EATING BEHAVIOUR AND NEURAL REPRESENTATIONS OF HUNGER AND SATIETY IN PATIENTS WITH ACQUIRED STRUCTURAL HYPOTHALAMIC DAMAGE: A CLINICAL AND FUNCTIONAL NEUROIMAGING STUDY

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy by

Dr Caroline Ann Steele, MBChB, MRCPCH (UK)

December 2017

Institute of Ageing and Chronic Disease, Department of Obesity and Endocrinology, Clinical Sciences Centre, Aintree University Hospital, Liverpool, L9 7AL

CONTENTS

CONTENTS **PAGE**

Declaration

Candidate

Dr C A Steele

This thesis is the result of work performed whilst registered as a candidate for the degree of Doctor of Philosophy at the University of Liverpool. I declare that no portion of the work in this thesis has been submitted elsewhere for another degree or qualification in any other university or higher institute of learning.

This thesis is the result of my own work, with help and support in certain aspects as noted:

Dr Joanne Powell-Greig - Completion of final expert analysis of the fMRI data (including the initial statistical analysis undertaken using the fMRI data) and help with interpreting this

Professor Jason Halford and Dr Joanne Harrold - Expert guidance on the experimental design of the fMRI and eating behaviour studies

Dr Una Masic - Completion of final expert analysis of the UEM data and help with interpreting this

Miss Silvia Cicconi – Statistical advice including transformation of data where necessary and initial computation of some of the two-way ANOVAs undertaken and other statistical calculations

Research supervisors

Dr Christina Daousi Prof. J P H Wilding Prof. Andrej Stancak

List of abbreviations

3.0T	3.0 Tesla
α-MSH	α-melanocyte-stimulating hormone
ACTH	Adrenocorticotropic hormone
AgRP	Agouti-related peptide
al	Anterolateral
am	Anteromedial
ANOVA	One way analysis of variance
AO	Adult-onset
ACC	Anterior Cingulate Cortex
ARC	Arcuate nucleus
AUC	Area under the curve
BDNF	Brain-derived neurotrophic factor
BMD	Bone mineral density
BMI	Body mass index
BMR	Basal metabolic rate
BOLD	Blood-oxygen-level-dependent
CART	Cocaine- and amphetamine-related transcript
CI	Confidence interval
CRH	Corticotrophin-releasing hormone
CO	Childhood-onset
CP	Craniopharyngioma
CPAP	Continuous positive airways pressure
cpm	Counts per minute
DI	Diabetes insipidus
dl	Dorsolateral
dm	Dorsomedial
EE	Energy expenditure
ELISA	Enzyme-linked immunosorbent assay
EPI	Echo-planar imaging
FDR	False discovery rate
fMRI	Functional magnetic resonance imaging
fT4	Free thyroxine

FWHM	Full-width half maximum
GLP-1	Glucagon-Like Peptide-1
GH	Growth hormone
GHD	Growth hormone deficiency
Gy	Gray
HO	Hypothalamic obesity
HOMA-IR	Homeostasis model assessment of insulin resistance
HV	Height velocity
HVA	Homovanillic acid
IGF-1	Insulin-like growth factor 1
IGF-BP3	Insulin-like growth factor binding protein 3
IQR	Inter-quartile range
LAGB	Laparoscopic adjustable gastric banding
LH	Lateral hypothalamus
l-/m-	Lateral/medial
MC4R	Melanocortin-4 receptor
MCH	Melanin concentrating hormone
MDEFT	Modified Driven Equilibrium Fourier Transform
MNI	Montreal Neurological Institute
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NFPA	Non-functioning pituitary adenoma
NPY	Neuropeptide Y
NTRK2	Neurotrophic tyrosine kinase receptor type 2
OFC	Orbitofrontal cortex
OGTT	Oral glucose tolerance test
OR	Odds ratio
OSA	Obstructive sleep apnoea
PFC	Pre-frontal cortex
POMC	Pro-opiomelanocorticotropin
PVN	Paraventricular nuclei
PWS	Prader-Willi syndrome
PYY	Peptide-YY

QoL	Quality of Life
QoL-AGHDA	Quality of Life Acquired Growth Hormone Deficiency Assessment
QUICKI	Quantitative Insulin Sensitivity Check Index
rCBF	Regional cerebral blood flow
ROI	Region of interest
RR	Relative risk
RTx	Radiotherapy
SD	Standard deviation
SE	Standard error
SMR	Standardised mortality rate
SO	Simple obese/obesity
SPM8	Statistical parametric mapping
T2DM	Type 2 diabetes mellitus
TBI	Traumatic brain injury
TSH	Thyroid-stimulating hormone
UEM	Universal eating monitor
VAS	Visual analogue scale
VFD	Visual field defects/deficits
vl/vm	Ventrolateral/ventromedial
VMA	Vanillylmandelic acid
VMH	Ventromedial hypothalamus
VTA	Ventral tegmental area
WHR	Waist-to-hip ratio
yr	Year

Abstract

Eating behaviour and neural representations of hunger and satiety in patients with acquired structural hypothalamic damage: A clinical and functional neuroimaging study

Dr Caroline Anne Steele

Hypothalamic obesity (HO) is a relatively rare cause of obesity within the population as a whole, but studies of patients with hypothalamic damage show there is a significant prevalence of obesity within the patient group. Additionally, the obesity is difficult to prevent and manage and increases morbidity and mortality in an already at risk patient group.

The work of this thesis had two main objectives. Firstly, to examine the prevalence of obesity and associated morbidities in patients with acquired, structural hypothalamic damage with a descriptive cohort study (n=110). A separate descriptive study quantified obesity and metabolic risk factors in young patients with pituitary tumours, with or without hypothalamic damage (n=41), as they were also identified as a possible at risk group during clinical observations. The second objective was to investigate the underlying pathophysiology of HO using various complementary techniques to study patients with hypothalamic damage who remained weight-stable (HWS), patients with HO and age- and BMI-matched controls. These cross-sectional case-control studies used functional magnetic resonance imaging (fMRI; 9 HO, 7 HWS, 20 controls), the universal eating monitor (UEM; 6 HO, 6 HWS, 9 obese controls [OC], 10 non-obese controls [NOC]), Three-Factor Eating Questionnaire (TFEQ; 8 HO, 6 HWS, 9 OC, 11 NOC) and three-day food diaries (6 HO, 7 HWS, 8 OC, 11 NOC) to assess eating behaviour.

The first descriptive study included 110 adults with tumours causing hypothalamic damage attending a specialist neuroendocrine clinic. There was a significant prevalence of weight gain and obesity; 81.8% were overweight/heavier, 56.4% obese and 13.6% morbidly obese, despite proactive assessment and treatment during routine clinic visits. Hypertension (30.9%), dyslipidaemia (54.5%), type 2

diabetes mellitus (T2DM) (14.5%) and cardiovascular disease (9.1%) were also prevalent. In 41 patients with childhood/adolescent-onset pituitary adenomas there was also a relatively high prevalence of obesity (39.0%) and cardiovascular risk factors (2 receiving antihypertensive medications, 2 with T2DM and 4 with treated dyslipidaemia), despite the majority of tumours being microadenomas (i.e. too small to cause hypothalamic damage and indicating the need for long-term follow-up of these patients.

The first study into the pathophysiology of HO involved the use of functional MRI in 9 patients with HO, 7 HWS and 20 age- and BMI-matched controls. Participants underwent fMRI scans in a fasted state, as well as one hour and three hours following a fixed-load breakfast (25% of their calculated basal metabolic rate [BMR]). At each scan session participants viewed alternating blocks of photographs of high- or low-calorie food, with non-food photographs also viewed to use as a baseline comparison, to allow purely visual activation to be subtracted from any BOLD signal differences which occurred. Whole-brain statistical analysis revealed significantly lower BOLD signal in HWS participants compared to HO (and to controls) in the food motivation and reward-related brain regions of the posterior insula and lingual gyrus ($p=0.001$) when viewing high-calorie food photographs (compared to non-food photographs). These differences in reward-related brain regions may be implicated in the development of HO/the ability to remain weight-stable despite hypothalamic damage.

Eating behaviour studies were undertaken on a separate study day where participants were asked to eat an unlimited pasta meal until adequately full, while seated at the UEM. This allowed monitoring of total intake, eating duration, eating rate and intra-meal on-screen ratings of hunger, fullness and meal pleasantness using visual analogue scales. Additionally Three Factor Eating Questionnaires (TFEQs) and three-day MRC-Human Nutrition Research diaries were completed at home to assess more long-term real-world eating habits. None of the eating behaviour studies identified significant statistical differences between HO and HWS, but this may have been due to lack of statistical power. There was however an unusual pattern of eating rate in those with HO on visual ascription. This involved an initial tendency towards a higher eating rate, followed by a reduction in rate, with a further increase towards the end of the meal. Further investigation of this pattern with larger numbers of

participants would be important to determine whether it is a significant finding or merely an anomaly due to the small group size. Interestingly c*ontrols* ate significantly more at the UEM (even when intake was adjusted according to fat-free mass or as a proportion of the estimated BMR) and for significantly longer than participants with *hypothalamic damage*, who reported lower hunger at the start of the meal, but there was no difference between *obese* and *non-obese* participants. In keeping with previously published research disinhibition scores measured using the TFEQ were higher in participants with simple obesity [1], with no evidence of increased disinhibition in HO participants [2], although the small numbers studied should be noted.

Finally, during both of the study days blood sampling was undertaken to look for biochemical/hormonal variances between the groups. Fasting and area under the curve (AUC) active ghrelin concentrations were significantly higher in *controls* than in *patients,* in keeping with some (but not all) previous studies [2-4]. Consistent with previously published research leptin concentrations were significantly higher in *obese* compared to *non-obese* participants

The small size of this study was a significant limitation and limits the generalisability of the findings, particularly in the eating behaviour studies. This was due in part to the rarity of the condition - the occurrence of acquired, structural hypothalamic damage is relatively rare and the number of individuals with hypothalamic damage who remain weight stable is small, leading to particular difficulty in recruiting patients for the HWS group. Whilst there were some interesting findings further larger studies should enable greater clarification and would allow correlation between clinical and biochemical findings. Further studies in larger cohorts could explore the unusual eating pattern seen in those with HO when studied using the UEM and the apparent lack of disinhibition seen in this group.

Although this research provides some preliminary novel evidence to support differences in BOLD signal in reward-related regions of the brain as a possible driver of HO, the mechanisms through which these differences exert their effects to contribute to the development of HO require further elucidation and further study with larger numbers of patients is needed.

Acknowledgements

I would like to start by sincerely thanking my supervisors Dr Christina Daousi, Professor Andrej Stancak and Professor John Wilding for their continued help, support and guidance in undertaking this research and in preparing this thesis. I also thank my former supervisor Professor Ian MacFarlane (now retired) for his help and encouragement.

Several people have helped immensely with the work contained within this thesis. I thank Professor Graham Kemp, Miss Val Adams, Mr Bill Bimson and Dr Laura Parkes for their help with setting up and undertaking the fMRI study, Dr Joanne Powell-Greig for finishing the fMRI data analysis and to her and Professor Andrej Stancak for help with interpretation of this data (Chapter 5). I also thank Professor Jason Halford, Dr Joanne Harrold, Dr Emma Boyland and Mr Peter Taylor for their help in designing and setting up the research described in chapter 6. I thank Dr Una Masic for help her with completing the final stages of the UEM data analysis and both her and Professor Halford for help with interpreting this data. I am grateful to Dr Andy Cross for analysis of the blood samples taken on the fMRI and UEM study days. I would like to particularly thank Miss Shirley Cooper whose help and support during the patient visits was immeasurable. I would also like to thank Mr Neil Molyneux for his help in preparing the food for the food photographs used in the fMRI study and Mrs Helen Steele for entering anonymised data into a spreadsheet.

I would like to thank my consultant colleagues at Leeds Children's Hospital for their support and encouragement in finishing this thesis.

Finally, my greatest thanks must go to my daughter Bethany, husband Neil, parents Helen and Richard and sister Jenn who have shown both patience and understanding while I finished this thesis and for their constant love and incredible support.

Chapter 1

Introduction

Hypothalamic Obesity (HO): causes, definitions and brief historical perspective

Although "simple" nutritional obesity is increasingly prevalent in the population some other types, such as those secondary to underlying medical conditions, remain relatively rare; one of these is hypothalamic obesity (HO). HO describes obesity occurring secondary to hypothalamic damage. This damage can be acquired (due to structural lesions and/or their subsequent treatment, for example with surgery or radiotherapy), genetic (for example Prader-Willi syndrome [PWS] or congenital leptin deficiency), following intracranial infections, trauma, or vascular insult, occur as a side-effect of certain psychotropic medications, or may be idiopathic (Table 1.1) [5-8].

Definition of Hypothalamic Obesity (HO)

HO is defined as an acute increase in body weight following a clear hypothalamic insult [7], with weight gain faster than any expected age-related increase in body mass index (BMI) [9] and despite appropriate replacement of any hormone insufficiencies. In the case of adults with acquired, structural hypothalamic damage secondary to tumours in the hypothalamus or in the surrounding region (causing hypothalamic invasion/distortion) and/or their subsequent treatment, this is further refined as BMI $\geq$ 30 kg/m^2 which has increased by $\geq$2 kg/m^2 since tumour diagnosis, with weight gain faster than any expected age-related increase and despite adequate treatment of any associated pituitary hormone deficiencies [6]. The work in this thesis will concentrate mainly on acquired structural hypothalamic damage causing HO.

Historical perspective

HO was initially described in patients who had undergone surgical excision of tumours in the hypothalamic region, with subsequent significant polyphagia and weight gain. The first case reports date back to 1900 and 1901, when Babinski [7, 9, 10] and Frolich et al. [7, 9-11] respectively described obesity (and sexual immaturity) associated with hypothalamic tumours. Numerous case reports and case series followed [5, 9]. The naming of solid and cystic tumours of the incompletely

Table 1.1. Causes of hypothalamic obesity. Adapted from Hochberg and Hochberg, Expanding the definition of hypothalamic obesity, Tables 1-3 [7]

Anatomic/Acquired Structural Causes	
Tumours	Craniopharyngioma, epithelioma, angiosarcoma, cholesteatoma, pinealoma, germinoma, endothelioma, hamartoma, chordoma, colloid/epidermoid cysts, ganglioneuroma, ependymoma, glioma, pituitary macroadenoma/prolactinoma (with suprasellar extension), meningioma, teratoma, leukaemia, Langerhans cell histiocytosis, metastasis.
Inflammatory	Sarcoid, tuberculosis, arachnoiditis, histiocytosis X, encephalitis.
Head trauma	
Neurosurgery	
Cranial radiotherapy	
Cerebral aneurysm	
Genetic Causes	
Single gene mutations and genetic syndromes	Leptin, leptin receptor, CART, POMC, Prohormone convertase-1, MC4R, BDNF, TrkB, Single-minded 1, Rapid-onset obesity with hypoventilation, hypothalamic, autonomic dysregulation and neural tumour (ROHHAD-NET), Prader–Willi syndrome, Bardet–Biedl syndrome
Psychotropic drugs	
Antidepressants	Amitriptyline, doxepin, imipramine, clomipramine, maprotiline, nortriptyline, trimipramine, paroxetine, mirtazapine, desipramine, isocarboxazid
Mood stabilizers	Lithium, valproate, carbamazepine
Antipsychotic	Clozapine, olanzapine, zotepine, quetiapine, chlorpromazine, thioridazine, perphenazine, trifluoperazine, risperidone, clopenthioxol, sulpiride.
Idiopathic	

closed hypophyseal-pharyngeal ducts as craniopharyngiomas by Cushing in 1932 was an important event, as was the improvement in mortality associated with them in the 1950's [12]. Increased survival has meant that craniopharyngiomas account for a substantial proportion of HO secondary to tumours [10]. More recently, with the discovery of the impact of certain hormones, genes and medications on the function of the hypothalamus (Table 1.1) it has become clear that HO does not affect only those with acquired, anatomical damage and that the definition needed to be augmented to include these other causes [7, 11].

In the first half of the 20th century the hypothalamus was identified as the critical region in HO, rather than the pituitary as had previously been thought [13]. Between 1927-1940 animal models in dogs and rats delineated the role of the hypothalamus, particularly the ventromedial hypothalamus (VMH), in HO [7, 10, 14]. These models were later substantiated in humans [14]. In 1969 and 1970 rat studies demonstrated that although hyperphagia (defined as "the extreme unsatisfied drive to consume food" [15]) was a factor in developing HO, it was not a prerequisite [16].

The "neuroendocrine" era of appetite regulation started with the identification of leptin in 1994 [7, 10]. Further studies explored the interaction of multiple new (e.g. leptin) and previously identified (e.g. insulin) hormones on weight regulation and food intake. Their effects on the hypothalamus and vice-versa have helped further knowledge of how HO may develop, although the exact pathophysiology remains ambiguous.

Anatomy of the hypothalamus and surrounding region

The hypothalamus forms the ventral part of the diencephalon and lies inferior to the thalamus, superior to the pituitary stalk and pituitary gland, posterior to the optic chiasm and medial to the temporal lobes and optic tracts (Figure 1.1). It has three regions (anterior, tuberal and posterior), each with medial and lateral areas containing multiple nuclei (Figure 1.2) with varying functions.

Figure 1.1. Midsagittal view of the brain [17]

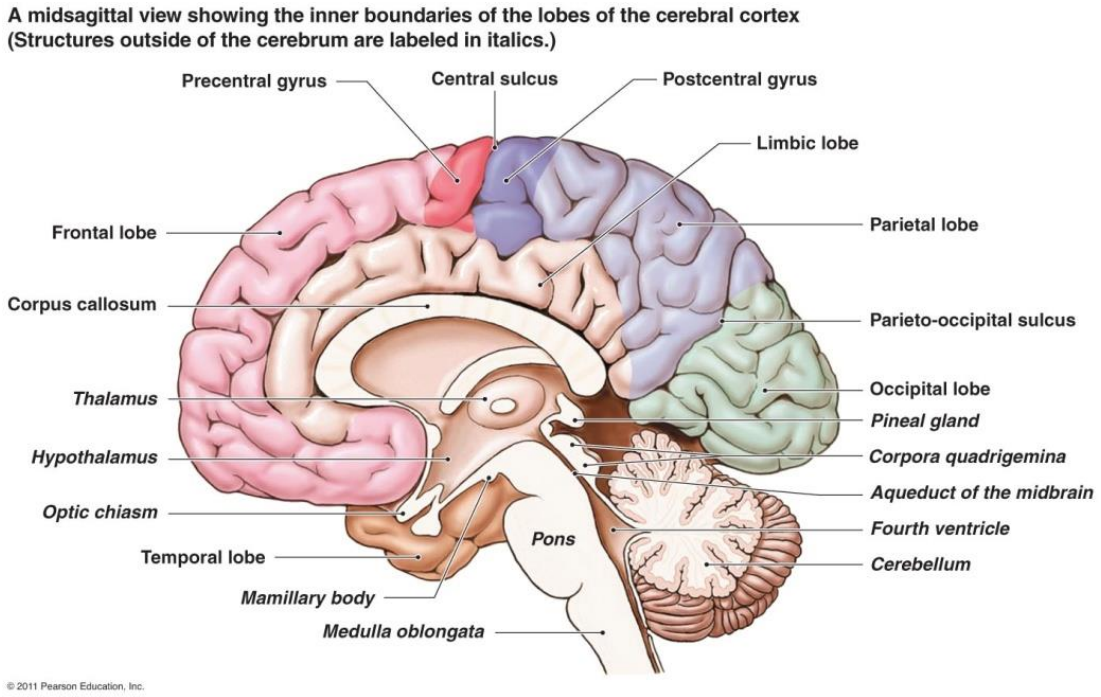
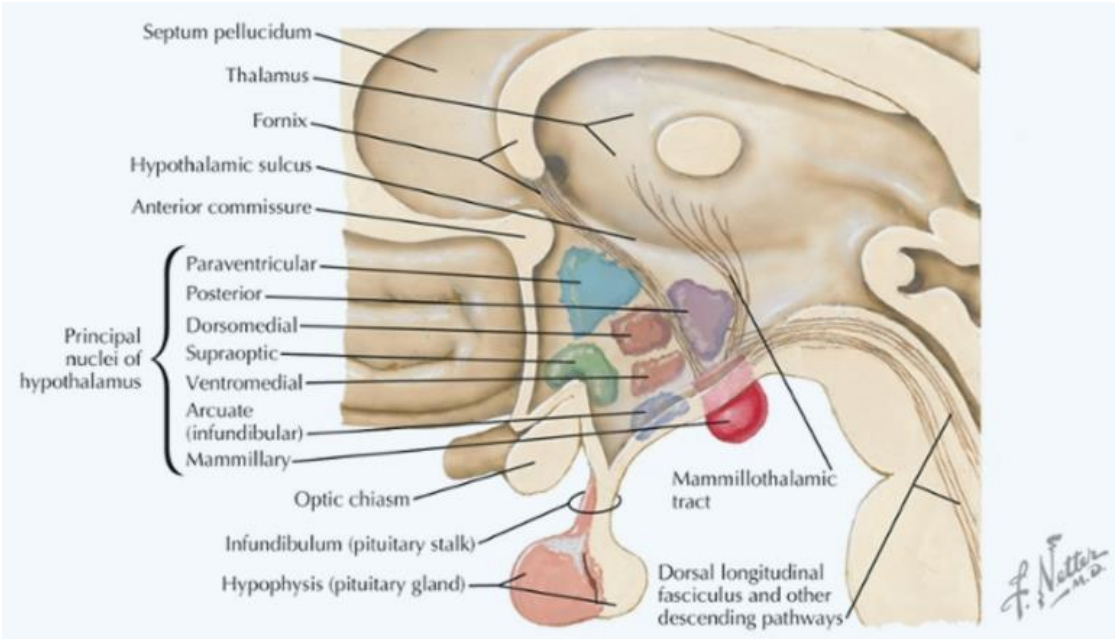


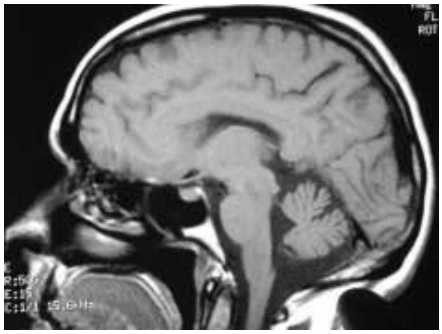
Figure 1.2. A midsagittal schematic illustrating the nuclei of the hypothalamus [18]



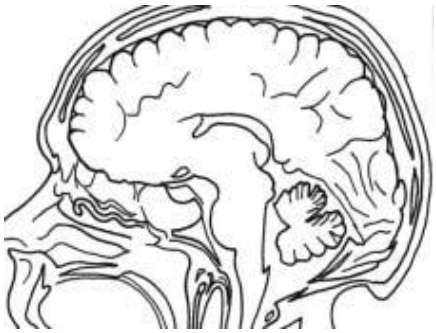
The primary function of the hypothalamus is to control the release of hormones from the pituitary gland, however it also facilitates homeostasis by controlling many autonomic functions, the sleep-wake cycle and energy regulation (balancing energy intake with energy expenditure and body fat stores). Although encroachment onto and damage of the hypothalamus by midline lesions (such as craniopharyngiomas) are easily seen on MRI scanning (Figure 1.3), the number and extent of hypothalamic nuclei affected cannot be precisely determined.

Figure 1.3. Coronal and sagittal MRI scans (left), with paired line drawings (right) showing: A and B - normal human hypothalamus at the level of the optic chiasm; C and D - normal pituitary gland and hypothalamus; E and F - a suprasellar glioma with invasion of the mediobasal hypothalamus and 3rd ventricular distortion; G and H - suprasellar extension into the medial hypothalamus [5].

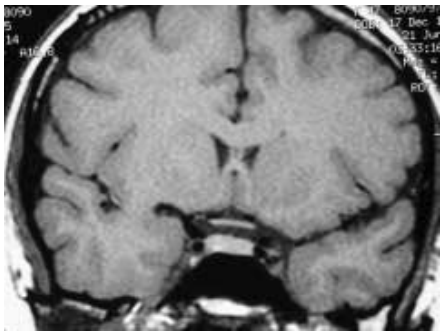
A



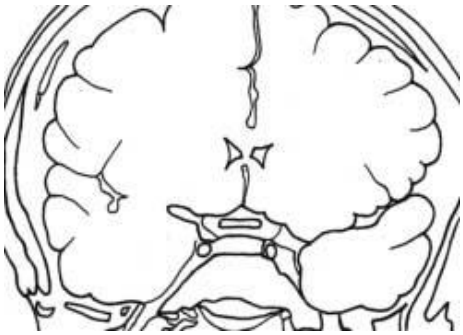
B



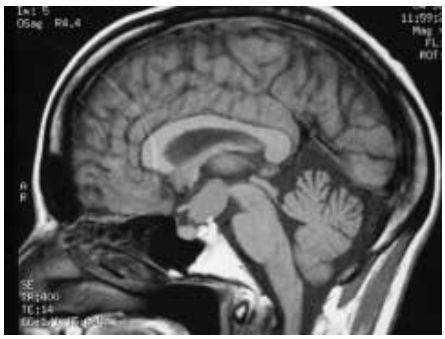
C



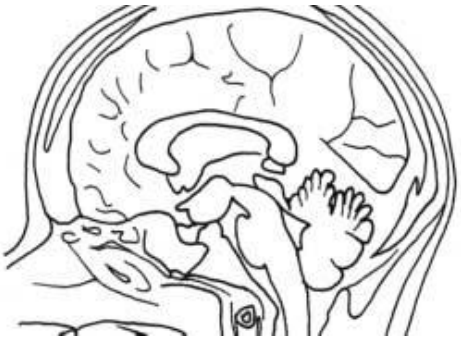
D



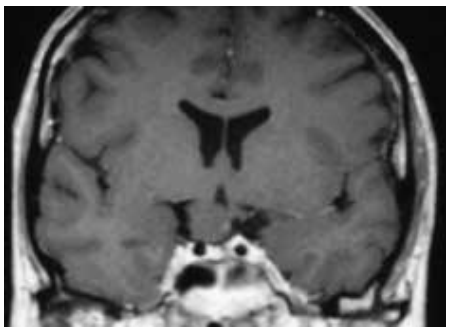
E



F



G



H

Prevalence of HO

The prevalence of weight gain and obesity varies in different series. Studies use a variety of methods to report weight (actual weight, body mass index [BMI], BMI standard deviation score [SDS], percentage overweight, weight gain) and apply varying definitions of obesity (Table 1.2, Appendix 1). Many studies exclusively describe patients with craniopharyngioma (CP), often with no clarification regarding hypothalamic damage, and information on weight-gain/obesity may only contribute a small part to the overall paper (Table 1.3, Appendix 1). The majority of literature relates to children with CP. A few papers describe adults with HO, usually including both patients with childhood-onset (CO) disease studied as adults, as well as those with adult-onset (AO) tumours.

Studies of children and adults with HO secondary to varying pathologies

A retrospective study from University Hospital Aintree described *adults* with acquired, structural hypothalamic damage [6]. Most were diagnosed with either CP (22/52) or pituitary macroadenoma with suprasellar extension (24/52). Forty-two patients had serial weight data; 52% had HO after a median follow-up of 5 years. This was more than double the rate of obesity at tumour diagnosis (24%). Median BMI had increased from 27.8 kg/m^2 at diagnosis to 30.4 kg/m^2 over the same time period ($p<0.0001$). Rates of overweight (BMI $\geq$ 25 kg/m^2) had increased from 67% to 88% and one patient was morbidly obese (BMI $\geq$ 40 kg/m^2). Another study compared 70 *adults* with CP (24 CO) to 89 with non-functioning pituitary adenoma (NFPA) requiring surgery and 29 with hypopituitarism after traumatic brain injury (TBI) (Table 1.2) [19]. After a median of 8 years follow-up 66% of patients with CP were obese, compared to 47% with NFPA and 31% post-TBI. CP patients had the highest median BMI, despite a significantly younger age at diagnosis than the NFPA group. The BMI in the post-TBI group was significantly lower than both the CP and NFPA groups. Unfortunately, it is not clear at what point after diagnosis BMI was recorded, or the length of follow-up in the NFPA or TBI groups. These factors could have influenced the findings. Additionally, the presence of hypothalamic damage was not specified, making the prevalence of obesity due to hypothalamic damage difficult to assess.

A Japanese study described 23 *children and adolescents* aged 2-22 years with suprasellar tumours, primarily CP (10 patients) or germinoma (7 patients), after 2-13 years of follow-up [20]. At latest follow-up 52% were obese, with no significant difference between tumour types. Another retrospective review studied 46 *children* aged ≤ 16 years at diagnosis of suprasellar tumour with radiological evidence of hypothalamic tumour extension [21]. The most common diagnosis was CP (Table 1.2). The prevalence of obesity increased from 6% at diagnosis to 43% after a median follow-up of 3.9 years.

Collectively, across both adult and childhood studies the prevalence of obesity at latest follow-up ranged from 43-75%.

Studies of children and adults with craniopharyngioma

Much of the literature describes weight gain and obesity in patients with CP. Some, but not all studies, specify the presence of hypothalamic damage. By including some patients without hypothalamic damage the prevalence of HO may be underestimated.

A study of 98 *adults and children* with surgically-resected CP between 1974-1991 reported that morbid obesity increased from 10.2% at diagnosis to 35.7% at latest follow-up [22]. As height and BMI are not included this study may underestimate obesity prevalence, particularly in those diagnosed during childhood where growth may have been affected. A retrospective review of UK *adults* (79 patients aged 16-83 years) and *children* (42 patients aged 2.5 to < 16 years) diagnosed with CP between 1964-2003 found obesity prevalence increased from 19.8% 5-years after diagnosis to 38.6% at 10-years and 66.3% after 20-years of follow-up in 101 patients with data available [23]. A similar study of 171 French patients diagnosed between 1972-2009 included 33 patients diagnosed with CP when under 10 years old, 32 patients diagnosed between 10-18 years and 106 with AO CP [24]. Suprasellar extension was present in 43.3%, 14.6% had a tumour ≥ 2 cm and 13% had polydipsia/polyuria/proven central diabetes insipidus (CDI), making hypothalamic damage more likely. In those diagnosed under 10 years old 67.9% were obese, 14.3% overweight and only 17.9% had a normal BMI. In the cohort 10-18 years at diagnosis 24% were obese and 30.3% overweight, compared to 42% and

36% respectively of those with AO. In keeping with other studies most weight gain occurred within the first 12 months of diagnosis. The largest study used data from the KIMS (Pfizer International Metabolic Database) growth hormone database of 393 patients with CP and GH deficiency (GHD, peak GH response to stimulation <3.0 micrograms/L) who were either GH naïve or untreated for at least six months prior [25]. Most had AO CP (> 18 years; 241 patients) compared to 152 with CO (< 18 years) (Table 1.3, Appendix 1). Given the potential detrimental effects on height of GHD in childhood, it was surprising that the prevalence of obesity was greater in AO patients (CO 39.1%, AO 51.8%, p = 0.02). It is unknown how many patients had hypothalamic involvement as there was no data regarding MRI scanning, although almost 60% had panhypopituitarism and over 60% had CDI - both risk factors for hypothalamic involvement. Additionally, length of follow-up is unknown and the possibility of shorter follow-up in the CO cohort may account for the greater prevalence of obesity in AO. Use of the KIMS database as a data source also excludes any patients not treated with GH, giving an incomplete picture. In adults in the UK, impaired quality of life (QoL) as well as low peak GH on stimulation testing is needed to qualify for GH replacement. The cohort studied may therefore be biased to include a greater proportion of obese and overweight adults, as these factors can lead to decreased QoL. Previous treatment of GHD during childhood for reduced height velocity and low peak GH may have influenced the rates of obesity in the CO cohort. These significant differences between the cohorts make direct comparison difficult.

Across adult and childhood studies of patients with craniopharyngioma the prevalence of obesity ranged from 20-68%.

Studies of children with craniopharyngioma

Studies exclusively including patients with CO CP also describe weight gain and obesity (Table 1.3) [26-34]. The largest series is a retrospective review of 185 children [27]. The authors found severe obesity (BMI > 3 SD) in 44% of patients, BMI between +2 and +3 SD in 13% and normal weight (BMI < 2 SD) in 43%. They also describe the early-onset weight-gain (within the first three years) noted by others. A further study identified risk factors and prognosis associated with severe obesity (BMI > 7 SD) [28]. Out of 183 patients in the multicentre HIT-ENDO

database 44.3% had a normal BMI (BMI SD < 2) and 55.7% were obese (BMI SD >2), including 30 (16.4%) with severe obesity (BMI ≥ 7 SD). The corresponding prevalence of hypothalamic involvement on intraoperative inspection and/or imaging illustrates the strong association between hypothalamic damage and obesity (Table 1. 4). For those with morbid obesity without hypothalamic involvement it is possible that damage may have occurred later (for example secondary to radiotherapy) or possibly may not have been recognised.

Table 1.4. Prevalence of obesity and hypothalamic involvement

	Number (%) patients	**% with hypothalamic involvement**
BMI SD < 2	81 (44.3)	33
BMI SD ≥ 2 <7	72 (39.3)	76
BMI SD ≥ 7	30 (16.4)	96

Smaller studies collectively show an increase in obesity from 15-30% pre-operatively to 53-77% after varying lengths of follow-up [26, 29, 33, 34], with a weight increase of over 10% in the first three post-operative months in 14/21 patients in one study (Table 1.3) [26]. Another study noted an increase in hyperphagia (a possible mechanism for HO) from 26% at diagnosis to 70% after a mean follow-up of 7 years and morbid obesity (not defined) in 18% of the 66 patients studied [32]. BMI was positively correlated with hypothalamic involvement but 32% had no hypothalamic involvement on post-operative MRI.

In studies of CO CP the prevalence of post-operative obesity ranged from 24-77%. Four studies noted the presence/absence of hypothalamic damage, however the others gave no indication [28, 32-34]. If patients with CP without hypothalamic damage were excluded the prevalence of HO may be even greater than described.

Mechanisms underlying the development of HO

The hypothalamus regulates weight by integrating afferent inputs such as gut-derived appetite hormones and initiating efferent stimulation to the periphery to control energy expenditure (EE) and physical exertion, thus acting as the homeostatic

control centre balancing energy intake (EI), EE and body fat. The proposed mechanisms underlying the development of HO include: i) decreased responsiveness to afferent signals leading to increased EI (for example impaired gut-brain satiety and hyperphagia), ii) increased efferent sympathetic activity leading to decreased EE (such as decreased resting metabolic rate and voluntary EE) and iii) increased vagal tone (leading to hyperinsulinism). These potential mechanisms will be described subsequently. Additionally, in simple obesity the limbic, paralimbic and higher cortical brain regions which process non-homeostatic environmental cues and the rewarding properties of food influence body weight. The interactions between the hypothalamic homeostatic centre and these other centres in humans remains poorly understood and little investigated in those with hypothalamic damage.

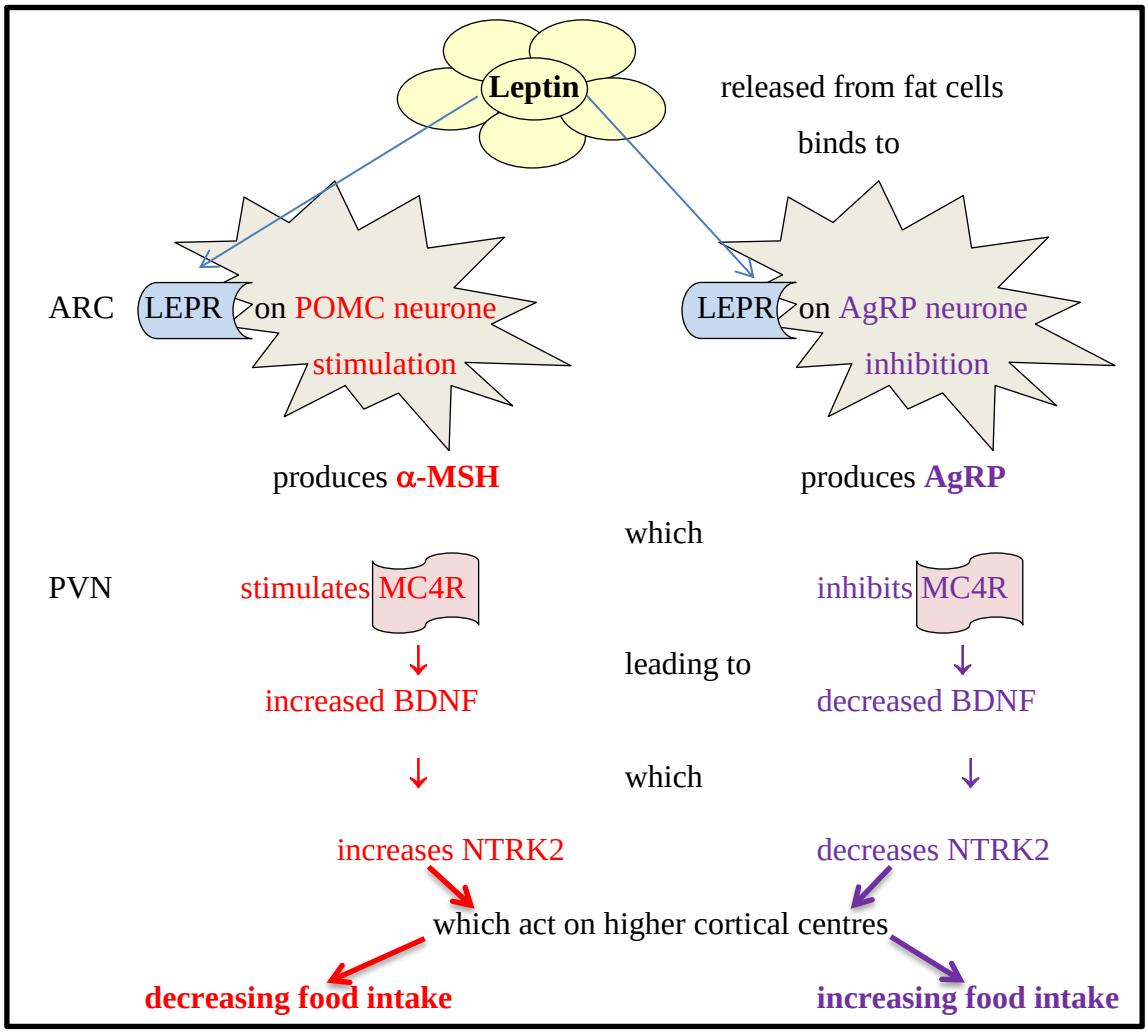
Key hypothalamic regions and their hormonal associations

The key hypothalamic regions associated with control of appetite are:

1. the **arcuate nucleus** (ARC) (Figure 1.4) which secretes agouti-related peptide (AgRP) and neuropeptide Y (NPY) with orexigenic effects and pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART) which are anorexigenic [7]. Both sets of neurones have receptors for the peripherally-secreted hormones leptin (Figure 1.4) and insulin, which stimulate POMC and suppress AgRP/NPY [11, 35]. Damage to these neurones may lead to disordered responses to leptin and insulin, causing appetite dysregulation and HO. Activation of the neurones' ghrelin receptors stimulates NPY and inhibits POMC, causing orexigenic effects. The ARC is connected directly to the paraventricular nucleus (PVN), lateral hypothalamus (LH) and other homeostatic brain regions [11].
2. the **paraventricular nuclei** (PVN) which express melanocortin-4 receptors (MC4R, Figure 1.4); these are stimulated by α-melanocyte-stimulating hormone (α-MSH) cleaved from POMC [11, 35] and inhibited by AgRP by competitive binding with α-MSH [35]. Acting through effector neurones which synapse in higher cortical brain centres, stimulation of MC4R has anorexigenic effects and inhibition has orexigenic effects [11, 35]. MC4R stimulation also results in increased EE [11]. Other neuropeptides including the anorexigenic corticotrophin-releasing hormone (CRH) and oxytocin are secreted from here [7].

3. the **ventro-medial hypothalamus** (VMH) has glucose- and insulin-sensing neurones and numerous other neuropeptide receptors (Figure 1.5) [11].
4. the **lateral hypothalamus** (LH) contains hypocretin and melanin concentrating hormone (MCH) neurones [11].

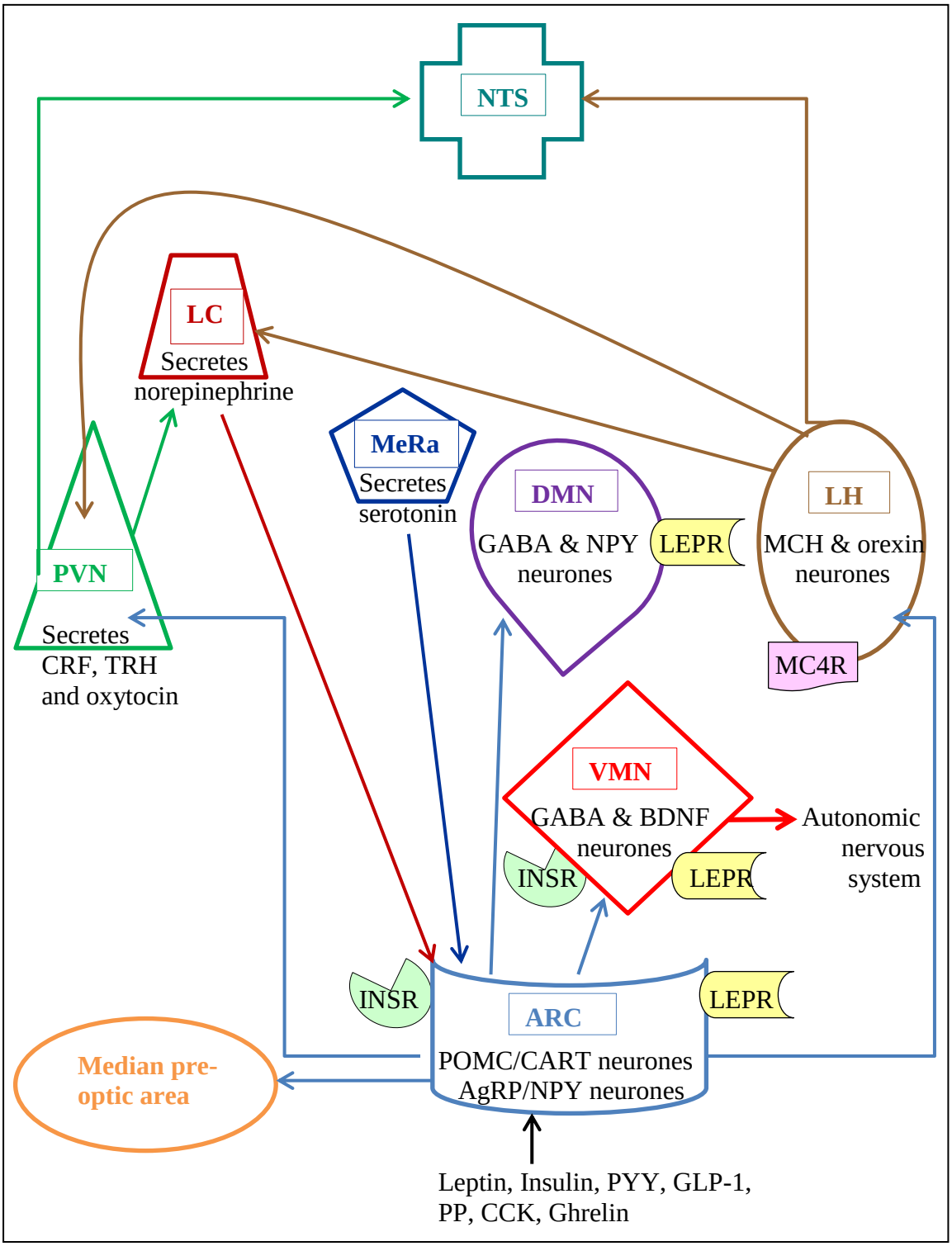
Figure 1.4. Effects of leptin (adapted from Walley et al [35]).



ARC: arcuate nucleus, LEPR: leptin receptor, POMC: pro-opiomelanocortin, AgRP: agouti-related peptide, α-MSH: α-melanocyte-stimulating hormone, MC4R: melanocortin-4 receptor, BDNF: brain-derived neurotrophic factor, NTRK2: Neurotrophic tyrosine kinase receptor type 2.

Other important hormones include cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1) (Figure 1.5). There are also mechanical influences on the hypothalamus, such as the stretch receptors of the stomach and visual and odour stimuli. In addition to the hypothalamus, the amygdala, limbic system, cerebral cortex and brainstem all play a role in appetitive neural circuitry [36].

Figure 1.5. Key neural and hormonal interactions regulating appetite and food intake. Adapted from text and figures by Roth [37] and Lustig [38].
NTS – Solitary tract nucleus, LC – Locus coerulus, MeRa – Median raphe, DMN – Dorsomedial nucleus, LH – Lateral hypothalamus, PVN – Paraventricular nuclei, VMN – Ventromedial nucleus, ARC – Arcuate nucleus, MC4R – Melanocortin-4 receptor, INSR – Insulin receptor, LEPR – POMC – Pro-opiomelanocorticotropin, CART – Cocaine- and amphetamine-related transcript, AgRP – Agouti-related peptide, NPY – Neuropeptide Y, GLP-1 - Glucagon-Like Peptide-1, PP – Pancreatic polypeptide, CCK - Cholecystokinin



While the hypothalamus is the critical homeostatic centre regulating appetite and eating, the limbic, paralimbic and higher-cortical brain regions process non-homeostatic environmental cues and the rewarding properties of food.

Hormones and hyperphagia

Hyperphagia has been reported in patients with both non-structural HO [39] and those with acquired, structural forms [11, 23, 40, 41]. A study by Daousi et al found that compared to 15 age-, BMI- and body fat-matched simple obese (SO) controls, 14 participants with HO reported significantly more hunger and desire to eat on visual analogue scale (VAS) ratings three hours after eating [2]. This suggested that post-prandially those with HO are full for a shorter period than those with SO. Interestingly though, their responses to the Three Factor Eating Questionnaire (TFEQ) indicated lower long-term hunger than controls. HO has also been reported in the absence of increased food consumption however, indicating this is not the only causative factor [42, 43]. Several different mechanisms have been postulated as the cause of hyperphagia (leptin insensitivity, hyperghrelinaemia, hyperinsulinaemia due to vagal disinhibition).

Impaired gut-brain signalling? The role of gut hormones

Ghrelin

Ghrelin is a gut hormone which consists of 28 amino-acids and is cleaved from preproghrelin. It exists in different forms within the human body – active (acyl-ghrelin) and inactive (desacyl-ghrelin). Post-translational acylation is necessary for ghrelin to cross the blood-brain barrier [44] and enable appetitive effects. Assays can measure either active acyl-ghrelin or total (acyl- and des-acyl) ghrelin [45]. Ghrelin increases when fasted, leading to hunger and meal initiation, and falls post-prandially due to inhibition by insulin and PYY which increase once fed [39]. Fasting ghrelin is lower in the obese population and increases following weight reduction [36], however is increased in patients with PWS compared to normal-weight controls [39, 46]. In patients with HO the presence of hyperghrelinaemia has been investigated [2-4, 39, 47].

In 16 children with HO secondary to craniopharyngioma or other structural lesions fasting total ghrelin concentrations were not different to SO controls (Table

1.5, Appendix 1) [47]. Both groups had significantly lower total ghrelin than normal weight controls. The same study showed no significant difference in fasting insulin, HOMA-IR or leptin between HO and SO but fasting glucose was lower in HO. A study of adult patients measured both fasting and post-prandial total ghrelin concentrations in patients with HO secondary to craniopharyngioma (BMI > 28 kg/m^2 and confirmed hypothalamic damage), non-obese patients with craniopharyngioma (CrNW), PWS, SO and non-obese controls (NOC) [39]. The authors found that after adjustment for age and gender, fasting ghrelin was lower in nine patients with HO than in 15 NOC ($p<0.05$) and 26 PWS patients ($p<0.001$, Table 1.5) [39]. Ghrelin concentrations were similarly reduced in 16 SO controls. Fasting plasma ghrelin was negatively correlated with fasting insulin and percentage body fat in HO and CrNW but not in PWS. In a separate fasting and fed arm, following a 522-kcal breakfast trough ghrelin concentration was significantly lower in HO than NOC with a significantly delayed trough. Postprandial ghrelin AUC was lower in HO than NOC but with a similar mean post-prandial fall in both groups. Hyperghrelinaemia was evident in PWS patients but not HO patients whose ghrelin concentrations were comparable to SO.

Two studies found *lower* ghrelin concentrations in HO compared to SO. Fasting total ghrelin was significantly lower in 14 patients with HO compared to 15 healthy age- and BMI-matched SO controls ($p = 0.03$, Table 1.5) despite other markers such as percentage body fat, fat-free mass and WHR being comparable between the groups [2]. In response to a test-meal of 600 kcal both groups demonstrated a similar decrease in plasma total ghrelin concentrations, but with a significantly lower AUC in HO. Fasting leptin and HOMA-IR were similar in both groups. Forty-two adults with childhood onset craniopharyngioma (CP) also had significantly lower fasting total plasma ghrelin than 42 age- and gender-matched controls (Table 1.5) [3]. Those patients with third ventricular extension (TVE), an indication of hypothalamic damage, had significantly lower fasting plasma ghrelin than those without ($p = 0.008$). As the median BMI was significantly higher in patients than controls and in those patients with TVE than those without, these differences would be expected.

Finally, lower fasting total ghrelin was demonstrated in 15 obese children with craniopharyngioma (CrO) and 15 SO age-, gender- and BMI-matched controls than in 12 CrNW and 12 normal weight (NW) controls [4]. However, whereas

ghrelin supressed significantly in SO and CrNW and NW there was a non-statistically significant decrease in the one-hour post-prandial ghrelin in CrO. Their baseline and post-prandial ghrelin concentrations were lower than SO. Only 87% of CrO and 58% of CrNW had tumours extending into the hypothalamus. Those with hypothalamic involvement had a higher BMI SDS and a smaller percentage decrease in post-meal ghrelin than those without ($p<0.05$).

None of the studies demonstrated a role for hyperghrelinaemia in the pathophysiology of HO and in fact three [2-4] found lower ghrelin concentrations than in healthy age- and BMI-matched controls.

Peptide YY (PYY)

Peptide YY (PYY) is another gut hormone and consists of 36 amino-acids. Its release is stimulated by the presence of nutrients in the gut lumen and regulated by other peptides such as GLP-1. It also exists in two different forms – PYY(1-36) and PYY(3-36). PYY(1-36) shows high affinity to all known Y-receptor subtypes through which it acts, however PYY(3-36), formed from cleavage of PYY(1-36) by dipeptidyl peptidase IV, shows high affinity only to the Y2 receptors [48]. Both forms cross the blood-brain barrier but have differing effects – whilst PYY(1-36) stimulates appetite and therefore promotes weight gain, PYY(3-36) is associated with meal termination via its actions on the arcuate nucleus to inhibit NPY release [49].

PYY(3-36) is reduced in those with SO compared to non-obese [48]. In a study of 14 patients with HO, fasting total PYY concentrations were similar to 15 healthy age- and BMI-matched SO controls ($p = 0.06$, Table 1.5) [2]. After a 600-kcal meal those with HO lacked the sustained rise in PYY normally seen following food ingestion, although they displayed a similar post-prandial AUC to SO, who also did not display a typical post-prandial PYY rise. The increase occurred only 180 minutes after food ingestion, whereas this would be expected to occur from about 15 minutes. Another study found similar concentrations of fasting PYY(3-36) in nine patients with HO, six CrNW, 16 SO and 15 NOC (Table 1.5) [39]. There was no correlation between percentage body fat and fasting PYY(3-36) in HO or CrNW. After a 522-kcal breakfast, post-prandial peak PYY(3-36) and AUC PYY(3-36) concentrations were similar in six HO, nine SO, eight NOC and ten patients with

PWS. In contrast, Roth et al's study of 12 CrNW, 15 CrO, 12 NW and 15 SO-controls found that total PYY concentrations increased post-prandially in all groups (figures not given), however craniopharyngioma patients (normal weight and obese) had a lower percentage increase than BMI-matched controls [4]. BMI SDS correlated negatively with postprandial percentage change in PYY but there was no relationship with tumour size and no difference in either fasting or postprandial PYY concentrations between those with hypothalamic involvement and those without.

There is no conclusive evidence to suggest PYY variations as a pathophysiological mechanism for weight gain in those with hypothalamic damage.

Other hormones/enzymes influencing appetite

Leptin

Leptin is produced by fat cells and is anorexigenic, primarily via the hypothalamus [50]. Leptin increases with rising adiposity, however this only appears to have a limited effect on food intake and leptin resistance is thought to be an evolutionary mechanism to prevent starvation [35].

