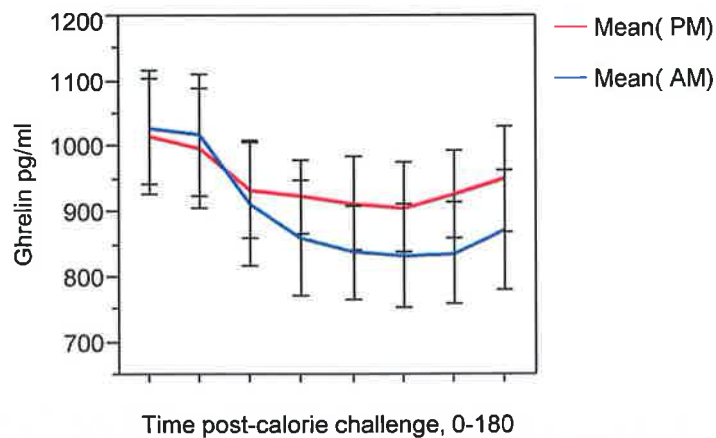
Normally-distributed data were described using the mean and standard deviation. Non-parametric data were described using the median and interquartile range, or total range where clinically appropriate.

Continuous variables were compared using Wilcoxon's test and were sub analysed using t test with Bonferroni correction where appropriate.

Correlation was assessed with Spearman's Rho and logistic regression was used for multivariate analysis. Statistical significance was accepted where the p value was less than 0.05. Statistical analysis was performed using JMP software (SAS Institute, California, USA).

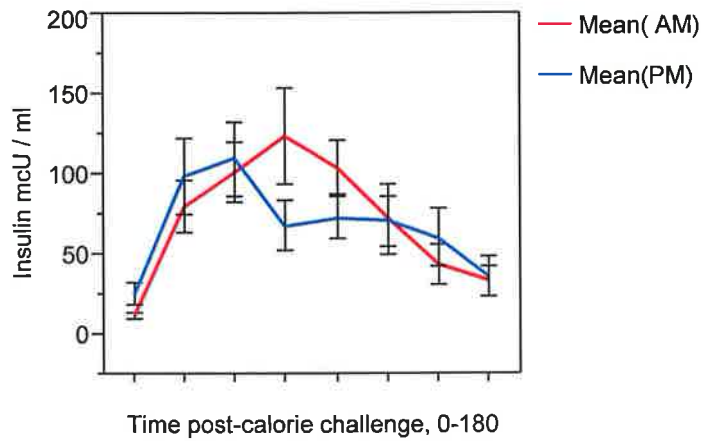
### 7.3 Results

Three patients had compromised appetite challenge studies; 2 were found eating food they had brought in to the hospital during the evening study and 1 had concealed food provided the night before and was eating immediately prior to commencement of the morning challenge. Analysis of the studies of the remaining 17 subjects revealed no difference between ghrelin and insulin levels in the evening studies and the morning studies (Figures 7.1 and 7.2); therefore the results for the evening study of the patient with a compromised morning study, were included with the morning studies for the purpose of analysis of effect of sleep apnoea.



**Figure 7.1.**

Comparison of CP subjects' ghrelin response to appetite challenge at night and in the morning,  $p = 0.2$  ( $n = 17$ )



**Figure 7.2.**

Comparison of CP subjects' insulin response to appetite challenge at night and in the morning,  $p = 0.5$  ( $n = 17$ )

The results for the comparison of fasting hormone profiles between CP subjects and control subjects are shown in Table 7.1. There was no difference between the groups in fasting ghrelin levels; fasting leptin was higher in CP patients than normal weight controls, but was comparable to the fasting leptin of obese controls (Table 7.1).

**Table 7.1.**

Comparison of craniopharyngioma and control groups in appetite study.

BMI = body mass index, M:F = ratio male:female.

$p < 0.001$  compared to normal controls \*

$p < 0.05$  compared to normal controls ^

Parameter	CP n = 20	Obese Controls n = 8	Normal weight Controls n = 14	p value
BMI kg/m <sup>2</sup>	35.6 ± 2.9*	34.1 ± 12*	20.7 ± 2.8	
Gender M:F	11:9	5:3	4:10	> 0.05
Ghrelin pg/ml	938 ± 277	951 ± 406	1111 ± 392	0.2
Leptin ng/ml	30 ± 23.7 <sup>^</sup>	28.6 ± 29	8.6 ± 8	

The results for the comparison of fasting hormone profiles between CP patients who had sleep apnoea and those who did not are shown in Table 7.2. There was a significant difference in both fasting ghrelin and fasting leptin levels between CP patients with apnoea than those without apnoea (Table 7.2). One outlier was excluded from analysis of leptin levels in the apnoea group, because the leptin measurement for this patient was 78 ng/ml, a result 10 standard deviations higher than the mean and 8 standard deviations higher than the next highest measurement. The difference in fasting insulin levels between the two groups did not achieve statistical significance,  $p = 0.07$  (Table 7.2).

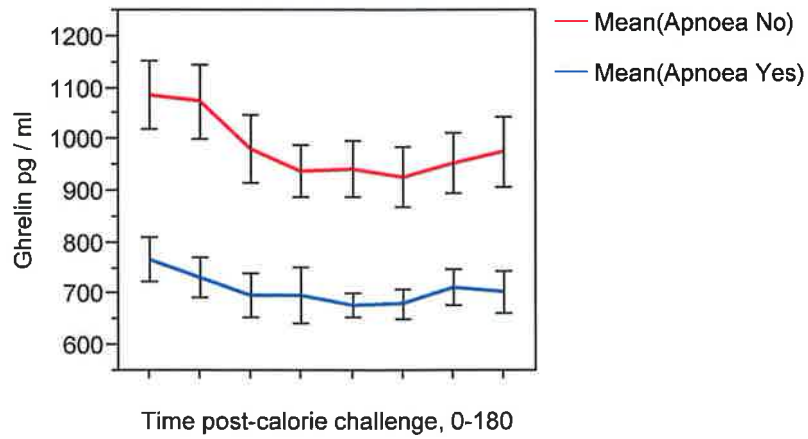
**Table 7.2.**

Comparison of appetite study in craniopharyngioma patients with and without sleep apnoea. M:F = male:female, AHI = apnoea hypopnoea index, BMI = body mass index, HC dose = hydrocortisone dose / equivalent dose of prednisolone, IGF-1 SDS = insulin-like growth hormone standard deviation score

Parameter	CP with apnoea n = 9	CP without apnoea n = 11	p value
Gender M:F	5:4	6:5	0.9
AHI Events / hour	17.5 ± 13	3.2 ± 1	<0.001
BMI kg/m <sup>2</sup>	36.1 ± 5.2	35.1 ± 9.3	0.6
Fasting Ghrelin pg/ml	794 ± 154	1056 ± 307	0.03
Fasting Insulin mcU/ml	19.0 ± 8.7	10.6 ± 6.0	0.07
Fasting Leptin ng/ml	14.7 ± 6.5	36.8 ± 23.9	0.03
T4 pmol/l	11.1 ± 4.2	10.4 ± 2.9	0.8
HC dose mg	22.1 ± 3.9	18.6 ± 5.6	0.2
IGF-1 SDS	0.8 ± 2.1	-0.2 ± 1.9	0.2

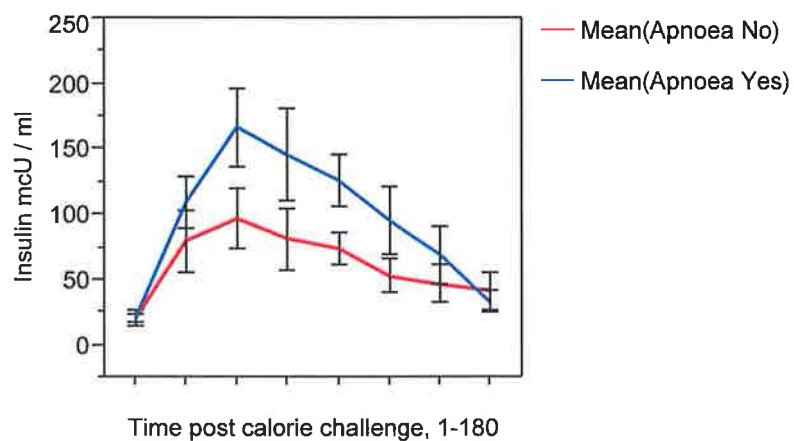
There was no difference in fasting ghrelin between CP patients with seizures ( $n = 5$ ,  $892 \text{ pg/ml} \pm 94$ ) and those who were seizure-free ( $954 \text{ pg/ml} \pm 319$ ,  $p = 0.5$ ). Fasting ghrelin levels did not correlate with AHI ( $r = -0.33$ ,  $p = 0.1$ ), nor with BMI ( $r = -0.09$ ,  $p = 0.7$ ) in CP patients.