In a study comparing patients with craniopharyngioma (obese and normal weight) and BMI-matched controls, leptin was positively correlated with BMI SDS, fasting insulin and HOMA-IR and negatively correlated with insulin sensitivity in all participants [4]. Holmer et al found higher leptin in 42 adults with CO CP than 42 controls not matched for BMI ($p<0.001$) with this difference persisting when corrected for fat mass ($p<0.001$) suggesting possible leptin insensitivity in CP [3]. When CP patients with TVE, a marker of hypothalamic involvement, were compared to those without TVE there was no significant difference in leptin concentration (Table 1.6, Appendix 1) or leptin corrected for fat mass, despite a significantly greater BMI in those with TVE ($p=0.001$). However, in a comparison of 14 children with CP (11 with suprasellar extension, 3 with intrasellar tumours) and 53 controls, after adjustment for BMI those with suprasellar tumours had higher leptin concentrations than controls, with similar concentrations in controls and those with intrasellar tumours [40]. The authors theorised that leptin insensitivity in patients with suprasellar craniopharyngioma led to increased NPY and decreased CRH

(which increase appetite), however assessments of hyperphagia and food consumption were not undertaken. Higher leptin in patients with hypothalamic involvement was supported in a second study of 23 obese children with hypothalamic-pituitary tumours (HPO), 16 non-obese children with hypothalamic-pituitary tumours (HPNO) and 22 SO controls [51]. The HPO group had higher serum leptin:BMI ratio and free leptin index than SO and HPNO (Table 1.6). In the 17/23 (73.9%) with HPO and tumour involving the thalamus/hypothalamus and the 4/16 (25%) with HPNO and thalamic/hypothalamic tumour serum leptin was higher compared to those without thalamic/hypothalamic involvement.

One study compared fasting leptin in 28 patients with HO to 18 patients with congenital hypopituitarism (CH) and 23 SO-controls [52]. Age and gender were similar in all three groups but SO were significantly taller and heavier than HO and CH, with a non-significant difference in BMI SDS. Leptin concentrations were highest in HO and this difference persisted after adjustment for fat-mass, gender, age and pubertal status (Table 1.7, Appendix 1). Even after adjustment for fat-mass leptin was 22% higher in HO than in CH and 50% greater than in SO. Other biochemical measurements found that fasting glucose was highest in SO, however no participants had diabetes mellitus or impaired fasting glycaemia and fasting insulin and insulin resistance (HOMA-IR) were not significantly different between groups. Adiponectin and resistin were highest in HO - the cause of which and their contribution to HO remains unclear and findings in simple obesity are inconsistent.

Another study compared serum leptin concentrations and leptin binding in 37 children with hypothalamic-pituitary axis (HPA) tumours and 85 healthy controls [53]. Participants were divided into BMI < 2 SDS (17 patients, 57 controls) and BMI ≥ 2 SDS (17 patients, 28 controls). Within each cohort there was no difference in gender, pubertal status or median age, however there was significantly greater BMI SDS in HPA than in controls in both groups. Both serum leptin and leptin adjusted for BMI were higher in HPA than controls, however this only reached significance in those with BMI SDS < 2. Serum leptin binding activity and BMI-adjusted concentrations were lower in HPA than controls so there was no evidence that leptin resistance was secondary to increased leptin binding in HO.

Only one study found no difference in fasting leptin concentrations between patients with HO and controls. In 15 children with CP and hypothalamic damage

with BMI > 95th percentile for age and gender and 15 age-, gender-, puberty- and BMI-matched SO controls leptin concentrations were not different [54].

With the exception of the last study [54], hyperleptinaemia has been consistently demonstrated in HO, however it remains unclear whether this is a pathophysiological mechanism causing HO, possibly secondary to leptin resistance caused by abnormal signalling; leptin insensitivity at the hypothalamus itself [52]; or a consequence of HO [29].

Insulin

The most researched mechanism for HO is hyperinsulinaemia. It has been proposed that hypothalamic damage leads to vagal nerve disinhibition. Increased vagal stimulation of the pancreas then results in excess insulin secretion leading to accumulation of fat and therefore weight gain and obesity [3, 55].

Studies linking hyperinsulinism and HO

Bray et al described increased insulin secretion early in the course of HO and found impaired glucose tolerance on OGTT in 4/8 [9]. More recently, in 17 children with panhypopituitarism following craniopharyngioma resection (not fully pituitary hormone replaced), insulin concentrations had already increased significantly one month post-operatively ($p<0.05$) [29]. Leptin had significantly increased 3-6 months post-operatively ($p<0.01$), as had BMI SDS ($p<0.01$). The authors concluded that as insulin increased first, it was the driver for weight gain and that hyperleptinaemia was a consequence of the increased body mass. Pre-operative fasting insulin concentration was positively correlated with pre-operative BMI, leptin and growth factors, and with post-operative weight gain ($p<0.01$) but not with BMI SDS at 12 months. Significantly one patient gained weight despite normal insulin concentrations. There are several weaknesses in this study. Firstly, although post-operative BMI SDS was analysed pre-operatively only BMI is noted. Secondly, hypothalamic involvement is not clear. Most importantly, the effects of untreated GHD and sex-steroid deficiency in some patients may have significantly influenced findings.

In a case-control study of 15 children with HO following surgically-resected craniopharyngioma (CP) and 15 SO age-, gender- and BMI-matched controls, their insulin dynamics and the prevalence of metabolic syndrome differed significantly [54]. Fasting insulin concentrations were not different between the groups nor was calculated whole body insulin sensitivity on OGTT. However OGTT did produce significantly greater first and second phase insulin secretion and AUC insulin and insulin sensitivity on frequently sampled intravenous glucose tolerance test *was* significantly lower in CP participants (Table 1.8, Appendix 1). The effects of OGTT on gut hormones such as GLP-1 may account for the differences found between the different methods. While none of the SO had impaired glucose tolerance on OGTT, 40% of CP did. The incidence of metabolic syndrome was also significantly higher in CP (73% vs. 20% in SO, $p = 0.03$). The main weakness of this study is that (as with many of the other studies) it was unable to distinguish cause and effect. As the mean time since CP diagnosis was 4.9 years, hyperinsulinaemia may have been a consequence of HO rather than causal. Notably, controls had a tendency towards being more advanced in puberty - a time of increased insulin resistance – therefore a more marked difference may actually have existed.

A study of 42 adults with childhood-onset CP (25 with possible hypothalamic damage, 17 without) and 42 age- and gender-matched controls found higher fasting serum insulin in patients than controls (Table 1.9, Appendix 1), with BMI, percentage fat mass and percentage fat-free mass all significantly higher in CP [3]. When the patients with and without possible hypothalamic damage were compared, those with hypothalamic involvement had significantly greater BMI, fat mass, fat-free mass and fasting insulin concentration.

Another case-control study found significantly higher fasting and post-prandial insulin, and HOMA-IR in 15 obese children with craniopharyngioma (CrO) compared to CrNW and age-, gender- and BMI-matched controls [4]. Quantitative insulin sensitivity check index (QUICKI) was significantly lower in CrO and BMI SDS was positively correlated with fasting and post-prandial insulin and negatively correlated with QUICKI. Hypothalamic extension was present in 93% of CrO and 57% of CrNW. Fasting insulin and HOMA-IR were non-significantly higher in those with hypothalamic involvement than those without however this sub-group analysis was relatively small (19 and 6 patients respectively) and not separated into obese or

normal weight. It is therefore difficult to conclude whether changes in insulin dynamics are a cause or a consequence of HO.

In a study by Goldstone et al, mean fasting and postprandial AUC insulin were higher in those with HO than in SO controls and those with PWS (Table 1.8) [39]. Both fasting HOMA-IR and post-prandial HOMA-IR were significantly higher in HO.

In two studies investigating the effects of the somatostatin analogue Octreotide on weight gain in HO [55, 56] Lustig et al found no increase in fasting insulin concentration before or following six months treatment with Octreotide (Table 1.10, Appendix 1) [56]. However 5/8 patients had impaired glucose tolerance on OGTT, with a delayed and higher mean peak insulin compared to non-obese controls. The second study of 20 patients also found an increased insulin response to OGTT at the start of the study [55].

Studies finding no association between insulin concentration and HO

In contrast, other studies have found no evidence for hyperinsulinaemia as the cause of HO. Shaikh et al found no significant difference in the prevalence of fasting hyperinsulinaemia, insulin resistance or median fasting insulin concentrations in 28 children with HO, 18 with congenital hypopituitarism and 23 with SO with similar BMI SDS [52]. All groups had elevated fasting insulin concentrations. There was a positive correlation between insulin concentration and fat mass ($p<0.01$). As only fasting insulin was measured, insulin response to OGTT may have yielded a difference [54].

In nine patients with CP compared to age-, gender and BMI-matched controls, similar fasting glucose and insulin concentrations, insulin sensitivity (Table 1.8) and rates of fasting hyperinsulinaemia were found [57]. Of note, not all the patients were obese and the study used an intravenous glucose tolerance test (IVGTT), which is less physiological than an OGTT. Another study compared 23 obese children with hypothalamic-pituitary tumours (HPO), 16 non-obese children with hypothalamic-pituitary tumours (HPNO) and 22 simple obese controls (SO) [51]. Fasting glucose, fasting- and 2-hour insulin following OGTT, and HOMA-IR were lower in HPO and HPNO than SO controls, however there was a significantly greater increase in insulin following glycaemic load in HO (3.5-fold increase) than

HPNO (3-fold) and SO (2.5-fold increase). The authors concluded that insulin dysregulation, rather than insulin resistance was a feature of HO. Similar findings have also been reported in another study [58].

Pinto et al [29] describe weight gain in one patient with acquired hypothalamic damage despite a normal fasting insulin concentration and in a study of "growth without growth hormone" 2/9 patients had weight gain despite a lower peak insulin response to OGTT than found in normal controls [59]. This may imply that other mechanisms, either alone or in combination with hyperinsulinism are responsible for weight gain in HO. In most of the obese patients studied however, hyperinsulinism was found [29, 59] and other studies of "growth without GH" in children with craniopharyngioma [60] have found a correlation between hyperinsulinism and good height velocity in patients with GHD [60-62].

There are no studies using clamp techniques to investigate insulin resistance in patients with HO and many of the studies described do not assess fat- or muscle-mass. As BMI is a relatively crude marker, ideally a measure of body composition, such as bio-electrical impedance or MRI assessment of fat/muscle proportion and fat distribution would be undertaken.

An alternative hypothesis to hyperinsulinism as a mechanism for HO is hyperinsulinaemia secondary to obesity. In conclusion, the role of hyperinsulinaemia – whether it is a causative factor or consequence of HO - remains unclear.

11β-hydroxysteroid dehydrogenase (HSD) 1 activity

The enzyme 11β-hydroxysteroid dehydrogenase (HSD) 1 converts inactive cortisone to active cortisol. One study investigated whether increased enzyme activity in patients requiring replacement glucocorticoids for cortisol deficiency secondary to pituitary or hypothalamic damage might result in abnormal glucocorticoid metabolism and obesity [42]. This was based on observations that the pattern of obesity in HO was similar to that in Cushing syndrome and that weight gain did not always seem proportional to reported food intake. The authors compared ten patients with HO and ACTH-deficiency needing glucocorticoid replacement with six requiring glucocorticoid replacement for other reasons. The BMI SDS of HO

patients was wider (-0.02 to +8.36) than controls (-1 to +1) and both groups contained participants with below average BMI SDS. Activity of 11β-HSD was not measured directly, but estimated using the ratios of various urine steroids and steroid metabolites. The authors found increased ratios of active cortisol/inactive cortisone (and their respective metabolites) in HO than controls and a significant correlation between the conjugated cortisol/cortisone ratio and the visceral fat/total adipose tissue ratio on a single slice CT at the umbilicus, but no correlation with BMI or BMI SDS. They conclude that these differences identified in patients with HO compared to controls indicate altered conversion of pharmacological glucocorticoid replacement in HO, potentially promoting obesity. The same ratios measured in simple obesity have been inconsistent [42] and further studies in the HO population are needed to establish whether this finding is reproducible and consistent.

All Hormones

In summary, there is no evidence to support either hyperghrelinaemia or abnormalities in PYY concentration as the underlying mechanism leading to HO. Hyperleptinaemia and hyperinsulinism have both been demonstrated in studies (although not consistently in all studies) and whether their presence are the cause or effect of HO remains unclear.

Reduced efferent sympathetic activity

Reduced sympathetic activity is another mechanism postulated to cause HO, possibly through decreased lipolysis resulting in lower resting metabolic rate and voluntary energy expenditure (EE) as well as symptoms such as lethargy [38].

Sympathoadrenal activity was assessed in eight patients with CP and hypothalamic damage by measuring plasma epinephrine and norepinephrine and assessing neurogenic and neuroglycopaenic symptoms and heart rate during induced hypoglycaemia [63]. Responses were compared to four patients with hypopituitarism due to other causes (HP) and six healthy controls. Baseline epinephrine did not differ between groups but some CP patients had a poor epinephrine response to hypoglycaemia, therefore CP patients were subdivided into two groups: poor responders (CPPoor) and good responders (CPGood, similar to that of controls). Plasma norepinephrine was similar in all groups at baseline and in

response to hypoglycaemia. Heart rate response to hypoglycaemia was lower in CPPoor with fewer adrenergic symptoms (tremor, anxiety, heart pounding), reflecting lower epinephrine response. There was no correlation between either biochemical markers or symptoms of hypoglycaemia and BMI. An orthostatic test of catecholamine and heart rate demonstrated a comparable increase in all groups. The authors concluded that the brainstem mediated sympathetic response was intact and that the reduction in heart rate response, adrenergic symptoms and epinephrine rise in CPPoor indicated damage to hypothalamic neurons needed to mount a counter-regulatory response to hypoglycaemia. These findings support the hypothesis of decreased sympathetic tone in some patients with hypothalamic damage, although the absence of a correlation with obesity/increased BMI does not support this as a mechanism for HO.

A second study measured urinary catecholamines and their metabolites (homovanillic acid [HVA] and vanillylmandelic acid [VMA]) and assessed physical activity, resting heart rate, blood pressure and treatment of hormone deficiencies in 109 patients with CP [64]. Hypothalamic involvement was present in 69%, but rose to 81% in 28 obese patients (BMS SDS 2-4) and 91% in 44 with extreme obesity (BMI SDS > 4). Norepinephrine/creatinine ratio was no different in those with and without hypothalamic damage. Both HVA and VMA were lower in those with BMI SDS > 4 than in those with BMI SDS 2-4 and highest in those with BMI SDS < 2. The decrease in catecholamine metabolites in those with obesity/extreme obesity suggested decreased sympathetic activity. Physical activity questionnaires demonstrated lower self-reported activity in patients with hypothalamic involvement than those without and was negatively correlated with BMI SDS. Decreased EE due to reduced sympathetic nervous activity secondary to hypothalamic damage is supported by these findings.

Energy expenditure (EE) and energy intake (EI)

Lower levels of physical activity have been described in nine children with CP compared to eleven controls in a home setting and 10 patients compared to 15 controls during hospital admission for a weight-reduction programme (Table 1.11, Appendix 1) [43]. Not all CP patients had hypothalamic involvement. On accelerometry, movement count was lower in CP than controls (Table 1.11). Seven

day self-reported food diaries showed the lowest reported calorie intake in patients with intrasellar CP (mean 1916 ± 677 kcal/day), slightly greater in those with hypothalamic CP (mean 2075 ± 877 kcal/day) and greatest in controls (mean 2476 ± 815), however these were completed by only 48% of patients. This significant limitation may have led to an underestimate of intake in patients. Under-reporting in the completed food diaries, either intentional or unintentional, is also possible. Additionally, accelerometry data does not reflect total EE which requires assessment of resting energy expenditure and non-exercise activity thermogenesis.

Another study assessed basal metabolic rate (BMR) and physical exertion in 18 children with CP and HO, 13 with congenital hypopituitarism (CH) and 16 with SO [65]. BMR was measured at rest wearing a ventilated hood and physical exertion by seven day accelerometry. Despite no significant difference in BMI SDS between groups, mean BMR was significantly higher in SO (Table 1.11) and after adjustment for gender, free T3 and lean body mass the differences persisted, with BMR lowest in HO. Accelerometry data was inconsistent due to equipment error. Total EI was not different between groups and compared to average estimated requirements reported EI was low in all groups, indicating possible under-reporting.

The final study of 42 adults with CO CP and 42 age- and gender-matched controls found the CP group were less physically active and had higher weight, BMI, fat and muscle mass, waist and WHR than controls, with lower ghrelin and fT3 and higher leptin, leptin/kg fat mass and free T4 concentrations [3]. Although BMR was significantly higher in CP than controls, the calculated percentage of expected BMR was lower (Table 1.11). EI and EI/BMR ratio were significantly lower in CP. TFEQ indicated similar disinhibition and hunger but higher restraint in CP, reflecting deliberate food restriction despite hunger. In summary, CP patients had greater fat accumulation, lower than expected percentage BMR and were more sedentary but consumed fewer calories, with little difference in eating behaviour. Subgroup analysis of CP patients with third ventricular involvement, a marker of hypothalamic damage, found higher BMI, WHR, fat mass and fat-free mass in those with third ventricular involvement than those without. There was no difference between the groups in BMR, percentage BMR of expected, or EI, although there was a significantly lower ratio of EI/BMR in those with third ventricular involvement than those without. Ghrelin was significantly lower, and insulin and insulin/kg fat mass higher in those with third ventricular involvement than those without. Percentage

calorie intake from fat, protein and carbohydrate and TFEQ scores were similar in both groups. One of the main disadvantages of this study is the duration of time since initial diagnosis and therefore the study may be measuring the effects rather than the cause of the HO.

In addition to reduced sympathetic activity other factors may effect physical activity following hypothalamic damage: physical factors such as restricted vision secondary to tumour impingement on the optic chiasm, neurological deficits, disturbed melatonin production leading to daytime somnolence [66] and finally, undertreated hormone deficiencies such as thyroxine, hydrocortisone, GH and testosterone. These three studies demonstrated lower physical exertion with a similar EI in patients with HO compared to controls. BMR in those with HO was lower than controls in one study and greater in another, although with a reduced percentage BMR of expected. The small number of studies, with differing methods and different findings make it difficult to conclude whether reduced efferent sympathetic activity leading to reduced EE (either through physical activity or through resting energy expenditure) could contribute to HO.

In summary, the mechanisms underlying the development of HO remain poorly understood and in some cases contradictory and are far from conclusive.

Clinical features and sequelae of hypothalamic obesity

The pattern of weight gain in HO is well described, with significant and rapid post-operative weight increase in the first 6-12 months following treatment [27, 33] which then stabilises but does not remit [3, 33]. The sequelae of simple obesity have been described in the medical literature for well over half a century, with long-term morbidities such as cardiovascular, renal and liver disease and type 2 diabetes mellitus [67] which not only impair quality of life, but also increase mortality. More recent reports describe sleep-disordered breathing, non-alcoholic fatty liver disease and an increase in certain cancers [68]. An increase in the prevalence of some of these sequelae and risk factors have also been described in patients with hypopituitarism [69, 70], although in most cases not exclusively in patients with

hypothalamic involvement. Increased mortality rates in craniopharyngioma are also described.

Mortality rates

An early study (1951-1988) described increased mortality following craniopharyngioma (CP) diagnosis, higher in females than males and remaining raised after excluding early postoperative deaths (Table 1.12, Appendix 1) [71]. All patients had suprasellar tumour extension and 88% had third ventricular involvement therefore the proportion with hypothalamic damage is likely to be significant although it was not specifically defined. Only 12 patients were treated for GHD and as untreated GHD is associated with increased cardiovascular risk factors this may have increased morbidity, although a later study found no difference in mortality between those with treated and untreated GHD [19]. Subsequent studies also reported an increase in mortality (18-30%) [19, 72], with a higher SMR in females than males [19, 72-75]. Postulated mechanisms include delayed diagnosis, oestrogen supplementation and oral oestrogen supplementation leading to GH resistance [76]. A meta-analysis of six studies containing patients with pituitary disease (with or without pituitary insufficiency) found an increased SMR in all patients with weighted SMR significantly higher in women compared to men ($p<0.0001$) [74].

A large UK study of patients with hypopituitarism of varying causes found that even after excluding deaths in the first postoperative month there was increased mortality in those who underwent pituitary surgery [73], however Pereira found no effect of treatment modality (surgical approach, use of radiotherapy) [72]. Other risk factors identified are diagnosis of CP, treatment with radiotherapy, younger age at diagnosis of hypopituitarism and untreated gonadotrophin deficiency [73]. For those treated with radiotherapy cerebrovascular mortality (SMR 4.4 [2.5-7.7] vs. 1.6 [0.9-3.0], $p=0.001$) and other malignancies are significantly increased (SMR not stated, $p=0.036$).

None of these studies specify the prevalence of obesity or include obesity/BMI but a large cross-sectional study did consider both obesity and hypothalamic damage [28]. Differences in overall survival were not significantly different between those with and without hypothalamic involvement, however 20-year survival was significantly lower in those with severe obesity compared to moderately obese and normal weight patients ($p = 0.03$).

Two studies focussed on cerebrovascular and cardiovascular mortality (Table 1.12) [77, 78]. The first found increased mortality from cerebrovascular disease (higher in females than males), with no relationship to duration of pituitary insufficiency but increased risk where pituitary insufficiency was diagnosed when under 55 years [77]. Overall cardiovascular mortality was also increased. None of the patients were reported to have received GH replacement. The wide study period (1952-1992) means there would have been considerable variation in the definition and treatment of pituitary hormone deficiencies. The second study found an increased estimated relative risk of death compared to that expected based on age, gender and period-specific UK mortality rates [78]. Pituitary or hypothalamic disease or other brain tumours were the biggest cause of death, but cerebrovascular disease was also increased.

All of these studies (Table 1.12) are retrospective and the hormonal supplementation of hypopituitarism has changed over time - GHD was previously untreated/infrequently treated and higher doses of corticosteroids were used, not reflecting current practice. In fact Nielsen et al found a normal SMR in males diagnosed from 1985 onwards [74]. Treatment of the underlying disease has also changed, with trans-sphenoidal surgery increasingly utilised and evolving radiotherapy techniques aiming to spare normal surrounding tissue and reduce vascular injury [76]. This may be particularly important since the risk of increased mortality in patients treated with standard radiotherapy remains inconclusive [73].

Cardiovascular risk factors and disease

Studies report increased prevalence of cardiovascular risk factors in adults with hypothalamic-pituitary disease (Table 1.13, Appendix 1), with at least one risk factor in 57% of patients in one study [72]. In addition, these are often inadequately treated, with sub-optimal treatment of hypertension and hyperlipidaemia in 44.6% and 69% respectively in a study undertaken at University Hospital Aintree [79]. Reported prevalence of treated hypertension ranges from 15-37%, dyslipidaemia treated with lipid-lowering therapy from 28-47%, diabetes mellitus from 9-18% and metabolic syndrome in 47% [19]. Atherosclerotic cardiovascular disease has been reported in 11.1% [79]. In studies of patients with pituitary adenomas, the inclusion of those with acromegaly or Cushing's disease, which have additional mechanisms

influencing cardiovascular risk factors confer an additional increased risk of cardiovascular risk factors and disease.

In children, Srinivasan found an adverse lipid profile and increased abdominal body fat in 9 patients with craniopharyngioma compared to 9 age, gender and BMI-matched healthy controls [57]. There was no difference in height, BMI SDS, WHR, abdominal or total body fat, insulin sensitivity or lipid profile between those with treated and untreated biochemical GHD. A Japanese study found hyperlipidaemia in 58% (7/12) of obese children with suprasellar tumours compared to just 39% (9/23) of the total group (lean and obese), with increased small dense LDL, which was significantly correlated with BMI and percentage fat mass and a high Apo B/A1 ratio in 50% (6/12) of obese patients compared to only 1/11 non-obese patients [80]. These authors also found no difference between treated and untreated GHD. In 39 patients followed-up in a comprehensive care clinic for children with brain tumour at risk of HO, despite amelioration of weight gain compared to that seen during follow-up in standard clinic (31/39 patients previously followed-up in a standard endocrine clinic) there was no change in blood pressure or biochemical markers of cardiovascular risk such as fasting glucose, triglycerides or LDL by the end of the study [81].

Pereira found increased cardiovascular morbidity in 22% (11/54) of patients after a median follow-up of 10 (range 0.5-37) years, at a median age of 49 years, with myocardial infarction in 3, transient ischaemic attack in 1 and cerebrovascular events in 7 patients [72]. The prevalence was higher in pre-menopausal women untreated with oestrogen (40%) compared to only 10% in sex-hormone replete patients ($p = 0.03$). In the general Dutch population of a similar age (20-50 years) the prevalence was 4.8%.

Non-Alcoholic Fatty Liver Disease (NAFLD)

Non-alcoholic fatty liver disease (NAFLD) has been described in case reports in children with hypopituitarism [82, 83] as well as several case series. An American series found NAFLD in 21/879 (2.3%) of adults and children) with hypopituitarism, HO or craniopharyngioma [69]. All had raised liver enzymes and hepatic steatosis on imaging. Cirrhosis was found in 6/10 who had a liver biopsy, non-alcoholic steatohepatitis (NASH) with fibrosis in two and simple steatosis in two. Two

patients required liver transplantation and 2/6 deaths were liver-related, both in patients with cirrhosis. Not all patients had hypothalamic damage, however five had hyperphagia, ten had experienced significant weight gain, and nine were receiving DDAVP. The study may under-report prevalence as in many cases it was based on a one-off measurement of liver enzymes.

A study of 12 children with craniopharyngioma (CP) and hypothalamic damage (9 severely obese, 1 obese) found NASH (not biopsy confirmed) in six, a median of 2.4 years after CP surgery [58]. As all CP had untreated GHD, this may have increased their prevalence of fatty liver. In 69 adults with untreated GHD secondary to hypopituitarism raised liver enzymes improved in 19 patients (including 11 with NAFLD) after GH replacement [84]. In five who underwent liver biopsy before and 6-12 months after GH replacement an improvement in inflammatory and fibrotic markers, steatosis and fibrosis were seen. The prevalence of NAFLD (diagnosed by ultrasound) was 77% (53/69), compared to 12% in 83 age-, gender- and BMI-matched controls ($p<0.001$). A further case-control study of 34 men with hypopituitarism and 40 age-matched male controls also found increased prevalence of NALFD on liver USS in patients (70.6%) than controls (32.5%, $p = 0.001$) [85]. The potential impact of GH deficiency or insufficiency and effects of GH replacement on NAFLD is therefore an important consideration.

Daytime somnolence

Daytime somnolence was explored in 79 patients with craniopharyngioma (CP), 19 with hypothalamic pilocytic astrocytoma (HPA) and 30 controls [27]. Melatonin concentration was significantly correlated with BMI category (normal weight: BMI SDS <2, overweight: BMI SDS 2-4, obese: BMI SDS >4, $p = 0.004$) and patient group ($p = 0.03$) in the early morning (06.00-08.00) and at night (23.00-03.00; BMI $p = 0.001$, patient group $p = 0.03$), with no difference in daytime melatonin concentrations. Severely obese patients with CP and HPA had similar melatonin concentrations, as did normal weight patients (BMI SDS <2) and controls. Increased daytime sleepiness (a higher Epworth Sleepiness Scale [ESS] score) was found in 31 obese CP patients compared to 17 overweight and 31 normal weight CP patients (median ESS 10, 7 and 3 respectively, $p<0.001$). An ESS >10 denotes severe daytime sleepiness and was reported in 42% with CP and BMI SDS >4, compared to

24% with CP and BMI SDS 2-4 and 11% with CP and BMI SDS <2 ($p<0.001$). Two out of four patients with HPA and BMI SDS >4 had an ESS > 10.

The ESS of those with CP was negatively correlated with both morning and nocturnal melatonin concentrations, suggesting the possibility that disrupted sleep may account for daytime somnolence. As the authors suggest, hypothalamic damage could be responsible for both increased risk of obesity and disordered melatonin secretion, however there was no assessment of sleep disordered breathing, a consequence of severe obesity that may have impacted on sleep. Assessment of sleep disordered breathing would have added useful information to this study.

Quality of Life and Neuropsychological Outcomes

There is evidence from the large, multinational KRANIOPHARYNGEOM 2000 database that both quality of life (QoL) and self-reported functional capacity are affected by HO and that increasing obesity inversely correlates with functional capacity [28]. Pediatric QoL (PEDQOL) questionnaires completed by 120 patients 36 months after diagnosis of childhood CP found that the degree of hypothalamic involvement correlated negatively with QoL, possibly influenced by their greater weight gain (median BMI increased by +3.22 compared to +0.45 in those with no hypothalamic involvement). Social function and family aspects were particularly affected. BMI SDS correlated negatively with QoL ($p<0.001$) in 185 patients studied and severely obese patients (BMI SDS > 3; 44%, more common in those with hypothalamic involvement) had lower QoL than non-obese patients with the exception of a few areas [27]. In a cross-sectional study of 183 patients with childhood- or adolescent-onset CP, functional capacity was significantly lower ($p<0.001$) in those with BMI SDS 2-7 (median score 46, range 5-100) and BMI SDS $\geq$ 7 (median score 33, range 4-64) than those with BMI SDS <2 (median score 50, range 1-95) [28].

Information on adults alone is limited to one small study of 13 patients and hypothalamic involvement and BMI were not taken into account [86].

In summary, there is evidence that hypothalamic damage secondary to structural lesions reduce quality of life, perhaps secondary to the increase in obesity, with stronger evidence in children than in adults. Reduction in the ability to perform activities of daily living and psychosocial morbidity may also occur.

Pituitary adenomas in children and adolescents

Whilst this thesis mainly concentrates on hypothalamic obesity (HO) it was noted during clinical discussions that some of the sequelae of hypothalamic damage (obesity, endocrine deficiencies, increased cardiovascular risk factors) can also be seen in children and adolescents with pituitary adenomas (even in the absence of hypothalamic damage). As pituitary adenomas *can* lead to hypothalamic damage (and therefore HO) it was of interest that even in those *without* hypothalamic extension these sequelae can be seen. The sequelae of obesity and increased cardiovascular risk factors are not well described in the literature relating to pituitary adenomas in children and adolescents and due to the overlap of these sequelae with HO further investigation of this cohort was felt to be relevant to this research.

Pituitary adenomas typically occur in the fourth and fifth decades of life [87] and are uncommon in childhood and adolescence, accounting for only 3–6% of all surgically treated adenomas [88] and constituting less than 3% of childhood supratentorial tumours [89]. Many of the childhood adenoma case series are small (≤ 21 cases [88, 90-92]), although a few larger series, often encompassing patients from multiple centres, contain between 26 and 44 patients [93-96]. The two largest series (of 136 [97, 98] and 150 [99] patients) describe children and adolescents who were entirely surgically managed and these patients represent a very different group.

Whilst the clinical features at presentation [88, 94, 98, 99], surgical outcomes [92, 97, 100] and endocrine outcomes [95, 96] are relatively well-described in these papers, other long-term sequelae described in adult patients with hypopituitarism, such as impaired fertility, premature mortality due to increased cardiovascular and cerebrovascular events and increased cardiovascular risk factors are generally poorly reported. Impaired fertility has been described in adult patients with pituitary adenomas [101], patients with hypopituitarism from various causes (including various types of adenoma, craniopharyngioma and Rathke's cleft cyst) [102] and in female survivors of childhood cancer who had received ≥ 30 Gy of radiation to the hypothalamic/pituitary axis [103]. Premature mortality has been reported in those with hypopituitarism, particularly in females, those diagnosed at a young age and those who have received radiotherapy [73]. There may however, be confounding factors resulting in this increase in mortality, for example it is unknown whether those presenting with pituitary tumours at a younger age have a more aggressive

pituitary disease and at the other end of the spectrum, it is possible that those undergoing radiotherapy may be older/otherwise unfit for surgery, which may of course also influence the mortality rate. Adults with hypopituitarism (with or without hypothalamic damage) have been found to have an increased prevalence of factors such as obesity, dyslipidaemia and hypertension [6, 79], which confer an increased cardiovascular risk. The prevalence of these sequelae in children and adolescents with pituitary adenomas (with or without hypothalamic damage) is therefore an important area for further exploration.

Obesity in children and adults with acquired structural hypothalamic damage: endocrine and neuroanatomic risk factors

Endocrine

Cranial Diabetes Insipidus (DI)

Cranial diabetes insipidus (DI) is associated with an increased risk of HO in many studies, which is unsurprising if hypothalamic-pituitary anatomy is considered. Cranial DI is caused by inadequate vasopressin release from the posterior pituitary; this area receives neuronal projections directly from the paraventricular nucleus (PVN) of the hypothalamus, an area where energy balance is controlled, therefore it is not surprising that damage to the neurohypophysis affecting vasopressin release may also effect PVN neurones involved in energy homeostasis [6]. In adults with tumours causing hypothalamic damage, DI requiring treatment with desmopressin significantly increased the risk of new or worsened obesity (odds ratio [OR] 13, $p = 0.007$) [6]. In a retrospective case-note review of 148 children less than 14 years old when diagnosed with a brain tumour and taking what authors considered to be a physiological dose of cortisol replacement (hydrocortisone < 12 mg/m^2/day) the most significant weight gain was seen in patients with DI [104]. However only 5.8% of patients were treated with DDAVP and no details of statistical significance are given. The presence/absence of hypothalamic damage was not an inclusion/exclusion criterion for the study but considered as one of the risk factors for weight gain. In a study of 393 adults with GHD secondary to CP, a correlation between DI and increasing BMI SDS ($r = 0.168$, $p = 0.001$) remained significant only in those with CO and not AO (aged 18 years or over) CP, when separated out [25]. Another study

which compared 171 patients diagnosed with CP in adulthood, late-childhood (between 10-18 years) and early-childhood (< 10 years of age at diagnosis) found the highest prevalence of both obesity (19/28, 68%) and DI (27/28, 96%) in the youngest cohort (< 10 years at diagnosis) [24]. In those with adult-onset CO only 39/94 (42%) were obese and 70/103 (68%) had DI. In those diagnosed between 10-18 years of age 8/34 (24.2%) were obese and 28/34 (82.3%) had DI. Those diagnosed in early childhood had an increased risk of obesity compared to the other groups (OR 3.81, 95% confidence interval 1.51-9.62). Not all patients had hypothalamic involvement - only 74/171 (43.3%) had extrasellar tumour extension and there was no further clarification of hypothalamic involvement, therefore it is possible that differences in the prevalence of hypothalamic involvement, rather than age at diagnosis, may have accounted for the difference in outcomes [24].

In children with CP Ahmet et al found that 38/43 (88%) had DI, 25/43 (58%) were obese and 22/25 (88%) of those obese patients had DI [33]. However, as only 58% (22/38) of those with DI were obese, it may be more appropriate to suggest that obese patients are more likely to have DI, but not necessarily the reverse. In a separate study of 58 children with CP there was no correlation between the prevalence of HO and DI, however nearly all the patients (53/58, 91%) had DI [105]. Even in the group reported to have no post-operative hypothalamic damage (intrasellar tumour only), 89.4% (17/19) had DI, which may call into question the accuracy of the assessment of hypothalamic damage in this study.

Growth Hormone Deficiency (GHD)

Growth hormone deficiency as a risk factor for HO is not as consistently reported and there are several possible reasons for this variance. Firstly, GHD does not necessarily reflect hypothalamic involvement in the same way as a diagnosis of DI. In children with CP Muller et al found no difference in the prevalence of GHD in those with and without hypothalamic damage [106]. Secondly, GHD is difficult to compare between studies due to variations in the definition between adults (peak growth hormone < 3 micrograms/l) and children (varies between different laboratories/hospitals/studies), lower peak GH response to stimulation in those with obesity [107], different GH stimulation tests (glucagon-, arginine-, clonidine-, GHRH-stimulation and insulin tolerance tests), recent increased sensitivity of GH assays and even a change of the units in which GH assays are reported in some

countries (mU/L to micrograms/L), necessitating a conversion when comparing older studies with more recent ones. Additionally, in the UK for adult patients to meet the criteria for GH treatment, in addition to proven biochemical severe GHD, the presence of impaired quality of life (QoL) must be demonstrated using the validated self-assessment QoL-AGHDA questionnaire [108]. This multitude of factors makes assessing the presence of GHD as a risk factor for HO much more challenging.

The risk of obesity in patients with GHD (treated or untreated) was increased in a study of 42 adults with hypothalamic damage secondary to varying tumour types (OR 7.6, p=0.04) [6]. In comparison, in 148 children with various brain tumours there was no association between increased BMI and GH replacement, although the need for replacement of any pituitary hormone including GH *was* associated with a significant increase in BMI compared to that expected for age [104]. Another study found no difference in the prevalence of obesity in children with untreated GHD secondary to suprasellar tumour (4/11 obese) and those with treated GHD (7/11 obese, 63.6%), although the numbers involved were small [20]. The prevalence of hypothalamic damage may have been similar in both cohorts and treatment with GH (or lack of it) may have had little effect and a comparison between those with and without GHD may have been more useful.

A large study compared three-year longitudinal data of pre-pubertal children with GHD on the KIGS (Pfizer International Metabolic) database of children receiving GH [109]. Patients were divided into three groups - children who underwent surgery and/or irradiation for CP, children who underwent surgery and/or cranial irradiation for tumours other than CP (other tumours) and those with GHD not due to tumour (no tumour, e.g. septo-optic dysplasia). Mean peak GH on stimulation was significantly lower in CP (2.1 mcg/L) than the other groups (both >4 mcg/L) and the incidence of DI was higher (CP 72%, other tumours 61%, no tumour 28%, $p<0.0001$), possibly indicating greater likelihood of hypothalamic involvement in the CP group. Those with CP had the highest mean BMI SDS and greatest incidence of BMI SDS >2 at both the start of GH replacement and after three years, despite a decrease in mean BMI SDS over time. The fall in BMI SDS occurred despite weight increase, due to an increase in height, consistent with GH treatment. In a subgroup comparison of all patients with peak GH < 2 micrograms/L the mean BMI SDS of the CP group remained the highest, even when compared to those with

hypothalamic-pituitary tumours, suggesting the possibility of different mechanisms in obesity secondary to CP.

Another study of 58 children with CP found BMI SDS four years after surgery was unaffected by the need for GH replacement [105] and a smaller study of 17 children with CP and post-operative panhypopituitarism (including GHD) found no correlation between peak GH and BMI, although a positive correlation between pre-operative IGF-1 concentration and post-operative BMI *was* found [29]. Two other studies examined IGF-1 and BMI in CP and GHD. One study of adult-onset CP and GHD (peak GH following stimulation <3.0 micrograms/L) found a positive correlation between IGF-1 SDS whilst untreated with GH and BMI SDS [25]. The second study compared five children with treated GHD secondary to CP to ten children with CP and untreated GHD with adequate height velocity (HV) [57]. The authors found no difference in BMI SDS, WHR or body composition between the groups, despite a significantly greater IGF-1 concentration in those treated with GH (treated: median 21 nmol/L untreated: median 7.5 nmol/L, $p = 0.01$). IGF-1 concentrations are affected by nutrition as well as GH concentrations, making interpretation more complex; whether this is a cause or effect of the differences found between studies illustrates the complexity of the association between GHD and HO.

In childhood, the phenomenon of *growth without growth hormone* can result in GHD being left untreated [57]. Stahnke et al investigated 10 children with CP who had normal post-operative HV despite GHD and 12 with GHD and poor post-operative growth [62]. In the first group 8/10 rapidly gained weight and were obese one year post-resection; mean weight-for-height increased to + 8.38 kg (SD ± 2.23 kg) above expected at one year. A more modest increase was seen in patients with poor post-operative growth, which increased to + 3.78 (± 1.39 SD) kg at one year from a lower pre-operative baseline. In another study, 32/73 children with untreated GHD and "catch-up" growth also had increased risk of weight gain one year post-resection (BMI SDS +5.0) compared to those with normal growth (BMI SDS +3.2) or inadequate growth (BMI SDS +1.6; $p<0.00001$) [110]. The underlying pathophysiology of *growth without growth-hormone* remains unknown, however increased concentrations of insulin, prolactin or leptin and the extent of hypothalamic involvement have all been postulated as possible mechanisms [109]. These factors overlap with the possible pathophysiological mechanisms leading to HO.

Inclusion of patients without hypothalamic damage in some studies may account for differences between them. Additionally, there may be a difference between adult- and childhood-onset hypothalamic damage and in treated vs. untreated GHD, especially in those with *growth without GH*. Alongside the difficulties described in accurately diagnosing GHD, these factors make the presence of GHD as a risk factor for HO more difficult to unravel than that of DI.

Other pituitary hormones

Several studies have examined the relationship between HO and other pituitary hormone deficiencies. Daousi et al found an increased risk of new/worsened obesity in adults receiving hydrocortisone, thyroxine, or GH [6] and Lustig et al found that the presence of any hormonal deficit in children was associated with a greater increase in BMI over time [104].

In children with CP, Pinto et al found no correlation between BMI and prolactin, plasma free thyroxine concentration, dose of thyroxine supplementation or post-operative peak GH [29] and Park et al found that the number of hormone deficiencies had no effect on obesity prevalence [105]. In a comparison of 90 patients with childhood CP with (n = 48) and without (n = 42) hypothalamic involvement, no difference was found in the prevalence of endocrinopathies or dose of hormone replacement required, although there was a difference in BMI SDS at diagnosis and on annual follow-up between the two groups [106]. In 171 patients with CP, those under 10 years of age at tumour diagnosis all had three or more anterior pituitary deficits (28/28) and 68% were obese (BMI > 30 kg/m^2) at latest follow-up, compared to the adult-onset cohort in which only 76/104 (74%) had three/more anterior pituitary deficits and 39/104 (41%) were obese [24].

Some studies have reported that prolactinomas may lead to weight gain, even without hypothalamic involvement, although the literature is divided on this [111, 112]. In Lek's cohort none of the patients with prolactinoma were obese at latest follow-up [21]. Daousi et al included a small number of patients with prolactinoma in their study but found no association between this diagnosis and increased BMI [6].

As with GHD, the link between HO and deficiencies in other pituitary hormones, or the presence of multiple pituitary hormone deficits remains unclear.

Dose of hydrocortisone replacement

Two studies found no association between the dose of hydrocortisone replacement and BMI despite the use of supraphysiological doses of steroid up to 15 mg/m^2/day [20, 29]. Other studies excluded patients on doses of replacement hydrocortisone considered supraphysiological (> 12 mg/m^2/day) [104] or use a dose considered physiologically equivalent (6-10 mg/m^2/day) [33]. As supraphysiological doses of glucocorticoids can result in obesity, it is advisable to keep doses of steroid replacement within normal physiological ranges.

Neuroanatomical aspects

Identifying hypothalamic damage is crucial for the definition of HO, however whether the extent of the damage can predict the risk of obesity is uncertain. In paediatric CP series all but one found an increased risk of HO with greater hypothalamic damage on MRI brain scans.

Post-operative MRI scans of 63 children with CP were graded 0-2 grade 0 – no visible damage, 1 – intermediate damage, or 2 – severe hypothalamic damage [113]. Those with the most significant hypothalamic damage (i.e. grade 2) had a greater increase in median BMI SDS (+5.5) at latest follow-up than those grade 0 (BMI SDS +1.1) or grade 1 (BMI SDS +2.5). A study of 27 children with CP found that whilst pre-operative BMI SDS was comparable between all patients (grade 0-2), at one year post-resection those with grade 2 pre-operative hypothalamic involvement (12 patients) had a significantly higher BMI SDS (4.0 ± 1.3) than 7 patients grade 0 on pre-operative assessment (BMI SDS 2.7 ± 1.1) [114].

Several studies graded both pre- and post-operative hypothalamic involvement on MRI scans. The first described a significant correlation between BMI at latest follow-up and the extent of hypothalamic involvement on both pre- and post-operative MRI scans [32]. An increase in hyperphagia was also reported (26% at diagnosis, 70% at latest follow-up). In a separate study, BMI SDS at diagnosis was significantly higher in those where the hypothalamus could no longer be identified on pre-operative MRI scan (mean BMI SDS 1.8), compared to those with no

involvement (mean BMI SDS 0.34, p<0.02) [115]. A year after surgery, whilst the group without hypothalamic involvement had no significant change in BMI SDS, patients with some involvement or complete involvement had a significant increase in BMI SDS (p <0.002 and p=0.0003, respectively). This increase persisted at latest follow-up, at a mean age of 14.9 years (mean age at surgery 7.4 years, range 0.9-14).

Three further studies used the Saint-Rose classification grade 0 (no hypothalamic damage) to 2 (hypothalamus unable to be identified) [32]. A retrospective review of 66 patients found significantly higher BMI SDS with increasing grade of tumour damage (p=0.007 preoperatively, p=0.001 postoperatively) [116]. The prevalence of both grade 1 and grade 2 involvement decreased from pre-operative (36% and 42% respectively) to postoperative scans (30% and 38% respectively), possibly indicating an overestimate in the amount of hypothalamic involvement on pre-operative MRI scans, although there may have been persisting damage despite subsequent decompression. In the second study, in 58 children with CP the prevalence of obesity rose from 8/58 (14%) at diagnosis (2 patients grade 1, 6 grade 2 on pre-operative MRI brain scan) to 22/58 (38%) at latest follow-up (2 patients grade 0, 10 grade 1, 10 grade 2 on pre-operative MRI brain scan) [105]. BMI SDS after four years of follow-up was significantly associated with both BMI SDS at diagnosis and the pre- and post-operative hypothalamic grade. After adjusting for pre-operative BMI and pre-operative hypothalamic involvement, postoperative hypothalamic grade was the only significant predictor of BMI SDS (other factors considered were not stated). A third study used pre-operative MRI grading to determine treatment in 22 prospectively studied CP patients [116]. The median postoperative BMI SDS was +1.3SD in this group, compared to a median post-operative BMI SDS of +2.5 in a retrospective group. The size of the prospective group was smaller than the retrospective cohort, however, the proportion of patients in each category (preoperative grade 0-2) was similar. Whilst the use of preoperative MRI assessment to determine patients' treatment may have ameliorated hypothalamic damage and subsequent weight gain in the prospectively studied patients, it is important to note the differences in the length of follow-up (prospectively studied patients mean follow-up 1.15 years, retrospectively studied patients median follow-up 7 years, range 1-19).

Muller et al used their own grading system to describe hypothalamic involvement in CP: grade 0 remained no hypothalamic damage, grade 1 described

anterior hypothalamic damage not involving the mammillary bodies or beyond and grade 2 described anterior and posterior hypothalamic damage extending beyond the mammillary bodies [117]. In 120 patients from 76 centres grade 2 pre-operative hypothalamic involvement was associated with a greater increase in BMI SDS (+ 3.22 SDS) than grade 0 (BMI SDS + 0.45, p = 0.03) or grade 1 involvement (BMI SDS +0.74, p=0.01). The presence of grade 2 hypothalamic damage pre-operatively was the only risk factor associated with a change in BMI SDS after 36 months of follow-up out of seven considered (p=0.002). A further study using this classification did *not* find an association between the grade of hypothalamic involvement (either pre- or post-operatively) and the risk of weight gain or obesity in 33 patients with CP [118].

Two studies considered hypothalamic involvement, without using a grading system. In 43 children with CP, the 30 with post-operative hypothalamic damage had a +0.74 BMI SDS increase at one year (p=0.014) [33]. In another study, hypothalamic involvement (based on MRI and/or operative findings) was found in 36/45 patients (80%), with the prevalence of obesity (BMI SDS >2) rising from 15.6% (7/45) at diagnosis to 70.5% (31/44 survivors) at latest follow-up and significantly associated with hypothalamic damage (p<0.001) [34].

In childhood brain tumours of varying types, Lustig et al found tumour location to be important, but only compared relatively gross findings, for example comparing the risk of obesity in those with hypothalamic versus lateral ventricle tumours [104]. In adults with hypothalamic damage secondary to various tumour types, tumour width from midline (coronal views), thalamus or temporal lobe involvement, distortion of the third ventricle at the infundibulum (coronal views) and infringement of the tuber cinereum were assessed [6]. No correlation was found between the risk of HO and the degree of hypothalamic damage on neuroimaging (26 pre-operative and 39 post-operative scans). However another adult study found that greater hypothalamic involvement on pre-operative MRI scans positively correlated with post-operative weight gain (p = 0.022) in 28 adults with CP after two years of follow-up [119]. Although there was a correlation between left-sided hypothalamic involvement and post-operative weight gain but not right-sided hypothalamic involvement, the study only included a small number of patients and therefore the

significance of this finding is unclear. Additionally, there was already a significant prevalence of obesity (BMI > 27.3 kg/m^2 in 61%) pre-operatively in this study, none of the patients were treated with GH replacement and the prevalence of GHD is not described. These factors may have influenced the findings.

The usefulness of grading hypothalamic involvement on neuroimaging in adults with tumours causing hypothalamic damage to predict the risk of weight gain therefore remains equivocal.

The presence of hydrocephalus or intracranial hypertension requiring a ventriculo-peritoneal (VP) shunt as a marker of hypothalamic damage is used in some studies, with varying findings. In childhood CP studies three found that hydrocephalus was significantly associated with post-operative obesity [24, 27, 33], with OR 15.28 in one study of 43 children [33]. Two other studies found no relationship between hydrocephalus and post-operative BMI [104, 116]. One adult series also found no association between obesity and insertion of a VP shunt (although numbers were small) [6].

Treatment modality

There are mixed findings regarding the risk of HO and surgical management. Avoiding total gross surgical resection of CP in order to minimise hypothalamic damage and long-term post-operative morbidity had no effect on the overall prevalence of obesity (BMI > 95th percentile for age and gender) reported by Cohen et al (53.5% between 1990-2001; 54.8% between 2001-2011) [30]. However, less aggressive surgery did result in a decrease in the prevalence of *severe* obesity (BMI > 99th percentile for age and gender) from 34.8% to 17.6%. Potential confounding factors include the size of the two cohorts (numbers of patients not provided), expertise of the surgeon, age and BMI at diagnosis and use of adjunct therapies. Another paper described 51 children with CP, of whom the majority underwent a conservative resection with additional radiotherapy [31]. After a median of 7.6 years (range 5.0-21.3) 24% were obese and 41% overweight. Dose of radiotherapy is not stated and there is no comparison of obesity prevalence when using more aggressive surgical management strategies.

A study of 66 children demonstrated a significant increase in post-operative BMI SDS under the care of a less experienced surgeon, with no effect of extent of

resection or route of approach [116]. Another study found increased risk of obesity at latest follow-up (BMI SDS >2, 28/45 obese) after subtotal resection of CP, second operation and with increasing number of surgeries [34]. These overlap with tumour recurrence and number of recurrences which were also associated with increased risk of obesity. Hypothalamic involvement was considered as a risk factor, and therefore not present in all patients in the study. Another study also found a small but significant increase in the median number of resections in those with obesity (median 1.4 operations in those with BMI < 2 SD, median 1.44 operations with BMI 2-7 SD, median 1.7 operations with BMI >7SD; $p<0.05$), although 41% (48/90) had no hypothalamic involvement [28]. In 171 children increased obesity was found with multiple surgeries (OR 1.4), recurrence (OR 2.3) and tumours needing a pterional approach, which indicates a larger initial tumour (OR 6.2). A third study found a correlation between the number of surgeries and BMI SDS, but not the type of surgery undertaken [25]. Other series considered extent or type of surgery, number of surgeries and recurrences in varying study populations and found no effect [6, 23, 104, 105]. The effect of these factors on the risk of HO therefore remains unclear.

The effect of radiotherapy is also inconclusive. In one study a significantly greater increase in BMI occurred in those receiving >51 Gy of hypothalamic irradiation ($p=0.002$) [101]. This remained significant after excluding patients with hypothalamic/thalamic tumours. Another study also found an increased risk of obesity with radiotherapy (OR 1.79) [20], however other studies failed to find any correlation [25, 105, 116].

Other risk factors

Many studies unsurprisingly show a correlation between increased BMI/BMI SDS before surgery and greater post-operative weight [29, 33, 34, 105, 115, 118], although not all studies specify hypothalamic damage. One study found that the risk of severe obesity (BMI > 3 SDS) was significantly increased in children with CP where BMI SD was > 2 at diagnosis (OR 16.4, $p<0.05$) [27], however an adult study found no correlation between preoperative BMI and postoperative weight gain [119]. Higher maternal BMI has also been identified as a risk factor for the development of severe obesity following CP (OR: 4.6; $p<0.05$) [27]. These pre-existing risk factors for weight gain/obesity may have some additional effects on the risk of HO.

While three studies found a correlation between younger age at diagnosis and the risk of weight gain/obesity [24, 33, 104], a comparison of AO and CO CP found a greater prevalence of obesity in adult-onset compared to child-onset CP [25] and other studies found no effect of age at diagnosis [21, 105, 106, 116].

Two studies found an increased risk of obesity in female patients compared to males [21, 25], however other studies have found that the risk of obesity was unaffected by gender [105, 106, 116].

In summary, the impact of these other risk factors for HO remains inconclusive.

Management of hypothalamic obesity: pharmacological and non-pharmacological treatments

Current options for weight management in simple obesity include diet and exercise modification, treatment with the medication orlistat (a lipase inhibitor which prevents fat absorption from the gut) and bariatric surgery. Some previous medications such as rimonabant (a selective cannabinoid CB1 receptor blocker) and sibutramine are no longer available due to health risks (an increased risk of psychiatric problems including suicide and cardiovascular events/strokes, respectively). Treatment of simple obesity, however, is often ineffective. Some case studies and interventional trials describe the utilisation of these treatments for simple obesity in patients with hypothalamic obesity. Other medications specific to HO have been investigated, including octreotide, dextroamphetamine and melatonin. Incretin therapy is a relatively new treatment for type 2 diabetes which alongside improvement in glycaemic control, also results in weight reduction and its use in HO has been described in several case reports. Bariatric surgery is becoming increasingly accepted in the treatment of simple obesity and its use in HO has also been described in several case reports and small case series.