Ghrelin was suppressed in all CP patients in response to the appetite challenge, with nadir at 120 minutes (mean  $983 \text{ pg/ml} \pm 277$  at 0 minutes,  $809 \text{ pg/ml} \pm 231$  at 120 minutes,  $p < 0.001$ , Figure 7.3). Insulin levels in CP patients rose during the appetite challenge, with a peak at 30 minutes (mean  $13.6 \text{ mcU/ml} \pm 8$  at 0 minutes, mean  $108.8 \text{ mcU/ml} \pm 78$  at 30 minutes,  $p < 0.001$ , Figure 7.4). There was a clear difference in ghrelin levels in response to appetite challenge between those CP patients who had sleep apnoea (ghrelin peak  $794 \pm 154 \text{ pg/ml}$ , nadir  $655 \pm 94 \text{ pg/ml}$ ) and those who did not (ghrelin peak  $1056 \pm 307 \text{ pg/ml}$ , nadir  $893 \pm 243 \text{ pg/ml}$ , ANOVA  $p = 0.02$ , Figure 7.3). There was also a difference in insulin levels (Figure 7.4) between those who had sleep apnoea (insulin nadir  $19.0 \pm 8.7 \text{ mcU/ml}$ , peak  $155.5 \pm 99.0 \text{ mcU/ml}$ ) and those who did not (insulin nadir  $10.6 \pm 6 \text{ mcU/ml}$ , peak  $83.3 \pm 53.8 \text{ mcU/ml}$ , ANOVA  $p < 0.05$ , Figure 7.4).



**Figure 7.3.**

Comparison of ghrelin response to appetite challenge in patients with and without sleep apnoea,  $p = 0.02$ . Error bars show standard error of the mean



**Figure 7.4.**

Comparison of insulin response to appetite challenge in patients with and without sleep apnoea,  $p < 0.05$ . Error bars show standard error of the mean.

#### **7.4 Discussion**

We demonstrated that there was a difference in ghrelin response to appetite challenge between patients with abnormal sleep due to sleep apnoea and those with normal sleep. Ghrelin suppressed appropriately following food intake in all CP patients; it was interesting to note that in CP patients without apnoea, ghrelin in the fed state was higher than ghrelin in the fasting state in patients with apnoea. There was no difference in the time at which ghrelin suppressed to nadir in patients with apnoea compared to those without apnoea; this was in contrast to the findings of other studies in which delayed ghrelin suppression, which implies prolonged appetite signalling and the potential for increased food intake at each meal, has been implicated in the development of obesity in CP patients (101, 223).

In the study published by Roth et al, obese CP patients did not suppress ghrelin after a similar 500 kcal challenge to that used in our study and it was postulated by Roth that the failure of ghrelin suppression in their CP cohort was due to insulin resistance at the level of the hypothalamus (101). Insulin levels during our dynamic test were significantly higher in CP patients with apnoea than those without apnoea and ghrelin levels were significantly lower in apnoea, which was an appropriate ghrelin response to hyperinsulinaemia and indicated that some sensitivity of the hypothalamus to insulin and ghrelin signalling appeared to be preserved in our patients. The difference in leptin levels between the group with apnoea and that without apnoea also suggests that the sensitivity of the



hypothalamus to leptin was preserved. HOMA levels in CP patients both with and without sleep apnoea were high, suggestive of insulin resistance, but HOMA is considered to be a measure of peripheral resistance and may not reflect hypothalamic sensitivity in these patients (209). There is no peripheral marker of which we are aware, that could be used to measure insulin resistance at the hypothalamus alone. The results from our study are indirectly suggestive that hypothalamic sensitivity to appetite signalling is preserved but are not definitive because they do not reflect receptor activity in response to these signals. Perhaps functional MRI studies could clarify what is happening in the hypothalamus of CP patients in response to food intake, by demonstrating that hypothalamic receptor activity remains intact in response to appetite signalling.