There are no studies in humans of the effects of diet or exercise alone on weight loss in patients with HO outside the setting of a dedicated multi-disciplinary clinic and no reports on the use of orlistat or rimonabant (now withdrawn from the market) specifically in the HO population.

Medications

Treatment with medications used in simple obesity

Sibutramine is a sympathomimetic which exerts its effects through the arcuate nucleus. It increases anorexigenic hormones, decreases orexigenic hormones, increases thermogenesis and prevents the normal weight-loss induced drop in resting energy expenditure [120]. In simple obesity (SO) its effects generally last for the first six months of treatment, with subsequent weight regain in many cases. A reported increase in MI and cerebrovascular accidents in patients treated with sibutramine led to its withdrawal by the European Medical Association [120].

Treatment of hyperinsulinaemia

Octreotide is a somatostatin analogue which inhibits insulin secretion. As hyperinsulinaemia is a proposed mechanism for HO some researchers have proposed that octreotide could ameliorate weight gain in HO by decreasing insulin release. Lustig and colleagues found an amelioration of weight gain with octreotide treatment (Table 1.10), with either weight maintenance (3 patients) or weight loss (5 patients), in eight patients with HO compared to weight gain in the previous six months [56]. After 12 months, two patients had lost 21.7% and 9.8% of their initial body weight. No participants developed diabetes mellitus secondary to insulin suppression and fasting insulin concentrations remained within normal limits throughout. A second study (Table 1.10) found ongoing weight gain in those randomised to placebo compared to weight stability in those on octreotide, with weight loss in months 4-6 [55]. Calorie intake was lower with a greater decrease in serum leptin concentration in those on octreotide than placebo, although neither reached statistical significance. Neither study considered the effects of octreotide discontinuation, or alternatively its long-term suitability and safety for treatment beyond the 6-12 month period studied.

Another study attempted to ameliorate hyperinsulinism with diazoxide and metformin (Table 1.14, Appendix 1) [121]. Diazoxide is used in children with hyperinsulinism to supress insulin secretion. Metformin primarily decreases hepatic gluconeogenesis but also increases insulin sensitivity. After six months of diazoxide and metformin, all seven participants had significant amelioration in weight gain compared to the previous six months. Three patients lost weight and four gained less than previously, although this may have represented their natural plateau years after

hypothalamic damage. The study was a pilot and limited by its small size. It is not possible to determine whether diazoxide or metformin alone, or both medications are necessary for weight reduction. The effects of stopping treatment were also not studied. Given the potential side-effects of diazoxide, such as gallstones and oedema, its long-term use may be impractical and therefore the effects of discontinuing it should be considered.

Treatment with sympathomimetics for possible impaired sympathoadrenal activation

In addition to sibutramine, other sympathomimetics studied in HO include dextroamphetamine, phentermine and combined caffeine and ephedrine.

Dextroamphetamine was studied after weight stabilisation was noted in a child started on this for attention problems (Table 1.15, Appendix 1) [41]. Its use has been described in adult obesity, with anorectic effects the proposed mechanism underlying weight loss. The authors expected to replicate this decreased appetite resulting in reduced calorie intake, however diet logs showed no difference in calorie intake before and after treatment. There was, however, an increase in physical activity. There was no information on subsequent continuation or effects of discontinuation. A second study [122] also found either weight stabilisation/loss with dextroamphetamine.

A conference abstract explored the use of phentermine with either *dl*-fenfluramine or fluoxetine and behaviour modification (Table 1.16, Appendix 1) [123]. The authors report weight loss with no significant side-effects, but no details are given of how many patients lost weight, percentage weight loss or cardiovascular effects. Greenway et al studied the effects of caffeine and ephedrine, which they postulated would lead to weight loss by increasing sympathetic tone and metabolic rate (Table 1.17, Appendix 1) [124]. All three women studied lost weight, although one regained a significant amount and was referred for bariatric surgery. No details of duration of treatment were available for either study and no further studies of these medications have been published.

Other hormonal interventions

Supraphysiological doses of triiodothyronine (T_3) have been explored after a patient was treated for symptoms consistent with hypothyroidism, despite adequate serum free T_4 and T_3 levels (Table 1.18, Appendix 1) [125]. Two children with hyperphagia and HO were subsequently treated. In all patients baseline thyroid function tests were normal and introduction of T_3 resulted in raised serum T_3 concentrations. None exhibited any symptoms of hyperthyroidism except weight loss. The first patient had bone mineral density (BMD) monitored due to the known sequelae of decreased BMD in patients with hyperthyroidism, however this improved after starting T_3. All patients reported increased energy levels and decreased appetite. No other reports of treatment with T_3 are found in the literature.

Incretin mimetics are a more recent treatment possibility. Secreted from the L-cells of the gastrointestinal mucosa, glucagon like peptide-1 (GLP-1) is an incretin hormone that increases post-prandially after oral glucose ingestion, acting both centrally and peripherally [126]. Although its mechanisms remain only partially understood, peripheral actions include potentiation of glucose-dependent insulin secretion and suppression of glucagon release, with inhibition of gastric emptying and gastrointestinal motility. Centrally, activation of hypothalamic GLP-1 receptors inhibits appetite and receptors in the wider central nervous system, such as the brainstem, may influence appetite, with delay in gastric emptying leading to a secondary effect of increased satiety through central mechanisms [126]. The incretin-mimetics exenatide and lirgalutide, analogues of GLP-1, have been used in patients with type 2 diabetes mellitus (T2DM) and latterly in treatment of SO without diabetes [126]. Their use has more recently been reported in patients with HO both with [127-129] and without [129] T2DM (Table 1.19, Appendix 1). A small case series showed that incretin mimetics were beneficial in HO although mean BMI remained within the obese range even at the end of the study period [129]. The effects of treatment discontinuation in one patient studied was maintenance of weight loss, postulated to be secondary to increased physical activity as reported by the patient, however as treatment was restarted after a period of six months, the longer-term effects of discontinuation on weight maintenance are unknown. Despite disruption of one of the potential mechanisms of action (i.e. hypothalamic damage) incretin mimetics seem effective in HO, although further investigation is needed. Gastro-intestinal side-effects have limited their use in some cases.

Bariatric Surgery

Until lately, only individual case reports (Table 1.20, Appendix 1) [130-134], or small series [135, 136] have described bariatric surgery in HO. More recently a larger study of nine patients with HO [137] and a systematic review and meta-analysis of the literature were published [138].

The individual case reports describe reduction in BMI in six patients from between 42-67 kg/m^2 pre-operatively to between 32-53 kg/m^2 post-bariatric surgery, with improvement or resolution of co-morbidities. There were no reported side-effects in four patients followed-up for 6-30 months, but two developed post-operative complications of diarrhoea, dumping and acute gallstone pancreatitis (follow-up 6 years) and intestinal stenosis requiring laparotomy and unremitting bradycardia requiring a pacemaker (follow-up 2 years) [132]. A small series described laparoscopic adjustable gastric banding (LAGB) in four children with HO secondary to craniopharyngioma (Table 1.21, Appendix 1) [135]. Self-assessed quality of life and functional capacity were similar before and after LAGB, but pre-occupation with food lessened post-insertion and BMI fell. In four adults who underwent bariatric surgery (Table 1.21) one patient had ongoing weight gain post-bypass [136], however as bariatric surgery was undertaken only 1.5 years following initial tumour diagnosis when weight gain is known to be greatest it is possible that even more weight gain would have occurred without the bypass. Alternatively it is possible that bariatric surgery is ineffective in preventing initial weight gain in HO. In the largest reported case series only the two patients who underwent gastric bypass had good results; no weight loss was seen following LAGB insertion or sleeve gastrectomy (Table 1.22, Appendix 1) [137]. This retrospective review of nine patients with HO (including one previously described in a case report [131]) compared their outcome to that of 154 individuals who had undergone bariatric surgery for SO. Complications were the same in both HO patients and controls. There are no details of any earlier weight-loss strategies for any of the HO patients however weight gain had slowed in the previous year.

The recently published systematic review and meta-analysis includes the published case-reports and case-series (17 patients) and four previously unpublished patients (Table 1.23, Appendix 1) [138]. The mean age at CP surgery was 16 years and at bariatric surgery was 24 years. After one year, a third of patients (6/18 with data available) had lost > 20% of their baseline weight (none after LAGB). Only

patients who underwent RYGB or BPD had ongoing weight loss at this time; weight increased between 6 and 12 months in the other groups. The prevalence of diabetes had decreased from 31.6% at baseline to 8.3%. The overall effects of bariatric surgery in this meta-analysis may be disappointing, with a pooled mean weight loss of -15.14 kg (-31.68 to 1.39) at 12 months (excluding the patient who underwent BPD), however in comparison to the inexorable weight gain often seen in HO, these are positive findings.

There are no long-term studies of bariatric surgery in HO, the optimal surgical method is unclear and the underlying physiological mechanisms leading to weight loss are not yet fully understood. Future trials of bariatric surgery would benefit from a multi-centred collaborative effort, with standardised pre-operative assessment of clinical and biochemical risk factors and guidelines regarding which method of bariatric surgery should be undertaken.

Multi-disciplinary dedicated clinics

Finally, the setting in which patients at risk of HO are followed-up is important. Children attending a dedicated comprehensive care clinic had lower weight-gain than those in "standard care" clinics [81]. In the specialist clinic, children could attend monthly (as opposed to six monthly) and meet with a multi-disciplinary team (including endocrinologist, dietician, psychologists and exercise consultant). Oncology, psychiatry and surgical care were accessible if needed and patients could contact the MDT by phone or email in-between clinics. Initial assessment was extensive, lasting half a day and consisted of diet, exercise and QoL assessments. Treatment encompassed psychosocial aspects, diet and fitness advice and medication (where indicated). Thirty-nine children attended the specialist clinic, including 31 previously cared for in standard follow-up clinic. Most had a diagnosis of CP (33/39). The eight newly-diagnosed patients exclusively cared for in the comprehensive care clinic had a decrease/stabilisation in their percentage weight-gain from their first attendance. The percentage weight gain in the 31 children previously followed-up in the standard clinic also decreased, although this may have reflected the typical slowing of weight-gain in HO after a few years and it is impossible to separate out the effects. Other limitations of this study are the lack of

clarification of hypothalamic damage and the limited duration of follow-up in some patients (mean follow-up 0.97 years, range 3–41 months).

Conclusion

As with simple obesity, weight-loss treatments in patients with HO have limited success and advice regarding a healthy diet and physical activity remains the main-stay of treatment. As there is currently very little effectual treatment, further research into both prevention and management are imperative.

Contribution of this thesis to knowledge and development of studies

As can be seen from the literature presented, whilst HO is well described in children, particularly in those with craniopharyngioma, it is less well described in the adult population and knowledge of the underlying pathophysiology in all cases is uncertain. This thesis therefore aims to build on the existing retrospective studies of the prevalence of obesity in adults and children with acquired structural hypothalamic damage in order to better understand the prevalence and also to identify any underlying clinical risk factors for developing HO, to allow clinicians to better guide and support those patients at particular risk.

Clinical experience gained during specialist pituitary clinics reviewing patients with pituitary tumours also indicated another group of patients with an increased prevalence of obesity and other cardiovascular risk factors compared to the general population of a similar age - children and adolescents with pituitary adenomas. These tumours are uncommon in this age group and existing literature typically describes small case series and concentrates on their presentation and endocrine and surgical outcomes. Any evidence of increased obesity and cardiovascular risk factors in this young population would be clinically important to uncover. In addition, as many of these patients have microadenomas too small to encroach on the hypothalamus if this group of patients were confirmed to also have an increased prevalence of obesity it may indicate that additional mechanisms contribute to weight gain and obesity both in the presence and absence of hypothalamic damage.

In addition to the empirical studies planned to address these questions, as optimal treatment of HO remains uncertain and HO is often refractory to treatment, investigation of its underlying pathophysiology was important. Elucidation of this could give further insights into ways to either prevent HO occurring, or possibly aid with the development of more efficacious treatments. There are many hypotheses regarding the underlying pathophysiology/pathophysiologies of HO (hyperphagia due to hyperinsulinaemia, hyperghrelinaemia, leptin resistance or reduced PYY; increased 11β-HSD activity; reduced sympathetic activity; reduced physical activity) with evidence supporting and refuting all of these to some extent, as can be seen in the literature described in this thesis. In simple obesity theoretical models have been developed to consider appetite control and the development of obesity [139, 139-142]. These models explore the interactions of hormonal, neural and behavioural aspects of weight regulation, for example the relationships between the classic homeostatic regulatory systems (both involving the brain and hormonal/nutrient signals), those brain regions responsible for reward, cognition and higher executive functions, external/environmental stimuli and energy expenditure. Berthoud describes how two interacting regulatory networks – one homeostatic, the other non-homeostatic – may influence food intake [143, 144]. The former integrates appetitive hormones such as leptin and ghrelin and involves brain regions extending from the hypothalamus to the caudal medulla. The non-homeostatic network integrates metabolic, hedonic, behavioural and autonomic aspects in cortico-limbic regions such as the nucleus accumbens (NA; food reward), insula, striatum and hippocampus (reward expectancy/learning/memory) and pre-frontal cortex (PFC; executive decision making). Berthoud hypothesized that interactions of the non-homeostatic network with the homeostatic network through NA/hypothalamic pathways might allow the homeostatic network to be "overridden". The interaction of the neural networks controlling appetite and food intake is a mechanism which has not been explored in patients with acquired, structural hypothalamic damage. Destruction of the hypothalamus in these individuals may mean that these other alternative areas assume increased importance and just as in Berthoud's model the cortico-limbic areas may become dominant over the homeostatic regulators of food intake and appetite. In addition to identifying any changes in the neural networks of patients with hypothalamic damage, their effects on eating behaviour were also important to explore. Investigative techniques to explore both neural networks and eating

behaviour in patients with acquired, structural hypothalamic damage (both those becoming HO and those remaining weight-stable despite hypothalamic damage) were therefore researched to allow development of studies which considered both of these aspects and add novel information to the existing literature. The use of functional MRI (fMRI) scanning to investigate neural networks, the universal eating monitor (UEM) to examine the microstructure of eating behaviour and both Three Factor Eating Questionnaires (TFEQ) and three-day food diaries to examine the macrostructure of eating behaviour have been employed in studies of simple obesity as well as in studies of participants with an underlying medical disorder, however the first two have not yet been utilized to study adults with acquired, structural hypothalamic damage. Their use in obesity research is discussed in the subsequent pages and their application to the study of HO explained. In addition, measurement of appetitive hormones such as insulin, ghrelin and leptin during the study days was planned to augment existing knowledge.

Investigative Techniques

Use of fMRI in obesity research

In recent years, functional neuroimaging (positron emission tomography [PET] and functional magnetic resonance imaging [fMRI]) has identified brain areas differentially activated by appetite and food consumption in different clinical and experimental conditions. PET works by injecting a radioisotope tracer into the peripheral circulation, combined with either glucose (^{18}F-FDG) or water (^{15}O). Radioisotope decay in various brain regions (as a marker of metabolic rate or blood flow, respectively) can then be detected. PET has reasonable spatial resolution and specific radioisotopes for various neurotransmitter receptors or transporters can be used. The disadvantages/limitations are exposure to ionising radiation administered via an invasive procedure (injection/cannulation) [145] and spatial resolution (5mm, compared to fMRI which can be as little as 1 mm). Functional MRI (fMRI) is a non-invasive modality that can detect transient haemodynamic changes in the brain in response to a variety of stimuli by utilising the different magnetic properties of oxy- and deoxy-haemoglobin. These changes in blood-flow are representative of underlying neuronal activation and compare two states (e.g. looking at food- vs. non-food pictures). The advantages over PET are its non-invasive nature, no radiation exposure and a higher spatial and temporal resolution [146] which is important in monitoring a dynamic process such as feeding. One disadvantage is the difficulty in imaging certain areas, for example the brainstem due to motion/other artefacts caused by respiratory and cardiac cycles [145] or the orbito-frontal cortex (OFC) as air in the nasal cavity/sinuses can lead to signal loss, causing areas of drop-out [147]. Neither technology measures neuronal activity directly and therefore cannot identify the specific neuron activated, only a region where increased blood flow is likely to indicate increased neuronal activity. Small areas or brain structures are therefore difficult to identify as it would require large changes in blood flow to detect a signal difference large enough to survive contrast and further statistical analysis [146, 148]. Both are costly and are somewhat restrictive in the size/weight of obese participants that can be studied, however have been successfully used to explore the homeostatic and non-homeostatic brain regions involved in regulating appetite and eating.

Whereas PET studies examine resting brain activity or metabolism, fMRI studies compare responses to tasks, such as viewing different types of food pictures.

Cerebral regions of interest in appetite and eating identified from neuroimaging studies

Multiple brain areas related to appetite and eating have been identified in fMRI and PET studies, reflecting the many different aspects of feeding-related cerebral processes and also the differences in the study protocols and paradigms. Tataranni undertook the seminal PET study of hunger and satiety in humans (using ^{15}O-labelled water), with many subsequent studies identifying both common and novel brain regions (Table 1.24, Appendix 1) [149]. Many early studies contrasted fasting and fed states, with later studies using food images under different conditions, e.g. after eucaloric- vs. over-feeding or in lean vs. obese participants. Variations in study design make direct comparison between studies difficult and may account for some of the apparent discrepancies in findings. Carnell et al categorise these design differences into three areas - imaging methodology, study paradigms (including the physiological state of the participant) and participants involved [150]:

1. Imaging modality: PET scanning (which uses different radioisotopes, most commonly ^{18} F-FDG, but also ^{15}O) offers a more direct assessment of neuronal activation than fMRI but has inferior spatial resolution which must be considered when comparing studies using these two different modalities. Physical issues such as the size of the hypothalamus, where there can be difficulty in distinguishing between functionally distinct areas, and signal drop-out in areas such as the OFC (close to sinus air cavities) may make it difficult to detect variations in some areas without using specific techniques to modify image acquirement or analysis which specifies these areas as *a priori* regions of interest (ROIs). In order to include some small ROIs which may be of interest the field of view may need to be narrowed to allow for adequate image quality or thinner "slices" acquired.

2. Study paradigms: Whilst some studies use resting-state scanning, others involve task-related paradigms. There is not only a difference between the cues used (real food, liquid meal, food images, imagined food), but in the ways that they are

presented (taste of a food/liquid meal, feeding to satiety, images of high- vs. low-calorie foods, high- vs. low-fat foods, palatable/unpalatable/disgusting foods, with comparison to neutral object images, food-related images or images matched for emotional valence). Some study methodologies include only fasting or fed states, while others compare fasting with fed states. One problem in studies comparing fasted and fed states is the potential confounding of scan order (satiation must always follow fasting if a significant period of fasting is required) [151] or alternatively the need to undertake scans on separate days, which also may introduce bias. Variations in the length of fasting will also have an impact on physiology – where some fasting periods were as short as 1.5 hours, others were significantly longer with the potential that prolonged fasting may have led to increased salience of the stimuli across all groups, therefore potentially negating differences between participants in different study groups.

3. Participants: Physical size and technical limitations may restrict studies to participants with a BMI <40 kg/m^2. Another inadequately studied group are those with BMI 25-30 kg/m^2 who are neither lean (BMI <25 kg/m^2) or obese (BMI >30 kg/m^2). Varying definitions of obese and non-obese/lean have been used in different studies. Individual factors such as age, gender, handedness, phase of the menstrual cycle of female participants and pubertal stage of adolescents studied may also account for variations between (or within) studies. A few papers describe repeated fMRI studies of within-participant changes in weight over time, which also has advantages and disadvantages.

Additionally, data analysis methods vary between studies, for example whole-brain analysis vs. *a priori* ROIs, different software packages (SPM, brain voyager) are used and varying statistical thresholds are employed when reporting significance.

In spite of these difficulties with variations in study design, review papers do identify with some consistency several brain areas in which food cues are associated with activation differences [145, 150, 152-157]. These are not only homeostatic areas which regulate appetite, but also reward-related regions like the insula, orbitofrontal

cortex (OFC) and dorsal striatum. Differences in cerebral activation with food/food cues are often more significant when fasted compared to fed, with more palatable/pleasant food and with greater hunger [145].

Many studies demonstrate increased activation in reward-related regions in obese compared to lean participants, as summarised in several review papers [145, 150, 152-156] (Table 1.25), however individual studies vary, possibly due to the reasons described above.

Table 1.25. Brain regions frequently associated with increased activation in obese compared to lean participants/predictive of weight gain and their described function.

Brain regions	Function	Reference
Anterior cingulate cortex (ACC)	Conflict monitoring/cognitive inhibition /reward-based learning/decision making	[150, 152-155]
Amygdala	Emotion/aversion	[150, 152-155, 158]
Anterior insula/insula	Taste/interoception/emotion/ gustatory processing	[150, 152-156]
Caudate/putamen (dorsal striatum)	Reward/motivation	[150, 152-156]
Hippocampus	Memory	[150, 153]
Medial/lateral orbitofrontal cortex (OFC)	Reward/emotional decision-making	[150, 152-156, 158]
Nucleus accumbens (NA)/ventral striatum	Reward/anticipation/motivation/expectancy	[150, 152-155, 158]
Parietal cortex	Spatial attention	[150]
Pre-frontal cortex (PFC)/medial PFC/ dlPFC/vmPFC	Motivation/executive function/ processing of cues into behaviour/ self-control/expectation	[150, 153]
Ventral pallidum	Reward/incentive motivation	[152, 153]
Ventral tegmental area (VTA)	Dopaminergic projections to limbic areas	[153]

Although many studies identify *hyperactivation* in reward-related regions in obese compared to lean participants, there remains a seemingly opposing hypothesis of *hypoactivation* of reward-related systems underlying the development of obesity [152, 154, 158-160]. Differences in study paradigms (as described above) may account for these divergences, for example several review papers describe an increased striatal response with palatable food *cues* (i.e. where there is the *prospect of ingestion*), but a decreased response to *actual ingestion* [153-155, 158].

Hypoactivation in areas responsible for homeostasis, cognitive control and attention have also been identified in obese compared to lean participants [156], for example reduced inhibitory control from the PFC [157, 158].

The most recent review paper describes the introduction of techniques such as functional connectivity and brain network analysis, which have led to further developments [160]. It also conveys the evolution of functional neuroimaging in investigating obesity, describing the latest studies where consideration of the interaction between hormonal, behavioural and neuroimaging data has allowed studies to explore the underlying mechanisms of obesity based on hypotheses formed using disease models. Longitudinal studies investigating the neural control of ingestive behaviour *preceding* weight gain/obesity are important as cerebral responses to food/food cues identified in participants who are already overweight may be due to neural adaptations to the adiposity which has developed and represent a consequence of obesity, rather than a cause [145, 160].

Food presentation during fMRI studies

Early neuroimaging studies using PET scanning demonstrated differences in brain activation in various regions before and after a liquid meal [149, 161, 164, 166, 168, 169]. A few studies have used imagined [167] or actual food [170], but most recent studies have used photographs of food and non-food items . The non-food items are used to distinguish brain activation due to any visual stimuli from activation due to food stimuli.

Some studies have investigated whether differences exist in cerebral activation between different types of food; high- vs. low-calorie [175-177, 179],

high- vs. low-incentive [167], high- vs. low- hedonic [178] and high- vs. low-fat foods [180]. These categories are similar, but not identical, making comparison between studies difficult.

On review, these studies used non-standardised images, for example the size and quantity of food in the photographs was different, or they contained distracting backgrounds such as a checked table cloth. This potentially influenced their outcomes and was important to consider when designing the fMRI study described in this thesis and when analysing and comparing these studies.

Hormone, nutrient and appetite correlates in functional neuroimaging studies

Several studies have measured nutrients and hormones, such as glucose, free fatty acids, insulin, leptin, GLP-1, gastrin and pancreatic polypeptide alongside functional neuroimaging [169]. Plasma insulin in particular has often been correlated with differences in brain activation [168]. Peptide YY 3–36 (PYY3-36), insulin, leptin and ghrelin are known to be involved in cerebral regulation of appetite [182], with receptors present in areas such as the amygdala and hippocampus (ghrelin receptors) [183]. The hypothalamus in particular has numerous hormone receptors including ghrelin, leptin, NPY, AgRP, insulin and glucose in hunger and satiation-signalling nuclei [184].

Both observational studies of ghrelin concentrations and BOLD signal [185] and the effects of infused ghrelin on brain activation have been studied using fMRI [186-188]. The observational study of 26 normal weight (mean BMI 21.1±2.0 kg/m2) young adults (mean age 24.4±3.4 years, 13 male) found a correlation between endogenous fasting unacylated ghrelin concentrations and fMRI BOLD signal when viewing food (as opposed to control) pictures in the bilateral caudate, pallidum and midbrain (reward-related regions), middle and superior occipital/temporal/fusiform gyri (visual processing regions), rolandic operculum, amygdala, thalamus and anterior cingulate gyrus (taste regions) and right hypothalamus [185]. The authors found no significant correlations between BOLD signal and a decrease in endogenous ghrelin concentrations seen following an oral glucose load. The first study involving infused ghrelin compared BOLD signal whilst viewing food images before and after ghrelin infusion in 12 normal weight males

(mean age 24.1±1.1 years, mean BI 22.2±0.5 kg/m2) with that of 8 normal weight male controls (mean age 23.2±1.3 years, mean BI 22.3±0.7 kg/m2) who received a saline infusion [186]. Imaging commenced three hours after a standardised breakfast, when endogenous ghrelin would be at its lowest, with the relevant infusion given between two scanning sessions. Those who received ghrelin had significantly greater BOLD signal in response to food compared to scenery photographs in reward-related regions - bilateral amygdala and anterior/mid-dorsal insula, left OFC and caudate, right substantia nigra/VTA and hippocampus, as well as visual areas (pulvinar and fusiform gyrus). The increase in BOLD signal in the bilateral amygdala, left OFC and left pulvinar following ghrelin administration correlated with self-reported VAS ratings of hunger. The control group were included to exclude order effects. Examination of the interaction effect between the groups found significantly greater increases in BOLD signal in the bilateral anterior insula and fusiform gyrus, left OFC, mid-insula and pulvinar, and right substantia nigra/VTA in those who received ghrelin. A physiological/ pharmacological fMRI study of 20 non-obese adults (mean BMI 25.1, range 21-28 kg/m^2) showed that a post-prandial ghrelin bolus caused a decrease in the BOLD signal in the medulla, midbrain and pons, cerebellum, superior hypothalamus, insula, para-hippocampal (amygdala/hippocampus) and post-central gyri and thalamus compared to a saline infusion during a 30 minute physiological/pharmacological fMRI scan, likely signifying a blunted response to feeding following ghrelin administration [187]. When ghrelin was given alongside an intra-gastric infusion of C12 fatty acid, the CCK-induced hypothalamic, brainstem, insula and amygdala/ hippocampal activations were reduced. A pre-prandial ghrelin infusion led to a small increase in BOLD signal in similar areas (also motor cortex and inferior hypothalamus) during a 54 minute physiological/pharmacological fMRI. Another study compared 22 normal weight adults (17 male) under different physiological conditions, whilst undergoing an fMRI picture-evaluation task – a) following an overnight fast and subsequent saline infusion (fasted-saline) where ghrelin would be endogenously increased; b) following a standardised breakfast meal with subsequent saline infusion (fed-saline) where ghrelin would be suppressed; and c) following a standardised breakfast meal followed by a ghrelin infusion (fed-ghrelin) where ghrelin would be exogenously increased [188]. Whole-brain analysis found no significant differences between these three states for the contrasts any food, high-energy or low-energy food photographs compared to objects however

examination of *a priori* ROIs showed significant differences in the OFC and hippocampus. In the OFC, BOLD signal was significantly greater with higher ghrelin concentrations (fasted-saline and fed-ghrelin) than with lower concentrations (fed-saline) when viewing both high-energy foods and all foods (high- and low-energy) compared to objects. In the hippocampus there was significantly greater BOLD signal with higher ghrelin concentrations (fasted-saline and fed-ghrelin compared to fed-saline) when viewing all foods compared to objects. Additionally, when viewing high-energy or low-energy foods compared to objects there was a significant difference in BOLD signal between fasted-saline and fed-saline for both picture types, and fed-ghrelin and fed-saline for low-energy foods. There was no significant difference in any of the other ROIs studied (NA, caudate, anterior insula or amygdala) with any of the photograph contrasts (high-energy, low-energy or all foods compared to objects) in any of the conditions (fasted-saline, fed-saline, fed-ghrelin). There was no correlation between BOLD signal and food appeal rating or ghrelin concentrations in any of the conditions in either the OFC or hippocampus. Similar results were found when female participants were excluded and data from only males examined.

The differing effects of glucose and fructose on the brain have been investigated. Glucose ingestion significantly decreased cerebral blood flow to the hypothalamus (an *a priori* region of interest) compared to fructose when given to 20 participants (10 female, 10 male; mean BMI±SD 22.5 ± 2.5 kg/m^2) following an overnight fast in a blinded, randomised cross-over manner [148]. Participants underwent an MRI scan, followed by ingestion of a drink containing either glucose or fructose, with a repeat scan after 60 minutes. Participants then received the alternate sugar in a second episode between one week and two months later. Whole brain analysis showed that whilst glucose decreased hypothalamic, thalamic, insula, anterior cingulate and striatal activation, fructose lessened activation in the thalamus, hippocampus, posterior cingulate, fusiform and visual cortex. Functional connectivity analysis, using the hypothalamus as the starting point (seed region) showed increased connectivity between the hypothalamus and the thalamus, caudate and putamen with glucose, but only with the thalamus following fructose ingestion. In further analysis, changes in the striatum (caudate and putamen) correlated with changes in plasma insulin in response to glucose ingestion. These areas are involved with motivation and reward [148].

Feelings of hunger and satiety experienced by individuals during neuroimaging studies have been evaluated using Visual Analogue scale (VAS) ratings [189]. These quantify participants' subjective feelings of appetite by asking them to mark their hunger, fullness and desire to eat on a straight 10 cm line, which is then measured. VAS ratings have been used to confirm hunger and fullness when comparing fasted and fed states [147, 161, 164, 166, 167, 178, 179, 183, 190]. Long-term eating behaviour and attitudes are also important and the Three Factor Eating Questionnaire (TFEQ) [191] has been used to quantify long-term hunger, whether participants actively restrict their diet or if they have disinhibited eating [164, 167, 171, 190, 192]. These are particularly important where disordered eating (such as Prader-Willi Syndrome or bulimia) may exist.

Use of functional neuroimaging in participants with an underlying medical disorder

Of particular interest and relevance are functional neuroimaging studies undertaken in participants with disordered eating such as leptin deficiency, Prader-Willi Syndrome (PWS) and melanocortin 4-receptor (MC4R) mutations.

Individuals with congenital leptin deficiency are significantly hyperphagic. This resolves with leptin supplementation. An fMRI study of three leptin-deficient adults examined participants in a fed state after 57 months of leptin supplementation, subsequently stopped for 33 days, then re-introduced before final data was gathered [193]. Self-reported hunger was higher without leptin treatment (statistically significant difference in 2 participants and approaching significance in the third), as was BMI (mean increased from 27.7 to 29.6 kg/m^2, $p = 0.009$). Viewing high-calorie compared to low-calorie foods in the leptin deficient state led to greater activation of the bilateral insula, right limbic lobe/parietal postcentral gyrus/occipital precuneus and left parahippocampus/parietal supramarginal and temporal fusiform gyri. Leptin supplementation resulted in greater activation of the left superior-, bilateral medial- and right middle-frontal gyri of the PFC, left cingulate gyrus/occipital cortex, right frontal lobe/pons and bilateral cerebellum. Increased insula activation during leptin deficiency was particularly noted, correlating with increased reported hunger and the

known insatiable appetite of those with leptin deficiency. The effects of leptin supplementation in greater activation of the PFC may reflect the satiation-induced inhibitory processes described in other studies. Another study of two adolescents with leptin deficiency compared VAS ratings of hunger, satiety and food-photograph "liking" and differential brain activation using fMRI before and after seven days of leptin treatment [194]. The short duration of treatment meant that no changes in BMI had occurred, but a significant effect on food intake was documented. Leptin treatment resulted in decreased fasting hunger, increased post-meal satiety and decreased liking of food photographs, compared to pre-treatment. Comparison of fMRI-measured brain activation showed that before treatment, food photographs (vs. non-food images) resulted in increased activation of the NA, caudate, putamen and globus pallidus. This was not evident in the leptin-replaced state. Food-photograph liking ratings were also decreased in the leptin-replaced fed state, compared to when untreated. Activation of the NA and caudate were positively correlated with liking ratings in both the fasting and fed states pre-treatment, but post-treatment only in the fasted state. This reflects changes in neuronal activation in the leptin-treated state in an area responsible for food reward, suggesting that leptin is important for this aspect of eating behaviour. Both studies are limited by the extremely small number of participants, which reflects the extreme rarity of the condition. The first study reported uncorrected whole brain analysis, with the second corrected for multiple comparisons, although it is not stated whether ROI or whole brain analysis was undertaken.

In Prader-Willi Syndrome (PWS), extreme hyperphagia leads to morbid obesity. A study of 13 adults with PWS compared PET findings (with ^{15}O tracer) whilst viewing food photographs in fasted and fed (400 kcal or 1200 kcal breakfast) states [189]. The authors found that when fasted there was greater activation in the hypothalamus, amygdala, basal ganglia and ACC (similar to their findings in non-obese individuals [167]), as well as the lateral OFC and inferior temporal cortex. In contrast to individuals without PWS [149, 167-169], there was no increased activity in areas associated with satiety (PFC, OFC and temporal cortices) when comparing fed to fasted states. VAS ratings before scanning showed significantly higher hunger and desire to eat and lower fullness on the fasted study day than when fed a 1200 kcal breakfast. However, ratings taken immediately after scanning on both of these

days were not significantly different, indicating that not all participants remained satiated following breakfast, which may have influenced the findings. Post-hoc analysis found increased activation in the medial OFC of the 6 participants who remained significantly full, compared to the 6 other participants. The authors also found that the medial OFC was greater activated following a 1200 kcal breakfast, compared to the lateral OFC which showed increased activation after a 400 kcal breakfast. They hypothesized that the medial OFC evaluates reward, whereas the lateral OFC has a separate function associated with punishment leading to behaviour change. The study is limited by a lack of direct comparison between participants with and without PWS.

This has been undertaken in other studies. One study compared 9 adolescents with PWS to 9 age-matched, normal-weight controls, undertaking fMRI whilst viewing images of food or animals [195]. In the PWS group, following a 500 kcal meal there was greater activation in the right amygdala/OFC/fusiform gyrus, left parahippocampus and bilateral medial PFC/insula when viewing food photographs compared to controls (Table 1.26a, Appendix 1). A second study (also fMRI) compared 14 young overweight/obese participants with PWS to 14 age-matched SO controls and 15 lean controls [171]. There was significantly greater reported hunger, disinhibition and dietary restraint in SO than in lean controls and in PWS than in both other groups using a modified TFEQ. ROI analysis demonstrated significantly greater activation (uncorrected for multiple comparisons) in the NA and amygdala in PWS compared to SO when viewing food stimuli before a meal (Table 1.26b, Appendix 1). Post-prandially the PWS group had greater hypothalamic and hippocampal activation compared to SO, with persistence of increased amygdala activation. Greater post-prandial dorso-lateral PFC activation in SO compared to PWS participants remained significant when corrected for multiple comparisons. This study supported many of the findings of the earlier paper by Hinton [189].

The authors concluded that in PWS there was hypoactivation of the left dlPFC, however the same area also had greater activation in SO compared to LCo, therefore hyperactivation in SO rather than hypoactivation in PWS may be present. One possibility is that individuals with SO need greater dlPFC activation post-prandially to inhibit areas responsible for the initiation of feeding, as found by Gautier [168], whereas in PWS the hyperphagia is due to increased activation of the hypothalamus, amygdala and hippocampus (all greater activated post-meal compared

to both SO and LCo). As PWS is associated with hyperghrelinaemia and ghrelin receptors are prolific in these areas [171], the increased activation present here seems logical. This is consistent with a different underlying pathophysiological mechanism for obesity in PWS compared to SO.

Melanocortin 4-receptors (MC4R) are found in significant numbers in the hypothalamus, brainstem, striatum and other brain regions [196]. As part of the leptin pathway stimulation of these receptors by α-MSH has anorexigenic effects and inhibition by AgRP has orexigenic effects [11, 35]. They are also affected by ghrelin and PYY. There is evidence from rat models that they are important in food reward. An fMRI study compared BOLD signal in eight patients with MC4R mutations to 18 controls (10 overweight/obese, 8 lean) one hour after a standardised lunch meal, calculated as a proportion of estimated daily energy requirements [196]. As the authors were interested in the effects of food reward, they primarily conducted an ROI analysis of the caudate/putamen and ventral striatum. This revealed lower BOLD signal in the left dorsal striatum of SO controls than in those with MC4R mutations and lean controls when viewing appetising compared to non-appetising foods. The same contrasts also showed lower activation in the left ventral striatum in SO controls than in those with MC4R mutations and increased activity in the inferior parietal cortex in MC4R participants than SO and lean controls during exploratory analysis (at a statistical threshold of $p<0.001$ uncorrected). When contrasting BOLD responses to appetising food photographs against non-food photographs, again there was decreased activation in the left dorsal striatum of SO controls compared to those with MC4R mutations and lean controls. There was no difference detected in the amygdala or OFC between the groups at $p<0.001$ uncorrected. Pleasantness and disgust were measured using VAS ratings and did not differ between the groups. The authors speculate that the reduced responsiveness of the reward-related striatum in a satiated state seen in SO controls may lead to over-eating to compensate, resulting in obesity. As the decrease is not seen in those with MC4R mutations, the authors argue that this implies that the decreased striatal response is not secondary to overconsumption, but that the alterations in neural response occur with the weight gain and need a working melanocortin pathway to arise. The reason for the somatosensory changes in MC4R participants is unclear and it is interesting to note the lack of differences except for this between them and lean controls.

Conclusions

The use of functional neuroimaging in participants with congenital leptin deficiency, PWS and MC4R mutations and the differences found between these groups and lean or SO controls demonstrates its utility in studying participants with disordered eating. Functional neuroimaging therefore lends itself to the study of adults with HO, a group in whom it has not yet been used. This thesis therefore describes the novel use of functional neuroimaging in adults with acquired, structural hypothalamic damage and explores the differences between those who develop HO and those who remain weight-stable (HWS).

Study of human eating behaviour: Assessment of the microstructure and macrostructure of feeding behaviour

Eating behaviour is an important consideration when investigating the pathophysiology of HO as hyperphagia has been described in both humans [11, 23, 32, 40, 41] and animal models, although is not considered essential for the development of HO [16, 42, 43]. Eating behaviour can be considered in terms of its macrostructure *and* microstructure. The macrostructure of eating behaviour describes patterns of food consumption throughout an entire day as well as longer-term behaviours, whereas the microstructure of eating behaviour relates to the pattern of food consumption during individual eating episodes [197]. Studying the micro- as well as the macro-structure of eating behaviour allows additional information, such as eating rate and changes in eating rate to be gathered [198], alongside intra-meal subjective assessments of appetite feelings. Since both single meal and longer-term intake may lead to excessive weight-gain, it is important to consider both in patients with hypothalamic damage and subsequent obesity.

Eating behaviour has different components, including physiological, behavioural and cognitive factors. Assessment of all these various factors is important however is not an easy undertaking [199] and can never be error-free [200], with advantages and disadvantages to all methods. Whether to assess eating behaviour in the laboratory or the "real-world" is another consideration. The reliability and accuracy of laboratory-based assessments must be balanced with its

potential to influence findings, whereas assessment of eating in participants' everyday environment, where normal influences and patterns have been established, may be less accurate [201].

Study of within-meal microstructure of human eating behaviour

The study of the microstructure of eating behaviour involves assessing food intake within (and/or immediately before or after) a meal and recording of subjective appetite measures such as hunger and satiety using visual analogue scale (VAS) ratings [202, 203].

Microstructure studies have demonstrated differences between groups with different characteristics, under different experimental conditions and influenced by disease. The first direct measurement of within-meal food intake involved liquid-food consumed through a straw from a hidden reservoir [204]. The system accurately recorded intake in 14 individuals studied, with good correlation between subjective measures of hunger and the rate/amount of liquid ingested. A similar system compared obese, normal-weight and under-weight subjects (described in detail below) [205]. Assessment of semi-solid food consumption was first undertaken in 1980 [206]. The authors compared liquid and semi-solid meals of the same caloric value consumed on different study days. Computer-connected scales recorded intake every 3 seconds until no significant change occurred for 15 minutes. There was no difference in the total amount consumed between conditions, however rate of consumption, initial eating rate and deceleration in eating rate were greater with liquid. Both meals were equally well-liked. The authors concluded that their equipment could be used with either semi-solid or liquid food.

Kissileff et al named their equipment the Universal Eating Monitor (UEM) [206]; other similar equipment with differing names (Endogram, Sussex Meal Pattern Monitor and VIKTOR) has been described by others since [197, 207-209]. For consistency, all papers described in this thesis will be referred to as UEM studies.

The UEM works by measuring food intake via sensitive scales connected to a computer, hidden under a thin plastic mat under the bowl/plate of food (Figure 1.6). To allow continual monitoring, the food must be liquid or semi-solid, for example yoghurt [203, 206, 210-212], Swedish hash [213-216], chocolate pudding [217, 218] or pasta [197, 207]. Foods such as sandwiches (picked up to eat) or meat and

vegetables (requiring cutting and eating in chunks) are not suitable for several reasons. Firstly, cutting of food may affect the accuracy of the scales, due to pressure exerted. Secondly, solid foods which are picked up whole may not be entirely consumed and need to be returned to the plate, which may not always occur, leading to inaccuracies. Even if the entire portion picked up is eaten, this may take a varying amount of time, leading to less accuracy than using food consumed in a single mouthful. UEMs continually record intake, enabling generation of cumulative intake curves in addition to accurate measurement of total consumption and meal duration. Contemporary systems allow subjective assessment of appetite ratings of hunger, satiety and food pleasantness with VAS measures by regularly interrupting participants (time- or quantity-based) to complete on-screen ratings. The advantages over paper-based VAS ratings are that the participant can be accurately interrupted without need for an experimenter and each rating is individual as it cannot be compared to previous ratings [219].

Figure 1.6. Photograph of participant consuming food at the UEM.

Validity

An important consideration is whether laboratory eating is reflective of real world intake. Kissileff and Thornton found a significant correlation between UEM consumption and real world intake when comparing food diaries and laboratory intake. Mean lunch intake eaten in the laboratory was 888kcal (±387 SD) in males, compared to 850kcal (±547 SD) eaten in a non-laboratory setting and 492kcal (±143) compared to 381kcal (±112 SD) in females, respectively. The variability in intake outside of the laboratory however (reported in food diaries) was greater, reflecting the multitude of influences on eating in everyday life such as nutritional variability and social interactions [220].

Reliability

Also important are consistency and reliability. Several papers describe good consistency within individual participants [204, 218, 220, 221], with greater inter-participant variability than intra-participant [205, 222]. One study found that after two days of familiarisation with the equipment, little variation in individual intake occurred over subsequent visits (3-7 days) [205], while another found it took between 3-5 visits before participants demonstrated a consistent intake (within 5-35% of their mean ingestion) [204]. Familiarisation is important as other studies have found lower intake on the first occasion compared to subsequent episodes (376.82 g vs. 419.21 g, $p<0.04$) [212], with no significant difference in subsequent sessions [220]. Hubel found that whilst the test-retest correlation for intake amount was good, the mean intake at each episode was significantly different (i.e. all individuals ate more on the second occasion, giving a consistent increase) [212]. These findings suggest that unfamiliarity with the laboratory environment and UEM equipment at the first episode may falsely lower intake, with a more authentic consumption on subsequent episodes. This is important when drawing conclusions from studies based on two eating episodes and also in study design.

Not all factors are so consistent. Hubel found that while intake amount and duration, initial eating rate, number of bites and bite size were significantly reproducible on re-testing, change in eating rate was not well correlated [212]. The authors speculated that changes in eating rate may be influenced by external stimuli, rather than inherent. Given this, studies showing changes in eating rate under

different experimental conditions need to be interpreted with caution. Other factors speculated to influence reliability are intrinsic differences between participants, although in 61 participants studied, differences such as gender, BMI, disinhibition and cognitive restraint did not affect the reliability of food intake across two episodes [212]. These may, however, be causes for variations between different groups, as discussed below.

UEMs have therefore been shown to be *both* reliable and reproducible in investigating the microstructure of eating behaviour and can determine differences in the rate of food consumption across a meal, although comparisons between eating rate on different occasions must be interpreted with caution.

Analysis

Finally, the method of data analysis must be considered. Various methods of analysing UEM data have been used, which makes comparison across studies difficult and may lead to differences in data interpretation [207]. Kissileff's first paper used graphs, termed cumulative intake curves, to measure food intake [206]. Subsequently, 11 different methods of data analysis were compared to find the best model to fit the data of 16 participants [220]. The quadratic equation accounted for 96.3-99.9% of variance in cumulative intake and provided the simplest model. Cumulative intake curves were useful for visual comparison of eating patterns, but could be up to 30% different from the mean actual intake over several meals. Quadratic equations were more accurate and allowed more precise inter- and intra-participant comparison. The parts of the quadratic equation fit two distinct parts of the curve - initial intake rate (linear coefficient) and rate of deceleration at the end of the meal (quadratic coefficient). These correspond with the stimulatory/excitatory aspect of eating (hunger) and negative feedback/inhibition of eating (satiety) respectively [220]. A negative quadratic coefficient indicates a decrease in eating rate (seen with a decelerated eating curve), a positive coefficient describes an accelerated eating rate and where the quadratic coefficient is zero, the eating rate has stayed constant (a linear or non-decelerated curve).

Other investigators have compared the amount eaten in different segments of the meal (temporal quarter analysis). Temporal quarter analysis of data from 92

participants found that in 39 participants who had decelerated intake curves deceleration was secondary to decreasing bite size rather than eating rate, which remained constant [223]. Decrease in consumption began in the third quarter but only became significantly different from the initial rate during the 4^{th}. Fixed time analysis undertaken for 55/92 did not identify a particular time when eating rate changed. There was no difference in the total meal size, meal length, or percentage change in the cumulative intake slope (i.e. rate of deceleration) between the 86 normal-weight and 50 overweight females studied.

Yeomans concluded that both the quadratic equation method and meal quartiles approach should be used due to a lack of evidence regarding the best method [219]. Dovey et al analysed a single data set using three different methodologies; visual ascription, area under the curve and coefficient approaches [207]. They found that whilst the first two methods did not demonstrate any difference between data obtained in a control condition (relaxation before the eating episode) and an experimental condition (a stressor consisting of submerging a hand in cold water before the eating episode), the coefficient approach revealed differences in VAS ratings of fullness. They describe a modified coefficient approach where data may be quadratic (eating rate changes at one point over the course of food ingestion) or cubic (eating rate changes at more than one point). Whilst the authors acknowledge the possibility of a Type I or II statistical error leading to the difference found when using this method, they argue that the highly significant nature of the difference on MANOVA analysis ($F_{(2,20)}=5.621$; $p = 0.012$) makes this unlikely. This paper demonstrated that although the total cumulative intake was similar between two experimental conditions, the pattern of subjective rating of fullness differed.

Intrinsic and extrinsic factors influencing eating behaviour

Initial eating rate is felt to reflect hunger however can also be influenced by factors such as food palatability or duration of fasting [220]. Eating rate at the end of a meal, reflecting satiety, may be influenced by factors such as the energy density of food consumed or the effects of gut hormones. While meal intake could be proposed to be a balance of hunger and satiety, termination of eating may not necessarily

reflect merely a physiological equilibrium of the two. In a comparison of predicted and actual mean intake, one study found that actual meals terminated before that predicted [220]. The authors proposed several possible reasons for this: i) use of a single homogenous food resulted in sensory specific satiety; ii) participants' previous experiences may result in meal termination (knowledge that continuing to eat will result in discomfort); iii) cognitive factors (awareness of calorie intake and effects on weight). Additionally, as there is a significant correlation between initial eating rate (hunger) and rate of deceleration at meal end, the deceleration may reflect a decline in hunger rather than increasing satiety. It is therefore important to consider both intrinsic and extrinsic factors influencing eating behaviour.

Intrinsic factors

1. Bodyweight

One study reported increased intake in obese/overweight participants [217], whilst most report no difference [205, 222, 224]. Laessle et al found a significantly greater mean intake in overweight compared to normal-weight participants (both genders), with larger spoonful size and faster initial eating rate [217]. The duration of eating and deceleration in intake was not significantly different. Another study found no difference between lean and obese participants in total intake or intake duration, but that obese participants ate faster ($p<0.05$) [224]. Binge-eating was seen in 22 obese individuals compared to only 4 lean participants.

Initially it was proposed that overweight/obese individuals fail to slow their eating rate towards the end of a meal, with two distinct patterns of consumption described –

1: 80% of intake in the first half of the meal, with a *decelerating* eating rate at the end;

2: linear intake with ≤ 50% consumed during the first half, giving a *non-decelerating* eating pattern [205].

Non-overweight individuals were more likely to eat in the first pattern, while those overweight were more likely to have a non-decelerated pattern. The authors postulated that normal-weight individuals feel satiated and decrease their eating rate at the end of a meal, but in overweight individuals a loss of normal satiety signals leads to lack of deceleration. Mean total intake was similar across the groups, but

there was greater variation in the overweight group, with a tendency to hyperphagia during stressful stimuli. Another study of 20 obese and 20 normal-weight participants found that those with normal-weight had a greater decrease in eating rate and significantly higher proportion of decelerated curves than obese [216]. This may suggest that although there was no significant difference in the amount eaten, obese individuals have blunted satiety awareness. Whilst these findings have been replicated in some studies [225, 226], other studies have found that overweight individuals have a decelerated eating pattern [197, 214, 217, 223, 224] with accelerating eating patterns found in a third of obese participants [227, 228].

One difficulty when comparing studies is the lack of consistency (definitions of obese vs. overweight, length of fasting, type of test-meal and study design) which should be taken into account in future research to allow more robust conclusions and comparisons.

2. Bodyweight and restraint

Several studies have found no relationship between BMI and change in terminal eating rate but have found eating rate and cognitive restraint closely linked [202, 222, 223]. These authors argue that restraint, rather than physiological factors, influences eating behaviour. The first study found greater cognitive restraint of eating, disinhibition and hunger in overweight compared to normal weight women ($p<0.001$ to $p<0.05$) [222]. Cognitive restraint and disinhibition were negatively correlated with decelerated eating in normal-weight women, but slightly positively correlated in overweight women. In those with greater disinhibition, normal-weight women were consistently non-decelerated eaters whilst overweight women were decelerated eaters. Differences in BMI alone were unrelated to the pattern of intake however restraint and disinhibition were significant factors. In a second study, there was no significant difference in intake between the groups normal-weight restrained (NWR), normal-weight unrestrained (NWU) and overweight (OW) (Table 1.27), however intake was negatively correlated with cognitive restraint on TFEQ [202]. Herman-Polivy (HP) restraint was positively correlated with TFEQ cognitive restraint and BMI. Change in eating rate was negatively correlated with TFEQ and HP restraint, age and BMI, however as BMI and HP restraint were positively correlated it was not possible to disentangle these factors further.

Table 1.27. Comparison of mean intake in normal-weight restrained (NWR), normal-weight unrestrained (NWU) and overweight (OW) women.

	NWR	**NWU**	**OW**
Number	9 women	9 women	6 women
BMI (kg/m^2)	< 25	< 25	> 25
Mean BMI (kg/m^2)	21.3 ± 1.9	21.1 ± 1.0	27.3 ± 1.4
Cognitive restraint:			
TFEQ	> 9	≤ 9	> 9
Herman-Polivy [HP] restraint	> 15	≤ 15	> 15
Mean Intake ± SD	365 ± 118 g	481 ± 144 g	424 ± 161 g

Both studies found that restrained eaters had a non-decelerated intake, with those of normal weight terminating eating before they were full. The second study found that the more restrained the eating behaviour (i.e. higher cognitive restraint on TFEQ), the more linear (non-decelerated) the intake. Those with unrestrained eating had decelerated intake curves where physiological factors (such as satiety) are likely to have played a role in terminating food intake. There was a positive correlation between age, non-decelerated intake and restraint. Significantly, eating rate was positively correlated with palatability.

These studies indicate that in some individuals, restraint may be more important in terminating eating than physiological factors, making it important to consider when analysing eating behaviour.

3. Gender

Only one study has found no significant difference in total intake between genders [229], with most studies finding that males ingest more than females [217, 224, 226]. Males have also been shown to eat faster initially, whether normal-weight [220, 224] or overweight [217]. Food diaries support a greater real-world intake in males [220]. Given these findings, it is possible that studies comparing groups unbalanced in gender may erroneously identify differences which in fact only reflect gender differences between the groups.

4. Age

Only one study has reported the effects of age and found this was positively correlated with a decelerated eating pattern [222]. This needs further investigation and should be considered when analysing data with significant differences in age between groups.

5. Perception of consumption

One study found no significant difference in intake when eating from closed and open containers and speculated that perceived intake, rather than actual intake (amount or calorie), influenced ingestion [220]. Another study however found poor correlation between actual and perceived amount ingested, suggesting that other factors play a role, for example hunger correlated with the amount ingested in the subsequent 5 minutes [204]. A study of 16 lean men with unrestrained eating found no difference in total intake due to soup labelling, however the actual fat content did effect ensuing intake, with less food consumed following a high-fat soup preload [209]. Whilst intake amount was not affected by labelling, this did alter the soup's perceived characteristics, while the actual fat content had no effect. There was no effect of soup labelling on hunger and fullness. The authors concluded that although food labels affected expectation, the effect did not persist. In contrast the actual fat-energy content led to a difference in intake. Labelling of foods and expectation in eating behaviour studies may therefore be less important than feared.

6. Vision

It would be expected that vision would have a significant impact on eating behaviour and as vision may be affected by midline brain tumours in patients with hypothalamic damage, it is an important factor to consider. Individuals with long-standing blindness and no visual memories of food have been found to eat more slowly than controls (both blindfolded and un-blindfolded, Table 1.28), with no difference in other outcomes [215]. As the controls ate less and for a shorter period when blindfolded, the authors concluded that vision influenced their eating behaviour, although unfamiliarity with the situation (blind-folded) and the UEM equipment and the small number of participants may have influenced the findings.

Table 1.28. Comparison of eating behaviour in blind participants, blindfolded and un-blindfolded controls.

	Blind participants	**Controls**		**p-value**
Number	6 female, 3 male	6 female, 3 male		-
Mean age (range) years	49 (41-59)	55 (40-58)		NS
Mean BMI (range) kg/m^2	23.4 (21.2-34.6)	25.0 (22.1-34.2)		NS
		With blindfold	*Without blindfold*	
Median eating rate (range) g/minute	30 (21-53)	39 (21-59)	39 (28-77)	<0.05 between blind participants and controls unblindfolded
Median intake (range) g	281 (195-717)	248 (117-317)	316 (190-440)	<0.05 within control groups
Median intake time (range) minutes	8.9 (6.8-27.8)	6.5 (4.8-10.4)	7.0 (4.6 to 10.3)	<0.05 within control groups
Median rate of deceleration (range) g/minute^2	-1.5 (-4.1 to 1.7)	0.5 (-4.2 to 3.0)	1.2 (-3.5 to 1.5)	<0.05 within control groups

NS = non-significant

Extrinsic factors

Extrinsic influences such as food palatability, duration of fasting, presence of another individual, or consumption of different preloads may affect food consumption and have been considered in UEM studies.

1. Food palatability

Several studies have manipulated the palatability of test-meals, influencing initial eating rate, total intake and duration of eating. Two different approaches have been used: making a pleasant food unpleasant by adding cumin [230] and making a

bland meal more palatable by adding seasoning [231, 232]. Palatability was hypothesised to influence initial eating rate (hunger), but not rate of deceleration (satiety) [230]. Adding cumin reduced total intake, eating duration, post-prandial satiety and palatability, with a slower initial eating rate and higher post-prandial hunger. The amount consumed was positively correlated with post-prandial palatability. In studies with soup preloads, the palatability of test-meals affected the total amount consumed, rate of initial intake and post-prandial satiety [231, 232]. Total daily intake was also higher on days where a more palatable meal was served.

2. Interruptions

As many studies interrupt participants to complete appetite ratings, it is important to consider whether this may affect outcomes. One study found increased intake when participants were interrupted, compared to continuous eating [233]. Duration of eating was also longer, although this may have reflected time taken to complete the ratings. Despite greater mean intake when meals were interrupted hunger remained higher and fullness lower than for meals eaten continuously, however as fullness was inexplicably lower before interrupted meals post-prandial differences may have been coincidental. A second paradigm found that the reason for interruption (to complete appetite-related VAS ratings or anagrams) had no effect in 9 normal-weight males. Duration of interruption (5-60 seconds) also had no significant effect on total intake or eating duration in 16 non-obese males, however hunger was significantly greater post-prandially with interruptions of 30 or 60 seconds and fullness greatest with 5 second interrupts. These results are important when designing and analysing UEM studies.

3. Eating Rate

An adapted UEM showing eating rate in real-time has been used to study the effects of manipulating eating rate on total intake and satiety (Table 1.29) in normal-weight women [234].

Table 1.29. Difference in total intake compared to control condition by eating type.

	Amount of total intake compared to control condition		Satiety compared to control condition	
Condition	Decelerated eaters	Non-decelerated eaters	Decelerated eaters	Non-decelerated eaters
40% Shorter than original meal*	Less	More	Lower	NS
Increased rate*	Less	More	Lower	NS
Decreased rate	NS	Less	NS	Higher
Interrupted*	NS	More	NS	Higher

NS = non-significant. For all cases, where a difference was found $p<0.001$.

* = significant difference between decelerated and linear eaters.

Satiety was greater in decelerated compared to non- decelerated eaters in the control condition. Decelerated eaters consumed less during a shorter meal and when eating more quickly, despite reporting lower satiety at the end of the meal. There was no difference in hunger between the groups. This study demonstrated a difference in total intake and satiety by using an adapted UEM to influence eating rate. Eating for a shorter period, or more slowly or quickly than normal may therefore influence study outcomes and participants must feel unrushed in their meal consumption during UEM studies.

4. Preloads

Preloading is a standard appetite-research methodological technique. It enables the effects of a manipulation in food given before a meal on subsequent appetite and food intake to be assessed. One of the earliest UEM studies considered preload effects [204]. Ingestion of a preload at any time-point decreased subsequent ingestion, but the timing which affected each participant most was variable. Even water preloads affected subsequent intake, although not for as long, or as much, as milk or Metrecal preloads. A study examining the effects of different carbohydrate preloads at breakfast found that whilst a bread-based breakfast resulted in higher post-breakfast insulin and glucose concentrations than a pasta-based breakfast, there

was no difference in the total amount eaten, eating rate, or change in eating rate of the lunch meal, or differences in VAS ratings of hunger and satiety [235]. Preload volume is also important, with lower mean hunger, increased fullness and significantly lower intake following a higher-volume, higher-energy soup preload [236].

In another study, authors hypothesized that non-decelerated eaters ate too quickly to feel satiated and used a preload followed by an 8 minute break to assess whether a break allowed satiation to develop [223]. All 10 non-decelerated eaters remained non-decelerated, therefore individuals who do not vary their eating behaviour in different situations may be at increased risk of weight gain.

Although not all studies involve a preload, the effects of earlier food intake (e.g. a breakfast meal) may affect subsequent food intake at the UEM and therefore should be controlled, or at least accounted for, when designing the study and interpreting the data.

5. Energy content of food

In contrast to food volume, a calorie increase of 25-30% had no effect on the shape/type of eating curve in one study [223]. The hypothesis that energy enrichment would lead to earlier satiation and therefore an increase in the prevalence of decelerated eating patterns was not demonstrated. The major determinant of satiation is volume, which is not surprising as fullness is important towards the end and immediately post-meal. It also explains the increase in obesity seen over recent decades with the increase in availability of energy-dense foods.

6. External influences

A study comparing the eating behaviour of overweight to normal-weight children found that in the presence of the child's mother, although the quantity eaten was no different between the groups, there was a significantly faster eating rate, larger bite size and acceleration of eating rate at the end of the meal in overweight compared to normal-weight children [226]. It may be important, therefore, in studies of childhood obesity for mothers to be present during eating to more accurately reflect the usual situation at home. The authors postulate that the mother's presence

may reflect learnt behaviour, where she has perhaps previously encouraged a child to "eat up", despite no prompting during the test-meal.

A study into stress while eating found no difference in cumulative intake between occasions when eating was preceded by a social stress test and that without, which is important as it is quite commonly perceived that some participants may feel stressed in study situations [217].

7. Influence of the experimental setting

Studies have used a variety of methods to try and minimise variability and/or distractions for participants, for example using recorded instructions [210, 230], playing background music [204], or eating in a windowless room [222, 223]. Another consideration is whether overweight/obese participants might artificially restrict their intake if they are aware that eating is being monitored and is the focus of the experiment [217]. A recent study examined whether awareness that food intake was being monitored led to differences in consumption at the UEM in 20 female university students who were told that their intake was being monitored, compared to 19 who were unaware [237]. The authors found that there was no significant effect on total intake, eating duration, time between mouthfuls or eating rate whilst consuming a pasta meal. When eating chunks of cookies following the pasta consumption those who were aware that intake was being monitored ate significantly more slowly (10.2 g/min) than those unaware (13.4g/min, $t_{37} = 2.39$, $p<0.05$) but there remained no difference in total intake, eating duration or time between mouthfuls. VAS ratings of fullness were significantly lower during the pasta meal in those aware that their eating was monitored (55 vs 63mm, $t_{37}=2.12$, $p<0.05$) and hunger was significantly higher when consuming the cookies (18 vs 9mm, $t_{37}=-2.48$, $p<0.05$). Given that 72 participants were initially recruited to the study, but data had to be discarded from 25 participants who were unaware of the use of the UEM due to leaning on the scales, compared to only five who were aware of its use, this may offer obvious benefits to informing participants of the purpose/techniques involved in UEM studies. This may not be generalisable to the study of all populations however as the study was restricted to females, with a mean (SEM) age of 20.0(0.4) years in the unaware group and 19.4(0.2) years in the aware group (t=1.39, p=0.17) and more importantly for obesity studies mean BMI was 21.9(0.5) and 21.8(0.5) kg/m^2 (t=0.06, p=0.95), respectively.

As UEM studies use a single food-type, sensory-specific satiety may occur, with a lower intake than in the normal free-living eating environment where multiple foods are freely available [233] and this must also be considered.

Participants with Additional Pathology

UEM studies have been undertaken in participants with atypical eating, such as Prader-Willi Syndrome (PWS), bulimia nervosa and binge-eating disorder (BED).

A study comparing 9 children with PWS, 20 normal-weight controls (NWC) and 20 obese controls (OC) found no significant differences in total consumption, but PWS participants ate for longer with a slower initial rate than NWC and OC (Table 1.30, page 91) [216]. PWS participants ate more slowly throughout the entire meal than OC, with less deceleration in eating rate than NWC, signifying a constant eating rate over a longer time. Limitations of the study included a set amount of food served (550g), differences in pubertal stage and a wide range of age and BMI SDS in the PWS group. The lack of an initial rapid eating rate indicated no evidence of increased hunger in the PWS group, with a longer eating time and constant rate of ingestion reflecting loss of satiety commonly described in PWS.

Women with bulimia may represent another group with aberrant satiety. Compared to 11 controls, 11 bulimic women consumed more of a test-meal (1597 g vs. 1004 g, $p = 0.02$), with no difference in food questionnaire responses before or after the yoghurt meal [203]. Bulimic women consumed more to reach maximal fullness and intake was higher at 50% and 75% of total intake, implying delayed satiety rather than eating past satiety. Other studies have found that ingestion rate accelerates during eating [227, 228].

Table 1.30. Comparison of food intake in participants with PWS, NWC and OC.

	PWS	NWC	OC	p-value for comparison between all 3 groups
Number	7 male, 2 female	11 male, 9 female	8 male, 12 female	-
Mean age (range) years	9.9 (5-17.5)	11.8 (5.9-18)	12.3 (6.4-17.8)	-
Mean BMI SDS (range)	3 (-0.1 to 6.4)	0.4 (-1.2 to 1.8)	6.3 (4.3 to 9.2)	-
Median intake (g)	267	270	314	NS
Median duration (range) minutes*	21.4 (5.8 - 43.9)	9.9 (4.0-28.7)[a,c]	7.7 (3.8-19.0)[b,c]	0.04
Initial rate (range) g/minute*	19 (3-46)	40 (12-93)[c,d]	47 (17-79)[c,d]	<0.01
Median eating rate (range) g/minute	16 (4-38)	28 (8-68)[c,e]	42 (15-65) [c,e]	<0.01
Deceleration in eating rate (range) g/minute^2	0 (–4.6 to 3.6)	–2.4 (–14.3 to 0.7)[d,f]	–0.9 (–9.3 to 3.7)[e,f]	<0.01
% of decelerated curves	4/9, 44%	18/20, 90%	14/20, 70%	0.03 (Fisher exact)

a: p=0.05 compared to PWS, b: p<0.02 compared to PWS, c: non-significant difference between NWC and OC, d: p<0.01 compared to PWS, e: non-significant difference compared to PWS, f: p=0.05 between NWC and OC.
Significant differences between groups identified on Kruskal-Wallis analysis of variance and where significant Mann-Whitney U tests established where the difference lay.

In women with BED both initial eating rate and change in eating rate were affected by stress, whilst there was no significant difference in total intake, reported hunger (pre-meal) or satiety (post-meal) under stressful conditions (Table 1.31) [218]. Participants with BED had an increased initial ingestion rate under stressful- compared to the neutral-condition and a smaller decrease in rate at the end of the meal. Controls ate more slowly with greater deceleration at the end of the meal under the stressful condition. The increased rate in BED was due to larger spoonful size, rather than an increased frequency. Women with BED ate a similar volume of food more quickly when stressed and did not identify a feeling of increased hunger.

Table 1.31. Comparison of food intake in participants with and without BED.

	Participants with BED		**Controls**	
Mean BMI, kg/m^2	37		36	
	Stress condition	Neutral condition	Stress condition	Neutral condition
Mean initial eating rate, g/sec	0.53	0.33	0.47	0.53 g
Mean change in eating rate, g/sec^2 x 1000	0.04	2.1	0.74	0.17

Conclusions

In summary, the UEM has been used in multiple settings, is reliable and sensitive to changes in eating behaviour in different populations and contributes unique data to the study of the microstructure of eating behaviour. Using the UEM in participants adequately familiarised with the equipment can demonstrate differences in hunger/satiety between different groups. Variances in eating patterns due to intrinsic and extrinsic factors must, however, also be taken into account. There are currently no studies using the UEM in the HO population. If differences in eating rate are found to be associated with the development of HO, these findings may shed some light on the underlying pathophysiological mechanism(s) contributing to the development of HO.

Study of the macrostructure of human eating behaviour

Assessment of the macrostructure of eating behaviour is often undertaken in the "real-world" environment. A recent systematic review of 31 studies undertaken between 1985-2010 found that the most common method of reporting intake was using a food diary (13/31) [238]. One advantage of food diaries is that they do not rely on memory [239]. Three-day food diaries have been shown to have fewer absolute errors and less missed or erroneously included foods than 5-day diaries or 24 hour recalls [240, 241]. However they still include errors, with 25% of foods observed eaten not reported and 10% of foods reported eaten not actually consumed in an observational study of girls aged 9 and 10 years [240]. In addition to underreporting another disadvantage of food diaries is the work-load on both participants (perhaps leading to underreporting) and on those assessing them [239]. Eating away from the home, which is common, can make it more difficult to accurately report food intake and a certain level of both literacy and numeracy skills are required. Undertaking food diaries may itself lead to a change in eating [239], or participants may choose to underreport certain food groups, for example fatty foods in those who are overweight/obese.

While food diaries reveal eating behaviour over a period of days, reflecting fluctuating physiological states (hunger versus fullness) along with short-term behaviour, certain more persistent eating traits can influence eating [242]. These more stable traits tend to influence food choices over a long period of time and become eating habits, influencing cognitive choices and can supersede hunger and satiety cues. Traits such as eating restraint, self-control and internal and external factors affecting hunger can be measured. The Three Factor Eating Questionnaire (TFEQ) [191] is the most commonly-used questionnaire [201]. The 51-item questionnaire can be used for adults of both genders and consists of true/false questions and ones answered on a scale of 1-4 or 1-6, with dichotomous scoring even for the scale questions [243]. Three overarching traits or "factors" are considered: dietary restraint, disinhibition and hunger. Dietary restraint describes the amount of behavioural control someone has over their eating behaviour. This has been inconsistently associated with BMI and energy intake (both higher and lower intake has been described in restrained eaters). Disinhibition describes the likelihood of

eating under certain environmental conditions, for example when upset or in the presence of palatable food. High disinhibition scores have most consistently been associated with increased BMI and energy intake. Hunger questions show how sensitive participants are to feelings of hunger and their tendency to eat, but has been less strongly correlated with outcomes than the other factors in studies so far [201].

Scores for normal-weight and obese populations have been compared. The TFEQ has been used to study large cohorts, particularly in relation to obesity/weight-loss trials and has high internal and test-retest reliability in both laboratory and non-laboratory settings [191, 201]. In a study of 233 adults comparing TFEQ to laboratory measures (reinforcing value of food and explicit liking/implicit wanting) BMI was most strongly correlated with disinhibition, with hunger also significantly correlated and none of the other measures showing significant correlation. Energy intake was negatively correlated with restrained eating; disinhibition, hunger, liking and wanting were all positively correlated [201].

Studying the macrostructure, as well as the microstructure, of eating behaviour in patients who are HWS and those with HO may help to elucidate the underlying mechanisms and behaviours leading to the difference in outcomes in patients with hypothalamic damage.

Aims of Thesis

The primary aims of this research were:

1. To assess the prevalence of obesity and other cardiovascular risk factors in patients with hypothalamic damage.

2. To assess the prevalence of obesity and other cardiovascular risk factors in children and adolescents with pituitary adenomas.

3. To investigate the underlying pathophysiology of HO using the complementary approaches described above to determine whether disturbances in neural pathways affect food intake and eating behaviour, leading to weight-gain.

Hypotheses

1. Individuals with acquired hypothalamic damage would have a high prevalence of obesity and other cardiovascular risk factors, despite appropriate awareness and aims at prevention of these sequelae amongst the treating physicians. Also that patients with growth hormone deficiency and cranial diabetes insipidus would be particularly at risk of HO.
2. Children and adolescents with pituitary adenomas (both with and without hypothalamic damage) would have an increased prevalence of metabolic disturbances such as obesity, dyslipidaemia and hypertension (conferring an increased cardiovascular risk) and impaired fertility, as described in adult patients with pituitary adenomas and hypopituitarism.
3. That the fMRI study would demonstrate that compared to those participants with hypothalamic damage remaining weight stable (HWS), those with HO (and obese controls) would have increased activation in non-homeostatic brain regions which process food reward, such as the insula, orbito-frontal cortex (OFC) and striatum when viewing food photographs.
4. Patients with HO would consume more food (demonstrated in both the UEM experiment and in the 3-day food diaries) than those HWS and non-obese controls and have increased levels of disinhibition and hunger, and lower restraint than the other groups.

Chapter 2

Methods

Methods

Ethical Approval, Recruitment and Consent

For the two retrospective reviews (Chapters 3 and 4), the permission of the Audit Departments and Caldicott Guardians at University Hospital Aintree (UHA), Walton Centre for Neurology and Neurosurgery (WCNN) NHS Foundation Trust and Alder Hey Children's Hospital (all Liverpool, UK) were obtained and data retrieved from medical casenotes.

For the fMRI and eating behaviour case-control studies (Chapters 5 and 6) the study protocol was reviewed and given favourable ethical opinion for conduct in the NHS by Sefton Research Ethics Committee (REC Ref: 09/H1001/4, submission date 07/01/2009) and the University of Liverpool. Approval was also granted by the Research and Development Department at UHA.

Study patients (Chapters 5 and 6) were recruited from either the weekly joint pituitary clinic at WCNN, run jointly by a consultant neurosurgeon and consultant endocrinologist, or from the weekly endocrine follow-up clinic at UHA run by a consultant endocrinologist with a special interest in pituitary disorders, where patients with a more long-standing diagnosis not requiring further neurosurgical intervention were seen. All had acquired, structural, hypothalamic damage caused by a tumour within the hypothalamus or directly adjacent to it causing compression or invasion. Hypothalamic damage was assessed and scored by a Neuroradiologist [245]. Patients were defined as hypothalamic obese (HO) or as having hypothalamic damage but remaining weight-stable (HWS). HO was defined as BMI $\geq$ 30 kg/m^2 at latest clinic follow-up with an increase $\geq$2 kg/m^2 since tumour diagnosis, based on a previous study by Daousi et al [6]. HWS patients' BMI was < 30 kg/m^2 and had remained stable since tumour diagnosis. All patients were adequately replaced for all pituitary hormone deficiencies caused by their tumour/surgery as clinically indicated (hydrocortisone, growth hormone, thyroxine, sex steroids, desmopressin).

As part of their standard clinical care all patients diagnosed with hypothalamic damage secondary to a structural lesion underwent serial endocrine assessment following diagnosis and treatment. The glucagon stimulation test

(GlucaGen; NovoNordisk Pharmaceuticals, Crawley, UK) was used to assess growth hormone (GH) and adrenocorticotropic hormone (ACTH) reserve [246], alongside basal measurement of thyroid-stimulating hormone (TSH), free thyroxine (fT4), prolactin, oestradiol or testosterone (depending on gender) and insulin-like growth factor 1 (IGF-1). Patients found to have inadequate ACTH (and therefore cortisol) response to the glucagon stimulation test underwent standard dose short-synacthen testing (250μg) and if cortisol deficiency was verified then treatment with hydrocortisone was commenced. Biochemical severe growth hormone deficiency was defined as a peak GH ≤ 3 μg/L, with treatment of severe adult GHD indicated in those who fulfilled the NICE criteria of impaired quality of life, as assessed by the 'Quality of life assessment of growth hormone deficiency in adults' (QoL-AGHDA) questionnaire [247]. Treatment for thyroxine, oestrogen or testosterone deficiency was started as clinically indicated.

Healthy controls were recruited by advertisement within the University of Liverpool and from participants in previous obesity studies at UHA or the University of Liverpool. Healthy controls were matched to patients for age and BMI, with obesity defined as BMI ≥ 30 kg/m^2.

All interested participants were given a participant information sheet (non-patient information sheet in Appendix 5) and had the opportunity to ask questions before giving written informed consent. Those seeking consent were either previously and/or currently involved with other research studies and understood the ethical principles underpinning informed consent following appropriate training. The validity of informed consent was ensured by providing adequate information and potential participants were assessed to confirm that they had the capacity to decide for themselves whether or not to take part.

Inclusion and exclusion criteria for participants

Inclusion criteria:

1. Patients: evidence of hypothalamic damage (secondary to tumours such as craniopharyngioma, midline tumour with hypothalamic involvement, or pituitary macroadenoma with supra-sellar extension/optic chiasmal compression with visual field defects where hypothalamic damage was visible on MRI).
2. Controls: BMI- and age-matched controls with none of the exclusion criteria.

Exclusion criteria:

For all participants

- Presence of metal implants or objects in the body which cannot be removed (e.g. cardiac pacemaker, mini−defibrillator or neurostimulator, artificial heart valve; metal-containing surgical clips; history of injury which may have left metal particles in the body) which would pose a safety risk to the participant.
- Claustrophobia in the MRI scanner

For controls

- A history of eating disorder such as bulimia nervosa, anorexia nervosa or binge−eating disorder (according to DSM IV criteria).
- History of psychiatric disorder (schizophrenia, major depression, bipolar affective disorder).
- Diabetes mellitus (type 1 or 2).
- Current history of excess alcohol consumption or substance abuse/addiction.
- Current (or previous 3 months) use of centrally acting medication (such as psychotropic or antidepressant medication, sibutramine, rimonabant) which are known to influence feeding behaviour.
- History of traumatic brain injury (which may affect the hypothalamus and/or pituitary and therefore affect the validity of any findings).
- Genetic forms of hypothalamic obesity (Prader−Willi syndrome, Biedl−Bardet syndrome).

For control subjects, exclusion criteria were chosen as they would have a direct influence on eating behaviour and patterns.

Study visits (see Appendix 4 for timelines)

Study day 1 (screening visit): During the first visit informed consent was undertaken, anthropometry was collected (height and weight to calculate BMI using the standard formula, and estimation of percentage body fat by whole-body bioelectrical impedance analysis [Tanita Systems, Tanita Corp, Tokyo, Japan]) and participants were given the TFEQ and 3-day food diaries for completion (Appendix 2). All participants (patients and controls) were medically screened prior to MRI scanning, with adequacy of vision assessed in the patient group. Participants also ate a meal seated at the UEM. The fixed load breakfast meal (estimated to provide 25% of participants calculated daily BMR and eaten on both study days 2 and 3) was presented, to enable familiarisation with the UEM and also to ensure that participants were able to consume the entire portion provided.

Study day 2 (fMRI study day): Participants able to undertake this part of the study attended for fMRI scanning and blood sampling, as well as undertaking VAS ratings of appetite throughout the day (further details in Chapter 5 and Appendix 2).

Study day 3 (UEM study day): Participants attended for blood sampling, a fixed breakfast load and an ad-libitum lunch-meal (both eaten at the UEM) (further details in Chapter 6 and Appendix 4). TFEQs and 3-day food diaries (Appendix 2) were collected at this visit and those not returning these were given a stamped-addressed envelope in which to return them.

On all three study days participants ate a fixed load breakfast-meal of porridge and orange juice, consuming 25% of their calculated basal metabolic rate (BMR) using the Harris-Benedict formula. For men: 66.5 + (13.75 x weight) + (500.3 x height) - (6.775 x age) and for women: 655.1 + (9.563 x weight) + (185 x height) - (4.676 x age). The exact quantities served varied according to the calculated BMR, but in general portions of 45g porridge made with 340mL of semi-skimmed milk and 200mL quantities of orange juice were served in equivalent proportions. Details of the nutritional content of the breakfast meal are shown in Table 2.1.

Table 2.1 Nutritional content of the breakfast-meal

	Orange Juice Per 100mL	Porridge Per 100g	Semi-skimmed milk Per 100g
Energy (kcal)	47kcal	356	49
Protein (g)	0.5g	11.0	3.6
Carbohydrate (g)	10.5g	60.0	4.8
of which sugars	10.5g	1.1	-
Fat (g)	0.1g	8.0	1.8
of which saturates	Nil	1.5	-
Fibre (g)	-	9.0	-
of which soluble	-	4.0	-
of which insoluble	-	5.0	-

Most participants took part in all aspects of the study (fMRI and eating behaviour studies), however some participants were not able to take part in the fMRI study (due to claustrophobia or an inability to tolerate looking at the photographs whilst in the MRI scanner) or failed to return the TFEQ and 3-day food diary, therefore the groups were not identical. Further details of the methods for each study day, the participants involved and the analysis of the data are given in each relevant chapter (Chapters 5 and 6).

Plasma hormone analysis

All samples (taken on either the fMRI or the UEM study day) were assayed in duplicate and in one assay to eliminate inter-assay variation. Plasma insulin was taken into a serum separating tube (SST) and measured using a Siemens Immulite 2000 Immunoassay system. Blood for glucose determination was collected into a tube containing sodium fluoride and potassium oxalate and measured using YSI 2300 STAT Plus™ Glucose & Lactate Analyzer (YSI Life Sciences). Blood taken for measurement of active GLP-1, PYY(3-36) and active ghrelin was collected into chilled EDTA tubes containing 50 µl of aprotinin to prevent degradation by proteolytic enzymes, centrifuged at -4°C and stored at -80°C until assayed. Active GLP-1 and active ghrelin were determined using commercial enzyme-linked immunoassay (ELISA) kits (Millipore, Billerica, USA). The standard curve range for

GLP-1 ELISA was 0.8-100 pmol/L, inter and intra-assay precisions were 8% and 7% respectively. Active ghrelin was measured using the assay kit Millipore #EZGRA-88K. Although AEBSF [4-(2-aminoethyl) benzenesulphonyl fluoride hydrochloride] was not available aprotinin was used, which is also a good serine protease inhibitor. The standard curve range for active ghrelin ELISA was 10-2000 pg/mL, inter- and intra-assay precisions were 10-16% and 7-10% respectively. PYY(3-36) was measured using an enzyme immunoassay kit from Phoenix Pharmaceuticals Inc. (range 0.06-100 ng/mL). Leptin was assayed following collection in a chilled blood tube containing EDTA using a human leptin "Dual Range" ELISA (EZHL-80SK, Millipore, Billerica, USA). Inter- and intra-assay precisions were 2.6-6.2% and 2.6-4.6% respectively and the standard curve range was 0.5-100 ng/mL.

Chapter 3

Prevalence of Hypothalamic Obesity, Risk Factors and Metabolic Outcomes in Adults With Acquired Structural Hypothalamic Damage

Abstract

Objective: Children with acquired, structural hypothalamic damage have a high prevalence of obesity. Similar data are emerging in adults, although these patients are less well-studied. The aims of this study were: i) to review the prevalence of obesity in adult patients with acquired hypothalamic damage, ii) to determine the hormonal and neuroanatomical variables associated with weight gain and obesity and iii) to reflect on early instigation of weight-loss measures undertaken in clinics following an earlier published review of adult patients with hypothalamic obesity.

Study design: Retrospective case-note review of 110 adults with tumours causing hypothalamic damage attending a specialist neuroendocrine clinic. Serial BMI was calculated (at diagnosis and latest clinic follow-up in n=77), endocrine data (including hormone replacement), neurosurgical procedures, additional therapies (such as radiotherapy) and weight loss measures were recorded and all available brain imaging reviewed. The prevalence of hypothalamic obesity (HO) was determined, defined by a body mass index (BMI) ≥30 kg/m^2 at latest follow-up, which had increased by at least 2 kg/m^2 since tumour diagnosis.

Results: After a median follow-up of 9.5 years (range 1-49), data from 100 patients showed that 91% were overweight/heavier (BMI ≥ 25 kg/m^2), 63% were obese (BMI ≥ 30 kg/m^2) and 16% morbidly obese (BMI ≥ 40 kg/m^2). Multivariate analysis revealed that requirement for desmopressin (odds ratio [OR] = 3.5, p = 0.026), growth hormone [GH] (OR = 2.7, p = 0.031) and thyroxine replacement (OR = 3.0, p = 0.03) were associated with HO. No correlation was found between neuroimaging features and weight gain. Despite proactive treatments offered in clinic in recent years (counselling, dietetic and physical activity advice, and anti-obesity medications), patients have continued to gain weight.

Conclusions: Obesity is significantly more prevalent in adult patients with acquired, structural hypothalamic damage than in the general population. The risk of HO is increased in those requiring desmopressin, thyroxine and GH therapy, but neuroimaging findings did not predict the risk of HO. Despite an increased awareness of the risk of HO by clinicians in neuroendocrine clinics it remained

difficult to prevent and treat those with HO. Improved understanding of the pathophysiological mechanisms underlying HO and multicentre collaborative trials to examine the efficacy of novel obesity interventions would be advantageous.

Summary of Justification and Aims

The prevalence of obesity in patients with acquired, structural hypothalamic damage is high, however reports on prevalence and the risk factors associated with developing obesity vary between studies and within different groups, for example children with craniopharyngioma or adults with hypothalamic damage due to varying causes (see *Chapter 1, Introduction*). Most of the published studies concentrate on the endocrine sequelae of hypothalamic pituitary disease in childhood (including obesity), particularly in children with craniopharyngioma (as discussed in *Chapter 1*) and data from studies in adults are sparse [6]. Obesity is associated with adverse health outcomes such as cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) which lead to increased morbidity and mortality. Cardiovascular risk factors are highly prevalent and often inadequately treated in adult patients with hypothalamic–pituitary disease [71, 77, 79]. Aggressive treatment of these factors is essential to reduce mortality and morbidity from CVD in these patients [73], particularly as effective treatments for HO have not yet been developed or evaluated.

Aims:

(i) to assess the prevalence of HO in a large cohort of adult patients with acquired, structural hypothalamic damage attending follow-up in a single adult neuroendocrine service and to ascertain whether the prevalence of HO had changed since an earlier review of the service was undertaken in 2002 [6]. Following the service review in 2002 (published in 2005), a more proactive approach to the management of patients at risk of HO and its complications was adopted in the clinic with the aim to limit the severity of weight gain by early provision of dietary and behavioural advice, counselling, encouraging regular physical activity, use of anti-obesity medications (where appropriate) and referral to multidisciplinary weight management services if necessary.

(ii) to assess the endocrine and neuroanatomical risk factors for HO, as investigated in other studies containing mainly paediatric populations [6, 20, 24, 25, 32-34, 57, 104-106, 109, 114-119].

(iii) to examine the prevalence of treated cardiovascular risk factors, such as hypertension and dyslipidaemia, which are likely to be more prevalent in this patient group [79] than in the general population.

Hypothesis:

Individuals with acquired hypothalamic damage would have a high prevalence of obesity and other cardiovascular risk factors, despite good awareness and aims at prevention of these sequelae amongst the treating physicians. Those with growth hormone deficiency and cranial diabetes insipidus would be particularly at risk of HO.

Participants and Methods

Participants

Permission was obtained from the relevant audit departments for retrieval of data from the medical case notes and electronic records of adult patients attending the regional joint neurosurgical and neuroendocrine clinic at the Walton Centre for Neurology and Neurosurgery (WCNN) and the neuroendocrine follow-up clinic at University Hospital Aintree (UHA), both in Liverpool, UK. One hundred and ten patients who had tumours involving the hypothalamic region were identified. All patients had undergone treatment for either hypothalamic tumours, or adjacent tumours directly compressing or invading the hypothalamus until early 2011 and had a clinical diagnosis of hypothalamic destruction, with Grade 2 hypothalamic damage (as per Saint-Rose et al. grading [32, 115]) on objective MRI review by a Neuroradiologist. Tumour type, neurosurgical procedure(s) undertaken (including whether the approach was transphenoidal or via craniotomy), the need for ventricular drainage and the administration of conventional fractionated external beam radiotherapy for either residual tumour or recurrence was recorded.

Tumour types included: pituitary macroadenomas (53 patients, 48.2%); craniopharyngiomas (38 patients, 34.5%) and primary hypothalamic lesions including hypothalamic hamartoma, glioma and histiocytosis X (19 patients, 17.3%)

as documented in Table 3.1. All patients with pituitary adenomas had suprasellar extension with optic chiasm compression and visual field defects, with hypothalamic damage confirmed by a Neuroradiologist.

Table 3.1 Diagnoses and prevalence of tumour types for all 110 patients with hypothalamic damage.

Diagnosis	Frequency	Percentage
Craniopharyngioma	38	34.5
Hypothalamic lesion (e.g./ Histiocytosis X)	9	8.2
Other tumour with hypothalamic extension	4	3.6
Pituitary adenoma with suprasellar extension	40	36.4
Prolactinoma with suprasellar extension	13	11.8
Primary brain tumour in close proximity to hypothalamus, where hypothalamus included in irradiation field (e.g./ optic nerve glioma)	6	5.5
Total	110	100.0

Anthropometry

Where data were available height and weight at diagnosis and latest follow-up clinic visit were used to calculate BMI (kg/m^2). The presence of hypothalamic obesity (HO) at latest clinic follow-up was determined. This was defined as a BMI $\geq$30 kg/m^2, with an increase of at least 2 kg/m^2 since tumour diagnosis, based on previous work from Daousi et al [6]. Patients whose BMI remained <30 kg/m^2 or in whom this had not increased >2 kg/m^2 were defined as having hypothalamic damage remaining weight-stable (HWS). Serial anthropometric data (from the time of diagnosis to the most recent follow-up) were available for n=77 patients.

Endocrine assessment

All patients underwent routine hypothalamic-pituitary-target organ axis testing following diagnosis of their tumour and subsequent serial testing following treatment. Adrenocorticotropic hormone (ACTH) and growth hormone (GH) reserve

were assessed using the glucagon stimulation test (GlucaGen; Novo Nordisk Pharmaceuticals, Crawley, UK). Patients with an inadequate ACTH response to glucagon (as measured by low cortisol concentrations) underwent a standard dose (250 microgram) short synacthen test to confirm cortisol deficiency. Measurement of basal concentrations of thyroid-stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3, where indicated), prolactin, oestradiol or testosterone, cortisol and insulin-like growth factor-1 (IGF-1) were also undertaken on a routine basis during clinic visits.

Neuroimaging

A single Neuroradiologist blind to the clinical data reviewed and scored all available computed tomography (CT) and magnetic resonance imaging (MRI) scans (pre- and post-operative where possible) for each patient. The anatomical features noted on the scans were used to produce a score of hypothalamic damage for each patient (as per Daousi et al [6]) and the factors assessed included:

1. the primary site of the tumour
2. its maximum extent (in mm) from the midline to the right and left (on coronal views)
3. presence of suprasellar extension in cases of pituitary tumour
4. pituitary tumour encroachment on the optic chiasm
5. invasion/compression of hypothalamic tissue by extra-hypothalamic tumours
6. distortion of the third ventricle at the level of the infundibulum (on coronal views)
7. abnormality of the floor of the third ventricle (partial or complete deficiency of the floor)
8. breach of the tuber cinereum by the tumour
9. tumour infiltration of other brain areas (including thalamus and temporal lobes).

Statistical analysis

Statistical analyses were performed using SPSS, versions 18.0 and 24.0 (SPSS Inc., Chicago, Illinois). Data are expressed as mean (standard deviation [SD])

or median (IQR). Longitudinal changes in BMI were compared using the Wilcoxon signed-rank test in the 77 patients with serial weight data available. McNemar tests were performed to examine for any differences in the prevalence of patients in each BMI category at diagnosis compared to latest follow-up. Either the chi-squared test or Fisher exact test were used to examine categorical data (as appropriate) and continuous data were examined with the *t* test (if normally distributed) or the Mann-Whitney *U* test (if non-normally distributed).

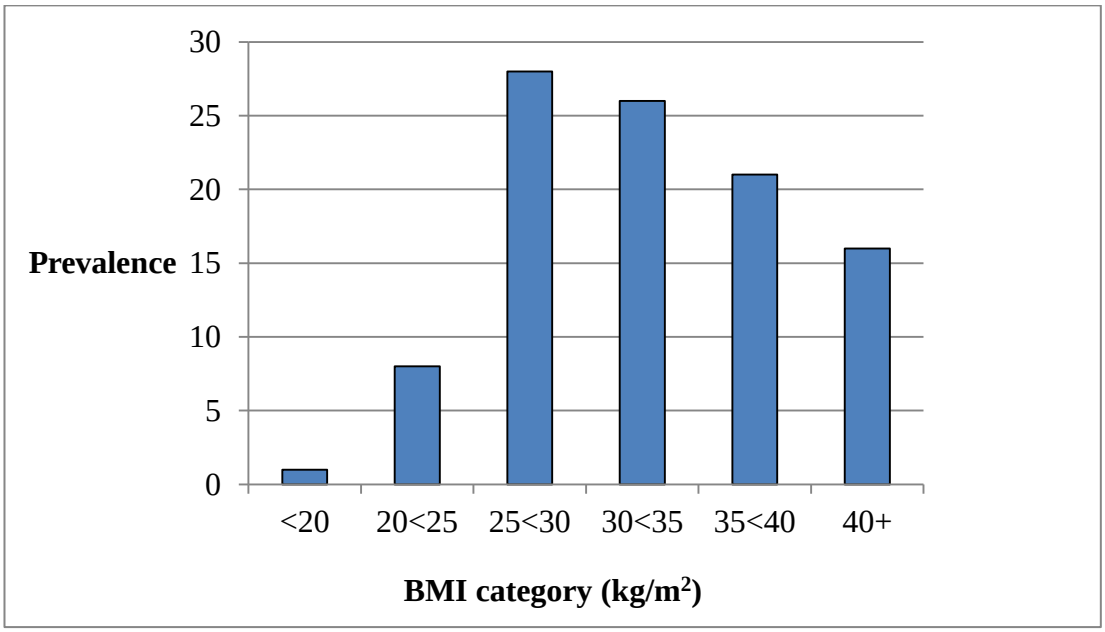
Features associated with weight gain were identified by multiple logistic regression analysis. For patients with HO, variables that differed significantly in univariate analysis were entered into the original model, along with current age and age at diagnosis of the tumour, and subjected to forward stepwise logistic regression analysis. Examination of a possible correlation between tumour size and subsequent weight gain was undertaken using the Spearman rank correlation coefficient for non-normally distributed data. Statistical significance was defined as $p<0.05$ (two-tailed).

Results

Prevalence of obesity in the entire cohort

At the time of study, 110 patients were attending the neuroendocrine clinics, with a median age of 55.5 years; 16 were diagnosed with a tumour during childhood. At the time of latest follow-up (data collected in 2010-11) BMI was available for 100/110 patients. Mean (SD) BMI was 33.7 (7.9, range 19-60) kg/m^2; 91 patients (91%) had a BMI ≥25 kg/m^2, making them overweight or heavier; 63 (63%) were obese (BMI ≥30 kg/m^2); 37 (37%) had a BMI ≥35 kg/m^2 and 16 (16%) patients were morbidly obese (BMI ≥40 kg/m^2). Figure 3.1 shows the prevalence of BMI by category. Within the general population of a similar age (aged 55-64), according to the Health Survey for England 2012 [256] the mean BMI was 28.3 kg/m^2 and 73% were overweight or heavier (BMI ≥25 kg/m^2), with the prevalence of obesity (BMI ≥30 kg/m^2) more than half (30.7%) of that seen in the patient cohort studied.

Figure 3.1. Prevalence of BMI category at latest follow-up in 100/110 patients with hypothalamic damage with full data available.



Treatment of obesity

Seventeen of the 110 patients had been treated with the weight loss medication orlistat and four had received multiple weight loss medications (orlistat,

sibutramine and rimonabant - the latter two before their withdrawal from the market due to drug safety concerns). Six patients were attending a hospital-based multi-professional weight management clinic where they had regular clinical review with a dietician and physician with a special interest in weight management. None of the patients had a clinically significant weight-loss following these interventions and many continued to gain weight. None of the patients in the study cohort had undergone bariatric surgery.

Endocrine replacement at follow-up

At latest clinic follow-up 103 (94%) of the 110 patients needed replacement therapy for one/more pituitary hormone deficiencies. These included 30 receiving desmopressin for cranial diabetes insipidus (DI), 63 with treated growth hormone deficiency (GHD), 53 on thyroxine replacement, 57 requiring hydrocortisone and 43 patients taking sex steroid replacement. These figures do not reflect entirely the prevalence of pituitary hormone deficiencies within the cohort. For example patients with severe biochemical GHD (defined as peak GH < 3 micrograms/L in response to provocative testing) require adequate replacement of all other pituitary hormone deficiencies and impaired quality of life as assessed by the QoL-AGHDA questionnaire before qualifying for a trial of GH replacement therapy. Additionally older patients with sex steroid deficiency may not have been treated as they were above the age commonly associated with menopause. Fourteen patients with severe GHD had declined treatment and five patients had suffered only transient post-operative DI.

Longitudinal changes in weight and BMI (n=77)

Height and weight measurement at both diagnosis and latest clinic assessment were available for 77 of the 110 patients (Figure 3.2). Their mean (±SD) age at latest follow-up was 44 ± 16.5 years; 43 (55.8%) were male (Table 3.3). Median BMI increased significantly ($p<0.0001$) from diagnosis (28.1 kg/m^2, IQR 24.3-32.4) to latest follow up (32 kg/m^2, IQR 27.7-38.4) a median of nine years later. The prevalence of obesity and severe obesity (BMI ≥35 kg/m^2) increased during the

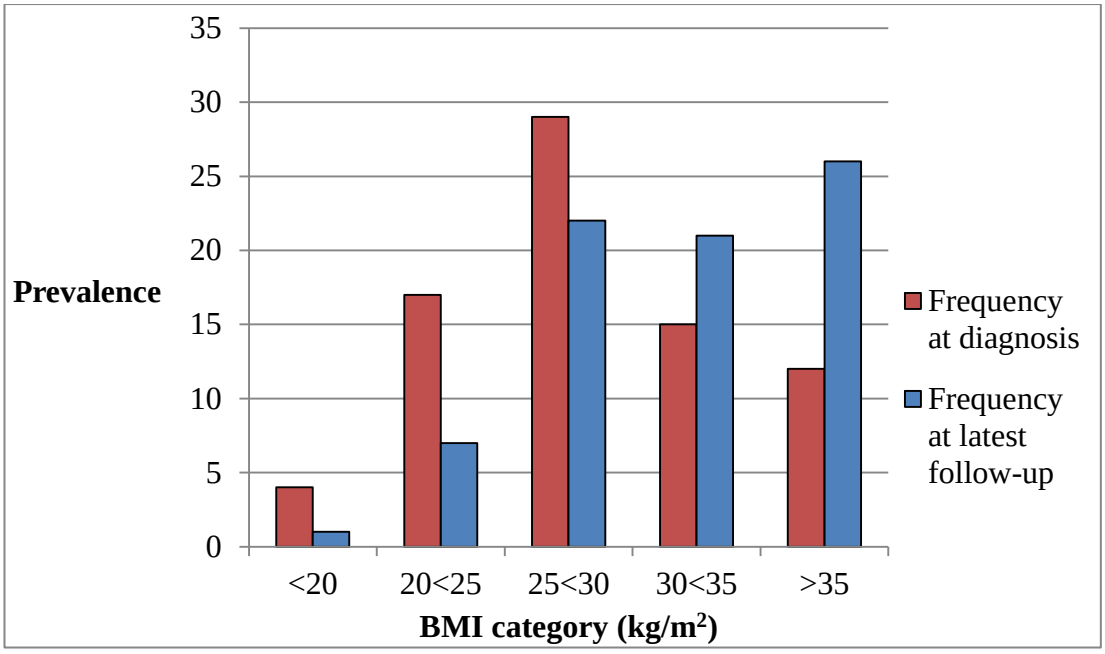
follow-up period (Table 3.2), with a statistically significant difference in severe obesity ($p<0.001$). As demonstrated consistently in studies in children the increase in weight was fastest in the year following diagnosis and treatment of their tumour (mean BMI increased to 30.4 kg/m^2 in this time, $p<0.0001$) with a subsequent deceleration in weight gain but no plateau.

Table 3.2. Prevalence of overweight/obesity/morbid obesity at diagnosis and latest follow-up in 77 patients with paired weight and height data.

Category (BMI [kg/m^2])	**Prevalence (%) at diagnosis**	**Prevalence (%) at latest follow-up (2010/11)**	**p-value**
Overweight (25-29.99)	29 (37.7)	22 (28.6)	0.23
Obese (30-34.99)	15 (19.5)	21 (27.3)	0.31
BMI ≥ 35	12 (15.6)	26 (33.8)	<0.001

p-value calculated using McNemar test.

Figure 3.2. Prevalence of BMI category at diagnosis and latest follow-up in 77 patients with hypothalamic damage with serial weight data available.



Endocrine replacement associated with obesity and weight gain since diagnosis

Patients with HO at latest follow-up were more likely to be receiving growth hormone replacement therapy (p = 0.007), thyroxine (p = 0.008), or desmopressin (p = 0.02) (Table 3.3). In a multivariate model, the development of HO during follow-up was associated with the use of desmopressin (odds ratio [OR] = 3.5; 95% confidence interval [CI]: 1.2 to 10.8; p = 0.026), growth hormone (OR = 2.7; 95% CI: 1.1 to 6.9; p = 0.031) and thyroxine (odds ratio [OR] = 3.0; 95% CI: 1.1 to 8.0; p = 0.03).

Table 3.3 Characteristics of patients with serial data (n=77), according to obesity status

	HO (n = 42)	HWS (n = 35)	
Characteristic	**Number (%) or Mean ±SD**		**p value**
Current age (years)	55.7 ± 13.1	54.2 ± 17.7	0.9
Age at diagnosis (years)	43.4 ± 15.7	44.5 ± 17.7	0.4
Male sex	22 (52.4)	21 (60)	0.4
Years of follow-up	10.8 ± 6.8	8.5 ± 5.9	0.6
Baseline BMI (kg/m^2)	30.9 ± 6.8	26.2 ± 4.8	0.001
Current BMI (kg/m^2)	38.6 ± 7.2	27.4 ± 3.6	<0.001
Hydrocortisone replacement	34 (81)	23 (66)	0.2
Hydrocortisone dose (mg)	22.3 ± 5.3	25.7 ± 6.2	0.04
GH replacement	29 (69)	14 (40)	0.007
Thyroxine replacement	35 (83)	18 (51)	0.008
Sex steroids	27 (64)	16 (46)	0.9
Desmopressin	15 (36)	6 (17)	0.02

HO - hypothalamic obese; HWS - hypothalamic damage remaining weight-stable; BMI - body mass index; GH - growth hormone. p-value calculated using the *t* test.

Neuroanatomic features associated with obesity and weight gain since diagnosis

Preoperative neuroimaging scans were available for 45 patients and postoperative scans for 67. None of the neuroradiological features considered were associated with weight gain or an increased risk of HO. On preoperative coronal views, no correlation was found between the width of the tumour from the midline and the change in BMI from diagnosis to latest follow-up (Spearman $r = -0.3$, $p = 0.09$). On postoperative coronal views, there was no correlation between the width of any remaining tumour from the midline and the development of HO (Spearman $r = -0.2$, $p = 0.14$). Radiological involvement of the thalamus or temporal lobe, distortion of the third ventricle at the level of the infundibulum (on coronal images), abnormalities of the third ventricular floor and breach of the tuber cinereum by tumour were not associated with weight gain (Table 3.4), neither was grade 1 (hypothalamus compressed/displaced) or grade 2 (hypothalamus unable to be identified) hypothalamic damage, assessed according to the Sainte-Rose grading system [32, 115].

There was no association between the risk of HO and either tumour type or treatment modality, including the need for transfrontal surgery (indicating a more extensive tumour and greater likelihood of perioperative hypothalamic damage), insertion of a ventriculo-peritoneal shunt and radiotherapy (where a greater theoretical risk of hypothalamic damage exists, in combination with the likelihood of a larger tumour which could not be fully resected at operation). Three of the patients were treated with radiotherapy alone.

Table 3.4 Neuroanatomic characteristics of patients with serial data (n=77), according to obesity status

	HO (n = 42)	HWS (n = 35)	
Characteristic	**Number (%) or Mean ±SD**		**p value**
Diagnosis: Craniopharyngioma	20 (39.2)	12 (27.9)	0.70
Hypothalamic tumour	4 (7.8)	2 (4.7)	
Pituitary adenoma	15 (29.4)	19 (44.2)	
Prolactinoma	7 (13.7)	7 (16.3)	
Hypothalamic	3 (5.9)	2 (4.7)	
Radiotherapy	2 (3.9)	1 (2.3)	
Other			
Mean maximum extent of tumour from midline in mm (right)	14.09 (SD6.63)	15.73 (SD7.39)	0.51
Mean maximum extent of tumour from midline in mm (left)	12.7 (SD6.33)	15.6 (SD10.3)	0.11
Suprasellar extension (for pituitary tumours)	28 (90.3)	26 (86.7)	0.65
Pituitary tumour encroachment on optic chiasm	21 (84)	22 (78.6)	0.61
Invasion/compression of hypothalamic tissue (extra-hypothalamic tumours)	18 (60)	20 (69.0)	0.47
Distortion of third ventricle (at infundibulum)	19 (59.4)	21 (67.7)	0.49
Abnormality of third ventricle floor/breach of tuber cinereum	15 (41.7)	17 (53.1)	0.35
Invasion of thalamus on MRI	3 (7.9)	1 (3.0)	0.38
Temporal lobe invasion on MRI	6 (16.2)	9 (27.3)	0.26
TSS	18 (43)	22 (63)	0.2
Transfrontal surgery	21 (50)	10 (29)	0.07
Radiotherapy	26 (62)	18 (51)	0.4
VP shunt	7 (17)	4 (11)	0.5

HO - hypothalamic obese; HWS - hypothalamic damage remaining weight-stable; MRI - magnetic resonance imaging; TSS - Trans-sphenoidal surgery; VP - Ventriculo-peritoneal. p-value calculated using the *t* test.

Cardiovascular risk factors in the entire cohort (n=110)

For 99 patients with data available, mean cholesterol was 5.3mmol/L (range 2.6–9.2 mmol/L), mean triglycerides 2.7 mmol/L (range 0.8-12.4), mean HDL 1.4 (range 0.7-4.0) and mean LDL 2.7 (range 1.1-4.8). Thirty-four of the 110 patients (30.9%) were treated for hypertension and 60 (54.5%) for dyslipidaemia; 16 patients (14.5%) had T2DM, 10 (9.1%) had known CVD and 2 (1.8%) cerebrovascular disease. Nineteen patients were known to smoke (17.3%), but smoking data was missing for 29 patients. According to the Health Survey for England 2012 [256], in the general population of a similar age (55-64 years) 22.3% had treated hypertension, 10.4% had doctor-diagnosed diabetes, 6.3% reported CVD, 3.2% reported having suffered a stroke and 18% were smokers.

Patients diagnosed with hypothalamic tumours between 2002 and 2011 (new cohort, n=35)

As noted above, following a review of patients attending the neuroendocrine clinics at WCNN and UHA, and subsequent publication of this study on the prevalence of HO and associated cardiovascular risk factors [6], a more proactive approach to the prevention of HO and its complications was adopted during clinic follow-up visits from 2002 onwards. Thirty-five of the 110 patients studied were diagnosed after 2002; assessment of these newly diagnosed patients was undertaken to see whether the increase in awareness had resulted in better weight outcomes. The mean (±SD) age at diagnosis was 50.9 (± 15.6 years) and mean age at latest follow-up was 54.8 years; 23 (66%) were male. Ten patients were diagnosed with craniopharyngioma, 1 with hypothalamic tumour and 24 with pituitary macroadenoma and suprasellar extension (including 7 with prolactinoma), all of whom had optic chiasmal compression and visual field defects. One patient referred due to significant weight gain during childhood was obese at presentation; further investigation then revealed the diagnosis of craniopharyngioma.