The finding of lower ghrelin levels in the patients with sleep apnoea suggested that the hormonal signal for appetite was markedly suppressed in the patients with sleep apnoea, which would be an appropriate physiological compensatory mechanism given the association of apnoea with obesity and the improvement in sleep apnoea reported in patients with weight loss. It is reasonable to speculate that in sleep apnoea, there would be physiological control mechanisms to reduce food intake such as suppression of the appetite stimulant ghrelin. Insulin, a satiety hormone, was detected in those with apnoea at levels that were not significantly higher in the fasting state but were higher during the appetite challenge than the levels found in patients without sleep apnoea,

which was further evidence that appetite and satiety signalling systems in CP patients with sleep apnoea were balanced in favour of signalling adequate energy reserve. Further studies of calorie intake differences and energy expenditure between these two CP groups would be required to demonstrate the clinical impact of these differences in appetite signalling.

It is clear from this study that in spite of appropriate appetite signalling in the CP population overall, there remain individual CP patients that suffer from excessive appetite. Three CP patients in our study (1 male) demonstrated abnormal behaviour related to food and eating, whereby 2 patients hid food and concealed food consumption from the investigators and a third patient hoarded food from the previous evening meal. All 3 were diagnosed with sleep apnoea during the course of the study and were obese (BMI 32.1, 32.8 and 43.3 kg/m<sup>2</sup>). All patients had been issued with a written description of the study protocol in the outpatient setting, more than 1 month in advance of admission and all had provided written consent, therefore there could be no possibility that 3 patients failed to understand the purpose or design of the study. These 3 patients represent the latest case series of CP patients with abnormal food-seeking behaviour suggestive of hyperphagia.

We did not find higher ghrelin levels in the CP patients with seizures. It has been suggested that the role of ghrelin in the pathogenesis of seizure disorders is mediated through its effects on the sleep cycle of patients

with seizures, and its effect on secretion of GH and prolactin in patients with epilepsy; ghrelin may not impact on seizures in the CP population because this is a hypopituitary cohort that cannot produce GH in response to ghrelin (222).

In summary, ghrelin levels in CP patients appear to be appropriate for the degree of obesity seen in these patients. Ghrelin is suppressed in CP patients with sleep apnoea, which may represent a physiological response to obesity and weight gain in this condition. Ghrelin suppression in sleep apnoea in patients with CP suggests that some hypothalamic sensitivity to peripheral signals is preserved in these patients. The findings of this study suggest that excess ghrelin and appetite signalling is not the major underlying pathology in hypothalamic obesity secondary to craniopharyngioma; some individuals with CP may suffer from excessive appetite but in the overall CP cohort other mechanisms for weight gain such as reduced energy expenditure secondary to somnolence, may be more significant. This study highlights again the importance of management of each CP patient on an individual basis, with careful attention to the possible underlying factors that contribute to weight gain.

## Chapter 8

### Summary and Discussion of Findings

In this study of hypothalamic disease in craniopharyngioma, I described the local cohort of CP patients and identified factors that could contribute to the increased morbidity and mortality reported in patients with this diagnosis worldwide.

The standardised mortality ratio in the local cohort was 8.75 (95% CI 5.4-13.3), which was comparable to the SMR reported in the West Midlands database (3). It was interesting that our SMR was the same as that in the West Midlands given that both SMRs were higher than the mortality reported by other groups, and given that our patients received growth hormone replacement and the English patients did not (2, 224). Our SMR data suggested that GH replacement did not reduce mortality in our cohort. Hydrocephalus was documented in over 40% of CP cases in our cohort and was associated with higher mortality; this was a novel association in the literature. Mortality in CP patients in our centre was secondary to cerebrovascular, cardiovascular and respiratory disease, which was in keeping with international reports (1, 2, 56).

The description of the four CP patients with adipsic diabetes insipidus highlighted the high rate of morbidity, such as obesity and sleep apnoea, and mortality associated with this small subgroup of CP patients. Adipsic diabetes insipidus appears to be a clinical marker of extensive

hypothalamic damage. The 4 cases in this study particularly highlighted the vulnerability of this group to hypernatraemia and to thromboembolic disease during episodes of hypernatraemia; this observation led to a new recommendation for the use of low molecular weight heparin prophylaxis in patients admitted with hypernatraemia.

I performed the largest sleep study in adult CP patients of which I am aware, and identified that sleep apnoea and somnolence are common in the CP population. Somnolence in CP patients was not always explained by sleep apnoea. Potential explanations for the somnolence observed in CP include secondary narcolepsy, melatonin deficiency, obesity itself, inadequate hormone replacement therapy (although this was not the case in our cohort), seizure disorders and anticonvulsant therapy, and disruption of normal circadian light cues secondary to visual loss (116, 117, 125, 140, 156). I documented successful treatment of sleep apnoea and somnolence with continuous positive airway pressure therapy and modafinil treatment in this cohort. The results from the sleep study suggested that somnolence and sleep apnoea were potential targets for treatment that could relieve some of the morbidity associated with a diagnosis of CP. Long term follow-up studies would be required to demonstrate whether treatment of sleep apnoea could reduce the respiratory and cardiovascular mortality reported in CP patients.

Dyslipidaemia and abnormal glucose tolerance were common in CP patients; however the rate of statin prescription was low in CP patients

and there was no recommendation in the literature for regular diabetes screening with oral glucose tolerance tests in the CP population. In the context of high mortality from cardio- and cerebrovascular disease in these patients, it would be appropriate to screen all CP patients for these cardiovascular risk factors. Again, only long term follow-up studies would prove definitively whether a policy of aggressive cardiovascular risk factor management would yield improvement in mortality rates in CP. Given the difficulty of collecting a large cohort of CP patients in order to conduct such a study, it seems reasonable to pursue an aggressive policy of cardiovascular risk factor reduction in these patients.

In the study of appetite signalling in CP, ghrelin levels were similar in CP to those of obese controls, which has also been reported by other investigator groups (86); this suggested that excess appetite signalling via high ghrelin levels was not the cause of hyperphagia and obesity in CP. CP patients had suppressed serum ghrelin levels in response to an appetite challenge, which demonstrated an appropriate reduction in appetite signalling after food intake; this was not found in a study in Germany where the failure of ghrelin suppression following food intake was attributed to hypothalamic resistance to peripheral appetite signals (101). The CP patients in my study appeared to have some preservation of hypothalamic sensitivity to ghrelin and insulin. Further evidence for preservation of hypothalamic sensitivity to peripheral signals was provided by the suppression of serum ghrelin levels throughout the appetite challenge in CP patients with sleep apnoea compared to those

without apnoea; this could be an adaptive mechanism by a functioning hypothalamus that could lead to weight loss in apnoea patients. It is not known why some areas of the hypothalamus are more vulnerable to damage from tumour or surgery than other areas; it is possible that some of the interaction between appetite signals occurs outside the hypothalamus.

The work performed for this thesis also confirmed some previous reports of morbidity in CP; more than 85% of our CP cohort were deficient in all anterior pituitary hormones and 81% were diagnosed with diabetes insipidus; these rates of hormone deficiency are also reported in other studies (1). The rate of recurrence of tumour in our cohort was 46%, which is in keeping with international reports and reflects the difficulty in achieving disease control in this condition (10); and the seizure rate was 49% which is higher than other reports (1). The high rates of hypopituitarism, seizure and hydrocephalus in our cohort reflect the damage caused by large tumours and the practice of gross total resection, often via craniotomy, pursued by our neurosurgical colleagues.

It is by no means clear how the high rates of morbidity and mortality identified in this study could be reduced in the future. The relative contribution of tumour bulk itself and damage from attempts at surgical resection, to hypothalamic sequelae in CP, remain poorly-elucidated. The answer to this question lies in evaluation of the patient by a multidisciplinary team before surgical resection is attempted. The

findings of this study suggest that a full evaluation of anterior and posterior pituitary dysfunction (including thirst dysfunction), ophthalmology review, oral glucose tolerance testing and sleep evaluation should be performed before and after surgery and radiotherapy, in order to identify the morbidity associated with a diagnosis of CP in a timely manner and in order to initiate treatment. Over time, such an approach would identify which tumour treatments were associated with increased morbidity and mortality, and would improve treatment planning for future generations of CP patients.

This study also illustrates the necessity for long term follow-up of CP patients with imaging for recurrence and assessment of any comorbidity. Serial measurements of BMI would help to identify the patients with excess weight who would benefit from more intensive intervention, including optimisation of cardiovascular risk factors and sleep assessment, although this should be offered to any somnolent patient regardless of BMI.

Craniopharyngioma is a rare tumour and it is likely that solutions to the problems of increased morbidity and mortality in this small patient group can only come from large, collaborative, international studies. In order to conduct large multicentre studies it is necessary to use standardised diagnostic tools and management plans. In this study I have identified some targets for treatment of morbidity in CP, such as dysnatraemia,



sleep apnoea and cardiovascular risk factors, which could be used in a multicentre study to improve outcomes for CP patients.

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