Prevalence of obesity in cohort diagnosed after 2002

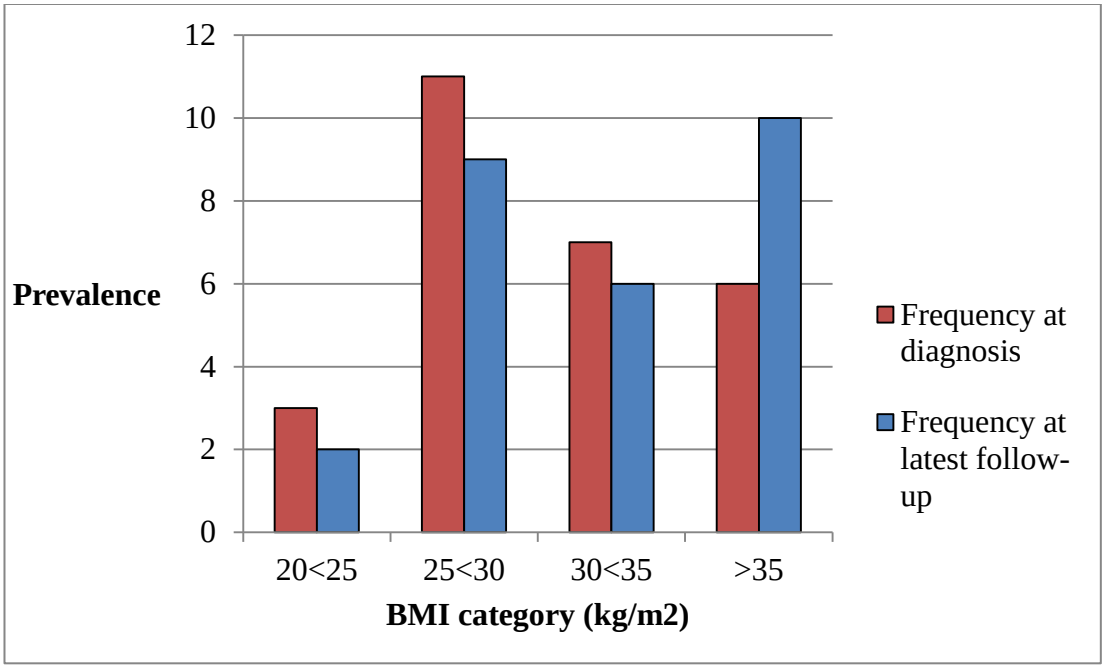
Height and weight measurements at both diagnosis and latest clinic visit were available for 27/35 patients (Figure 3.3). The prevalence of severe obesity increased during the follow-up period (Table 3.5; 22.2% at diagnosis, 37% at latest follow-up, p=0.13). Although this was not statistically significant (p=0.13), this may be due to the small number of patients available to study. Median BMI increased significantly (p<0.001) from 29.9 kg/m^2 (IQR 26.5-33.4) at diagnosis to 31.7 kg/m^2 (IQR 27.8-38.8) at latest follow-up, a median of 3 years later. Therefore despite the increase in awareness of obesity and strategies to curtail weight gain, patients diagnosed since 2002 (with adequately but not over-replaced pituitary hormone deficiencies) had gained weight.

Table 3.5 Prevalence of overweight/obesity/morbid obesity at diagnosis and latest follow-up in 27/35 patients diagnosed between 2002-2011 with paired weight and height data.

Category (BMI [kg/m^2])	**Prevalence (%) at diagnosis**	**Prevalence (%) at latest follow-up (2010/11)**	**p-value**
Overweight (25-29.99)	11(40.7)	9 (33.3)	0.73
Obese (30-34.99)	7 (25.9)	6 (22.2)	1.0
BMI ≥ 35	6 (22.2)	10 (37)	0.13

p-value calculated using McNemar test.

Figure 3.3. Prevalence of BMI category at diagnosis and latest follow-up in patients diagnosed and treated for a hypothalamic tumour during the period 2002-2011 (n = 27).



Treatment of obesity

Five of the 35 patients had received anti-obesity medications (4 orlistat, one orlistat then sibutramine) and one attended the hospital-based weight management clinic. None of the patients had a clinically significant weight-loss following these interventions and many continued to gain weight.

Discussion

This series is the largest study to date to investigate the pattern of weight gain and BMI trends in adult patients with acquired structural hypothalamic damage. Despite efforts during clinic follow-up to prevent/treat HO, patients continued to gain weight and the median BMI of the cohort increased. In the entire cohort followed-up for a median of nine years (77 patients with serial weight data) median BMI increased significantly from 28.1 kg/m^2 at diagnosis to 32 kg/m^2 at latest follow-up ($p<0.0001$), with McNemar's test showing a significant difference in the prevalence of severe obesity (15.6% at diagnosis to 33.8% at latest follow-up, $p<0.001$). Patients diagnosed since 2002, when there was increased awareness of obesity within the clinics, also had a significant increase in median BMI (29.9 kg/m^2 at diagnosis to 31.7 kg/m^2 at latest follow-up, $p<0.0001$), although there was no statistically significant difference in the prevalence of obesity or severe obesity within this group. This, however, may be due to the small number of patients involved and also the high prevalence of both obesity and severe obesity at presentation, meaning more patients were already in these BMI categories at diagnosis. It would have been interesting to directly compare weight change in the two cohorts (pre- and post-2002) however the median follow-up was 9 years and 3 years, respectively. It was not possible to compare the data from the two cohorts at the three year time-point as there was not enough data available, however if the data had been available, presuming the linear model assumptions were not violated, a linear model for BMI at 3 years could have been constructed (adjusted for BMI at diagnosis and whether the patient was diagnosed pre- or post-2002).

As discussed previously, obesity is associated with multiple co-morbidities, including CVD, T2DM, hypertension, NAFLD, sleep apnoea and certain cancers. Cardiovascular risk factors were found to be highly prevalent in this cohort, as in other studies of patients with hypothalamic–pituitary disease [79] and craniopharyngioma [57, 71]. Those who sustain hypothalamic damage at a relatively young age are particularly vulnerable to this increase in cardiovascular risk factors [257], which occurs despite adequate pituitary hormone replacement. The high prevalence of obesity, which is an independent risk factor for CVD [258], in a

population with already increased cardiovascular risk factors is therefore even more concerning.

Analysis of the data showed that the risk of developing HO was greater in those treated with GH, levothyroxine and desmopressin, which likely reflects a greater degree of hypothalamic damage, leading to multiple pituitary hormone deficiencies. The paraventricular nucleus (PVN) is responsible for vasopressin release (absence of which leads to cranial DI) and has a role in the neuroendocrine control of energy balance. It is likely therefore that damage to the PVN leading to DI would also result in some effect on the energy balance circuitry. In agreement with earlier findings from a smaller cohort of the same patients [6], there was no correlation between the risk of postoperative obesity and either pre- or post-operative MRI findings. This is in contrast to studies of children with craniopharyngioma, where all but one study [117] found an increased risk of HO in those with more extensive hypothalamic damage on either preoperative, or pre- and post-operative MRI scans [32, 115]. One study of adult patients with craniopharyngioma [119] found that greater hypothalamic involvement on preoperative MRI scan was positively correlated with greater postoperative weight gain ($p = 0.022$). This study included 28 adults with craniopharyngioma only, had a limited follow-up of two years and none of the patients were treated with GH replacement (the prevalence of symptomatic severe GHD warranting replacement is not stated), therefore this study is not directly comparable to the findings from the patient cohort described in this thesis and these factors may account for the differences in findings.

Some of the patients described in this cohort were prescribed orlistat (with variable results) however no clinical trial of orlistat has been undertaken in this patient population. At the time of study no patients were receiving GLP-1 agonists, although since then this treatment was introduced in one patient with concurrent T2DM whose weight fell from 148 kg to 135 kg three years after starting the medication, with a subsequent increase and stabilisation of weight to 140 kg four years after initiation [127]. The treatment of HO is even more challenging than that of simple obesity. HO is often refractory to conventional therapeutic interventions, possibly due to its underlying structural neurobiological nature, with limited evidence of success with a variety of pharmacological interventions [259]. Since

2007 a few case reports [130, 131], small series [135] and a meta-analysis [138] of bariatric surgery in patients with HO secondary to structural hypothalamic lesions have been published. Although the meta-analysis demonstrated a reasonable net weight-loss (6.1-20.2%) at 12 months, there is lack of long-term follow-up data and as surgery is often only undertaken in those with extreme morbid obesity (mean BMI 49.6 kg/m^2 in the meta-analysis), many of the patients remained in the obese category, even after successful bariatric surgery. One of the additional difficulties in studying weight-loss therapies and interventions in patients with HO is the possibility of multiple pathophysiological mechanisms underlying and contributing to the development of the syndrome.

Based on clinical experience and data from the series described here and the earlier published series [6] the standard practice in the neuroendocrine clinics at WCNN and UHA is to undertake weight measurement of patients at every clinic visit and to ensure that any pituitary hormone deficits are adequately (but not excessively) replaced. In those with no evidence of direct invasion of the hypothalamic nuclei by tumour at presentation, further surgical management aims to minimise the risk of hypothalamic damage wherever possible. In those who have already demonstrated weight gain and in those who are newly diagnosed, the problem of weight gain and obesity secondary to hypothalamic damage is discussed with the patient and dietary and exercise advice are offered as first-line measures. For those newly diagnosed the risk of weight-gain, particularly within the first year after diagnosis and treatment of a tumour causing hypothalamic damage, is emphasized. As lifestyle modification alone is unlikely to be preventive/curative of excessive weight-gain in those prone to HO referral to the regional multi-disciplinary weight management service at UHA is also considered, although patients are warned that their weight gain may be difficult to treat, even if an appropriately healthy diet and active lifestyle are followed. Whilst weight gain and obesity may be more refractory in this patient group, the limited current treatment options mean that it is even more important to ensure that diet and exercise are adequately addressed by the patient. Stabilisation of weight, rather than weight loss, may need to be the objective for many patients, particularly those who have experienced years of incessant weight gain.

Despite little evidence for improved outcomes in the prevalence of HO in patients with acquired, structural hypothalamic damage, the findings from this study

could be used to help to shape best-practice guidelines. Adoption of a multi-disciplinary approach would help to ensure that neurosurgical, endocrine and dietetic issues are considered. Routine monitoring of cardiovascular risk factors (blood pressure, lipids, smoking status, family history), with a low threshold for treatment of these may help to prevent the increased morbidity and mortality seen in this patient group [72], although long-term studies would be necessary to substantiate this approach. The complications of obesity (such as T2DM, NAFLD and sleep apnoea) should also be considered, screened for and adequately treated when identified.

There are many theories regarding the underlying pathophysiological mechanisms involved in weight gain and obesity in those with acquired structural hypothalamic damage. These include hyperphagia and increased energy intake, hyperinsulinism secondary to autonomic nervous system dysregulation, leptin resistance, reduced energy expenditure due to lower levels of physical activity and/or reduced basal metabolic rate, melatonin dysregulation leading to increased daytime somnolence and enhanced 11-β hydroxysteroid dehydrogenase-1 activity leading to increased concentrations of cortisol [11, 260-262]. As the hypothalamus is responsible for both integrating and initiating multiple pathways influencing body weight, it is likely that any, or in fact several of these mechanisms could contribute to the development of HO and that this may vary between individual patients [5, 7, 11]. To further investigate these underlying mechanisms, modalities such as functional neuroimaging (positron emission tomography [PET] and functional MRI [fMRI]) may provide useful insights, as seen in studies of simple obesity. Functional neuroimaging (PET and more recently fMRI) is non-invasive and has helped to identify neuroanatomical correlates of hunger, satiety and feeding in simple obesity [168, 169]. Functional neuroimaging studies have demonstrated how the brain is affected under different experimental conditions - fed versus fasted state [147, 149, 169, 175], with presentation of food [263] or food images and with high-calorie, low-calorie and neutral picture stimuli [175, 180]. A difference in brain activation between obese and lean individuals [168, 169, 175] has also been shown. These studies have demonstrated that regulation of hunger and satiety is complex, involving both homeostatic and non-homeostatic (e.g. cognition, emotion and reward) factors which are processed and integrated in different brain regions, including the hypothalamus, thalamus, orbito-frontal cortex, limbic and paralimbic areas. Studies

which identify potential brain regions of difference in those with HO (either with or without hormonal correlates) may suggest potential future therapeutic targets. Functional neuroimaging studies of patients with HO before and after treatment with incretin-mimetics or bariatric surgery, as have been conducted in patients with type 2 diabetes and simple obesity, may help to elucidate why therapies have been successful in some individuals but not others. Due to the relative rarity of HO, further research would ideally be undertaken in the form of multi-centre, possibly multi-national, collaborative trials involving specialist centres. Both medical and bariatric surgical studies, as well as trials of intensive lifestyle intervention compared to routine care, ideally conducted in a randomised manner, would be beneficial to facilitate improved management of patients with hypothalamic obesity. A UK database and epidemiological study of all patients with suprasellar tumours would be a useful resource, taking a lead from the HIT-ENDO and KRANIOPHARYNGEOM 2000 studies involving all children and adolescents in Germany, Austria and Switzerland diagnosed with craniopharyngioma since 1996 [28, 117].

In clinical practice, complete versus partial resection of hypothalamic tumours to facilitate preservation of the hypothalamus without compromising on long-term disease control or restoration of visual field defects, and timing of post-operative radiotherapy are some of the management dilemmas encountered by clinicians and neurosurgeons. An individualised approach within a multi-disciplinary setting is required to help determine the optimal management of patients, both at initial presentation and upon tumour recurrence, in order to minimise hypothalamic damage and aim to reduce the significant long-term morbidity and mortality associated with this.

Chapter 4

Pituitary Tumours In Children And Adolescents: Presentation And Long-Term Endocrine And Metabolic Outcomes

Abstract

Objective: To describe the long-term sequelae of pituitary adenomas diagnosed in children and adolescents, focussing on metabolic and endocrine outcomes.

Study design: A retrospective review of patients diagnosed with a pituitary adenoma aged 21 years or younger attending a continuous neuroendocrine service in Liverpool, UK.

Results: There were 41 patients (33 female) with mean age at diagnosis 17.3 years (range 11 - 21) and mean follow-up of 9.6 years. Adenomas were sub-classified by hormone type into prolactinoma (29 patients, 15 macroprolactinomas), non-functioning adenoma (NFPA; six), ACTH-secreting (Cushing's disease - CD; five) and growth-hormone secreting (acromegaly; one patient). Ten patients were receiving pituitary hormone replacement: hydrocortisone (9 patients), thyroxine (5 patients), sex steroids (7 patients) and growth hormone (5 patients). Another 7 patients had asymptomatic severe biochemical GH deficiency.

Thirteen patients had gained a significant amount of weight after diagnosis of their pituitary tumour (defined as an increase in body mass index [BMI] >2 kg/m^2 since tumour diagnosis); 16/41 were obese (BMI >30 kg/m^2) at latest follow-up. Five patients had been prescribed orlistat and one was attending a multi-disciplinary weight management service. Other cardiovascular risk factors present included two patients receiving antihypertensive medications, two with type 2 diabetes mellitus and four with treated dyslipidaemia. Three female patients were treated for infertility; 2/3 had successful pregnancies, the third patient was undergoing treatment. An underlying genetic cause was found in only two of the patients; both had multiple endocrine neoplasia (MEN) type 1 syndrome and a prolactinoma.

Conclusions: This is one of the largest case series reviewing patients aged 21 years or younger at diagnosis of a pituitary adenoma and followed up by a single service. Just over two-thirds of the patients had a prolactinoma. The sequelae of increased cardiovascular risk factors (obesity and dyslipidaemia) found in this patient cohort and infertility warrant long-term follow-up to allow active identification and treatment if necessary.

Summary of Justification and Aims

Pituitary adenomas typically occur in the fourth and fifth decades of life [87] and are uncommon in childhood and adolescence. These patients account for only 3–6% of all surgically-treated adenomas [88] and constitute less than 3% of childhood supratentorial tumours [89]. Pituitary adenomas are one differential diagnosis of sellar lesions, along with craniopharyngiomas (which account for nearly 50% of childhood suprasellar masses [264]) and other even less common diagnoses [7]. Pituitary adenomas can cause hypothalamic damage through suprasellar extension, however are most often microadenomas (<10 mm diameter) and therefore confined entirely to the sella, with no impingement on the hypothalamus. They are biologically different from craniopharyngiomas, but adult series have shown that they can still cause significant morbidity [73], including obesity - some of this occurs through hypothalamic damage, but as morbidity can also occur in those without hypothalamic damage, other mechanisms must also be involved.

Whilst clinical features at presentation [88, 94, 99], surgical outcomes [92, 97, 100], remission rates and endocrine outcomes [95, 96] are well described other long-term sequelae described in adult patients with hypopituitarism, such as impaired fertility, increased cardiovascular risk factors and premature mortality due to increased cardiovascular and cerebrovascular events [73] have not generally been reported in younger patients. Impaired fertility has been described in adult patients with pituitary adenomas [101], patients with hypopituitarism from various causes (including various types of adenoma, craniopharyngioma and Rathke's cleft cyst) [102] and in female survivors of childhood cancer who had received ≥ 30 Gy radiation to the hypothalamic/pituitary axis [103]. Premature mortality has been reported in those with hypopituitarism, particularly in females, those diagnosed at a young age and those who received radiotherapy [73]. There may however be confounding factors resulting in this increase in mortality, for example it is unknown whether those presenting with pituitary tumours at a younger age have more aggressive pituitary disease and at the other end of the spectrum, it is possible that those undergoing radiotherapy may be older/otherwise unfit for surgery, which may of course also influence mortality rate. Adults with hypopituitarism have been found to have an increased prevalence of metabolic disturbances such as obesity, dyslipidaemia and hypertension [6, 79], which confer an increased cardiovascular risk. It was therefore important to include a study of children and adolescents with

pituitary adenomas (both with and without hypothalamic damage) in this series of studies, to explore whether there is an increased prevalence of obesity and other metabolic risk factors in this cohort of patients, as is seen in adult studies [73].

The definition of adolescence in previous series has been variable, with the upper-age limit ranging between 17 and 20 years. Given this lack of consensus in the definition of adolescence and that symptoms of pituitary adenoma have been reported as early as 2-5 years before presentation [95, 97, 265, 266] patients diagnosed with pituitary adenoma up to the age of 21 years were included in this case-series.

Aims:

The main aim of this study was to identify the prevalence of obesity and other less well-described long-term sequelae of pituitary tumours (such as metabolic/cardiovascular risk factors for premature mortality) in children and adolescents with pituitary adenomas with and without hypothalamic damage, as has been described in adult patients with hypopituitarism. Secondary aims were to assess the presenting features, tumour type and treatment, endocrine sequelae (including impaired fertility) and underlying genetic diagnoses in this patient group.

Hypothesis:

Children and adolescents with pituitary adenomas (both with and without hypothalamic damage) would have an increased prevalence of metabolic disturbances such as obesity, dyslipidaemia and hypertension (conferring an increased cardiovascular risk) and impaired fertility, as described in adult patients with pituitary adenomas and hypopituitarism.

Participants and Methods

Participants

Permission was obtained from the Audit Departments at The Walton Centre for Neurology and Neurosurgery [WCNN], University Hospital Aintree [UHA] and Alder Hey Children's Hospital [AHCH] for retrieval of data from medical case-notes and electronic records of patients aged ≤ 21 years at diagnosis of pituitary adenoma. Sefton Research Ethics committee gave permission for some additional data to be gathered by contacting patients no longer attending follow-up to ask them to complete a questionnaire regarding weight and fertility status. All patients had attended either the local tertiary paediatric endocrine clinic (AHCH), with subsequent referral to the adult neuroendocrine clinic at UHA, or the joint neurosurgical/neuroendocrine clinic at the WCNN and UHA between 1984 and 2009.

Methods

Where available, data was obtained regarding clinical history, physical examination, auxology and baseline pituitary hormone results at presentation; treatment (medical, surgical, or both); ongoing endocrine problems, such as obesity/weight gain, growth hormone deficiency (GHD), panhypopituitarism and infertility; current medical problems and medications history. Height and weight at diagnosis and latest follow-up were used to calculate BMI (kg/m^2).

Endocrine assessment

Patients underwent routine hypothalamic-pituitary axis testing at tumour diagnosis and as further indicated during treatment and follow-up. The glucagon stimulation test (GlucaGen; Novo Nordisk Pharmaceuticals, Crawley, United Kingdom) tested for growth hormone deficiency (GHD) in the adult setting; severe adult-onset GHD was diagnosed in those ≥ 17 years with a peak GH < 3 μg/L who had completed growth. Patients in the paediatric setting aged ≤ 16 years who had not completed growth and puberty underwent either glucagon stimulation test, insulin tolerance test or arginine test (two separate tests were undertaken in some cases, as per standard practice in children with short-stature of unknown cause). Severe

childhood-onset GHD was defined as peak GH < 5 μg/L. Hormones not requiring stimulation testing were routinely measured during clinic visits; thyroid-stimulating hormone (TSH), free thyroxine (FT4), prolactin, sex steroids (oestradiol or testosterone), cortisol and insulin-like growth factor-1 (IGF-1).

Statistical analysis

Statistical analyses were performed using SPSS, version 15.0 (SPSS Inc., Chicago, Illinois). Longitudinal changes in BMI were compared using the Wilcoxon signed-rank test. Comparisons between groups were made with the Student's *t* test or the Mann-Whitney *U* test for normally and non-normally distributed data, respectively. Statistical significance was defined as $p<0.05$ (two-tailed).

Results

Of the 41 patients (33 female) diagnosed with pituitary adenoma, the mean age at diagnosis was 17.3 years (median 17, range 11 - 21) and mean current age 26.9 years (median 26, range 15 - 46). Mean follow-up was 9.6 years (range 0.1 – 26). All patients were symptomatic, with none presenting as a co-incidental finding of neuroimaging (Table 4.1).

Table 4.1 Presenting symptoms and signs of children and adolescents with a pituitary adenoma: overall and by tumour type.

	Total	**PRLma**	**ACTH-secreting**	**GH-secreting**	**NFPA/ apoplexy**
Number of patients	41	29	5	1	6
Gender (F/M)	33/8	28/1	1/4	0/1	4/2
Symptoms & signs					
Primary amenorrhoea	7	6	1	0	0
Secondary amenorrhoea	17	16	0	0	1
Oligomenorrhoea	4	3	0	0	1
Galactorrhoea	14	13	0	0	1
Weight gain	14	7	5	0	2
Short stature	4	1	1	0	2
Tall stature	1	0	0	1	0
Headache	11	5	2	0	4
Visual defects	6	3	0	1	2
Apoplexy	3	1	0	1	1

PRLma – prolactinoma; ACTH – adrenocorticotrophic hormone; GH – growth hormone; NFPA- non-functioning pituitary adenoma.

Prolactinoma was diagnosed in 29/41 patients (70.7%), 15 were macroprolactinomas (>10mm) and 14 microprolactinomas (≤ 10mm); five patients had Cushing's disease (CD), one a growth-hormone (GH) secreting adenoma and six were non-functioning pituitary adenomas (NFPAs). Seven of the 29 patients with prolactinoma reported weight-gain at presentation. The five with CD all presented with significant weight gain and reported recent slow growth, however only one had short stature. The six patients with NFPAs (three microadenomas, three

macroadenomas) presented with various symptoms, including obesity. The male patient with acromegaly presented with pituitary apoplexy (visual field deficits and seizures) aged 20. Tall stature was noted, but baseline GH levels were not available. Following craniotomy and conventional fractionated radiotherapy GH and insulin-like growth factor-1 (IGF-1) were within normal limits (0.7 μg/L and 16 ng/mL respectively).

Two patients had multiple endocrine neoplasia (MEN) type 1 syndrome. Both had a prolactinoma and a family history of MEN-1. The first patient had hyperparathyroidism requiring surgery and was diagnosed clinically; she developed a jejunal leiomyoma, a gastrinoma and a pancreatic lesion whilst under follow-up. The second patient was diagnosed on genetic testing and has developed a pancreatic body tumour and mild hyperparathyroidism. There was no family history of pituitary disorders in any of the remaining patients in the cohort.

Current endocrine and metabolic status

1. Hormone replacement

Of the 41 patients, ten currently receive pituitary hormone replacement (Table 4.2); five have anterior panhypopituitarism (GH, gonadotrophin, thyroxine and cortisol deficiency) but only one patient has central diabetes insipidus (a patient with CD). With the exception of oestrogen in one patient, none of the 14 patients with microprolactinoma have required any hormone replacement.

Thirteen patients were diagnosed with severe GHD during follow-up, four during childhood. At latest follow-up two of these were receiving GH replacement (GHR) for growth, one had adequate growth (50th centile for height) and declined GHR; the fourth had now reached adulthood but remained on GHR as they fulfilled the UK National Institute for Health and Clinical Excellence (NICE) criteria for continuing GHR into adulthood [247], including impaired quality of life assessed by the Quality of Life - Assessment of GHD in Adults (QoL-AGHDA). Of the nine patients diagnosed with severe GHD in adulthood, two were receiving GHR.

Table 4.2 Treatment and current pituitary hormone replacement of the entire cohort with pituitary adenomas

	Total (n=41)	**Prolactinoma (n=29)**	**ACTH-secreting (n=5)**	**GH-secreting (n=1)**	**NFPA (n=6)**
Tumour Treatment					
Dopamine agonists	30	29	0	0	1
Surgery – TSS	9	2	5	0	2
Surgery – craniotomy	4	1	0	1	2
Radiotherapy	4	1	1	1	1
Current hormone replacement					
Hydrocortisone	9	4	2	1	2
Thyroxine	5	1	1	1	2
Sex-steroid replacement	7	3	1	1	2
Growth hormone	5	2	2	0	1
Desmopressin	1	0	1	0	0

TSS: transphenoidal surgery.

Of the 29 patients with prolactinoma 28 were female and the fertility status of six is known. Three had spontaneous pregnancies (two had two children each and one a single child) and three required treatment for infertility - two had successful pregnancies (one following treatment with clomiphene, another where treatment was unknown) and one was awaiting fertility treatment. In five female patients with non-prolactin secreting adenomas there were no documented pregnancies, however it is not known how many were attempting pregnancy. In the general UK population fertility problems have been reported to affect 1:7 heterosexual couples [267], with 47,422 women undergoing in-vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI) and 2,265 women receiving donor insemination in 2012 [267]. At the time of the last national census (27 March 2011), there were 32.2 million women

in the UK [268]. As the numbers in this patient cohort are small and the proportion desiring pregnancy unknown, direct comparison would be misleading.

2. Auxology and obesity

Excluding patients with CD whose mean BMI, as expected, decreased from diagnosis to latest follow-up and two patients diagnosed less than 2 years previously (with inadequate follow-up data) the mean BMI/BMI-SDS of the cohort increased over the follow-up period (Table 4.3). Thirteen had a significant increase in weight (BMI increased > 2kg/m^2). At time of study 16 patients (39%) were obese (BMI ≥ 30 kg/m^2): 11 with BMI between 30-35 kg/m^2, two between 35-40 kg/m^2 and three had morbid obesity with BMI ≥ 40 kg/m^2. Data from the Health Survey for England 2012 [256] showed that within the general female population of a similar age (age 25-34 years) at that time 21% were obese (BMI ≥30 kg/m^2), nearly half that seen in the patient cohort.

Five patients were receiving treatment with the weight-loss medication orlistat and one was referred to the tertiary multi-disciplinary weight-management service at UHA. None of the patients had a clinically significant weight-loss following intervention and all had a higher BMI when last measured than at diagnosis (increase of between 3.6-9 kg/m^2 since diagnosis).

In the patients with CD (4 with serial measurements) mean BMI fell from 27.6 kg/m^2 at diagnosis to 24.5 kg/m^2 at latest follow-up (p=0.18). In the 29 patients with prolactinoma (21 with serial weight measurements) mean BMI increased from 27.9 kg/m^2 at diagnosis to 30.9 kg/m^2 at latest follow-up (p = 0.04). The five patients with the highest BMI (33.8-41.3 kg/m^2) at diagnosis all had prolactinomas; three lost weight following diagnosis and treatment of prolactinoma, however two gained significant weight (BMI increased from 35 to 41.2 kg/m^2 and 33.8 to 39.5 kg/m^2) despite normalisation of prolactin to well within the normal range (130 mU/l and 11 mU/l).

3. Other cardiovascular risk factors

At latest clinic follow-up two patients were receiving antihypertensive medications and two had type 2 diabetes mellitus. Twenty-six patients had non-

Table 4.3 Distribution of BMI in the entire cohort

	At diagnosis	**At latest follow-up**	***t*(26)**	**p value**
BMI (kg/m^2) of the entire cohort (excluding those with CD and 2 patients diagnosed < 2 years ago)	Mean 26.9 Median 25 Range 17.2-41.3	Mean 30.2 Median 30.7 Range 17.4-46.7	2.75	0.01
BMI SDS of the entire cohort (excluding those with CD and 2 patients diagnosed < 2 years ago)	Mean 1.3 Median 1.2 Range -1.6 – 3.7	Mean 1.9 Median 2.2 Range -1.5 – 4.6	2.57	0.02
	At diagnosis	**At latest follow-up**	***t*(3)**	**p value**
BMI (kg/m^2) of patients with Cushing's disease	Mean 27.6 Median 26.6 Range 24.4-32.6	Mean 24.5 Median 24.4 Range 18.7-30.4	1.67	0.18
	At diagnosis	**At latest follow-up**	***t*(20)**	**p value**
BMI (kg/m^2) of patients with prolactinoma	Mean 27.9 Median 25.7 Range 17.2-41.3	Mean 30.9 Median 31 Range 17.4-46.7	2.17	0.04

p-values show where a significant difference exists between BMI at diagnosis and at latest follow-up. Calculated using paired samples *t*-test.

fasting serum lipids measured. Mean total cholesterol (TC) was 5.6 (range 3.2-9.5) mmol/l, mean HDL 1.3 mmol/l and mean LDL 3.2 mmol/l. Mean TC was higher than that found in the general population of a similar age, based on data from the 2003 Health Survey for England data [269], where mean TC was 4.5 mmol/l (males) and 4.6 mmol/l (females) in the 16-24 year old age group. In the general population, 65.9% of men and 66.4% of women had raised cholesterol (classified as a total cholesterol > 5 mmol/l), compared to 73.1% in this cohort of patients. Four of the patients in the cohort were treated for dyslipidaemia; TC reduced from mean 9.2 (median 7.7) mmol/l pre-treatment to mean 5.7 (median 5.5) mmol/l on lipid-lowering treatment. Mean TC in patients with severe GHD receiving GHR was 5.9 mmol/l, in those with untreated GHD was 5.7 mmol/l and in those GH-sufficient was 5.5 (range 4-8) mmol/l.

Discussion

Pituitary adenomas diagnosed in childhood and adolescence were frequently hormonally active (85%) in keeping with that reported in the literature (80-97% [90, 92, 95-97]). Presenting symptoms were typically in keeping with the hormonal sub-type, rather than visual field deficits which are seen commonly in older adults.

Weight gain before diagnosis occurred in all patients diagnosed with CD, but also in 7/29 (24%) patients with prolactinoma and 2/6 (33%) patients with NFPA. In contrast to the expected findings, the five patients in the series with the highest BMI at diagnosis (range 33.8-41.3 kg/m^2) had a prolactinoma and not CD; three have lost weight following treatment, however two gained further significant amounts of weight despite a decrease in prolactin to well within the normal range. In adult patients with prolactinoma there is conflicting evidence with regards the impact of normalisation of prolactin concentrations on weight loss [111, 112]. Weight gain and obesity are not only distressing sequelae of pituitary adenomas and/or their treatment, but can lead to significant morbidity and mortality. Given the increased prevalence of obesity found in this patient cohort (39%) compared to the general female population of a similar age (21%), despite the predominance of microadenomas and therefore lack of hypothalamic damage within this cohort, this provides some evidence that hypothalamic damage is not the only factor contributing to an increased prevalence of obesity in children and adolescents with pituitary tumours. This is important to bear in mind when considering the underlying pathophysiological mechanisms responsible for hypothalamic obesity as it suggests that there may be other contributing factors causative of weight gain, in addition to the hypothalamic damage, at least in young patients with pituitary adenomas.

Dyslipidaemia in overweight and obese adolescents has been associated with a higher carotid intima-media thickness in adulthood [270]. Carotid intima-media thickness is a surrogate marker of atherosclerosis, which can begin in childhood and progress with age [271] and may result in cardiovascular damage. Adachi et al found 39% of children and adolescents with suprasellar tumours had hyperlipidaemia (lipids >5.7 mmol/L), rising to 58% in those with BMI > 90th percentile [80]. The authors also found a higher incidence of Apo B/A1 elevation and higher sdLDL-C in obese patients with suprasellar tumours compared to obese controls, despite a lower mean BMI in patients and comparable levels of TC, triglycerides and LDL-/HDL-

cholesterol. These factors are associated with increased risk of vascular events [80]. NICE currently recommends that UK clinicians consider investigating lipid concentrations in children with BMI $\geq 98^{th}$ centile for age. In children and adolescents with pituitary adenoma however, the evidence suggests it is advisable to assess lipid profiles even in patients below this BMI cut-off. Both obesity and hyperlipidaemia are important, modifiable risk factors for cardiovascular disease and therefore in this at-risk patient group early identification and targeted dietary and weight loss interventions are extremely important.

As seen in other series [95, 96] prolactinoma was the commonest type of pituitary adenoma (71%) found in the patients attending the clinics at WCNN, UHA and AHCH and all but one patient were female. In contrast with many of these series [93, 95] all but two (4.9%) were treated with dopamine agonists (DA) as first-line therapy. DA therapy has been proven effective in treating prolactinomas and reduces the risk of hypopituitarism. Colao et al described 26 patients with prolactinomas; 15 had macroprolactinomas (5 with VF defects) and 11 microprolactinomas [93]. Nine underwent surgery prior to assessment by the authors for "symptoms of tumour expansion", despite VF defects being present in only two patients and presence of a microprolactinoma in seven without threat to the optic apparatus. Notably, in all 26 patients prolactin normalised within 6-12 months of DA initiation (both those who had undergone surgery and those treated with DA alone) and significant reduction in tumour size was seen in 10/26. The authors of the paper note the effectiveness of DAs and raise the possibility that surgery could have been avoided in the nine operated cases. This is significant as out of seven patients with pituitary insufficiency in their series, five developed this post-operatively. In other series all patients underwent surgical management. Kunwar and Wilson describe 78 patients with prolactinoma, all managed with TSS undertaken as first-line treatment due to a "good outcome" (although this is not defined) [99]. They reason that long-term surgical cure is reported in 82% of patients [97] compared to a need for lifelong DA treatment, however make no mention of subsequent hormone sufficiency/deficiency outcomes or relapse/cure rates in their patients. In a series of 18 patients with prolactinoma undergoing TSS (13 with suprasellar extension) only 7/18 (39%) normalised prolactin postoperatively and 6/18 had a reduction in prolactin [100]. The authors attribute a low rate of "cure" (normal prolactin concentrations) to higher

concentrations of prolactin at diagnosis. In the cohort of patients seen in our service, DA therapy has been successfully discontinued in 9 patients (7 with microprolactinoma, 2 with macroprolactinoma) with no evidence of recurrence after a median duration of follow-up of 4 years (range 1–10 years). Based on this experience, the clinical effectiveness of DAs, the frequency of recurrence of hyperprolactinaemia following surgery [93, 95, 100, 272] and the scarceness of VF defects at presentation, children and adolescents with both macro- and microprolactinomas could most effectively and safely be treated with DAs as first-line therapy, as is recommended in adults [272]. Given the increased risk of premature mortality associated with hypopituitarism [73] medical management of prolactinomas with DAs may decrease the risk which these young people are exposed to.

The prevalence of Cushing's disease was 12% (5/41) in the patients attending the joint service. All patients presented with the characteristic stigmata of significant weight gain and the single female patient in the group also had primary amenorrhoea. Weight gain and obesity are increasingly common in childhood and adolescence and identification of the relatively few patients with CD as the cause can be challenging, with reliance on other symptoms such as growth or pubertal arrest and headache. TSS for these adenomas can be technically challenging, with failure to achieve cure even in the most experienced hands. Cure rates vary widely (45-97%) between paediatric series [100, 273-278] and recurrence after initial cure is possible, therefore life-long follow-up is mandatory.

Pituitary adenomas are familial in about 5% of cases [279] and are known to be associated with multiple endocrine neoplasia (MEN) type 1 syndrome (present in two patients in our series), Carney complex and mutations in the aryl-hydrocarbon receptor interacting protein (AIP) gene. AIP mutations have been described in some cases of familial isolated pituitary adenoma (FIPA) and, significantly to this series, have been associated with earlier-onset, larger, more invasive/aggressive tumours [280-286], typically secreting GH, prolactin or both GH and prolactin [287]. AIP is a tumour suppressor gene, with more than 60 mutations reported and approximately 20% of FIPA families have a heterozygous germline AIP mutation [287]. Given these underlying genetic syndromes, routine questioning regarding family history of

pituitary adenomas is important in this age group and consideration should be given to biochemical screening in families where multiple members have pituitary adenomas.

Conclusions

This is one of the largest reviews of patients aged 21 years or younger at diagnosis of a pituitary adenoma seen in a joint neuroendocrine setting and followed up by a single, continuous service. In this age group hormonally active tumours are more common than non-functioning adenomas, particularly prolactinomas in females. DA treatment is both safe and efficacious and should be first-line treatment for prolactinoma. Surgery is required as first-line treatment only in CD or acromegaly, or in those with adenoma and threat of visual impairment. An underlying genetic cause should be considered by careful attention to tumour type, age of onset and by taking a careful family history.

The sequelae of pituitary tumours are important and include increased cardiovascular risk factors (obesity and dyslipidaemia) and infertility. Active identification and treatment of these sequelae is essential. In particular relevance to this thesis, the increased prevalence of obesity despite the lack of hypothalamic damage in many of the patients studied points towards the possibility of additional contributing factors causative of weight gain in patients with pituitary pathology, regardless of whether hypothalamic damage exists. These patients could be further studied to elucidate the possibility of alternative/additional mechanisms causing weight gain following pituitary and/or hypothalamic damage.

Chapter 5

Cerebral activations during viewing of food stimuli in adult patients with acquired structural hypothalamic damage: a functional neuroimaging study

Abstract

Objective: Acquired hypothalamic damage secondary to tumours commonly leads to obesity, known as hypothalamic obesity (HO). Brain regions controlling homeostatic and non-homeostatic processes of eating and appetite are well described, but their interaction following hypothalamic damage is unknown. It was hypothesized that in patients with hypothalamic damage who became obese (HO), aberrant processing of food stimuli leads to abnormal appetite and excessive food consumption and possibly hyperphagia, which has been described in some patients with HO. This study aimed to compare those with HO to patients with hypothalamic damage who remained weight-stable (HWS) and age- and BMI-matched controls without hypothalamic damage to ascertain any differences in activation of cerebral regions associated with food-motivation and reward.

Study design: Cross-sectional study of 9 patients with HO, 10 age and BMI-matched obese controls (OC), 7 patients who remained weight-stable (HWS) and 10 non-obese controls (NOC). Functional magnetic resonance imaging (fMRI) was performed when fasted, 1 hour and 3 hours after a fixed-load breakfast (25% of participants calculated basal metabolic rate [BMR]). During each scan session participants viewed alternating blocks of photographs of high- or low-calorie food, with non-food photographs also viewed to use as a baseline comparison, to allow purely visual activation to be subtracted from any BOLD signal differences which occurred. Appetite-associated hormones (glucose, insulin, active GLP-1, PYY(3-36) and active ghrelin) were measured throughout the experiment, as were self-reported visual analogue scale (VAS) appetite ratings of hunger, fullness and desire to eat.

Results: Whole-brain statistical analysis (corrected for multiple comparisons using a false discovery rate (FDR) of $p \leq 0.05$ and cluster size $k \geq 3{,}000$ voxels) found mean BOLD signal was significantly lower in HWS compared to the other 3 groups in the posterior insula and lingual gyrus ($p = 0.001$) when viewing high-calorie food photographs compared to non-food photographs. These brain regions are associated with food motivation and reward. There were no significant differences between the four groups in fasting or area under the curve (AUC) hormone concentrations, with the exception of ghrelin (both fasting and AUC) which was significantly higher in *controls* compared to *patients*. VAS ratings of hunger and desire to eat were

significantly higher in obese compared to non-obese participants throughout the study day, but there were no differences in fullness.

Conclusions: A difference exists in food motivation-related BOLD signal in patients with hypothalamic damage who remain weight-stable (HWS) compared to those who develop HO and *controls*. The differences in these neural pathways associated with food motivation and reward-related behaviour may be implicated in the pathophysiology of HO.

Summary of Justification and Aims

Introduction

Weight gain and obesity are common sequelae of acquired, structural hypothalamic damage, which can occur secondary to pathology such as hypothalamic tumours or craniopharyngiomas [6, 245]. Hypothalamic obesity (HO) is an acute increase in body weight following such damage, with weight gain faster than any expected age-related increase and occurring despite adequate treatment of any associated pituitary hormone deficiencies. Substantial weight gain often occurs, but predicting which patients will be affected and adequately treating them remains difficult [6, 245] and the underlying pathophysiology remains unclear. Exploration of the various factors controlling appetite and eating may help to elucidate answers to these problems and provide areas of investigation for future treatment methods. In addition to helping elucidate the pathophysiology underlying HO, areas of interest for further "simple obesity" research may be identified.

While the hypothalamus is the critical homeostatic centre, the nucleus accumbens (NA) and brainstem (particularly the ventral tegmental area [VTA] of the midbrain) are also important. Non-homeostatic factors, such as emotion, cognition and reward are regulated by limbic, paralimbic and higher cortical regions; these areas process environmental and hedonic cues and food reward. Interactions between homeostatic and non-homeostatic systems remain poorly understood. Studying patients with damage to the major homeostatic regulator (i.e. the hypothalamus) allows exploration of reward-related non-homeostatic areas influencing appetite and

feeding. Over the last 10 -15 years, human appetite and eating regulation has been studied using functional neuroimaging (functional magnetic resonance imaging [fMRI] and positron emission tomography [PET]) to explore both the homeostatic and non-homeostatic cerebral regions involved. Early neuroimaging studies using PET demonstrated differences in brain activation in several regions before and after a liquid meal [149, 168, 175]. A few studies have used imagined food [167] or actual food [170], but most recent studies have used photographs of food and non-food items [162, 175-178] to distinguish brain activation due to visual effects from activation due to food stimuli.

Aims: The main objective of the study was to identify differences in brain responses to visual food-stimuli using fMRI in participants with HO, compared to those with hypothalamic damage who remained weight-stable (HWS) and BMI-matched controls.

Hypothesis: Participants with HO would have greater activation of the reward-related brain regions than those HWS, where the homeostatic pathways may have remained more influential. If this hypothesis was supported by the findings then an increase in reward-related eating in those with HO would be reflected in an increase in food intake, as explored in other aspects of the study (food diaries, TFEQ and UEM study day, Chapter 6).

Participants and Methods

Participants

The recruitment of patients for the study is fully described in chapter 2. Thirty-six participants aged 18-75 were recruited (Table 5.1a); nine with HO, seven HWS and 20 BMI-matched, otherwise healthy controls (10 obese [OC], 10 non-obese [NOC]) of similar gender and age).

Patients were recruited from pituitary/neuroendocrine clinics at University Hospital Aintree (UHA) or the Walton Centre for Neurology and Neurosurgery (WCNN). All had undergone treatment of a tumour of the hypothalamus or a surrounding structure with direct hypothalamic compression/invasion (Table 5.2). All had grade 2 hypothalamic damage (Saint-Rose et al. grading [32, 115]), assessed

by a Neuroradiologist. HO was defined as body mass index (BMI) $\geq$ 30 kg/m^2 at latest follow-up, increased $\geq$ 2 kg/m^2 since tumour diagnosis. HWS participants had a BMI < 30 kg/m^2, within 2 kg/m^2 of that at tumour diagnosis. All patients were on adequate replacement of pituitary hormone deficits - 8 required hydrocortisone, 9 levothyroxine, 3 desmopressin for permanent cranial diabetes insipidus (DI), all pre-menopausal women and 3 men with secondary hypogonadism received sex steroids and 9/11 participants with GHD received growth hormone (two did not qualify for treatment as they were asymptomatic on QOL-AGHDA questionnaire).

Healthy controls were recruited by advertisement within the University of Liverpool and from previous obesity studies. Obesity was defined using the standard definition of BMI $\geq$ 30 kg/m^2. All participants were medically screened, with visual adequacy assessed in the patient group.

The study was approved by the local ethics committee (Northwest Research Ethics Committee, 09/H1001/4). Signed, informed consent was obtained from each participant prior to study participation.

Study design and methods

Participants were instructed to fast from 22:00 the previous night and to refrain from alcohol, strenuous physical activity, cigarettes and caffeine-containing drinks for at least 72 hours (see Appendix 4 for timeline). Participants attended the MARIARC (Magnetic Resonance And Image Analysis Research Centre), University of Liverpool, around 08:30. Blood samples were collected and participants asked to rate their feelings of hunger, fullness and the strength of their desire to eat using Visual Analogue Scale (VAS) ratings (Appendix 2), before undergoing two MRI scans (Figure 5.1). The first was an anatomical scan and the second an fMRI scan during which photographs were viewed using a mirror and screen inside the scanner (paradigm described in Figure 5.3). Participants were asked to try and remember each photograph for a recognition test at the end of each fMRI scan, when they were asked to look through a photo album containing photographs identical to those shown during the scanner session, presented in the same order, and rate on VAS scales how much they liked the food and how much they wanted to eat that food at that moment (Figure 5.2). This data was not analysed as it was purely to ensure that participants were concentrating on the food photographs shown during the fMRI scanning session.

Participants then consumed a breakfast meal of porridge and orange juice totalling 25% of their estimated basal metabolic rate (BMR), calculated using the Harris-Benedict formula (see Chapter 2, Methods for details). Details of the nutritional content of the breakfast meal are shown in Table 2.1 (Chapter 2).

A second and third fMRI scan were undertaken one and three hours after the breakfast meal whilst undertaking the same paradigm as during the first fMRI scan (Figure 5.3). Between scans participants rested in a quiet room whilst on-going blood samples and VAS ratings of appetite were completed. Blood samples were taken prior to breakfast and at 15, 30, 60, 120 and 180 minutes after the breakfast meal for measurement of glucose, insulin, active ghrelin, PYY(3-36) and active GLP-1. VAS ratings of hunger, fullness, and desire to eat were completed by the participants in the fasting state and at the end of each scanning session.

The protocol for collection, storage and measurement of these biochemical parameters is fully described in chapter 2 (*Methods*).

Figure 5.1. Timeline for participants.

Previous Day	Study Day				
22.00	08.30	09.00	09.30	10.30	12.30
Start fasting	Fasted blood sample	Anatomical MRI and baseline fMRI	VAS ratings prior to breakfast (porridge and orange juice equivalent to 25% of BMR)	2nd fMRI (1hPB)	3rd fMRI (3hPB)

Figure 5.2. Example of the food photograph rating undertaken by participants after each fMRI scanning session.

Photo 1

How **MUCH DO YOU LIKE THIS FOOD at this moment**?

Not at all ——————————————————— A large amount

How **MUCH DO YOU WANT TO EAT THIS FOOD at this moment**?

Not at all ——————————————————— A large amount

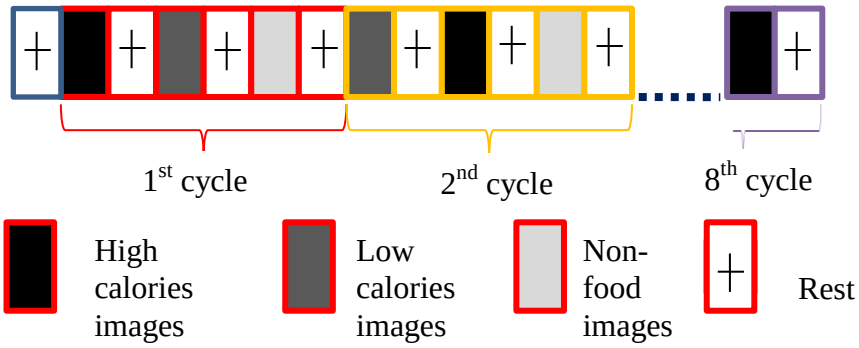
MRI and fMRI acquisition

MR images were obtained on a 3.0 Tesla Siemens Trio (Siemens Medical, Erlangen, Germany) scanner with [248] eight-channel head coil. Foam padding minimised head movement during each scan and alignment with the first scanning session was attempted as much as possible on subsequent scanning sessions. Whole-brain T1-weighted anatomical sagittal brain images were acquired using Modified Driven Equilibrium Fourier Transform (MDEFT) to minimise susceptibility effects [248] (TR7.92 ms, TE 2.48 ms, flip angle 16˚, FOV 256×240, 180 1mm slices, voxel 1×1×1 mm^3). Structural images provide anatomical representations against which functional scans (usually of low spatial resolution with poor tissue contrast) can be compared. fMRI sequences used echoplanar EPI (TR 3000 ms, TE 30 ms, flip angle 90˚, FOV 192×192 mm^2, 56 oblique 2 mm slices with slice gap 0.8 mm, voxel 3×3×3 mm^3).

Functional neuroimaging (fMRI): Activation paradigm

An activation paradigm was generated using 'Presentation' software (*https://nbs.neuro-bs.com*) which combined an initial on-screen written instruction, food and non-food photographs and a fixation cross (to be viewed during rest periods in between blocks of photographs). The software programme was used with each fMRI scan to ensure accurate timing and consistency of images and rest periods and was viewed via a mirror mounted in the scanner which reflected a projected computer screen. The presentation began with the written instruction "Please concentrate on the pictures and imagine what they taste like!", followed by a fixation cross shown for six seconds. The photographs (32 per category; high-calorie, low-calorie, non-food) were presented in blocks, with no repeat of any photograph *within* each scanning session (although the same photographs were used in subsequent sessions, but in differing orders). Each block contained photographs from a single category (high-calorie, low-calorie, non-food), with each photograph presented for four seconds and four photographs presented in one block (Figure 5.3). Each block was 16 seconds with a rest period (6 seconds) showing a fixation cross in-between. Each condition (high calorie, low calorie, non-food) appeared once in each cycle in a random order, with a total of eight cycles.

Figure 5.3. Schematic representation of the block experimental design. Each shaded rectangle represents a 16 second period during which 4 pictures of the same category were presented (4 seconds each).



A pilot study using this fMRI paradigm was undertaken in five healthy, lean or overweight individuals to ensure that it elicited a BOLD response in the regions that you might expect when viewing high and low calorie food images, as previously reported in studies using similar experimental paradigms. Data from these subjects were not included in the final analysis.

Food photographs

The photographs shown were a series of standardised pictures, composed specifically for use in this study to ensure that differences between photographs were not contributing to differences in cerebral activation. Food and non-food photographs were generated using a Nikon D80 camera with additional flash, taken on a non-distracting, white infinity curve background using freshly prepared food products and cropped so that the food or object filled almost the entire screen to ensure standardised images. The best photograph of each item was then selected and categorised as: high-calorie food (e.g. sausage rolls, doughnuts), low-calorie food (e.g. steamed salmon with fresh vegetables, mixed fruit salad) or non-food objects (e.g. calculator, candles) (Appendix 3). Of 92 food photographs taken, 64 were shown (32 in each category) based on responses to an online questionnaire of University of Liverpool staff and students who responded to an internal University advertisement (University of Liverpool Ethics approval PSYC07080300). Participants judged whether each food photograph was high or low calorie and rated its pleasantness (hedonic value). Photographs with the greatest agreement on calorie

content (high or low) and rated most pleasant were included. This allowed comparison between food-energy categories, with less influence of food pleasantness, although did not allow for individual food preferences as the same images were used for each participant. Low- and high-calorie food and object photographs were well matched for visual interest (variety, colour, size) in order to minimise other variability which might then account for differences found.

Image processing and analysis

Pre-processing and statistical analyses of fMRI data was undertaken using SPM8 (http://www.fil.ion.ucl.ac.uk/spm; Statistical Parametric Mapping software package, Wellcome Department of Cognitive Neurology, London, UK) as follows:

1. The first two images of each experimental run were collected with the participant at rest and discarded for noise.
2. A reference slice (number 25 of 1-50) was chosen for slice-timing correction and all slices shifted to that time-point using an interpolation method; changes in the BOLD (blood-oxygen -level-dependent) fMRI response then started at the same time for each voxel in each slice of the volume.
3. Realignment to the same reference image was undertaken to correct for head movement. Spatial transformation was performed using a least squares (rigid body) approach.
4. A mean functional image volume was created for each participant using the realigned images and coregistered with the EPI template supplied by SPM8 in Montreal Neurological Institute (MNI) space. The resulting pixel size in standard stereotaxic coordinates was 2 mm^2, with an interplane distance of 2 mm.
5. The normalized images were subsequently smoothed with an isotropic 6 mm^3 FWHM Gaussian kernel to compensate for normal variations in brain size and individual gyral pattern, filter out any high-frequency noise potentially introduced in the previous processing steps and to improve signal to noise ratio [288, 289].

Biochemical assays

These are fully described in Chapter 2.

Visual Analogue Scale (VAS) ratings

Unipolar VAS ratings to assess hunger and satiety consist of a question accompanied by a 100mm unmarked scale, with descriptors of extreme state (e.g. not hungry at all-extremely hungry) anchoring each end (Appendix 2). Unipolar scales such as these (as opposed to bipolar scales, where anchors are hungry to sated) are more accurate and less likely to skew the data [250]. VAS ratings have been found to be a reliable measure in appetite research [251].

Participants were asked to mark on a 100mm paper VAS scale a single, vertical line which best corresponded with their subjective feelings of appetite (hunger, fullness and their strength of desire to eat). The scales were anchored by "Not at all" and "Extremely" at either end. This allowed participants' subjective sensations to be quantified numerically by later measurement of the point along the scale and analysed. VAS ratings were made at various time points throughout the study day (as described above and in the timeline in Appendix 4).

Statistical analysis

Two-way ANOVA was used to compare age, body composition and VAS ratings of appetite between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* participants, *patients (HO/HWS) vs. controls* (*NOC/OC)* and the interaction between these factors. Levene's test was used to check the ANOVA assumption of equality of variances. For BMI and VAS ratings of hunger at three hours data required transformation to equalise the variances. A ladder of power [290] was performed in order to choose the best transformation for the data; the inverse function was used for BMI and the square for hunger at three hours. Further Levene's tests on the transformed data sets were non-significant ($p>0.05$), indicating that the problem of unequal variance was resolved. The two-way ANOVA undertaken on these transformed data and transformed means (SD) are reported.

Fisher's exact test was used to examine the categorical data of the patient groups (due to the small sample size) and continuous data (duration of diagnosis in the two patient groups) was examined using the Mann-Whitney *U* test, as it was non-normally distributed.

For the fMRI data statistical analysis tested for significant regional BOLD signal differences. Functional images were smoothed and normalised then included in the first level design matrix. For each participant the contrast for determining BOLD signal differences was the contrast between *all* foods (high- and low-calorie) and objects (and not a contrast with baseline). This contrast was selected to eliminate activation related purely to visual stimulation. Second level analysis using a one sample t-test of the single contrast images (one per participant) determined mean BOLD signal secondary to viewing *all* foods amongst all participants. Results were corrected for multiple comparisons using a false discovery rate (FDR) of $p \leq 0.05$. As it was an exploratory study it was decided to conduct whole brain analysis and not to use *a priori* regions of interest (ROIs) initially. The ROIs identified were delineated using MarsBaR (MARSeille Boîte À Région d'Intérêt, a toolbox in SPM used for ROI analysis; http://marsbar.sourceforge.net/ [249]) and those with a cluster size $k \geq 3000$ voxels subsequently analysed. A cut off of $k \geq 3{,}000$ voxels was chosen as this allowed for a reasonable but not excessive number of ROIs with significant BOLD signal, but did exclude smaller potential ROIs such as the amygdala or NA. In total six significant BOLD signal clusters were found (Table 5.3). Using MarsBaR, contrast values for both high- and low-calorie foods for each of the three fMRI scanning sessions were defined using each participant's first level design matrix, generating a total of six contrast values for each participant.

Further statistical analysis was performed using SPSS v.17, v. 22 and v. 24 (http://www.spss.com/uk/). Six linear mixed-effects models were computed - one for each of the six clusters identified. Linear mixed-effects models were chosen because they allow for the inclusion of both fixed factors (e.g. gender, patient/control group) and random factors (which account for the within-subject correlation in the data, e.g. correlation which occurs due to the inclusion of high- and low-calorie foods belonging to the same participant). Potential interactions between patient/control group, non-obese/obese group and high/low calorie foods were also considered in each of the models. Each interaction was incorporated in the model by adding the product of the corresponding two variables as an additional explanatory variable. These additional variables did not significantly improve the fit of the models ($p > 0.05$) and were therefore excluded. Contrast values for high- and low-calorie foods for each of the three sessions within each ROI were entered as the outcome variable in the first repeated-measures ANOVA. The grouping factors ***non-***

obese/obese (HWS and NOC/HO and OC), ***control/patient*** (OC and NOC/HO and HWS) and the ***interaction between these two***, along with the variables ***high-/low-calorie foods*** and ***fMRI session*** (fasting, 1hPB and 3hPB) were entered as the predictor variables. Identical analysis was performed for each of the six ROIs. As the assumption of sphericity is unlikely to be met in a repeated-measures ANOVA and violations of sphericity are detrimental to the accuracy of ANOVA, the Greenhouse-Geisser results for within-subject effects were interpreted, as this correction adjusts the degrees of freedom and decreases the likelihood of a Type I error.

Mean insulin, glucose, active ghrelin, active GLP-1 and PYY(3-36) across the timepoints were calculated, to reflect the amalgamated timepoints used in the fMRI analysis. Additional linear mixed-effects models were calculated using mean hormone concentrations as the outcome variable, with ***non-obese/obese***, ***patient/control*** and ***session*** (at all three fMRI timepoints i.e. fasting, 1hPB and 3hPB) used as predictor variables.

Further analysis of the hormonal variables glucose, insulin, PYY(3-36), active GLP-1 and active ghrelin was undertaken using Microsoft Excel 2013 to calculate mean fasting concentrations and area under the curve (AUC) by trapezoidal integration. Any values below the limit of quantification were set to zero. Differences between the groups in fasting and AUC hormone concentrations were assessed using two-way ANOVA. Levene's test was used to check the ANOVA assumption of equality of variances. For AUC insulin and fasting glucose, ghrelin and PYY, transformations were needed to equalise the variances. For each of these variables a ladder of power was undertaken to identify the best transformation for the data [290]. The inverse of the square root was used for AUC insulin, the inverse function for fasting glucose, the square root for fasting active ghrelin and the natural logarithm for fasting PYY(3-36). A further Levene's test on the transformed data was non-significant ($p>0.05$), indicating that the problem of unequal variance was resolved. The two-way ANOVA undertaken on the transformed data is reported and transformed means (SD) reported, where appropriate.

For all analyses $p\leq0.05$ (two-tailed) were taken to be significant.

Results

Participant characteristics

Nine patients with HO, seven who remained weight-stable following hypothalamic insult (HWS) and 20 age-matched obese (OC) and non-obese controls (NOC) were studied. There was no significant difference in age between *obese (HO/OC) vs. non-obese* (*HWS/NOC), controls* (*NOC/OC) vs. patients (HO/HWS),* or the interaction between these factors on two-way ANOVA (results of interaction in Table 5.1a). There was significantly lower transformed BMI (inverse BMI) in *obese (HO/OC)* than *non-obese* (*HWS/NOC)* (mean transformed BMI in obese 0.027, mean transformed BMI in non-obese 0.038, $F_{[1,32]}$= 68.0, p<0.001), however there was no significant difference between *controls* (*NOC/OC) vs. patients (HO/HWS)* ($F_{[1,32]}$= 0.25, p=0.62), or the interaction between these factors (Table 5.1a). There was significantly higher percentage body fat in *obese (HO/OC)* than *non-obese* (*HWS/NOC)* participants (mean percentage body fat in obese 44.4, mean percentage body fat in non-obese 31.3, $F_{[1,30]}$= 21.5, p<0.001), however there was no significant difference between *controls* (*NOC/OC) vs. patients (HO/HWS)* ($F_{[1,30]}$= 0.06, p=0.81), or the interaction between these factors (Table 5.1a).

None of the participants had glucose intolerance or diabetes. Hormone deficiencies and duration of diagnosis are noted for participants with hypothalamic damage (Table 5.1b).

Eleven patients (HO or HWS) had severe GHD (growth hormone deficiency) with nine receiving replacement therapy; two were asymptomatic on QOL-AGHDA questionnaire and did not qualify for treatment under UK guidelines. There was a significant difference in the prevalence of hypothyroidism (p=0.02). There was no significant difference in time since diagnosis of the hypothalamic lesion (p=0.48). The gender, current age and BMI, disease duration and diagnosis of participants with hypothalamic damage are noted (Table 5.2). The duration of diagnosis of hypothalamic damage ranged from 2 to 38 years.

Table 5.1a. Characteristics of the four participant groups. Mean (SD) reported for age, BMI and percentage body fat

	HO	HWS	OC	NOC	F value	p-value
Participants	9	7	10	10	-	-
Gender: F	4	4	8	6	-	-
M	5	3	2	4		
Age, years	45.2	57.9	45.5	47.6	F[1,32]	
	(14.6)	(17.1)	(11.7)	(20.2)	0.94	0.34
Median BMI	38.4	27.8	38.8	26.6	0.29[a]	0.59[a]
[IQR](kg/m^2)	[8.6]	[4.0]	[10.2]	[5.0]		
Percentage	43.9	31.1	44.9	31.5	F[1,30]	
body fat	(6.1)	(7.9)	(5.7)	(11.1)	0.18	0.90
Body fat category:						
Healthy	0	3	0	4	-	-
Overfat	0	2	1	4		
Obese	8	2	8	2		

F = female; M = male. F-ratio and p-values for the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* on two-way ANOVA. [a]BMI data required transformation (using the inverse function) to equalise the variances. Statistical comparison was performed using these values.

Table 5.1b. Characteristics of the two patient groups.

	HO Mean (SD)	HWS Mean (SD)	p-value
Number of participants	9	7	-
Duration diagnosis	12.1 (13.6)	13.1 (8.0)	U=21.5, z=-0.75, p=0.48
Cortisol deficiency	5	3	0.59
GHD	6	5	0.22
Hypothyroidism	7	2	0.02
Hypogonadism	2M, 2F	1M, 2F	0.79
Permanent DI	2	1	0.56

F = female; M = male. Statistical comparisons using two-sided Fisher's exact test, except for duration of diagnosis (Mann-Whitney test).

Table 5.2. Tumour types of patients with hypothalamic damage.

ID	Gender	Current age (yrs)	Current BMI (kg/m^2)	Duration (yrs)	Group	Diagnosis
HO1	F	70	28.1	12	HWS	PA
HO2	F	40	43	29	HO	HG
HO3	M	68	30	5	HWS	PA
HO4	F	52	38.4	4	HO	CP
HO5	F	18	34.7	9	HO	CP
HO6	F	66	28.96	14	HWS	PA
HO7	M	22	23.7	12	HWS	CP
HO8	F	52	26	26	HWS	PA
HO12	M	34	32.4	2	HO	PA
HO13	M	40	32.9	6	HO	Prolactinoma
HO14	M	67	27.7	3	HWS	Prolactinoma
HO15	M	60	48.2	5	HO	CP
HO16	M	56	32.3	38	HO	CP
HO18	M	64	39.4		HO	PA
HO20	F	58	24	20	HWS	PA
HO21	F	42	34.5	4	HO	Midline cyst

F = female, M = male. HWS = hypothalamic weight-stable, HO = hypothalamic obese. PA = pituitary adenoma, HG = hypothalamic glioma, CP = craniopharyngioma.

fMRI data

Effects of photograph category: food vs. object

Across all participants and all fMRI sessions, there were 6 ROIs with significantly greater BOLD signal for food images than objects (Table 5.3; activation maps Figure 5.3). These were: left-sided posterior insula and middle frontal gyrus; right-sided lingual gyrus, middle temporal (precentral) gyrus, anterior cingulate and posterior cingulate gyrus.

Table 5.3. ROIs identified where BOLD signal was significantly greater for all food images compared to objects, across all participants (results corrected for multiple comparisons using an FDR of $p \leq 0.05$ in areas with $k > 3{,}000$ voxels)

Region	Nearest BA	Talairach coordinates	Cluster volume (mm^3)	T-score
Lingual gyrus (R)	18	6, -88, -4	52640	11.37
Posterior insula (L)	13	-38, -4, 4	12448	8.0
Anterior cingulate gyrus (R)	32	2, 18, 42	45752	7.10
Precentral gyrus (R)	21	66, -16, 34	4640	6.18
Posterior cingulate gyrus (R)	32	4, -28, 32	17336	5.71
Middle frontal gyrus (L)	46	-42, 38, 22	6736	4.7

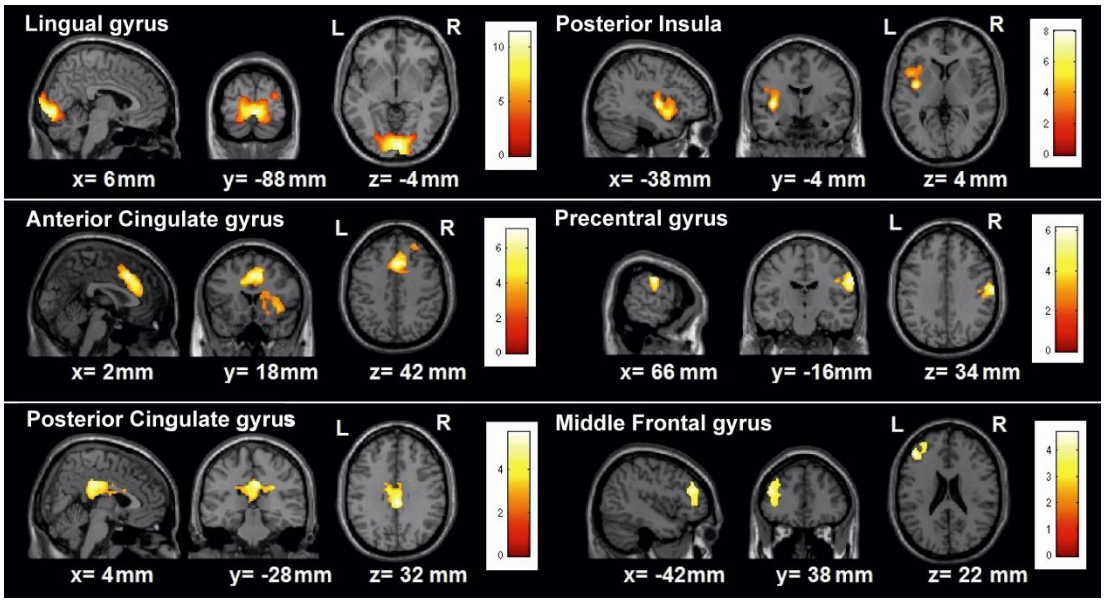
Abbreviations: L = left hemisphere, R = right hemisphere. BA = Brodmann Area. Talairach coordinates (x, y, z) are given for the most significant voxel in the cluster, however ROIs cover larger areas.

The mean BOLD signal values of these 6 regions for high- and low-calorie foods in each of the four participant groups are given (Table 5.4) and activation maps shown (Figure 5.4).

Table 5.4. Mean % BOLD signal (SD) and [minimum, maximum] BOLD signal *within each* ROI for ***high-calorie*** and ***low-calorie foods*** compared to objects.

Area	Calorie type	HWS	HO	NOC	OC
Lingual gyrus (R)	High	1.31 (0.42) [0.61,2.26]	1.64 (0.49) [-0.93,2.5]	1.7 (0.58) [0.81,2.96]	1.61 (0.25) [1.12,2.08]
	Low	1.34 (0.42) [0.61,2.22]	1.78 (0.57) [0.86,2.74]	1.78 (0.53) [1.14,3.07]	1.61 (0.40 [0.46,2.51]
Posterior Insula (L)	High	-0.04 (0.27) [-0.66,0.58]	0.28 (0.53) [-0.34,2.05]	0.19 (0.2) [-0.24,0.77]	0.18 (0.28) [-0.54,0.92]
	Low	-0.03 (0.25) [-0.59,0.39]	0.22 (0.58) [-0.16,2.45]	0.21 (0.17) [-0.23,0.44]	0.22 (0.35 [-0.33,1.58]
Anterior Cingulate gyrus (R)	High	0.08 (0.19) [-0.33,0.38]	0.19 (0.25) [-0.34,0.66]	0.02 (0.24) [-0.49,0.49]	0.14 (0.26) [-0.6,0.82]
	Low	0.12 (0.19) [-0.29,0.52]	0.14 (0.19) [-0.42,0.46]	0.11 (0.22) [-0.42,0.49]	0.12 (0.23 [-0.26,0.98]
Precentral gyrus (R)	High	0.09 (0.47) [-0.7,0.85]	0.18 (0.51) [-0.76,1.21]	0.07 (0.29) [-0.47,0.8]	0.1 (0.4) [-0.67,1.17]
	Low	0.21 (0.28) [-0.16,0.8]	0.05 (0.56) [-1.1,1.17]	0.12 (0.28) [-0.74,0.63]	0.08 (0.27 [-0.44,0.79]
Posterior Cingulate gyrus (R)	High	-0.05 (0.33) [-0.6,1.05]	0.11 (0.54) [-1.02,1.62]	-0.12 (0.19) [-0.5,0.21]	0.07 (0.41) [-0.46,1.4]
	Low	0.01 (0.27) [-0.53,0.5]	0.1 (0.42) [-0.55,1.44]	-0.08 (0.20) [-0.54,0.24]	0.09 (0.38 [-0.52,1.43]
Middle Frontal gyrus (L)	High	0.12 (0.22) [-0.32,0.52]	0.22 (0.45) [-0.68,1.36]	-0.002 (0.3) [-0.59,0.84]	0.13 (0.41) [-0.69,1.42]
	Low	0.15 (0.29) [-0.43,0.66]	0.21 (0.4) [-0.3,1.45]	0.15 (0.31) [-0.47,0.97]	0.16 (0.42 [-0.44,1.76]

Figure 5.4. Brain maps showing BOLD signal across the 6 ROIs for the contrast *all foods (high- and low-calorie) vs. objects* (results corrected for multiple comparisons using an FDR of $p \leq 0.05$ in areas with $k > 3{,}000$ voxels)



ROIs are shown in sagittal, coronal and axial planes on a single-subject template from SPM8. Talairach coordinates (x, y, z) for the most significant voxel in the cluster are given. Colour corresponds to T-scores. L = left hemisphere, R = right hemisphere.

Effects of photograph category: high- vs. low-calorie

There was no significant effect of high- vs. low-calorie food photographs in any of the 6 linear mixed effects models ($p > 0.05$). Interactions between group (non-obese/obese, patient/control) and food-type (high/low-calorie) were entered into each model by adding the product of the corresponding two variables as an additional explanatory variable. As none significantly improved the fit ($p > 0.05$) they were excluded.

As there was no significant difference between high- and low-calorie foods and as fMRI analysis cannot exceed three levels, only the data produced by viewing high-calorie foods was used for further analysis.

Between-group comparison: obese vs. non-obese

In five of the six areas obese participants (HO/OC) exhibited increased BOLD signal when viewing high-calorie foods compared to non-obese participants (HWS/NOC). Box plots show BOLD signal in each of the six ROIs in each participant group (Figure 5.5).

There was significantly lower posterior insula (p=0.001; coefficient -0.2, SE 0.06, 95% CI: -0.33, -0.08) and lingual gyrus (p=0.001; coefficient -0.34, SE 0.1, 95% CI: -0.53, -0.15) BOLD signal in non-obese *(HWS/NOC)* compared to obese *(HO/OC)* participants on the linear mixed-effects model. The BOLD signal cluster in the insula showed spatial maximum in the posterior insula, but spread to middle insular cortex.

Between group comparison: patient/control, obese/non-obese

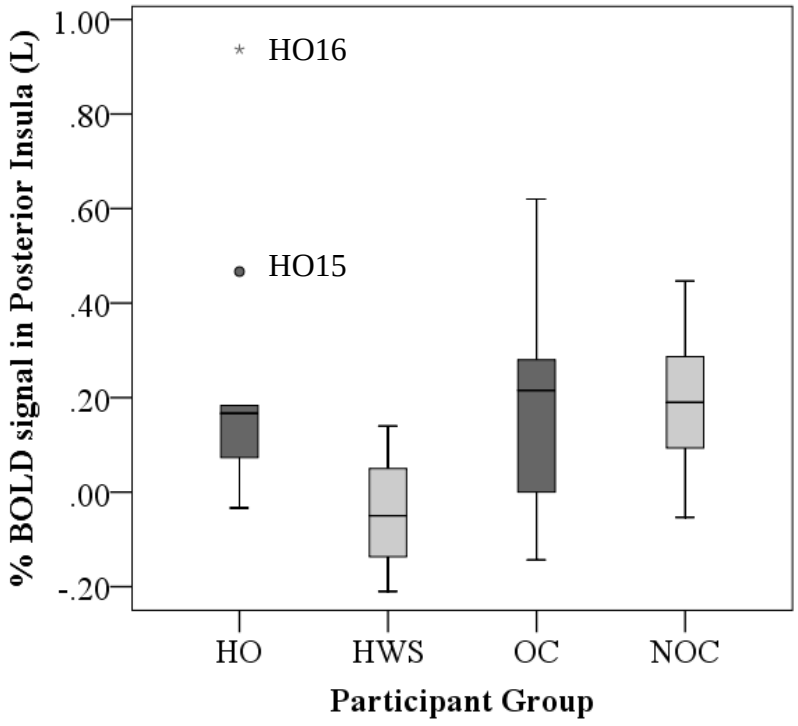
There was a statistically significant interaction between *non-obese* (*HWS/NOC) vs. obese (HO/OC)* and *controls* (*NOC/OC) vs. patients (HO/HWS)* in the posterior insula (p=0.028; coefficient 0.19, SE 0.08, 95% CI: 0.02, 0.35) and the lingual gyrus (p<0.001; coefficient 0.47, SE 0.13, 95% CI: 0.22, 0.73).

Viewing high-calorie food photographs resulted in a significant difference in insula BOLD signal ($F_{[1,103]}$=5.554, p=0.02) between the groups (Figure 5.5). Significantly lower BOLD signal was seen in HWS (mean=-0.042) compared to HO (mean=0.237), NOC (mean=0.186) and OC (mean=0.175).

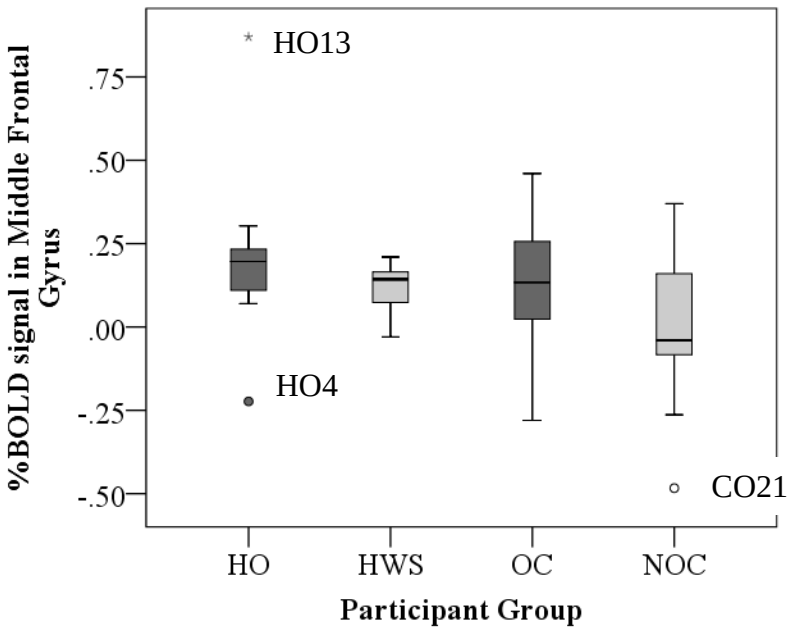
Within the lingual gyrus of the occipital lobe ($F_{[1,103]}$=5.176, p=0.025) there was significantly lower BOLD signal in HWS (mean=1.309) than in HO (mean=1.633), NOC (mean=1.698) and OC (mean=1.609) (Figure 5.5).

Figure 5.5. Box plots showing median % BOLD signal across all three sessions [(thick central line), interquartile range (upper and lower box edges) and minimum/ maximum (lowest/uppermost horizontal lines, with outliers depicted as stars/circles)] whilst viewing high-calorie foods (compared to objects) in the 6 ROIs, shown by participant group. A. Posterior Insula, B. Middle Frontal Gyrus, C. Anterior Cingulate, D. Posterior Cingulate, E. Middle Temporal Gyrus, F. Lingual Gyrus

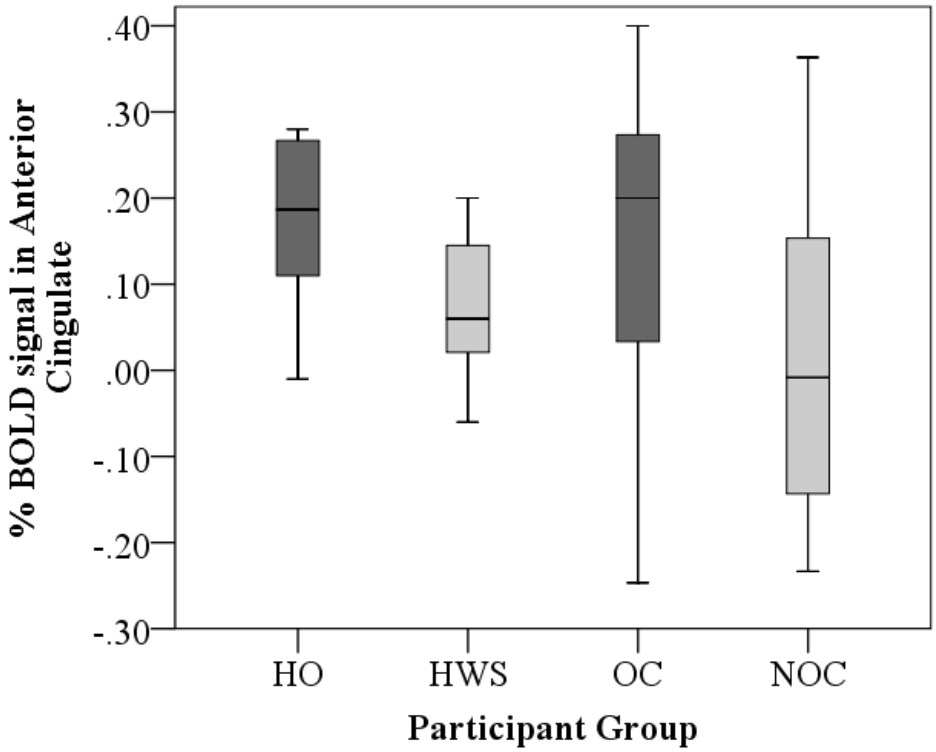
A.



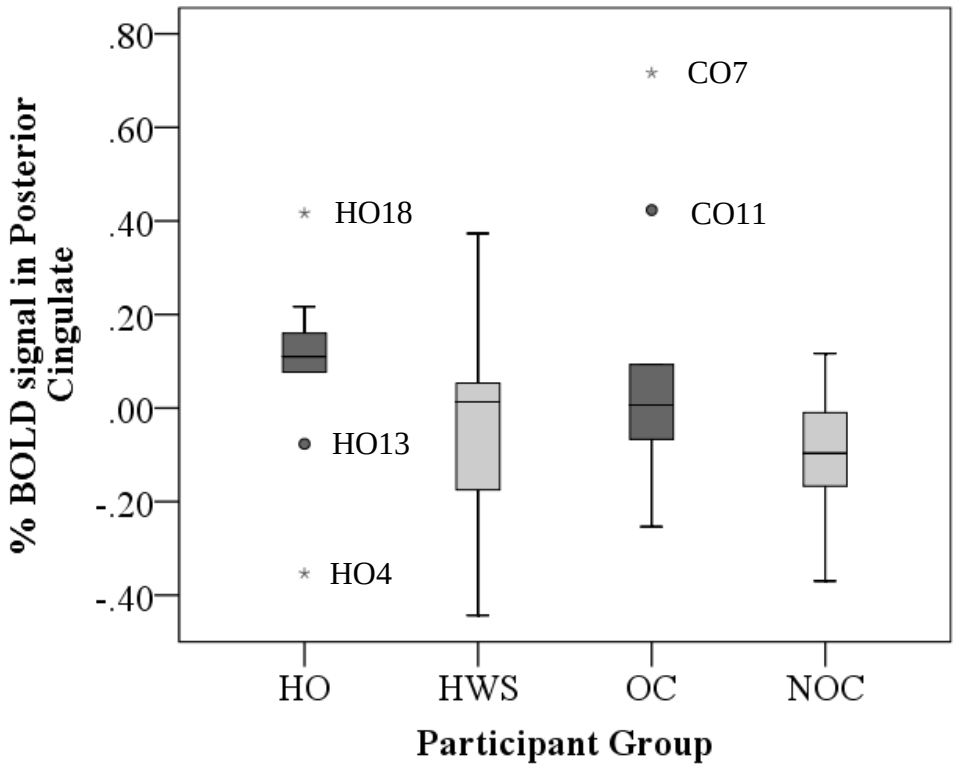
B.



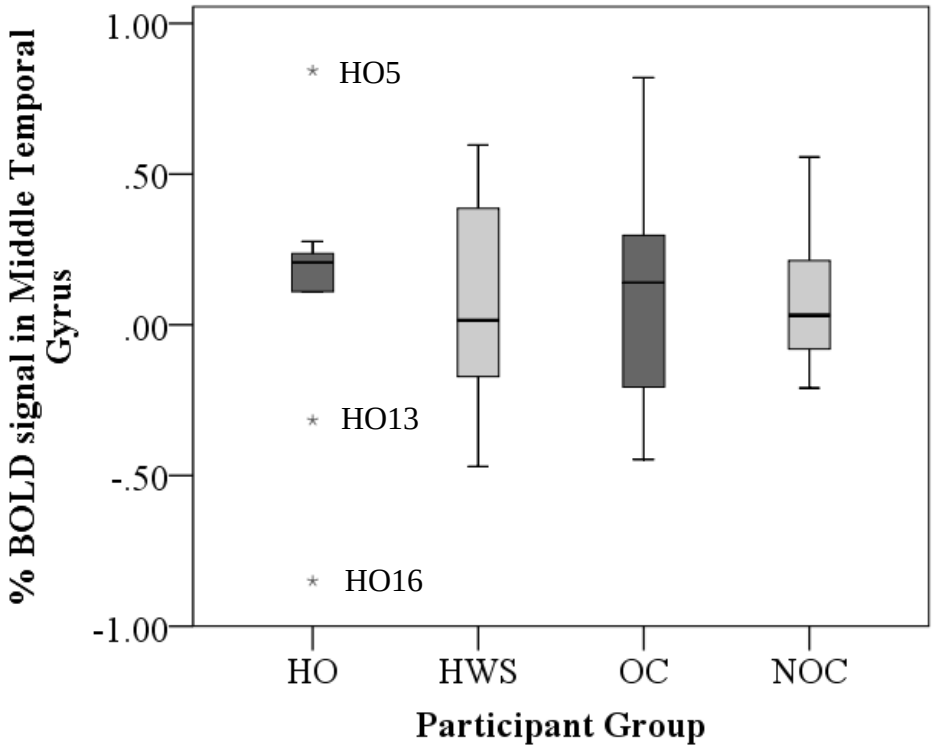
C.


% BOLD signal in Anterior Cingulate
.40
.30
.20
.10
.00
-.10
-.20
-.30
HO
HWS
OC
NOC
Participant Group

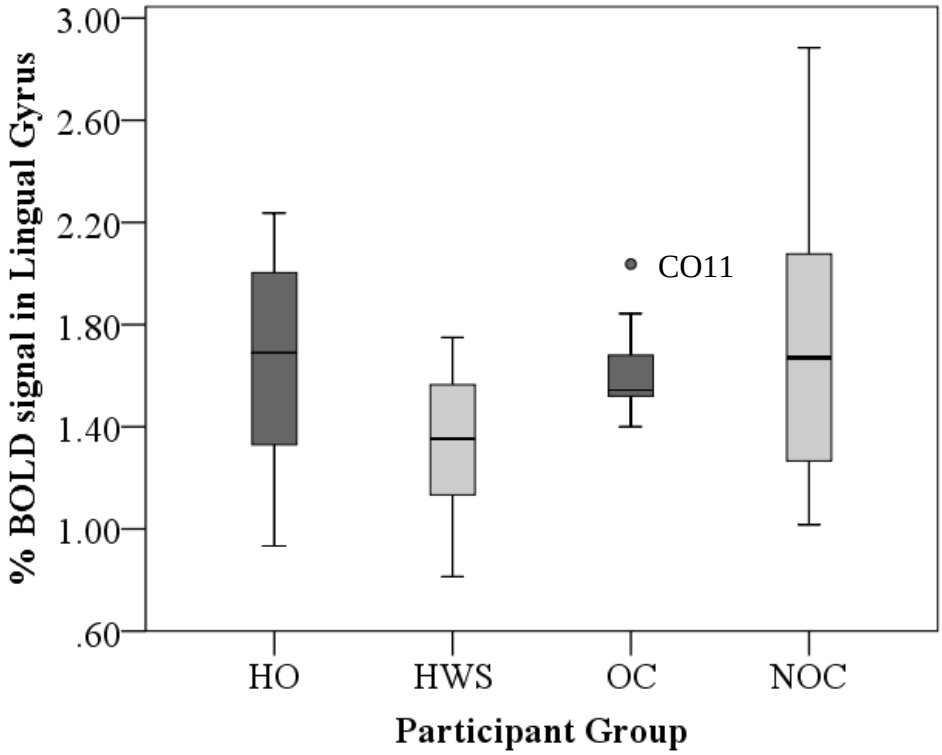
D.


% BOLD signal in Posterior Cingulate
.80
.60
.40
.20
.00
-.20
-.40
CO7
CO11
HO18
HO13
HO4
HO
HWS
OC
NOC
Participant Group

E.



F.



Light-grey bars = non-obese, dark-grey bars = obese. Outliers are labelled with their participant code.

Between session comparison: fasted, 1hPB and 3hPB

Post-hoc pair-wise comparisons were performed for the variable "session" (at three timepoints: fasted, 1hPB and 3hPB) in each linear mixed-effects model. The variable "session" was significant for posterior insula ($F_{[2,151]}$=3.024, p=0.05), lingual gyrus ($F_{[2, 138]}$=4.542, p=0.012) and posterior cingulate gyrus ($F_{[2,148]}$=3.556, p=0.03). Pairwise comparisons showed that the difference in posterior insula BOLD signal was between fasted and 3hPB (p=0.04; mean difference 0.12, SE=0.06, 95%CI: 0.004 to 0.23) and 1hPB and 3hPB states (p=0.05; mean difference 0.09, SE=0.05, 95%CI: -0.001 to 0.18), with greater posterior insula BOLD signal when fasted and at 1hPB compared to 3hPB. Lingual gyrus BOLD signal was greater fasted than 3hPB (p=0.003; mean difference 0.24, SE=0.8, 95%CI: 0.08 to 0.40), with lower posterior cingulate gyrus BOLD signal when fasted than 1hPB (p=0.012; mean difference -0.15, SE=0.06, 95%CI: -0.27 to-0.034).

There were no significant differences between the groups (patients/controls, non-obese/obese) when BOLD signal across sessions was compared (data not shown).

Hormonal analysis

The results of the mean (SD) area under the curve concentrations for glucose, active GLP-1, active ghrelin and PYY(3-36) and median insulin in each participant group are given (Table 5.5). Mean fasting concentrations for insulin and active GLP-1 and median glucose, active ghrelin and PYY(3-36) in each participant group are also given (Table 5.6, Figure 5.6).

There were no significant differences in AUC hormone concentrations between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* or *patients (HO/HWS) vs. controls* (*NOC/OC)*, or the interaction between these factors on two-way ANOVA (results of interaction in Table 5.5), except for ghrelin AUC where there was significantly higher AUC ghrelin in *controls* (*NOC/OC; mean 18,389, SD 18.055)* than in *patients(HO/HWS; mean 7,609, SD 5998)* ($F_{[1,30]}$= 4.56, p=0.04), but no significant difference between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* ($F_{[1,30]}$= 2.52, p=0.12) or the interaction between these factors (Table 5.5).

Table 5.5. Mean (SD) AUC hormone concentrations in each participant group

	HO	HWS	OC	NOC	F ratio	p-value
Median[IQR] insulin (mIU*180mins/mL)	5978 [1458]	5764 [6693]	5629 [8453]	7563 [3827]	F(1, 27) 0.10[a]	0.75[a]
Glucose (mmol*180mins/L)	1412 (617)	1438 (152)	1353 (255)	1257 (225)	F(1, 29) 0.21	0.65
Active GLP-1 (pmol*180mins/L)	563 (494)	795 (696)	689 (515)	695 (509)	F(1, 31) 0.37	0.55
Active ghrelin (pg*180mins/mL)	6957 (6839)	8786 (4548)	11484 (15489)	25295 (18502)	F(1, 30) 1.48	0.23
PYY(3-36) (ng*180mins/mL)	122.7 (22.0)	137.5 (25.1)	122.6 (31.3)	123.9 (28.5)	F(1, 26) 0.42	0.52

F-ratio and p-values for the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* on two-way ANOVA.
[a]AUC insulin data required transformation (using the inverse of the square root) to equalise the variances. Statistical comparison was performed using these transformed values.

There were no significant differences in fasting hormone concentrations between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* or *patients (HO/HWS) vs. controls* (*NOC/OC)*, or the interaction between these factors on two-way ANOVA (results of interaction in Table 5.6), except for transformed fasting ghrelin where there was significantly higher fasting ghrelin in *controls* (*NOC/OC; mean 81.4, SD 111.2)* than in *patients(HO/HWS*; mean 9.9, SD 14.7*)* ($F_{[1,31]}$= 9.48, p=0.004), but no significant difference between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* ($F_{[1,31]}$= 0.57, p=0.46) or the interaction between these factors (Table 5.6).

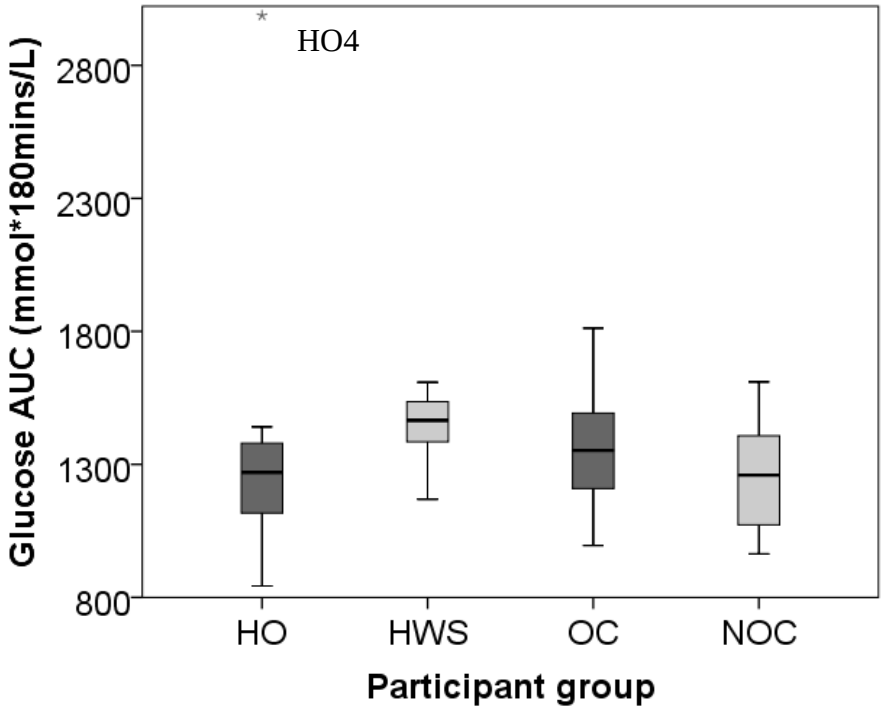
Table 5.6. Mean (SD) or median [IQR] fasting hormone concentrations in each participant group

	HO	HWS	OC	NOC	F(1,32)	p-value
Insulin (mIU/mL)	15.7 (20.1)	6.9 (3.3)	10.6 (4.4)	12.5 (4.2)	1.15	0.29
Median glucose (mmol/L)	6.9 [2.7]	7.2 [10.0]	6.1 [2.0]	7.7 [3.1]	1.34[a]	0.26[a]
GLP-1 (pmol/L)	1.7 (2.2)	1.9 (0.8)	3.2 (3.5)	3.3 (3.2)	0.003	0.96
Median active ghrelin (pg/mL)	8.13 [14.6]	0.50 [18.5]	16.3 [61.0]	82.1 [153.9]	F(1,31) 1.36[b]	0.25[b]
Median PYY (3-36) (ng/mL)	0.65 [0.17]	1.03 [0.72]	0.72 [0.55]	0.67 [0.43]	1.14[c]	0.29[c]

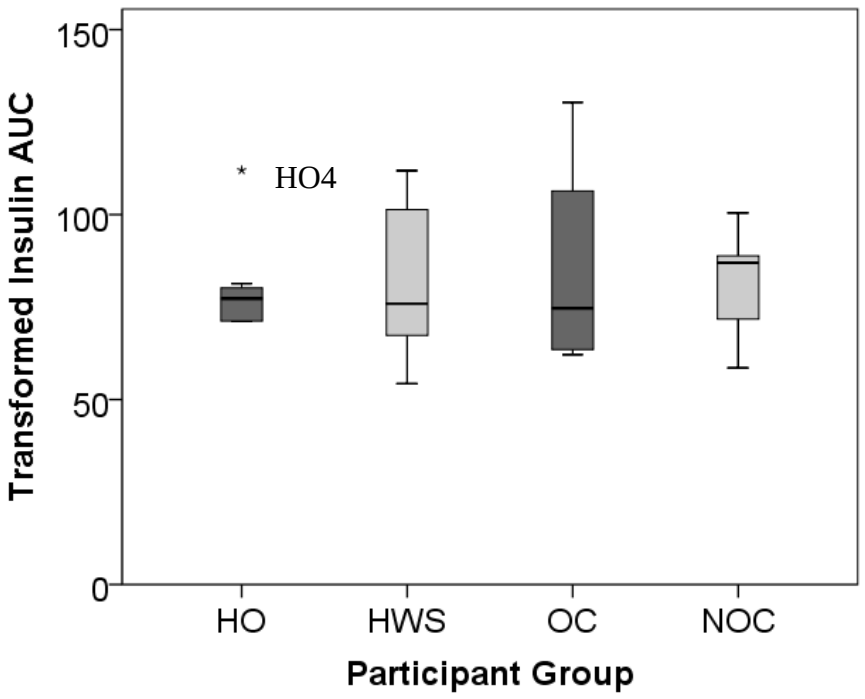
F-ratio and p-values for the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* on two-way ANOVA. [a]Fasting glucose, [b]active ghrelin and [c]PYY(3-36) required transformation to equalise the variances using the inverse function, the square root and the natural logarithm, respectively. Statistical comparisons (two-way ANOVA) were then performed using this transformed data.

Figure 5.6. Boxplots showing median (thick central line), IQR (upper and lower box edges) and minimum/maximum (lowest/uppermost horizontal lines, with extreme outliers depicted as stars/circles), for A. Glucose, B Transformed insulin, C. Active GLP-1, D. Active ghrelin and E. PYY(3-36) AUC concentrations in each of the four subject groups

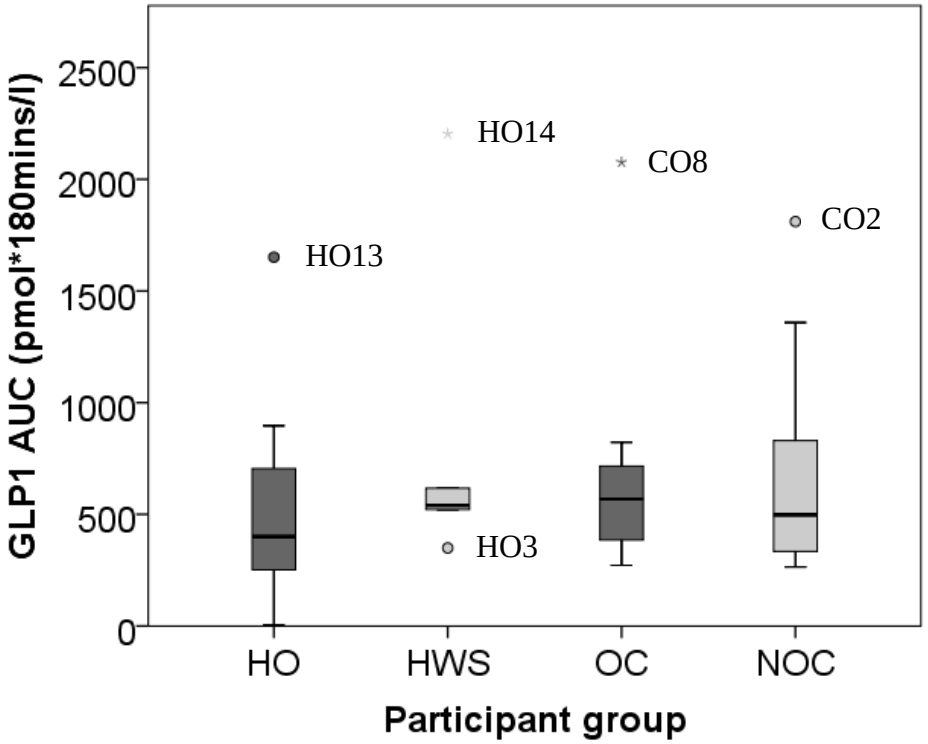
A.



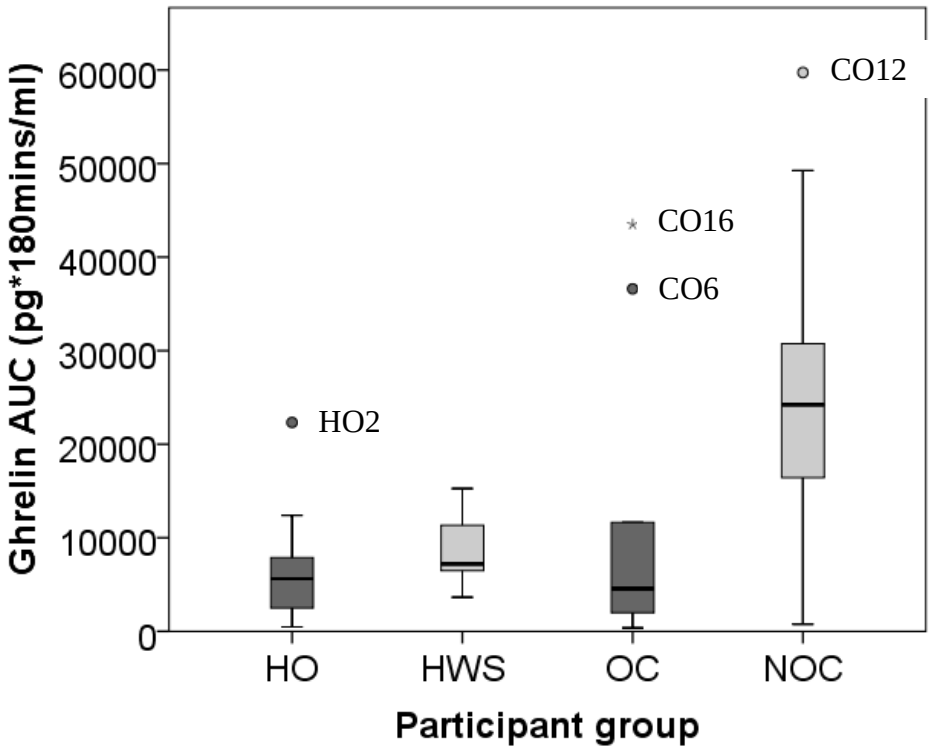
B.



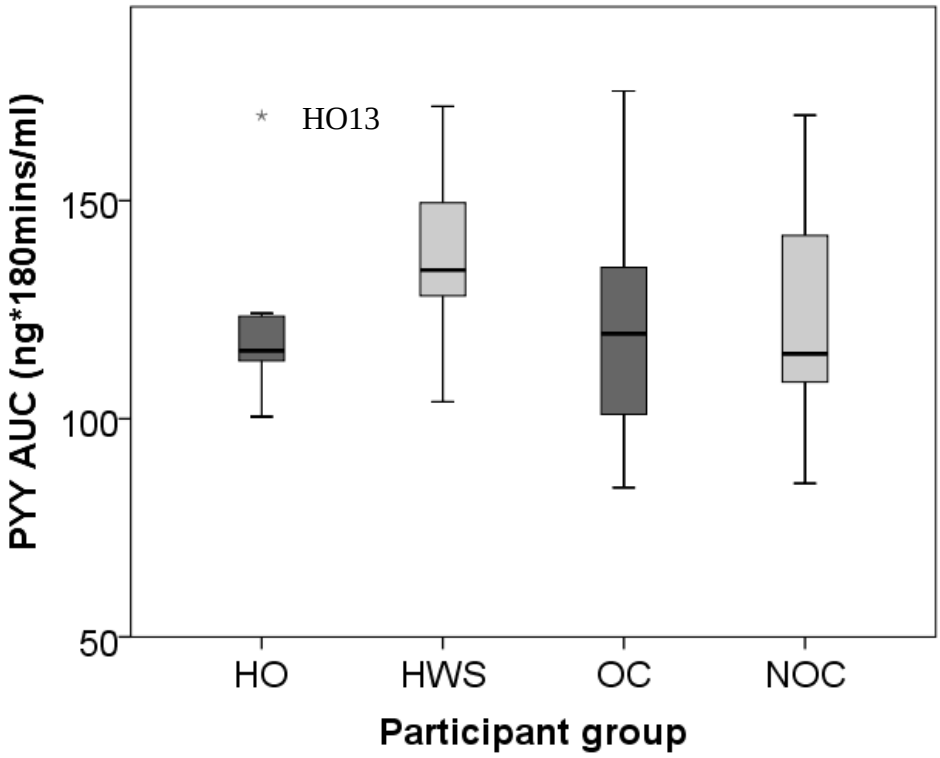
C.


GLP1 AUC (pmol*180mins/l)
2500
2000
1500
1000
500
0
HO14
CO8
CO2
HO13
HO3
HO
HWS
OC
NOC
Participant group

D.


Ghrelin AUC (pg*180mins/ml)
60000
50000
40000
30000
20000
10000
0
CO12
CO16
CO6
HO2
HO
HWS
OC
NOC
Participant group

E.



Between-group comparison of hormones: obese vs. non-obese

The effect *obese (HO/OC) vs. non-obese* (*HWS/NOC)* was statistically significant in the linear mixed-effects models where PYY(3-36) was the outcome variable ($F_{(1,27)}$=8.99, p=0.006); obese participants *(HO/OC)* had lower PYY(3-36) than non-obese *(HWS/NOC)* (coefficient -0.24, SE 0.1, 95% CI: -0.4 to -0.1). There was no group effect significantly associated with levels of active GLP-1, active ghrelin or insulin (p>0.05).

Between-group comparison of hormones: patient/control

The linear mixed-effects models showed a significant effect for *control vs. patient group* where glucose ($F_{[1,27]}$=5.25, p<0.03) and active ghrelin ($F_{[1,34]}$=5.65, p<0.03) were the outcome variables. Patients *(HO/HWS)* had higher glucose than controls *(OC/NOC)* (coefficient -1.06, SE 0.5, 95% CI: -2.0 to -0.1). There was no group effect significantly associated with concentrations of PYY(3-36), active GLP-1 or insulin (p>0.05).

VAS appetite ratings

The results of the mean (SD) VAS scores for each question regarding appetite at all three timepoints are given, according to participant group (Table 5.7). For

hunger at three hours the median [IQR] of the data is given, but the statistical comparison was undertaken on transformed data (square of the original data).

There were no significant differences in any of the VAS ratings between the groups in the comparisons between *obese (HO/OC)* and *non-obese (HWS/NOC)* participants, participants with *hypothalamic damage (HO/HWS)* and *controls (OC/NOC)* or the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* using two-way ANOVA.

Table 5.7. Mean (SD) VAS scores across the four groups (score out of 100)

	HO	**HWS**	**OC**	**NOC**	**F(1,32)**	**p-value**
Hunger (fasted)	56.0 (24.0)	70.0 (16.0)	57.2 (30.5)	67.5 (23.9)	0.18	0.9
Hunger (1hPB)	29.1 (15.8)	30.2 (20.6)	28.6 (21.6)	38.7 (21.8)	0.42	0.52
Median [IQR] hunger (3hPB)	60.0 [49.5]	71.0 [17.0]	70.0 [47.8]	67.5 [24.3]	0.001[a]	0.98[a]
Fullness (fasted)	23.4 (28.4)	19.2 (14.9)	11.3 (13.2)	21.3 (13.3)	1.84	0.18
Fullness (1hPB)	55.3 (21.4)	57.3 (23.1)	58.5 (26.1)	58.8 (25.8)	0.01	0.92
Fullness (3hPB)	36.6 (28.6)	26.2 (28.0)	31.8 (22.6)	28.0 (18.2)	0.26	0.61
Desire to eat (fasted)	57.4 (20.9)	71.3 (19.3)	60.1 (31.8)	70.0 (19.4)	0.12	0.92
Desire to eat (1hPB)	31.8 (13.7)	39.3 (20.2)	28.8 (21.6)	48.3 (23.3)	0.73	0.40
Desire to eat (3hPB)	46.9 (26.3)	40.5 (32.1)	53.2 (21.7)	67.4 (16.0)	1.14	0.29

hPB = hours post-breakfast. F-ratio and p-values for the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* on two-way ANOVA. [a]For the VAS ratings of hunger at three hours data required transformation (square of the data) to equalise the variances. Statistical comparison (two-way ANOVA) was then performed using these transformed values.

Discussion

Of the six regions with significantly greater BOLD signal when viewing food (vs. object) photographs, two (insula and anterior cingulate cortex [ACC]) were first identified in Tataranni's seminal PET study of human hunger and satiety [149], as well as in multiple further studies since [147, 161, 164, 166, 167, 170, 178, 180, 181, 190, 291-293]. The middle temporal gyrus (BA 21) [181, 294] and lingual gyrus of the occipital lobe (BA 18) [181] have also been identified as important in obesity-related neuroimaging studies. The multiple regions identified reflect the numerous aspects of eating-related cerebral processes and each region has discrete roles relating to appetite and eating; some determine the incentive/reward value of food (e.g. OFC [164, 167]), some are linked to meal termination (PFC [149, 161]) and satiation [149] and some with liking [161]. These regions have been shown to be differentially activated in obese and lean individuals [168, 169].

In the fMRI study presented in this thesis, in patients with hypothalamic damage remaining weight-stable (HWS) viewing high-calorie food photographs resulted in significantly lower BOLD signal in cerebral regions which process interoceptive inputs and promote food ingestion (i.e. posterior insula) [161, 164, 295, 296] or are responsive to food-related reward (i.e. lingual gyrus) [168, 177] compared to all other groups (HO, OC, NOC). This reduced activation may result in lower hunger and desire to eat, leading to reduced food intake compared to patients who gain significant weight (HO), despite significant damage to the homeostatic, energy-regulating hypothalamus in both groups. These findings may suggest the possibility of greater preservation of functional and anatomical connectivity between areas processing food-related reward/stimulating ingestion and extra-hypothalamic homeostatic areas (such as the midbrain VTA and NA) in HWS compared to HO, allowing continued coordinated response between homeostatic and reward-related networks regulating appetite/eating and energy balance.

Lingual gyrus (BA18)

There was significantly greater BOLD signal of the lingual gyrus (BA18) in response to high-calorie food photographs in all groups compared to HWS and

significantly greater BOLD signal when fasted than fed in all four groups. The lingual gyrus, part of the occipital lobe located immediately behind the parahippocampal gyrus, processes food reward [168, 177]. PET imaging has demonstrated that obese males (BMI ≥35 kg/m^2) have a greater decrease in lingual gyrus activation than lean males (BMI ≤25 kg/m^2) following satiation [168]. An fMRI study also found increased activation in the left lingual gyrus (and insula) in obese compared to lean individuals when viewing high-calorie foods, consistent with the two main areas of difference found in our study [177].

An fMRI study found that mood affected food-related lingual activity in lean females (BMI 22.1 +/- 2.4 kg/m^2) [297]. When viewing high-calorie food pictures, right lingual gyrus activation was correlated with positive affect (mood), on the Positive and Negative Affect Schedule. The lingual gyrus therefore seems to be activated in response to rewarding stimuli, with mood possibly influencing the salience of food cues. The authors note the link between visual cortex activation and other types of reward (such as financial reward in gamblers) described in other studies [244]. No assessment of mood was included in our study.

Insula cortex

The role of the human insula is not as well described as other brain regions, mainly due to its anatomical inaccessibility and more limited functional data compared to other brain regions [296], however it has been found to be involved in processing multiple different stimuli and regulating attention and emotion [163]. It can be divided into anterior and posterior sections based on macroscopic appearance (divided by the central sulcus), or three sections based on cytoarchitecture (anterior agranular cortex, intermediate dysgranular cortex and posterior granular cortex). Functional connectivity analysis has differentiated between anterior and posterior insular networks, which connect to the ACC and posterior cingulate cortex, respectively [163]. The posterior insula receives afferents from the amygdala, dorsal thalamus and sensory cortices, whereas the ventral/anterior insula mostly receives afferents from the limbic system, such as the posterior OFC and cingulate gyrus [296]. The mid-dorsal insula also receives input from the thalamus [298]

The posterior and middle insula, where significantly lower BOLD signal was observed in HWS compared to HO participants, has been identified as an important region in appetite and feeding, although much of the published functional neuroimaging literature lacks differentiation between the regions of the insula. Where differentiation between the different areas has been made there has been debate as to the location of the primary gustatory cortex; whereas the anterior insula has typically been described as the location for this in non-human primates [296, 299] Small argues that there is considerable evidence that the posterior insula may be the location in humans, with the possibility of multiple insula regions being involved [299]. An early review of ^{15}O-PET studies described activation peaks scattered throughout the insula, including several in the posterior insula, in regions previously identified as related to gustation in animal studies [295]. Subsequent studies have also found evidence of posterior insula involvement in appetite, although the methods involved in each were significantly different. In nine participants ^{15}O-water PET scanning showed significant decreases in bilateral posterior insula activation (and other brain regions) as chocolate was eaten to excess by chocolate lovers, with decreases in activation correlated with decreasing chocolate ratings [161]. In a study of cocaine users, food cues resulted in greater activation in the posterior insula than neutral cues (whilst cocaine cues resulted in lower activation compared to neutral cues) [300]. An electro-cortical stimulation study of five females with intractable epilepsy also indicated that the posterior insula is related to taste, as stimulation of both central and posterior insular electrodes resulted in gustatory (and other sensory) phenomena [296]. Differences between studies may be influenced by their differing study paradigms, as shown in a study of adolescents (mean age 15.2±0.8 years, mean BMI 22.8±4.4 kg/m^2) who underwent fMRI following a four hour fast. The authors found that whilst a taste of Coke resulted in greater activation in the midbrain extending into the insula than a tasteless solution, pictures of Coke products caused increased activation in the left putamen extending into the posterior insula (compared to non-food adverts) and viewing Coke adverts (which included the logo but not necessarily the Coke product) led to greater bilateral lingual gyrus activation (amongst other areas) compared to non-food adverts [301].

Differences in activation have also been shown to alter with BMI. A PET study using ^{15}O-water found that 21 obese participants had greater increases in mid-dorsal insula activation compared to 20 lean participants after a 2ml taste of a liquid

meal given following a 36 hour fast, with percentage body fat, disinhibition (measured on TFEQ) and plasma glucose concentration positively correlated with middle-dorsal insula activation on multivariate regression [164]. An fMRI study of gastric distension using a balloon inserted into the stomach and filled with water found that increased BMI was positively linearly associated with increased left posterior insula activation and was significantly higher in five obese compared to 11 lean participants [302].

One study did report conflicting findings, with greater activation of the posterior insula in five lean (mean BMI 22.0±2.9 kg/m^2) compared to five obese women (mean BMI 41.6±5.0 kg/m^2) on fMRI scanning whilst smelling food compared to non-food odours [303]. This study however was small, with the scans of 3/5 obese women not fully completed (two were not able to tolerate the complete protocol and one had excessive head movement) and the statistical threshold for comparison between the groups was $p<0.001$ uncorrected with an extent threshold of $k=5$.

The BOLD signal cluster identified in the posterior insula in the study reported in this thesis showed a spatial maximum in the posterior insula, but also spread to the middle insular cortex. Based on results from an fMRI study of nine healthy participants given solutions that were either pleasant or unpleasant and varied in intensity, Small et al proposed that the middle insula relates to taste intensity, with the dorsal insula representing affect [304]. The viscosity of food has also been linked to the mid-insula, where oral somatosensory stimuli are processed [305].

A meta-analysis of 13 functional neuroimaging experiments involving activation to tastants compared to a tasteless baseline found bilateral activation of both the anterior dorsal and dorsal mid- insula [298]. Another study of 23 obese individuals, 11 previously obese participants who had lost weight and 21 lean participants found increased activation in the middle insula of obese and previously obese participants, with no change in the lean participants on ^{15}O-water labelled PET scanning undertaken after 2ml of liquid food was given following a 36 hour fast [166]. The authors hypothesize that this persistence of "abnormal responses" in the insula and hippocampus (linked to memory processing, spatial learning and enteroception) in post-obese individuals and may indicate the pathophysiology of obesity/weight gain. However, since the individuals were not studied prior to

becoming obese, it is possible that the insula changes could be secondary to the weight gain. The authors postulate that increased middle insula activation in obese and post-obese individuals reflects an increased desire for the coming meal (as is described with other cravings, for example drug use), with either a greater craving of the anticipated satiating meal to follow, or greater anticipation of the reward of that meal in obese individuals [166].

Although the study described in this thesis is unique in that the patient groups have lost the influence of the main homeostatic control centre (the hypothalamus), the finding of significantly greater posterior and middle insula BOLD signal in obese (HO) compared to non-obese (HWS) adults with hypothalamic damage is in keeping with these previous findings described. These studies support the finding of lower insula activation in HWS vs. HO and may indicate a mechanism protecting against HO.

One hypothesis is that in HWS patients the insula connections with other feeding-related brain areas (particularly extra-hypothalamic homeostatic neurocircuitry like the VTA or NA) may be better preserved despite hypothalamic damage, explaining the pattern of reduced insula activation with high-calorie food stimuli, although there is no evidence from the work undertaken to corroborate this theory.

The only other study similar to that described in this thesis is a small study of 4 children with craniopharyngioma (CP) and hypothalamic damage (2 obese, 2 weight-stable; all at least one year post-surgery/radiotherapy) and 4 controls [306]. The 13-17 year olds were asked to fast overnight and then underwent an fMRI scan three hours after consumption of a standardised breakfast (totalling 10% of their estimated total daily calorie requirement), following which they consumed a milkshake totalling 20% of their estimated daily calorie requirement with a second fMRI scan 30 minutes afterwards. During the fMRI paradigm the participants viewed a total of three blocks of "fattening" (high-calorie), three blocks of "non-fattening" (low-calorie) and seven blocks of non-food photographs, with 10 photographs per block and each photograph shown for 2.4 seconds. The photographs were viewed in a mirror whilst lying in the 3T fMRI scanner. Food and non-food photograph blocks alternated and participants were told they would be tested on the photographs later.

In five *a priori* ROIs (bilateral insula, bilateral NA and medial OFC), those with CP had a tendency towards lower activation compared to controls before a test meal, but had a trend towards higher activation following the meal, compared to a reduction in activation seen in controls. While this trend was seen in all five ROIs, only the medial OFC showed a statistically significant difference in BOLD signal between CP patients and controls, with no differences between the patients of different weight groups (weight-stable or obese), possibly due to the small size of the study.

Hormonal/biochemical findings

There was significantly lower AUC active ghrelin and transformed fasting active ghrelin in *patients (HO/HWS)* compared to *controls*, but no difference between *obese (HO/OC)* and *non-obese* (*HWS/NOC)* participants. Previous studies have found mixed results [2, 3, 39, 47], although three of the studies found evidence of lower fasting total ghrelin in participants with HO/craniopharyngioma compared to those with SO/BMI-matched controls [2-4]. Whilst there was no difference between obese and non-obese groups (which would be expected as ghrelin is known to be lower in obesity [36]) it is important to note that whilst the mean BMI was lower in the non-obese (26.1 kg/m^2 and $27kg/m^2$) than in the obese groups (38.0 kg/m^2 and 37.7 kg/m^2) studied many of those in the non-obese groups fell into the "overfat" and "obese" percentage body fat ranges on Tanita assessment (1 overfat and 8 obese in the NOC group and 2 each in the HWS group), reflective of these groups not being "lean" participants.

PYY, which may influence postprandial satiety, has been shown to be similar when fasting in HO and obese controls, without an immediate or sustained post-prandial rise [2]. PYY concentrations have been lower in obese than lean individuals in previous studies [307, 308], however no difference was found here. Differences in experimental design and macronutrient and energy content of the breakfast meal (based on each individual's calculated BMR) may account for this disparity.

Leptin reduces the perception of food reward and increases the response to satiety signals [194, 309]. Leptin supplementation in leptin-deficient and 10%

weight-reduced obese participants reduces insula activation [150]. Although leptin concentrations were not analysed on the fMRI study day, fasting leptin concentrations have been found to be similar between HO and OC in a previous study [2] and on the UEM study day (Chapter6) while there was significantly higher leptin concentrations in *obese* compared to *non-obese* participants, there was no difference between *patients* and *controls,* or in the interaction between these parameters.

Strengths and limitations of the study

This study has its limitations:

- The small size of this study was a significant limitation. This was due in part to the rarity of this condition and particularly the difficulty in finding HWS participants. Whilst there were some interesting findings, further larger studies may give greater clarification and would allow correlation with other clinical and biochemical findings.

- Although the original intention had been to consider fMRI findings alongside biochemical/hormonal differences, statistical advice was that in order for regression analysis to be robust a minimum of 10 participants per group was required for each co-variate in the model [310, 311] and therefore the groups were not appropriately powered for this statistical analysis.

- It was not possible to personalise the food photographs to an individual's food preferences (likes/dislikes), therefore the foods shown may have had a different valence for each participant. The foods shown however, were chosen as they were the highest rated in each category (high- or low-calorie foods) for valence and had the greatest agreement on calorie category.

- Amongst participants there was likely to have been differing knowledge of the calorie-content of some of the foods. The photographs were shown in blocks of four, grouped according to calorie-content to try and prevent this causing a significant difference.

- It was not possible to control for handedness, gender, or timing of menstruation (females) due to the complex nature of the study group. Previous studies have

identified gender differences in various states: insula response to satiation [147, 292], increased anterior insula activation after eating chocolate to satiety in males but not females [147]; caffeine, sodium chloride, citric acid and sucrose causing greater change in insula activation in males than in females from hungry to satiated states [292]. As hypothalamic damage/HO is relatively rare it was not possible to study only a single gender; the mixed gender groups may therefore contribute to a lack of stronger pre- and post-prandial differences in our study.

- Weight-stable patients (HWS) were not lean (BMI < 25 kg/m^2), therefore the BMI-matched control group were also non-obese but not lean; many were overweight (and were classed as overweight or obese on the Tanita percentage body fat assessment) and this may account for the greater insula BOLD signal found in NOC.

- As with all fMRI studies, artefacts can cause lack of homogeneity in image quality in some cerebral regions [170]. The lack of a statistically significant difference between groups in hypothalamic BOLD signal does not exclude differences due to the spatial and contrast resolution required to detect changes in the hypothalamus [150].

- A stringent statistical threshold was used for analysis which may have reduced the quantity of ROIs, however this adds weight to the positive findings.

- Ideally the patient group would have been homogenous, with one underlying histological diagnosis causing hypothalamic damage. As noted, it is a relatively rare condition and therefore it was accepted at the outset that studying a more heterogeneous group would be necessary.

The strengths of this study are:

- A standardised, reasonably physiological overnight fasting period.

- All photographs (food and non-food) were taken on a standardised photographic background, producing images of similar size and visual interest (shape, colour) without distracting features as used in some other studies (e.g. chequered table cloth)

in order to minimise other factors which may have accounted for differences found in other studies, or additional areas of activation.

- Food photographs had comparable hedonic values, as rated by a normal population (unreported) with good visual variability in both low-calorie and high-calorie photographs and without repetition of photographs within a single scan session.

- The use of strict statistical thresholds.

- Use of fMRI gives better spatial and temporal resolution than PET [170, 291] and does not entail the toxicity of radionuclide injection.

Possible future analyses on existing data

Further analyses could be performed using the data generated from this fMRI study, including analysis of between-group differences in BOLD signal when viewing low-calorie/any food pictures compared with non-food pictures. Subcortical *a priori* ROIs such as the amygdala and nucleus accumbens (NA) could also be studied. These ROIs were too small to survive the threshold of k≥3,000 voxels used as a cut-off for analysis, but have been identified in numerous studies as differing between lean and obese participants [150, 152-155, 158]. In addition, a small fMRI study demonstrated lower activation of the NA pre-prandially and a trend towards higher activation post-prandially in five participants with craniopharyngioma compared to controls [306].

Conclusions

This is the first evidence of food motivation-related differential BOLD signal in adults with hypothalamic damage who remained weight-stable compared to those who developed HO. The hypothesis of greater activation in reward-related cerebral regions in those with HO compared to those with HWS was supported in two areas - lingual gyrus and posterior insula. It is clear that these regions, both influenced by food-reward, are an integral part of the brain network which processes food stimuli and are differentially activated in those remaining weight-stable. Comparatively

lower posterior insula and lingual gyrus activation in HWS may protect these individuals from the weight-gain seen in those who become HO. What leads to the differences in insula and lingual gyrus activation unfortunately cannot be elucidated from this study. Unravelling the underlying neurochemical/neuroendocrine mechanisms involved may provide vital clues in understanding the pathophysiology of weight-gain in both patients with HO and obesity in the general population. Further research into the underlying pathophysiology of weight-gain in this interesting group of patients should be encouraged, potentially in a large multi-centre trial.

Chapter 6

Microstructure and macrostructure of eating behaviour in adult patients with acquired, structural hypothalamic damage: a laboratory study of eating rate, total intake and within meal appetite ratings and a real-world assessment of eating behaviour and food intake

Abstract

Objective: Part 1: To examine the microstructure of eating behaviour in adults with hypothalamic obesity (HO) secondary to acquired, structural hypothalamic damage, compared to adults with hypothalamic damage remaining weight-stable (HWS) and age and BMI-matched obese and lean controls without hypothalamic damage (OC and NOC, respectively) using the Universal Eating Monitor (UEM) to explore any differences in eating behaviour within a single meal. Part 2: To assess the influence of day-to-day and longer-term eating behaviours in HO compared to HWS, OC and NOC.

Study design: Part 1: Cross-sectional study of 6 patients with HO, 6 HWS, 9 OC and 10 NOC. Participants consumed an unlimited pasta lunch at the UEM. Total intake, eating duration and visual analogue scale (VAS) ratings of self-reported hunger, fullness and food pleasantness were recorded. Eating rate and changes in eating rate were calculated. Appetite-associated hormones (glucose, insulin and leptin) were measured before lunch and glucose and insulin were measured post-prandially. Part 2: Three Factor Eating Questionnaires (TFEQs) and three-day MRC-Human Nutrition Research diaries were given to all participants at their screening visit and returned subsequently. Participants were asked to self-complete a full record of their food and drink consumption over three standard days, including at least one weekend day.

Results: Part 1: There was significantly higher total intake ($F_{[1,27]}$=7.74, p=0.01), calorie intake per kg of FFM ($F_{[1,26]}$=23.3, p<0.001) and amount of calories consumed as a percentage of estimated BMR ($F_{[1,27]}$=18.2, p<0.001) by *controls (OC/NOC)* than those with *hypothalamic damage (HO/HWS)*, but no significant difference between *obese (HO/OC)* and *non-obese (HWS/NOC)* participants or interaction between these factors. Eating duration was also significantly longer in *controls* than in participants with *hypothalamic damage* ($F_{[1,27]}$=6.43, p=0.02). There were no significant differences in overall eating rate between the groups on two-way ANOVA or on repeated-measures ANOVA however visual ascription showed a

tendency towards an initial higher eating rate in HO, followed by a reduction and later acceleration in rate.

There was significantly lower hunger at the start of the meal in participants with *hypothalamic damage* compared to *controls* ($F_{[1,27]}$=4.55, p=0.04), but no significant differences in fullness ($F_{[1,27]}$=3.43, p=0.08) or pleasantness ($F_{[1,27]}$=1.87, p=0.18) at baseline. There was no significant difference in baseline hunger, fullness or pleasantness after a taste of the pasta meal between *obese* and *non-obese* participants or in the interaction between *obese vs. non-obese* and *patients vs. controls* in any of the outcomes. Repeated-measures ANOVA showed no evidence of significant interaction between participant group and time or group effect on hunger, fullness or pleasantness of the pasta meal, however the outcome means changed over the quartiles of the meal for all three parameters, as would be expected. Pre-lunch leptin concentrations were significantly higher in *obese* compared to *non-obese* participants ($F_{[1,27]}$=7.78, p=0.01), but there was no significant difference between participants with *hypothalamic damage* and controls or in the interaction between *obese vs. non-obese* and *patients vs. controls*. There was no significant difference in pre-lunch glucose or pre-lunch insulin in any of the between-group comparisons undertaken. There was no significant difference in AUC glucose between *obese* and *non-obese* participants or between participants with *hypothalamic damage* and *controls,* however there was a significant difference in the interaction between *obese vs. non-obese* participants and *patients vs. controls* in AUC glucose ($F_{[1,26]}$=8.14, p=0.008). There was significantly higher AUC insulin in *obese* compared to *non-obese* participants ($F_{[1,26]}$=11.87, p=0.002), but no significant difference between participants with *hypothalamic damage* and *controls* or in the interaction between *obese vs. non-obese* and *patients vs. controls*. Part 2: Thirty-four participants returned the TFEQ (8 HO, 6 HWS, 9 OC, 11 NOC). Kruskal-Wallis testing showed a significant difference between the groups in age and BMI. Box-plots indicated that participants remaining HWS had a higher median age than the other participant groups (HO, OC and NOC) and that *obese (HO/OC)* participants had a greater mean BMI than *non-obese (HWS/NOC)*. Disinhibition also differed significantly between the groups on Kruskal-Wallis testing and boxplots demonstrated the median to be highest in OC. There were no significant differences between the groups in hunger or restraint. There was no correlation between BMI and restraint, disinhibition or hunger across all of the participants on Spearman's rho.

Thirty-two participants had a three-day food diary analysed (6 HO, 7 HWS, 8 OC, 11 NOC). There were no significant differences between the groups in age, BMR or fat-free mass (FFM) but Kruskal-Wallis testing showed a significant difference in BMI and percentage body fat between the groups. Boxplots indicated that *obese* participants had a greater median BMI and percentage body fat than *non-obese*. There were no differences between the groups in the number of reported eating episodes, total calories reportedly eaten (including when adjusted for FFM and as a percentage of BMR) or the amount of fat, protein, carbohydrate or sugar consumed.

Conclusions: The UEM failed to show any significant difference in the microstructure of eating behaviour in HO compared to HWS, although the patient groups consumed significantly less food at the UEM than controls. Visual ascription showed a tendency towards an unusual eating pattern in HO which warrants further investigation, but the small numbers studied must be noted and may account for this finding. The lack of increased hunger and total food intake in HO suggests that within-meal hyperphagia is not a key pathophysiological component of weight-gain in HO, however numbers studied were small. The trend towards lower hunger in patients at the start of the meal may account for their lower intake compared to controls, but does not help to explain the difference in BMI between those with hypothalamic damage who remain weight-stable and those who become obese (HO).

In relation to longer-term eating behaviour there was no significant increase in disinhibition reported by those with HO compared to those remaining HWS. Increased median disinhibition seen in OC is in keeping with other literature where this has been associated with over-eating and increased body mass index (BMI). The lack of greater disinhibition in HO compared to HWS may simply reflect the small size of the groups studied or perhaps reflects a regulatory disturbance in appetite accounting for the weight-gain/obesity in HO rather than an external drive to over-eat. Greater disinhibition leading to increased food intake was not reflected in the returned food diaries (or at the UEM), however under-reporting in food diaries is a well described phenomenon and the numbers studied were again small. The lack of difference in reported food intake in food diaries may reflect the inconsistencies inherent in this method.

Summary of Justification and Aims

Identification of abnormal eating behaviour in patients with hypothalamic obesity (HO) may help elucidate underlying pathophysiological mechanisms, particularly as some studies report hyperphagia [11, 23, 32, 40, 41] Eating behaviour can be considered in terms of microstructure (single-meal intake and immediate preceding/following-effects) and macrostructure (longer-term eating patterns). Either or both of these could be altered in HO.

By studying the microstructure of eating behaviour, aspects such as eating rate and changes in eating rate can be evaluated, in addition to intra-meal subjective appetitive feelings [202, 203]. Previous studies have demonstrated differences in eating patterns between groups with different characteristics (lean, obese), under different conditions (pre-loads, interruptions in eating) and with underlying pathology (Prader-Willi syndrome, bulimia). One method used to assess the microstructure of eating is the Universal Eating Monitor (UEM). This uses a hidden scale connected to a computer which frequently records food intake (every few seconds), allowing not only the total eating duration and amount consumed to be recorded, but also measures overall eating rate, initial eating rate and any changes in eating rate and allows cumulative intake curves to be calculated. The UEM has been developed over the past 35 years and can assess intake of a semi-solid food, such as chocolate pudding or pasta. The computer can also interrupt and prompt participants to complete on-screen ratings of hunger, satiety and food pleasantness using VAS scales, without involving the researcher [219]. Intake at the UEM has been shown to correlate well with real-world intake [220], and intake amount, meal duration and initial eating rate are consistent and reliable within each individual participant after a period of familiarisation with the equipment, whilst demonstrating variability between individuals [204, 205, 218, 220-222]. UEMs are therefore a reliable and reproducible research tool for studying the microstructure of eating behaviour. Additionally they have previously been used in participants with atypical eating behaviour due to underlying medical diagnoses such as Prader-Willi Syndrome (PWS) [216], bulimia nervosa [207, 227, 228] and binge-eating disorder [218].

To assess the macrostructure of eating behaviour food diaries are the most commonly used tool to assess daily intake in a real-world environment [238]. These have the advantage of not relying on memory (as needed in food recall assessments) [239] and three-day diaries have been shown to contain fewer errors (including missed or erroneously added foods) than 5-day diaries or 24 hour recalls [240, 241], although errors still occur [239, 240].

More persistent traits such as eating restraint, self-control and internal/external factors affecting hunger can be measured using the Three Factor Eating Questionnaire (TFEQ) [191]. These long-standing behaviours influence food choices over the longer-term and can influence cognitive choices and can surpass physiological hunger and satiety cues. Dietary restraint describes the amount of behavioural control someone has over their eating behaviour; disinhibition describes the probability of eating under certain conditions, for example when served palatable food; hunger demonstrates the sensitivity of individuals to feelings of hunger and their inclination to eat. High disinhibition scores in particular have been consistently associated with increased BMI and energy intake [201].The TFEQ has been used in large cohorts, particularly in obesity studies, and has high internal and test-retest reliability in both laboratory and non-laboratory settings [191, 201].

Aims: The aims of this study were to assess the eating behaviour of patients with hypothalamic damage and to evaluate whether they differed between those who were HO and those remaining HWS; additionally to compare eating behaviour between participants with hypothalamic damage to obese and non-obese controls. The objective was to assess both the microstructure and macrostructure of eating behaviour to assess what contribution each might play to the development of HO or the ability to remain HWS.

Hypothesis: Participants with HO would consume greater amounts (demonstrated in both the UEM experiment and in the three-day food diaries) than all other groups, especially those remaining HWS and have increased levels of disinhibition and hunger, and lower restraint than the other groups on TFEQ.

Participants and Methods

Participants

As described in detail in Chapter 2, patients were recruited through clinics at the Walton Centre for Neurology and Neurosurgery or University Hospital Aintree and controls through general advertisement within the University of Liverpool and from previous obesity studies.

Part 1: Assessment of the microstructure of eating behaviour with the Universal Eating Monitor (UEM)

Study design and methods

The microstructure of eating behaviour was assessed using the Universal Eating Monitor (UEM). This equipment consists of a hidden digital balance (Sartorius Model BP 8100, Sartorius Ltd., Epsom, UK; 0.1g accuracy) positioned underneath a thin, circular, plastic placemat 37cm in diameter, which conceals the upper surface of the scales from view. The balance underneath protrudes slightly above the surface of the table allowing accurate measurement of the bowl placed on top and measures weight change during consumption of a test-meal. Total amount eaten and eating rate are monitored without participants' awareness. The balance is connected to an Apple Macintosh computer (model 4G) with custom-programmed UEM software which records the weight output from the scales, producing raw data of cumulative intake and also interrupts the participant in real-time after a pre-set amount of food has been consumed [207]. The UEM software used was the Sussex Ingestion Pattern Monitor (SIPM), under license from its owner (University of Sussex). At each interruption (every 100g in this study) the participant completes on-screen VAS ratings of hunger, fullness and pleasantness of food to provide measurement of within-meal satiety ratings.

All UEM data were collected at the Clinical Sciences Centre, UHA. The first session was undertaken during the screening visit to allow familiarisation of participants with the UEM equipment and to ascertain that they would be able to consume the fixed-load porridge and orange juice breakfast-meal during subsequent

fMRI and UEM study days. A further breakfast meal of porridge was consumed at the UEM at the third visit to remind participants how to use the equipment, as it is important participants do not move the bowl or rest their spoon on it in-between mouthfuls to ensure accurate weight-measurement by the hidden scales. The data from these breakfast-meal sessions were collected but not used in the final analysis.

On the UEM study day participants attended the investigational unit in the Clinical Sciences Centre at around 09:00, having fasted from 22:00 the previous evening (Appendix 4). They were asked to refrain from smoking, alcohol and strenuous exercise in the 24 hours preceding the study day. They consumed an identical fixed-load breakfast to that eaten on the screening visit and fMRI study day (porridge and orange juice accounting for 25% of their calculated basal metabolic rate [BMR] see Chapter 2 for details). After three hours participants had blood samples taken (pre-lunch) and were then presented with a standardised lunch meal of pasta in a tomato-sauce (each 100g providing: energy 117kcal; fat 1.0g; protein 3.8g; carbohydrate 22.7g) which they were instructed to eat until they felt full. The UEM software interrupted them to complete on-screen VAS ratings of hunger, fullness and pleasantness after a taste of food and then every 100 grams of food that was consumed. At these interruptions the bowl was refilled with pasta if needed to allow participants to continue an otherwise uninterrupted consumption of the test-meal. After completing the test-meal further blood samples were continued hourly for a further three hours.

Measurements during UEM study day

Anthropometry: this is fully described in Chapter 2. The measured percentage body fat and bodyweight were used to calculate fat-free mass (FFM).

UEM: See Appendix 4 for flow-diagram of the study day. As noted, food intake at the UEM was measured from first bite to last and eating duration was also recorded.

VAS ratings: Three appetite-related questions were asked during the meal eaten at the UEM using a 0-500mm on-screen VAS scale. Participants were asked to use the mouse attached to the computer where they ate and drag a cursor to a place on the VAS scale which best corresponded with how hungry and full they felt and how pleasant the

food tasted. The scale was anchored by "Not at all" and "Extremely" at either end. This allowed participants' subjective sensations to be quantified numerically by computer calculation and analysed. The VAS ratings were measured on a 0-500 scale as this is the only scale available on the software. Assessments were made during both the breakfast and lunch meal eaten at the UEM, however only the data from the lunch meal was analysed. The ratings were only completed during the breakfast meal to allow participants to re-familiarise themselves with the UEM and the on-screen VAS ratings and there was never any intention to analyse this data.

Blood sampling and biochemical assays: Blood samples were taken for glucose, insulin and leptin measurement before the lunch meal. Further bloods were taken for glucose and insulin following the meal (see Appendix 4) and area under the curve was calculated. Details of the blood bottles used and biochemical assays undertaken are fully described in Chapter 2.

Statistical analysis

The raw data collected at the UEM during consumption of the pasta-meal was converted into an analysable format using Microsoft Excel. This allowed calculation of the total amount consumed during this free-eating session, generation of cumulative intake curves, calculation of eating rate (by dividing total amount eaten [grams] by time taken [seconds]) and assessment of VAS ratings of hunger, fullness and pleasantness of the food. Intake (in grams and kilocalories) and within-meal VAS parameters (reported at each interrupt) were reported or extrapolated to provide data at five incremental stages of the test meal (0%, 25%, 50%, 75% and 100% intake) for all participants. Extrapolation was undertaken using a simple interpolation rule.

Data were analysed using SPSS for Windows, versions 21, 22 and 24. Kolmogorov-Smirnov (K-S) testing revealed one outlier in terms of food consumed, who ate much more than all other participants (total intake 1169.2 g over 1142 seconds). Removing this person allowed for a more normal distribution of the sample population. After calculation of the quadratic equation the difference between groups for the intercept (appetite at the start of meal), coefficient (orosensory reward)

and quadratic (satiation throughout the meal) for hunger, fullness and pleasantness was assessed. The development of satiation describes the interaction of the change in appetite rating throughout the meal in relation to the calories consumed during it (pre-prandial hunger rating – post-prandial hunger rating/test-meal calories consumed).

Data are presented as mean (± SD) unless stated. They were further analysed using two-way independent ANOVA, except where two-way repeated-measures ANOVAs were undertaken to test for the effect of time and participant group, and the interaction between these for the VAS ratings hunger, fullness and pleasantness of the food. Time was the within-subject effect and participant group the between-subject effect. As the assumption of sphericity is unlikely to be met in a repeated-measures ANOVA and violations of sphericity are detrimental to the accuracy of ANOVA the Greenhouse-Geisser results for within-subject effects are reported, as this adjusts the degrees of freedom and decreases the likelihood of a Type I error.

Statistical significance was taken at $p<0.05$ in all cases.

Results

Thirty-eight participants undertook the UEM part of the study, however for one control participant and 5 participants with hypothalamic damage (3 with HO and 2 HWS) UEM data could not be analysed due to technical difficulties. At the analysis stage it became apparent that the UEM had not properly recalibrated during one episode of refilling during the meal and therefore the amount of food consumed was uninterpretable. Statistical analysis found an outlier in total intake (in the HO group) and data from this person was excluded from further analysis. The data presented therefore is for 31 participants.

Anthropometry

Between-group comparisons: obese vs. non-obese, patient vs. control and interaction between these factors

There was no significant difference in age between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* or *patients (HO/HWS) vs. controls* (*NOC/OC),* or the interaction between these factors on two-way ANOVA (results of interaction in Table 6.1).

There was significantly higher BMI in *obese* (*HO/OC,* mean [SD] BMI 37.0[5.4] kg/m^2) compared to *non-obese* (*HWS/NOC,* mean [SD] BMI 26.5[2.9] kg/m^2) participants ($F_{[1,27]}$= 40.9, p<0.001), as would be expected, however there was no significant difference between *patients (HO/HWS) vs. controls* (*NOC/OC)* ($F_{[1,27]}$= 0.02, p=0.88), or the interaction between these factors (Table 6.1).

There was also significantly higher body fat percentage in *obese* (*HO/OC,* mean [SD] 44.3[5.3]) vs. *non-obese* (*HWS/NOC,* mean [SD] 31.0[9.8]) participants ($F_{[1,26]}$= 16.3, p<0.001), with no significant difference between *patients (HO/HWS) vs. controls* (*NOC/OC)* ($F_{[1,26]}$= 0.12, p=0.73), or the interaction between these factors (Table 6.1). There was no significant difference in fat-free mass (FFM) between *obese (HO/OC) vs. non-obese* (*HWS/NOC), patients (HO/HWS) vs. controls* (*NOC/OC),* or the interaction between these factors on two-way ANOVA (results of interaction in Table 6.1).

Table 6.1. Mean (SD) demographics and anthropometry of UEM study participants

	HO	HWS	OC	NOC	F[1,27]	p-value
Number of participants	6	6	9	10	-	-
Gender: F	2	2	7	6	-	-
M	4	4	2	4		
Mean (SD) age, years	45.7 (17.7)	54.4 (20.1)	57.0 (19.1)	47.6 (20.6)	0.64	0.43
FFM (kg)	57.6 (7.3)	57.6 (7.3)	55.2 (11.4)	52.2 (10.1)	0.20	0.89
% body fat	42.3 (5.0)	31.1 (8.4)	45.4 (5.5)	30.7 (11.0)	0.40	0.54
Body fat category						
Healthy	0	2	0	6		
Overfat	0	0	1	2		
Obese	5	4	8	2		

F=female, M=male. F-ratio and p-values for the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* on two-way ANOVA.

Intake at the UEM

Amount consumed

Between-group comparisons: obese vs. non-obese, patient vs. control and interaction between these factors

There was no significant difference between *obese (HO/OC)* and *non-obese (HWS/NOC)* participants in total intake at the UEM ($F_{[1,27]}$=3.26, p=0.08), total intake per kg of fat-free mass (FFM) ($F_{[1,27]}$=1.67, p=0.21) or amount of calories consumed as a percentage of estimated BMR ($F_{[1,27]}$=0.79, p=0.38).

There was significantly higher total intake ($F_{[1,27]}$=7.74, p=0.01), calorie intake per kg of FFM ($F_{[1,26]}$=23.3, p<0.001) and amount of calories consumed as a percentage of estimated BMR ($F_{[1,27]}$=18.2, p<0.001) by *controls* (*OC/NOC*, mean

[SD] total intake 537.6[184.8]g, calories/kg FFM 11.7[3.3], calories as percentage of estimated BMR 37.4[10.1]) than those with *hypothalamic damage (HO/HWS*, mean [SD] total intake 345.3[207.7]g, calories/kg FFM 6.1[2.6], calories as percentage of estimated BMR 20.0[8.2]), however there was no statistically significant interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* in any of these parameters (Table 6.2). Figure 6.1 shows the mean total amount consumed over time by each participant group.

Figure 6.1. Mean cumulative intake curves from the lunch meal measured by the UEM for all participants, by participant group.

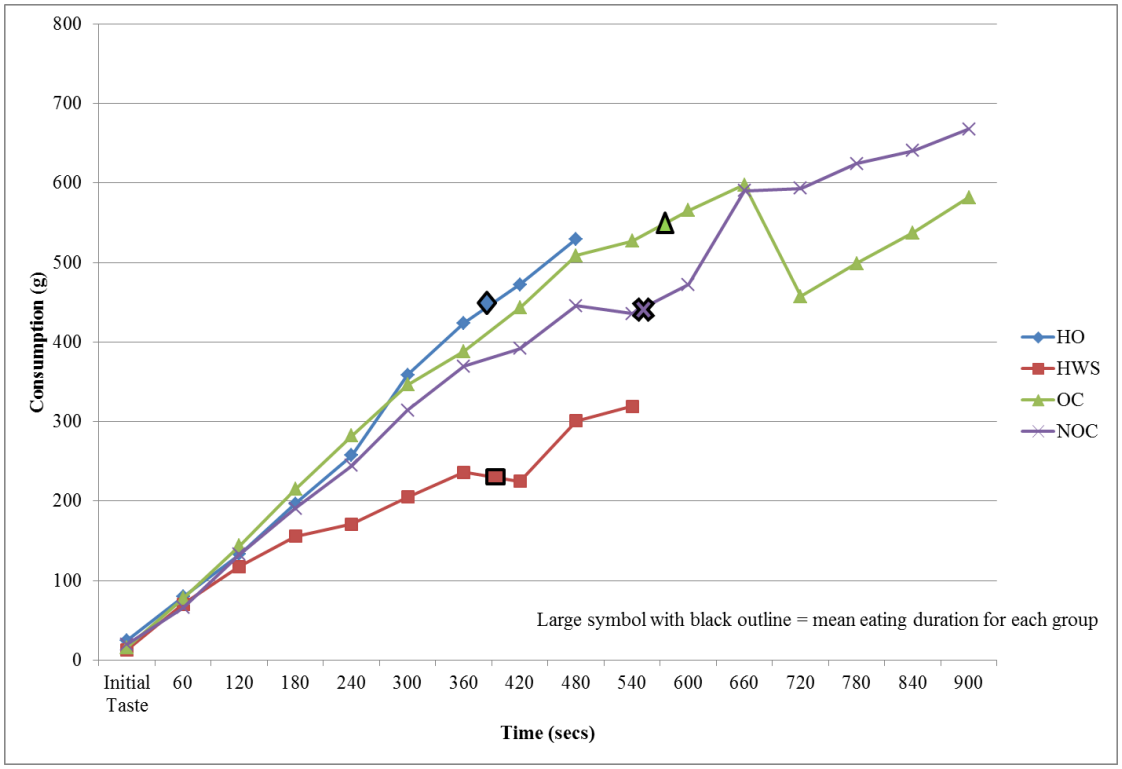


Table 6.2. Mean (SD) total intake at the UEM (in grams and kcal), calculated BMR and calories consumed adjusted for BMR and FFM

	HO	HWS	OC	NOC	F[1,27]	p-value
Intake (g)	430 (263)	260.5 (91.4)	581 (176.8)	498.4 (192.4)	0.38	0.54
Calories consumed (kcal)	503 (308)	305 (107)	680 (206)	583 (225)	0.38	0.54
BMR (kcal/day)	1982 (295)	1688 (270)	1819 (471)	1566 (266)	0.16	0.69
Calories consumed (as % calculated BMR)	24.1 (11.9)	19.0 (6.7)	38.2 (10.3)	36.6 (10.3)	0.23	0.64
% body fat	42.3 (5.0)	31.1 (8.4)	45.4 (5.5)	30.7 (11.0)	0.30	0.59
Body fat category						
Healthy	0	2	0	6		
Overfat	0	0	1	2		
Obese	5	4	8	2		
FFM (kg)	57.6 (7.3)	57.6 (7.3)	55.2 (11.4)	52.2 (10.1)	0.17	0.69
Calories consumed/kg FFM	6.9 (3.3)	5.4 (1.9)	12.5 (3.3)	11.0 (3.2)	0.001	0.97
Eating duration (secs)	383 (131)	406 (119)	574 (216)	546 (187)	0.15	0.70
Overall eating rate (g/sec)	62.4 (23.5)	42.0 (19.8)	65.2 (28.2)	59.9 (21.4)	0.74	0.40

BMR = basal metabolic rate, FFM = fat-free mass. F-ratio and p-values for the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* on two-way ANOVA.

Eating Duration

Between-group comparisons: obese vs. non-obese, patient vs. control and interaction between these factors

There was no significant difference in mean eating duration between *obese (HO/OC)* and *non-obese (HWS/NOC)* participants ($F_{[1,27]}$=0.02, p=0.97). Participants with *hypothalamic damage* (*HO/HWS*) ate for a significantly shorter mean duration (394.7[119.6SD] seconds) than *controls* (*OC/NOC*, mean [SD] 559.2[196] seconds) ($F_{[1,27]}$=6.43, p=0.02), however there was no statistically significant interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* in eating duration (Table 6.2, Figure 6.1 – mean eating duration shown as large symbol with black outline).

The graph in Figure 6.1 shows the mean intake every 60 seconds in all participants. At the later time points some of the participants had finished eating therefore the mean amount consumed drops despite ongoing food consumption in those who continue to eat, reflecting in these participants a longer duration of eating but not necessarily a greater amount consumed. Whilst all participants were eating at the earliest timepoints, many had finished eating before the latest timepoints.

Measures of eating rate

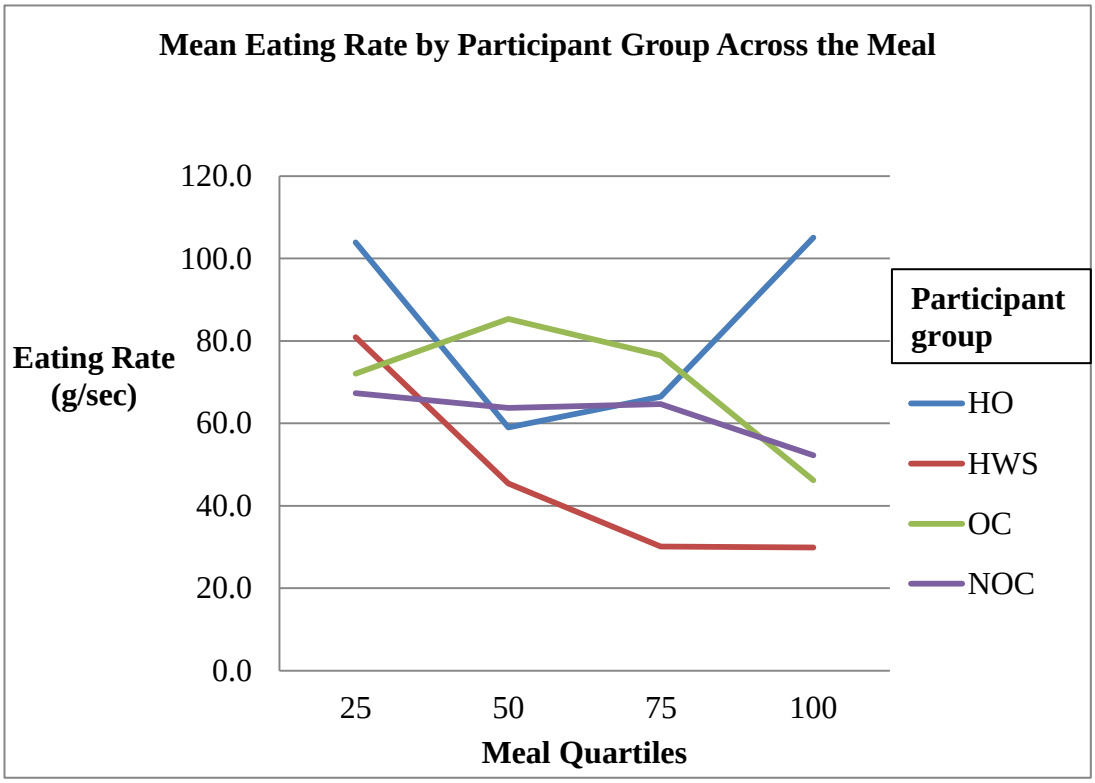
Between-group comparisons: obese vs. non-obese, patient vs. control and interaction between these factors

There were no significant differences in overall eating rate between *obese (HO/OC)* and *non-obese (HWS/NOC)* participants ($F_{[1,27]}$=2.16, p=0.15) or participants with *hypothalamic damage (HO/HWS)* and *controls (OC/NOC)* ($F_{[1,27]}$=1.40, p=0.25) and no significant interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* in overall eating rate (Table 6.2).

Repeated-measures ANOVA for cumulative changes in eating rate were not significantly different between groups ($F_{[4.3,39]}$=1.13, p=0.36) or over time ($F_{[1.4,39]}$=1.43, p=0.24), most likely due to the small numbers of participants. On

visual ascription however, those with HO tended to have a higher initial eating rate than the other groups, which then reduced, but was followed by an increase towards the end of the meal (Figure 6.2); due to low numbers this did not reach statistical significance.

Figure 6.2 Changes in eating rate across the meal



Within-meal measures of appetite

Between-group comparisons: obese vs. non-obese, patient vs. control and interaction between these factors

There was no significant difference in baseline hunger ($F_{[1,27]}$=0.30, p=0.60, Figure 6.2), baseline fullness ($F_{[1,27]}$=2.87, p=0.10, Figure 6.3) or baseline pleasantness after a taste of the pasta meal ($F_{[1,27]}$=0.32, p=0.57, Figure 6.4) between *obese (HO/OC)* and *non-obese (HWS/NOC)* participants.

There was significantly lower reported hunger at the start of the meal in participants with *hypothalamic damage* (*HO/HWS*, mean [SD] 284.7[98.0]) than *controls* (*OC/NOC*, mean [SD] 362.4[93.8]) ($F_{[1,27]}$=4.55, p=0.04), but no significant

differences in fullness ($F_{[1,27]}$=3.43, p=0.08) or pleasantness ($F_{[1,27]}$=1.87, p=0.18) at baseline.

There was no significant difference in the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* in any of the outcomes considered using two-way ANOVA (Table 6.3).

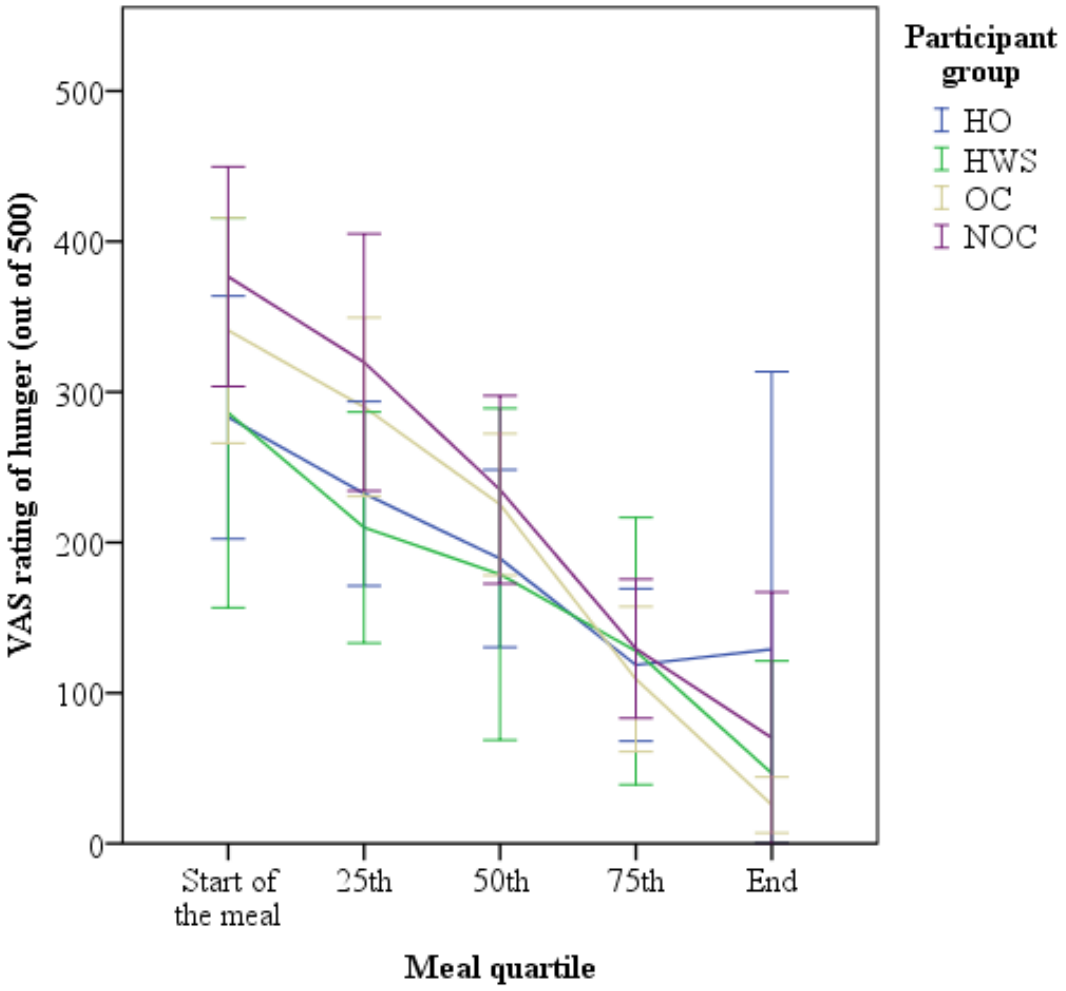
Table 6.3. Mean (SD) intercept of the on-screen VAS ratings (scored out of 500) at the start of the meal.

	HO	HWS	OC	NOC	F[1,27]	p-value
Hunger	283 (77)	286 (123)	341 (97)	381 (91)	0.28	0.60
Fullness	127 (82)	259 (124)	132 (59)	121 (113)	3.97	0.06
Pleasantness	280 (132)	319 (157)	356 (121)	370 (104)	0.06	0.80

F-ratio and p-values for the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* on two-way ANOVA.

Hunger: Repeated-measures ANOVA showed no evidence of significant interaction between participant group and time ($F_{[4.96,44.60]}$=1.12, p=0.36) or group effect on hunger ($F_{[3,27]}$=2.11, p=0.12). However, the outcome mean changed over the quartiles of the meal ($F_{[1.7,44.6]}$=45.6, p<0.001).

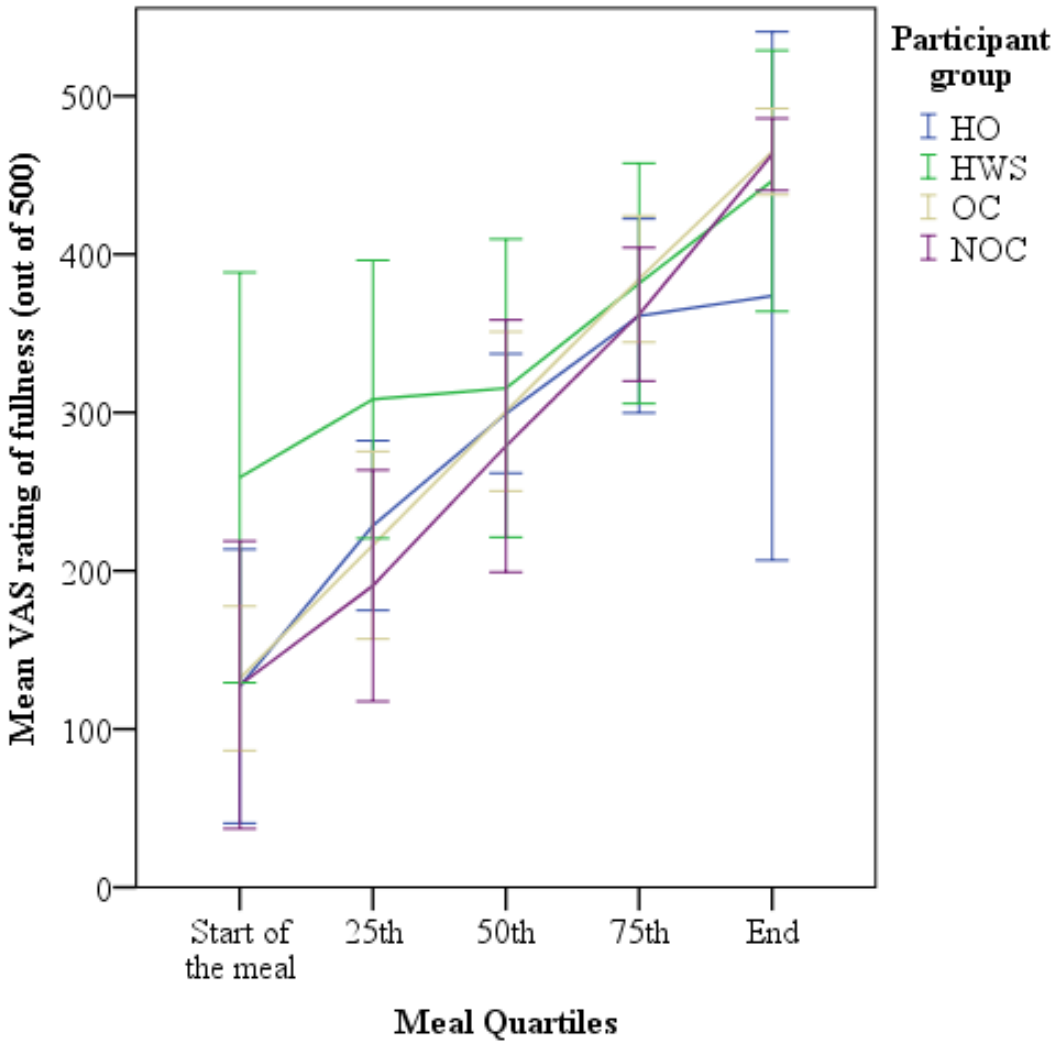
Figure 6.2 Changes in mean hunger rating across the meal (by participant group)



Fullness: Repeated-measures ANOVA showed no evidence of significant interaction between participant group and time ($F_{[6.18,55.66]}$=1.08, p=0.39) or group effect on fullness ($F_{[3,27]}$=1.91, p=0.15). However, the outcome mean changed over the quartiles of the meal ($F_{[2.06,55.66]}$=49.94, p<0.001).

There was no significant difference between the groups in the experience of orosensory reward, as reflected by the *change in fullness* ($F_{[3,30]}$=1.98, p=0.14) and no difference in the development of satiation throughout the meal ($F_{[3,30]}$=1.41, p=0.26) (Figure 6.3).

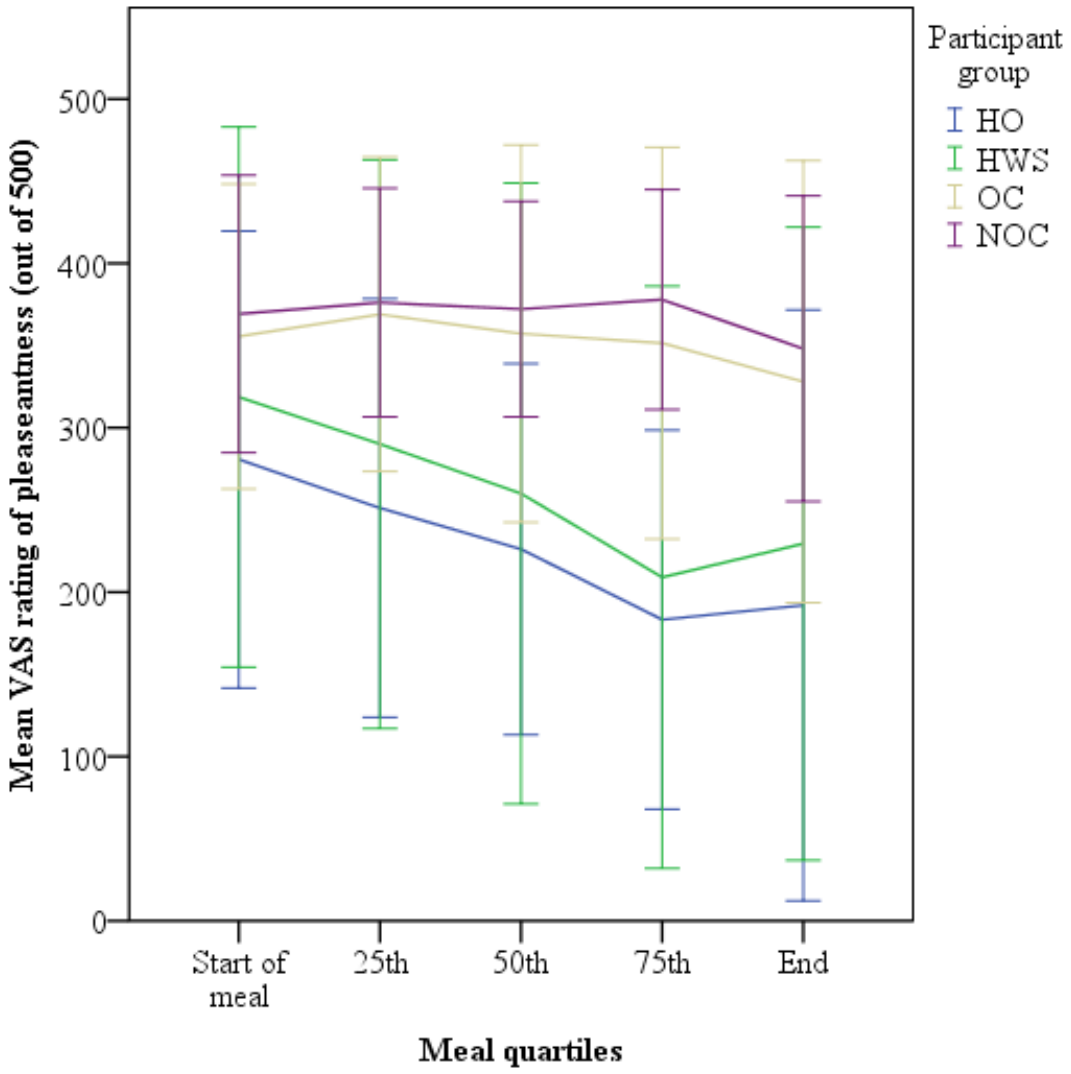
Figure 6.3 Changes in mean fullness rating across the meal (by participant group)



Pleasantness: Repeated-measures ANOVA showed no evidence of significant interaction between participant group and time ($F_{[6.61,57.26]}$=1.40, p=0.23) or group effect on pleasantness ($F_{[3,27]}$=2.08, p=0.12). However, the outcome mean changed over the quartiles of the meal ($F_{[2.2, 57.26]}$=6.73, p=0.002).

There were no significant differences between the groups in the orosensory reward of the meal ($F_{[3,30]}$=2.42, p=0.09) or the development of satiation ($F_{[3,30]}$=1.36, p=0.28). There were no significant differences in changes in pleasantness affecting hunger or fullness within the meal between the groups (Figure 6.4).

Figure 6.4 Changes in mean pleasantness rating across the meal (by participant group)



Hormonal analysis

Between-group comparisons of pre-lunch hormones: obese vs. non-obese, patient vs. control and interaction between these factors

There was no significant difference in pre-lunch glucose ($F_{[1,26]}$=0.20, p=0.66) or insulin ($F_{[1,26]}$=0.003, p=0.96) between *obese (HO/OC)* and *non-obese (HWS/NOC)* participants, however pre-lunch leptin was significantly higher in *obese* (*HO/OC*, mean [SD] 21.1[16.1] ng/mL) compared to *non-obese* (*HWS/NOC*, mean [SD] 7.6[7.0] ng/mL) participants ($F_{[1,27]}$=7.78, p=0.01).

There was no significant difference in pre-lunch glucose ($F_{[1,26]}$=0.10, p=0.76), pre-lunch insulin ($F_{[1,26]}$=1.87, p=0.19) or pre-lunch leptin ($F_{[1,26]}$=2.93, p=0.10) between participants with *hypothalamic damage (HO/HWS)* and *controls (OC/NOC)*.

There was no significant difference in the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* in any of these parameters (Table 6.4).

Table 6.4. Mean (SD) of the pre-lunch blood samples taken on the UEM study day.

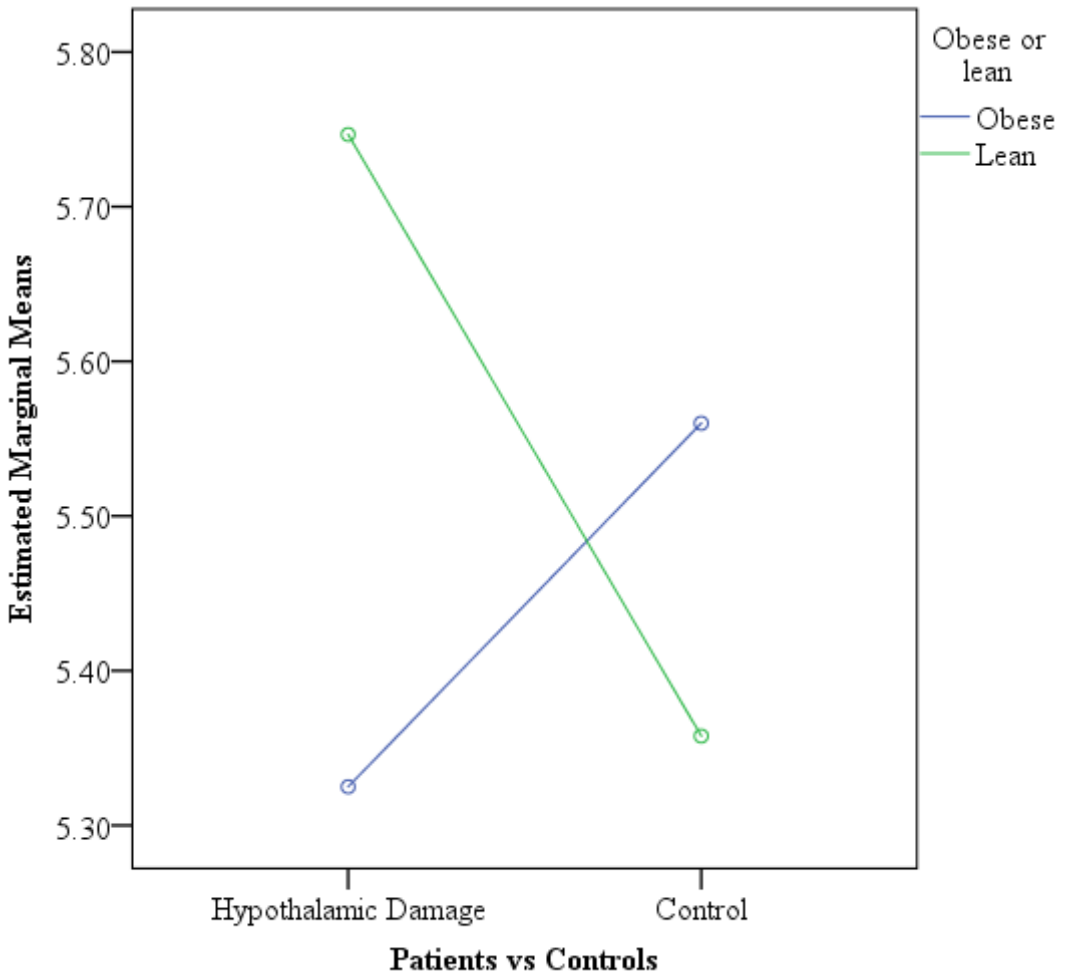
	HO	HWS	OC	NOC	F[1, 26]	p-value
Mean (SD) leptin (ng/mL)	13.2 (9.4)	6.4 (5.4)	26.4 (17.9)	8.4 (8.1)	1.59	0.22
Mean (SD) glucose (mmol/L)	5.3 (0.72)	5.7 (0.73)	5.6 (0.40)	5.4 (0.78)	1.60	0.22
Mean (SD) insulin (mIU/mL)	24.9 (11.8)	28.3 (18.7)	17.5 (17.8)	15.0 (26.9)	0.15	0.70

F-ratio and p-values for the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* on two-way ANOVA.

Between-group comparisons of AUC hormones: obese vs. non-obese, patient vs. control and interaction between these factors

There was no significant difference in AUC glucose between *obese (HO/OC)* and *non-obese (HWS/NOC)* participants ($F_{[1,26]}$=0.06, p=0.81) or between participants with *hypothalamic damage (HO/HWS)* and *controls (OC/NOC)* ($F_{[1,26]}$=1.46, p=0.24), however there was a significant difference in the interaction between *obese (HO/OC)* and *non-obese (HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* in AUC glucose (Table 6.5, Figure 6.5). As can be seen by the line graph and Table 6.5 AUC glucose was greater in HWS and OC and lower in HO and NOC.

Figure 6.5. Line graph showing the interaction of the mean AUC glucose by participant group



There was significantly higher AUC insulin in *obese* (*HO/OC*, mean [SD] 17,173[7,335] mIU*180mins /mL) compared to *non-obese* (*HWS/NOC*, mean [SD] 8,908[4,338] mIU*180mins /mL) participants ($F_{[1,26]}$=11.87, p=0.002), but no significant difference between participants with *hypothalamic damage (HO/HWS)* and *controls (OC/NOC)* ($F_{[1,26]}$=0.40, p=0.53) or in the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* (Table 6.5).

Table 6.5. Mean (SD) AUC of glucose and insulin concentration on the UEM study day.

	HO	HWS	OC	NOC	F[3,29]	p-value
Mean (SD) glucose concentration (mmol*180mins/L)	1111 (158)	1362 (269)	1440 (252)	1229 (169)	8.14	0.008
Mean (SD) insulin concentration (mIU*180mins /mL)	15029 (8463)	9318 (6002)	18603 (6607)	8634 (3194)	0.88	0.36

F-ratio and p-values for the interaction between *obese (HO/OC) vs. non-obese* (*HWS/NOC)* and *patients (HO/HWS) vs. controls* (*NOC/OC)* on two-way ANOVA.

Part 2: Macrostructure of feeding behaviour: Assessment of self-reported eating behaviour with the Three Factor Eating Questionnaire (TFEQ) and the MRC-Human Nutrition Research Diary

Study design and methods

Three Factor Eating Questionnaire (TFEQ)

The Three Factor Eating Questionnaire (TFEQ) is a self-assessment questionnaire which assesses restraint (21 questions), disinhibition (16 questions) and hunger (14 questions) to determine the long-term behavioural and cognitive aspects of eating (Appendix 2) [191]. There are 51 questions: 36 true/false (scored 0 or 1 as per the answer key), 14 Likert-scaled questions (scale 1-4, with ratings of 1 or 2 scored as 0 and ratings of 3 or 4 scored as 1) and one 0-10 scaled-question regarding eating restraint (scored 0 or 1 at analysis). The questions relate to the three categories described and scores are totalled for each category. Restraint questions reflect conscious control of food intake for bodyweight control; disinhibition questions reflect loss of control of eating, influenced by emotion and external triggers; hunger questions measure food cravings/chronic subjective hunger [252, 253]. Higher scores indicate greater restraint, disinhibition and hunger predisposition [254].

TFEQs were given to participants at their screening visit and they were asked to return these on either of their subsequent visits/by post. Participant responses were analysed using a Microsoft Excel spreadsheet which automatically scored the answers and subdivided them into the relevant categories of cognitive restraint of eating, disinhibition or hunger.

MRC-Three-Day Food Diaries

The MRC-Human Nutrition Research Diary (or three-day food diary, Appendix 2) is a self-completed record of individuals' food and drink consumption completed over three standard days in the "real-world" environment, with participants asked to include at least one weekend day. Participants were advised not

to undertake this on a day which did not reflect their routine diet, e.g. on a special occasion. Anything consumed (food or drink, including water) was to be noted. The food diaries were given to participants at their screening visit and they were asked to return these on either of their subsequent visits or by post. Participant responses were analysed using WinDiets, a specialist dietary software. The elements calculated were total calorie, fat, protein, carbohydrate (CHO) and sugar consumption per day for three days. This data was then averaged over the three days to give an average daily intake. The total calorie consumption was then compared to calculated basal metabolic rate (BMR) as a percentage and the total calorie consumption per kg of FFM was also calculated.

Statistical analysis

Further analysis of both the TFEQ data and the three-day food diaries was undertaken using SPSS for Windows, versions 22 and 24. A non-parametric approach was chosen for the analysis of this data as many of the variables considered did not have a normal distribution. Also the small number of observations and different sample sizes across the groups made it problematic to attempt suitable transformation of the data to allow parametric tests to be undertaken (i.e. two-way ANOVA, as undertaken for data in the rest of the thesis). Kruskal-Wallis tests were therefore used to explore the data. The adjusted statistic test and the relative p-value are reported in the presence of ties. For the TFEQ data correlation between BMI and the various TFEQ scores were examined using Spearman's rho.

For all data median (IQR) are reported and $p<0.05$ considered significant.

Results

TFEQ

TFEQ demographics

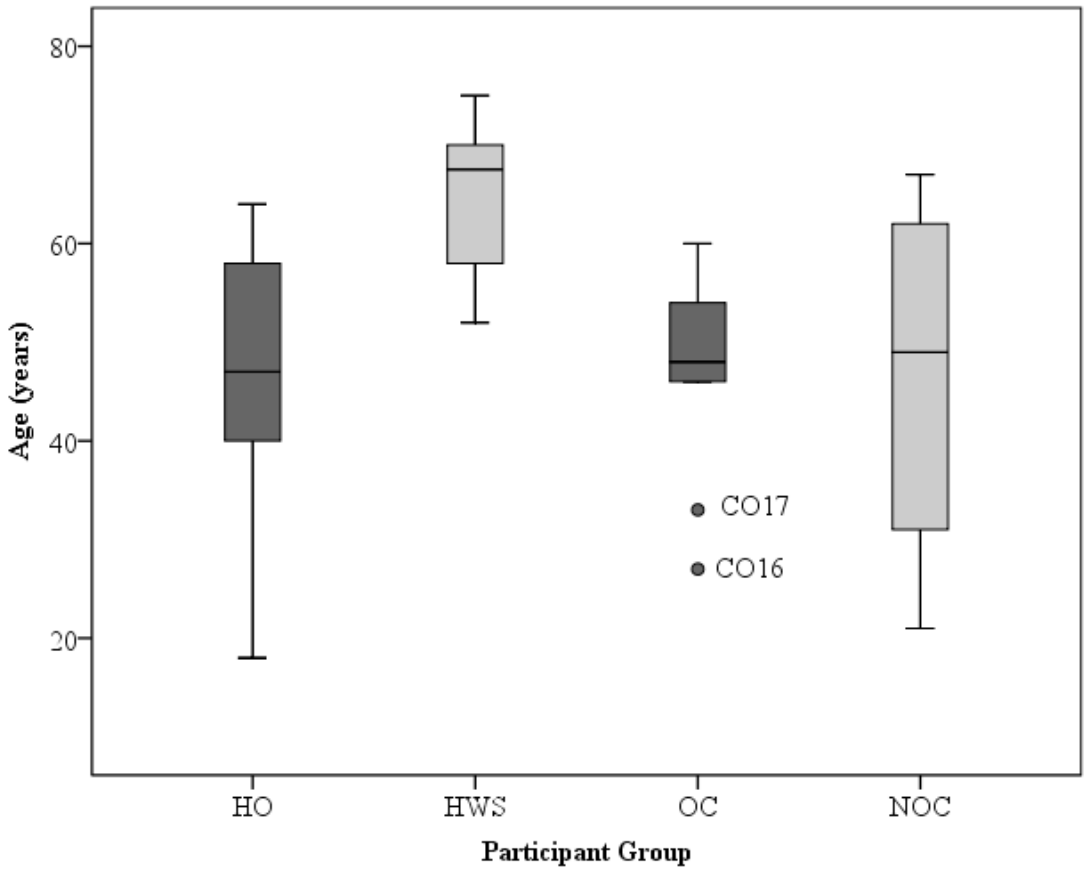
Thirty-four participants returned the TFEQ. The mean rank differed significantly across the groups for age and BMI on Kruskal-Wallis testing (Table 6.6). An explorative boxplot by group suggested that the median age was highest in the HWS group (Figure 6.6) and median BMI was higher in *obese (HO/OC)* than *non-obese (HWS/NOC)* participants (Figure 6.7).

Table 6.6. Median (IQR) demographics and anthropometry of TFEQ participants

	HO	HWS	OC	NOC	H(3)	p-value
Number of participants	8	6	9	11	-	-
Gender: F	4	7	3	6	-	-
M	4	2	3	5		
Age, years	47.0 (19)	67.5 (15)	48.0 (17)	49.0 (40)	8.5	0.04
BMI, kg/m^2	36.6 (8.8)	27.9 (4.6)	36.9 (12.1)	27.2 (4.7)	24.8	<0.001

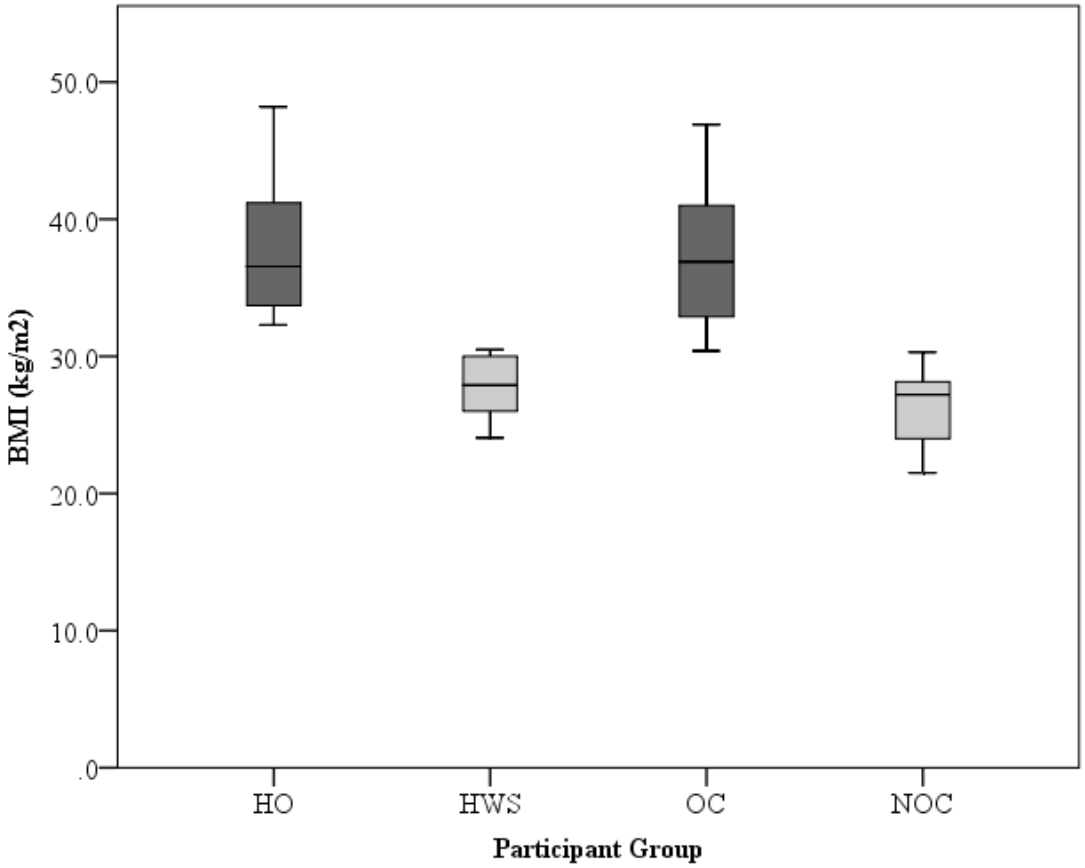
F = female, M = male. Comparison between groups using the Kruskal-Wallis test.

Figure 6.6. Box-plots showing differences in median age between the groups [(thick central line), interquartile range (upper and lower box edges) and minimum/ maximum (lowest/uppermost horizontal lines, with outliers depicted as circles)]



Light-grey bars = non-obese, dark-grey bars = obese. Outliers are labelled with their participant code.

Figure 6.7. Box-plots showing differences in median BMI between the groups [(thick central line), interquartile range (upper and lower box edges) and minimum/maximum (lowest/uppermost horizontal lines)]



Light-grey bars = non-obese, dark-grey bars = obese

TFEQ scores

There was no significant difference in restraint or hunger between the groups on Kruskal-Wallis testing however for disinhibition the mean rank differed significantly across the groups (Table 6.8). An explorative boxplot by group suggested that disinhibition was affected by patient characteristics, with a higher median score in OC than the other groups (Figure 6.8).

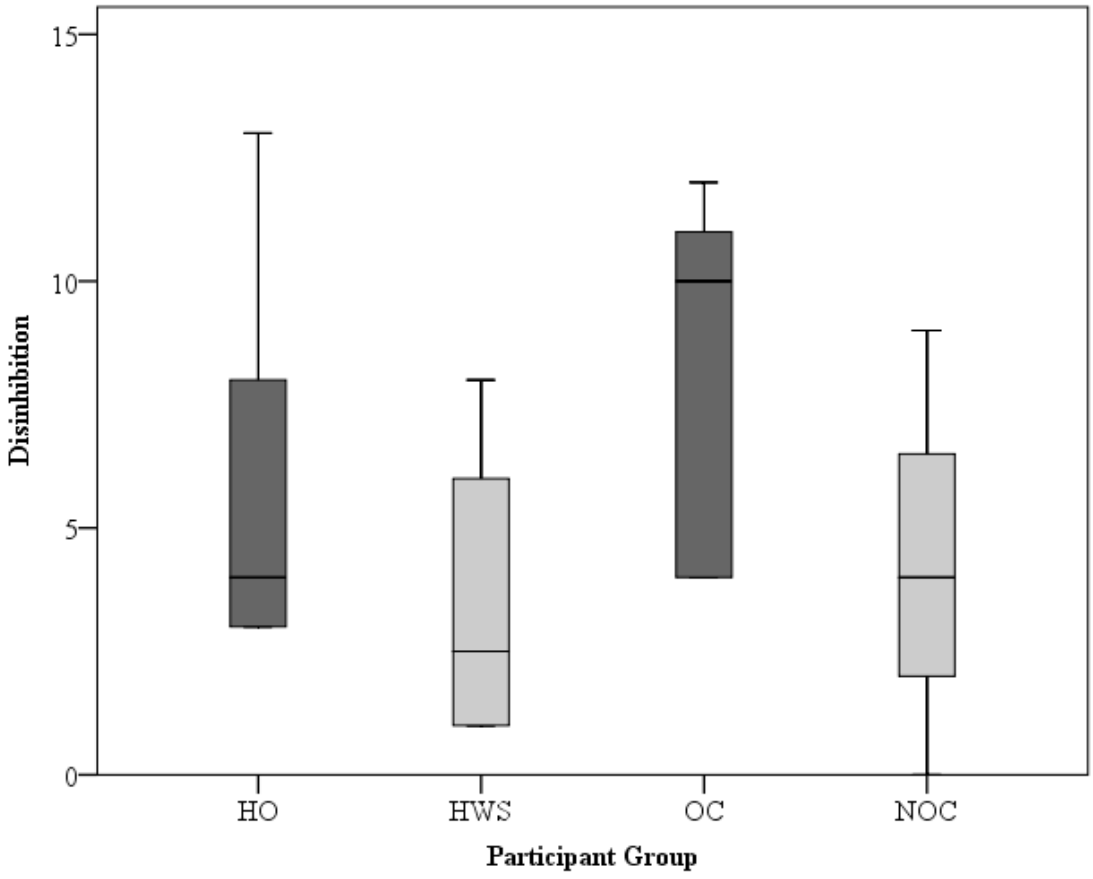
There was no correlation between BMI and restraint, disinhibition or hunger across all participants (*no*t separated by participant group).

Table 6.7. Median (IQR) TFEQ scores across the four groups

					Kruskal-Wallis	**Spearman's rho**
	HO	HWS	OC	NOC	H(3) [p-value]	r_s [p-value]
Restraint (max 21)	13.0 (14)	13.0 (1)	9.0 (8)	9.0 (11)	1.85 [0.61]	.03 [0.86]
Disinhibition (max 16)	4.0 (6)	2.5 (6)	10.0 (7)	4.0 (5)	8.96 [0.03]	.33 [0.06]
Hunger (max 14)	2.5 (7)	3.0 (4)	5.0 (6)	4.0 (4)	1.41 [0.70]	.09 [0.58]

Comparison between groups using the Kruskal-Wallis test. Spearman's rho examined correlation between BMI and TFEQ scores (across all participant groups).

Figure 6.8. Box-plots showing differences in disinhibition between the groups [median (thick central line), interquartile range (upper and lower box edges) and minimum/maximum (lowest/uppermost horizontal lines)]



Light-grey bars = non-obese, dark-grey bars = obese

MRC-Three Day Food Diaries

Thirty-three participants returned a 3-day food diary, however one HWS patient was excluded as reported total calories (TC) varied by up to 200% across the three days and on average was substantially lower than the estimated basal metabolic rate (only 42.1%) and the accuracy of the data was therefore uncertain. The data for 32 participants is therefore reported (Table 6.8).

Food Diary demographics/anthropometry

There was no significant difference in age between the groups on Kruskal-Wallis testing, however for BMI the mean rank differed significantly across the groups (Table 6.8). An explorative boxplot by group suggested that the BMI was affected by patient characteristics and HO and OC showed higher medians and a more widely spread distribution compared with the other two groups (Figure 6.9).

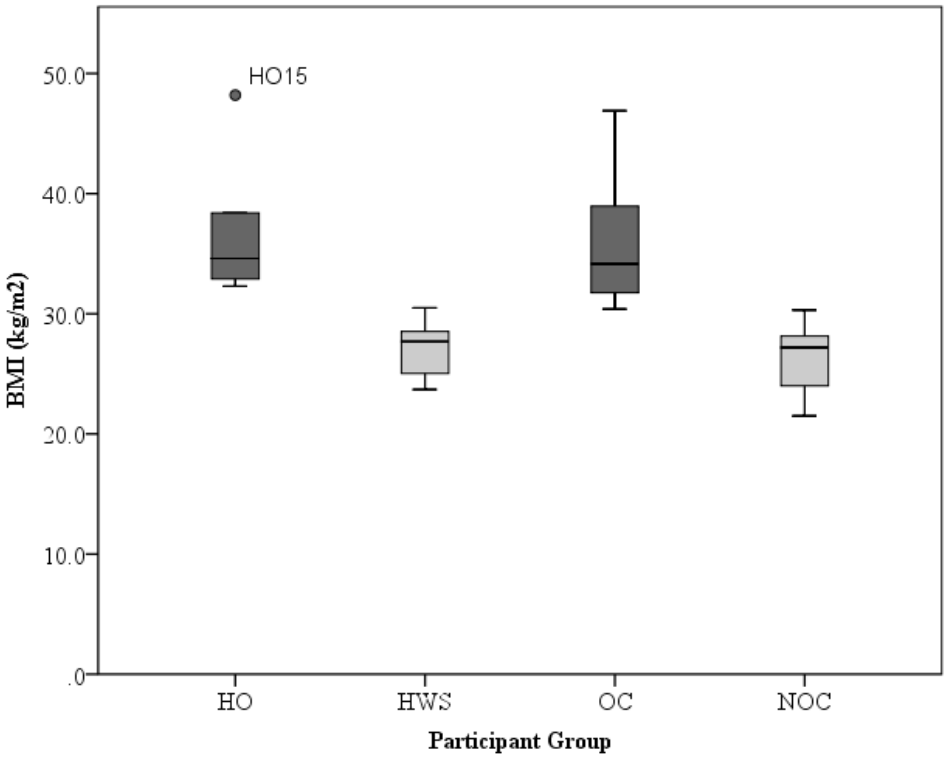
There was no significant difference in basal metabolic rate or fat-free mass between the groups on Kruskal-Wallis testing however for percentage body fat the mean rank differed significantly across the groups (Table 6.8). An explorative boxplot by group suggested that the percentage body fat was affected by patient characteristics and HO and OC showed higher medians than the other two groups (Figure 6.10).

Table 6.8. Characteristics of participants who returned food diaries, reported as median (IQR)

	HO	HWS	OC	NOC	H(3)	p-value
Number of participants	6	7	8	11	-	-
Gender: F	3	4	7	6	-	-
M	3	3	1	5	-	-
Age, years	47.0 (23)	66.0 (18)	48.5 (23)	49.0 (40)	4.81	0.19
BMI, kg/m^2	34.6 (8.1)	27.7 (4.9)	34.2 (8.8)	27.2 (4.7)	22.8	<0.001
BMR (kcal/day)	1843 (381)	1699 (556)	1596 (441)	1449 (387)	3.38	0.34
% body fat	45.5 (11.5)	32.5 (8.5)	44.0 (8.8)	31.0 (21.5)	13.46	0.004
FFM (kg)	51.7 (7.9)	50.5 (19.2)	50.6 (10.0)	48.0 (16.0)	0.83	0.84

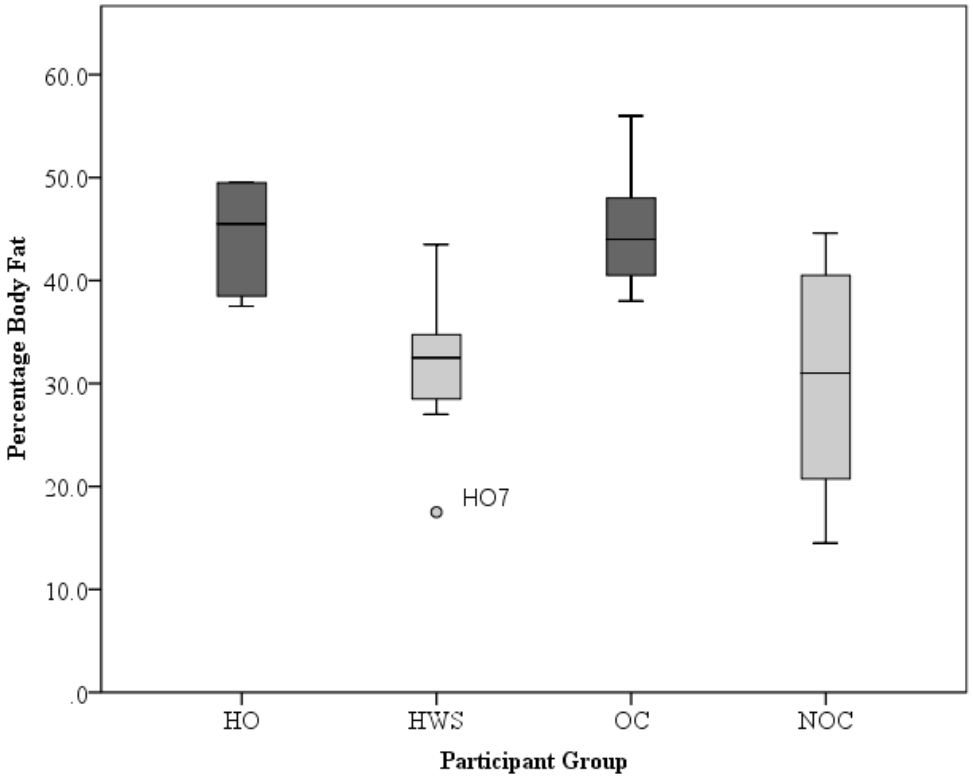
F = female, M = male. BMI = body mass index, BMR = basal metabolic rate, FFM = fat-free mass. For percentage body fat and fat-free mass data only available for 5 HO participants as one was wearing a compression stocking and therefore Tanita could not assess these parameters. Comparison between groups using Kruskal-Wallis test.

Figure 6.9. Box-plots showing differences in median BMI between the groups [(thick central line), interquartile range (upper and lower box edges) and minimum/maximum (lowest/uppermost horizontal lines]



Light-grey bars = non-obese, dark-grey bars = obese. Outliers are labelled with their participant code.

Figure 6.10. Box-plots showing differences in percentage body fat between the groups [median (thick central line), interquartile range (upper and lower box edges) and minimum/maximum (lowest/uppermost horizontal lines]



Light-grey bars = non-obese, dark-grey bars = obese. Outliers are labelled with their participant code.

Reported consumption

There was no significant difference in the number of eating episodes, total calories consumed, total calories/kg fat-free mass or total calories as a percentage of BMR between the groups on Kruskal-Wallis testing (Table 6.9).

Calculated macronutrient consumption based on food diary responses

There was no significant difference between the groups in the amount of fat, protein, carbohydrate or sugar consumed in weight (g), or as a percentage of total calories consumed on Kruskal-Wallis testing (Table 6.10).

Table 6.9 Median (IQR) consumption over three days, as reported in the MRC three-day diary

	HO	HWS	OC	NOC	H(3)	p-value
Eating Episodes	3.8 (0.5)	4.0 (1.8)	4.2 (1.9)	4.7 (0.7)	5.6	0.13
Total Calories [TC] (kcal/day)	3263 (1329)	3304 (2567)	3562 (1307)	3258 (639)	0.76	0.86
TC/kg fat-free mass (FFM)	52.5 (31.2)	67.0 (23.4)	61.6 (29.1)	54.4 (29.0)	2.08	0.56
TC as percent of calculated BMR	159 (90)	243 (131)	194 (120)	215 (88)	2.44	0.49

TC = total calories. Comparison between groups using the Kruskal-Wallis test.

Table 6.10 Median (IQR) macronutrient consumption over three days, as reported in the MRC three-day diary

	HO	HWS	OC	NOC	H(3)	p-value
Fat (g)	113.4 (72.6)	147.4 (71.8)	146.5 (71.6)	176.1 (101.8)	3.63	0.30
Fat% of TC	33.2 (5.5)	27.0 (23.0)	36.9 (11.3)	43.5 (21.9)	4.42	0.22
Protein (g)	136.9 (113.1)	145.4 (223.5)	119.1 (93.1)	143.8 (32.3)	1.83	0.61
Protein% of TC	18.4 (13.8)	15.7 (24.9)	14.4 (6.6)	15.4 (5.7)	1.76	0.62
Carbohydrate (CHO) (g)	309.2 (373.8)	601.6 (810.0)	390.1 (306.7)	443.0 (132.6)	2.03	0.57
CHO% of TC	47.6 (15.0)	44.2 (26.8)	49.1 (8.5)	44.0 (17.9)	2.58	0.46
Sugars (g)	44.7 (115.6)	106.2 (99.2)	87.8 (64.3)	66.1 (78.0)	4.06	0.26

Comparison between groups using the Kruskal-Wallis test.

Discussion

Hyperphagia

There was no evidence of hyperphagia in participants with HO compared to those who remained HWS, even when intake was adjusted for fat-free mass or calculated as a proportion of the estimated BMR of the participants. In fact whilst there was no significant difference in intake between *obese* and *non-obese* participants, there was significantly greater total intake, intake per kg of FFM and calories consumed as a proportion of BMR, as well as a longer eating duration in *controls* compared to participants with *hypothalamic damage*. These results were unexpected, as it was hypothesized that those participants with HO would be hyperphagic and therefore would consume significantly more than the other groups, or at least significantly more than HWS participants [32]. There were no significant differences in pleasantness ratings between the groups to suggest that the HO group found the meal less pleasant and therefore ate less than they would have with a different meal causing an erroneously low intake whilst eating at the UEM. It is possible that participants were aware that their intake was being monitored and that those with HO altered their intake more than the other groups, however as participants were not asked at the end of the session whether they were aware of the purpose of the UEM there is no evidence that this is the case and no reason to expect that those with HO modified their intake to any greater extent than the other groups. One outlier with HO did eat significantly more than all other participants (1169.2g) and was excluded from analysis to allow for a more normal distribution. It is possible that this excluded individual reflected the upper limit of a range of hyperphagia seen in the HO group and was therefore still representative of this group. It is also possible that only some of those with HO have hyperphagia. As there was significantly higher total intake, calorie intake per kg of FFM and amount of calories consumed as a percentage of the estimated BMR in *controls* than in *patients*, despite no significant difference in BMI or percentage body fat between them, this could point toward the possibility of differences in metabolic rate, which in this study were calculated (based on age, gender, height and weight) but not measured. It would be important to consider measuring basal metabolic rate in future studies. Most importantly, due to the difficulties encountered with the UEM, only a small number of patients with hypothalamic damage (6 HO, 6 HWS) had data available for

analysis. This makes it difficult to conclude that there were no differences between the groups and the lack of significant findings may have been due to power. The current data could be used to calculate the sample size necessary for future studies, including the possibility of assessing for hyperphagia and dividing the groups accordingly.

Monitoring of food intake at the UEM necessitates using a single, homogenous food and it is possible that sensory specific satiety [220] was greater in the HO group, leading to lower intake than might occur if a more varied meal had been offered. It may also be that while intake across a single meal is not increased in those with HO, across the course of a day food frequency may be increased (although this was not demonstrated in the food diaries undertaken as part of this study). A study comparing 15 age-, BMI- and body fat- matched simple-obese controls to 14 participants with HO found that three hours after eating those with HO reported significantly more hunger and desire to eat (VAS ratings, $p<0.01$) [2], suggesting a shorter period of post-prandial fullness. Food intake later in the day following the lunch-time UEM meal was not recorded in the study described in this thesis however eating patterns and food intake were assessed using three-day food diaries, as reported in this chapter.

Food intake before the lunch-time UEM meal was controlled by asking participants to attend fasting and then offering a breakfast meal based on 25% of their estimated intake, based on calculated metabolic rate. Basal metabolic rate may be altered in those with HO [3, 65], however the results are conflicting. This may have affected the calculation of an appropriate breakfast "load". As participants were at home before the breakfast meal, it also is possible that not all may have arrived fasting.

It is also possible that hyperphagia is not a consistent feature of HO in all cases, as in some studies obesity has been reported in the absence of increased food consumption [42, 43].

The pattern of eating rate

Although there were no statistically significant differences between the groups in eating rate across the entire meal, or differences for cumulative change in eating rate over time, the HO group initially tended towards a higher eating rate than

the other groups on visual ascription (Figure 6.2). Their eating rate then reduced, but was followed by an increase towards the end of the meal. This finding is unusual, but due to low numbers was statistically non-significant. Evidence varies within the literature as to whether those with simple obesity have an increased eating rate compared to lean individuals and whether they fail to slow their eating rate at the end of a meal. In this study, obese controls tended towards an increase in eating rate initially (on visual ascription), followed by a slower reduction compared to the other groups; however, most likely due to low participant numbers, this was statistically non-significant and does not contribute any further evidence to this literature.

Microstructure

The lack of significant difference between the groups in pleasantness ratings throughout the meal suggests that palatability did not influence total intake or eating rate. Similarly there were no significant differences in fullness at the start of the meal, or through the course of the meal between any of the groups. Baseline hunger was significantly lower at the start of the meal in participants with *hypothalamic damage (HO/HWS)* than in *controls (OC/NOC)* and food intake at the UEM lower (even once adjusted for calculated BMR), but there was no significant difference between *obese (HO/OC)* and *non-obese* (*HWS/NOC)* participants, or interaction between *hypothalamic damage vs. controls* and *obese vs. non-obese*. There was no evidence of increased hunger in those with HO compared to those HWS and no differences in orosensory reward, development of satiation or meal pleasantness to suggest that fullness was linked to feelings of a less pleasant meal, or other contributory factors.

The small size of the participant groups may have led to a lack of statistical difference between patients with HO and HWS as the study was powered to detect differences in brain activation to food stimuli, as discussed in Chapter 5 (the fMRI study). Further cross-sectional studies of larger groups of lean and obese participants both with and without hypothalamic damage may lead to more significant findings. Recruitment of participants with hypothalamic damage who remained weight-stable was particularly difficult, but if the study was restricted to investigation with a UEM only, this may potentially allow easier recruitment.

Self-reported eating behaviour had been assessed using the TFEQ, with the intention of considering this as a factor which might influence food consumption (as in other studies). The groups, however, were too small to be likely to yield significant differences if further separated according to self-assessed eating behaviour and this analysis was therefore not undertaken.

Biochemistry

Pre-lunch leptin was significantly higher in *obese* compared to *non-obese* participants, reflective of leptin resistance in simple obesity and hypothalamic obesity [35, 51].

There was no difference between the groups in pre-lunch insulin concentrations. AUC insulin was higher in *obese* compared to *non-obese* participants, but there was no significant difference between participants with *hypothalamic damage* and *controls*, or in the interaction between *hypothalamic damage vs. controls* and *obese vs. non-obese*. Hyperinsulinaemia after hypothalamic damage is a proposed mechanism of HO. Authors have hypothesized that excess insulin, secreted due to the effects of vagal nerve disinhibition on the pancreas, drives hunger/hyperphagia and accumulation of fat [3, 38, 55]. Results from studies measuring insulin concentrations have been conflicting – some have found evidence of increased fasting insulin concentrations [3, 4, 9, 29, 29, 39, 51, 52, 54, 57-59], others have found no difference in fasting insulin but that differences existed following OGTT [55, 56, 312] and others have found no difference in insulin using any study methods [51, 52, 57, 58]. As many of these studies were undertaken some time after the hypothalamic damage had occurred even in those studies where insulin differences were apparent it is not clear whether this was the causal mechanism or was secondary to weight gain, as is seen in individuals with simple obesity. There was no evidence on either of the study days undertaken for work presented in this thesis of greater fasting or pre-lunch insulin concentrations, or higher AUC insulin in those with HO compared to the other three groups. However as insulin concentrations were only available for six HO and six HWS participants it is very difficult to draw any conclusions given the small numbers involved. In fact, this is true for all of the reported data and previous studies have contained much larger numbers of patients [52, 58, 262].

In terms of the macrostructure of eating behaviour the TFEQ measures behavioural traits (restraint, disinhibition, feelings of chronic hunger) which influence food choice over time (eating habits) [242] and can outstrip more immediate hunger and satiety cues. As such, it was hypothesized that these factors may be of greater importance where homeostatic influences on hunger and satiety have been disrupted due to hypothalamic damage. In this study, OC had higher median scores for disinhibition than the other groups. Increased disinhibition has been reported in previous large studies of obese compared to lean individuals [1]. Interestingly, the HO group appear to be *less* disinhibited than OC, with a lower median score, although a large range of scores for reported disinhibition, as can be seen on the boxplots. Perhaps more pertinent is the lack of evidence of greater disinhibition in HO compared to HWS and NOC, in contrast to that seen in OC, suggesting that disinhibition is *not* a driver of obesity as occurs in the simple obese population. Once again however the small numbers of participants studied may have accounted for lack of difference found. A previous study comparing TFEQ scores in HO and OC [2] found significantly lower disinhibition in HO compared to OC, but non-obese participants (either with hypothalamic-damage or controls) were not studied. That study also found significantly lower hunger reported on TFEQ in HO than OC, a feature again not noted in the study presented in this thesis. More participants were included in Daousi et al's study (14 HO, 15 OC) [2] and it is possible that significant differences may have been found between the patients described in this thesis had the groups been larger. The study was powered to look for differences in areas of cerebral activation in response to food stimuli based on previous fMRI studies and not powered to detect differences in eating behaviour. Disinhibition reflects the likelihood of overeating secondary to external or emotional triggers (the theory of externality), for example if offered highly palatable food or when upset, as opposed to a homeostatic, physiological drive to eat [313]. There is some evidence from the two studies (Daousi et al [2] and the current study) that disinhibition is not an important behavioural component in HO, as is seen in simple obesity, which would be consistent with a more regulatory rather than externally driven theory of intake. Dietary restraint (which assesses behavioural control over eating) and hunger were not significantly different across the groups studied here and previous studies have shown these factors to be inconsistently and less strongly correlated with outcomes, respectively [201].

The only other use of the TFEQ in acquired HO in the literature is a case report of a 29 year-old male with a BMI of 52 kg/m^2 and history of craniopharyngioma resection aged 8 years, who underwent bariatric surgery [131]. TFEQ undertaken before and after surgery found a low level of pre-operative restraint (10/21) and disinhibition (8/16) and high hunger ratings (8/14). Hunger and disinhibition had reduced by 18 months post-bariatric surgery (2/14 and 4/16, respectively). Comparison with an individual case report is difficult and it is also unknown as to whether these changes persisted on a more permanent basis.

Although the TFEQ has been used in large cohorts and has high internal and test-retest reliability in both laboratory and non-laboratory settings [191, 201] it should be borne in mind that its use has not been validated in patients with structural brain lesions and therefore may not be reproducible in this group. It has, however, previously been used in studies of HO secondary to a non-structural, non-acquired genetic cause, such as melanocortin-4 receptor (MC4R) mutations. A literature review describes six studies, four of which use the TFEQ-51 questionnaire to assess associations between different MC4R mutations/variants and TFEQ factors, in some cases comparing patients with non-carrier overweight/obese individuals [314]. Only two of these studies found a significant difference between any TFEQ factors: disinhibition was higher in one study of 38 functional MC4R carriers (all obese) compared to 33 overweight related non-carriers [315] and hunger scores were higher in adults with a particular MC4R variant compared to those without in a study of 2,438 obese participants [316]. Therefore, despite much larger participant groups (71–2,438 individuals, 19-1,880 with MC4R mutations) there were no consistent associations between MC4R mutations/variants and TFEQ findings. Whether this implies that the TFEQ method is less reliable in patients with any form of HO, or whether this simply reflects this particular cohort where the mechanism of HO is very different to our cohort is a matter for debate.

The three-day food diaries revealed no significant differences between the groups for any of the parameters measured, including the number of eating episodes, mean total calories consumed/day or any of the nutritional components of the food eaten (CHO, fat, protein). This remained the case even when total calorie consumption was calculated against FFM and as a percentage of BMR, with median consumption across the four participant groups reported at 159-243% of that needed

to match their calculated BMR. Although participants were asked to complete the food diaries on a "typical" day, some were undertaken around the Christmas period and one participant had eaten out at an event (although this may have represented a typical day for that individual). There was a significant amount of variation in both the details and the quality of the completed diaries, with some participants (possibly those involved in previous weight-/obesity-related research) giving much greater detail. This introduces the likelihood of a large amount of error in assessing both the types and amounts of food consumed.

Food diaries, although widely used in research studies [238] can contain significant errors, with foods erroneously under-reported (25-37%) or added (10%) [240, 317]. The burden of completing food diaries, particularly when dining out, as well as the need for literacy and numeracy skills have been described, as well as the effects that completing a food diary may have on eating behaviour and reporting, particularly in certain groups such as obesity [239]. Under-reporting is more prevalent in females, obese participants, those dieting, with higher dietary restraint and with various psychosocial factors such as level of education [317, 318], although did not appear to be an issue in this study, given that the median total calorie consumption reported as a percentage of estimated BMR was greater than 100% in all four participant groups. If the food diary aspect of this research project was to be repeated with a larger group of participants it would benefit from additional support and information being given to participants by a dietician at enrolment of the study to enable more accurate completion of the diaries, as well as including eating behaviour (as assessed by TFEQ), gender and level of education as covariates in any statistical models to help correct for measurement error in reported dietary intakes.

It is interesting to note the lack of significant difference in calculated BMR between the groups, which may reflect the need to use a non-obese, rather than lean group of controls and HWS patients. BMR is also affected by age and differences between the groups in age may have played a role, although there were no statistically significant differences in age between the groups on analysis of the three-day food diaries. Finally, power will also have had an effect and due to the small amount of participants returning both TFEQs and food diaries it is difficult to conclude for certain any lack of difference between the groups. Any future study would benefit from a larger number of participants, with a greater difference in BMI

between the obese and lean groups. Data from the studies described in this chapter could be used to calculate the numbers needed for future studies.

Strengths and limitations of the study

This study has its limitations:

- The small size of this study was a significant limitation. This was due in part to the rarity of this condition and particularly the difficulty in finding patients with hypothalamic damage who remained weight stable. Whilst there were some interesting findings, further larger studies may give greater clarification and would allow correlation with other clinical and biochemical findings.

- There are variations in the group sizes (due to technical difficulties with some of the UEM recordings and inconsistent returns of the TFEQ and three-day food diaries) which makes the power of statistical tests weaker than with equal sized groups. Therefore even where there are significant p values these should only be used as an indication of possible differences and should be further investigated in larger studies.

- Weight-stable patients (HWS) were not lean (BMI < 25 kg/m^2), therefore the BMI-matched control group were also non-obese but not lean. Although the *obese* participants studied using the UEM had a significantly higher BMI and percentage body fat compared to *non-obese* participants, using the reported Tanita ranges to categorise participants into healthy, overfat and obese, a significant proportion of participants were either overfat (2/6 NOC) or obese (2/6 NOC and 4/6 HWS), even within the HWS and NOC categories. Larger studies, containing patients with hypothalamic damage who remained weight-stable with a normal BMI (< 25 kg/m^2) may help to give a clearer picture.

- Ideally the patient groups would have been homogenous, with one underlying histological diagnosis causing hypothalamic damage. As noted, it is a relatively rare condition and therefore it was accepted at the outset that studying a more heterogeneous group would be necessary.

- The basal metabolic rate in this study was calculated based on age, gender, height and weight and not measured. It would be important to consider measuring basal metabolic rate in future studies, especially as some studies have found lower BMR in participants with HO [65].

- The same homogenous food was used for all participants throughout the UEM meal. This may have been liked to a different degree by different study participants, although there was no significant difference in rated pleasantness reported on the VAS ratings. It is possible however that (given such a small study group) individual food preferences affected the findings of this study. As the food used was a savoury meal it is possible that the outcomes may have been different if a sweet food was used and that sensory specific satiety may have played a part in termination of eating.

The strengths of this study are:

- This is a unique study as the UEM has never before been used to study the microstructure of eating behaviour in patients with hypothalamic damage (either in those with or without obesity).

- This study is also unique in that it considers both the microstructure and macrostructure of eating behaviour in patients with hypothalamic damage.

- A standardised, reasonably physiological overnight fasting period was used.

- All participants were studied at the same research centre, with the same research staff conducting the study days.

Conclusions

There was no evidence of an increase in total food intake, overall increased eating rate or a failure to slow eating rate at the end of the meal consumed at the UEM in those with HO compared to the other groups. The lack of both greater hunger and total food intake in those with HO does not support within-meal hyperphagia as a key pathophysiological component of weight-gain in these patients, as both of these factors would be expected if patients with HO were hyperphagic. A meal eaten at the UEM therefore failed to demonstrate any significant differences between the microstructure of eating behaviour in those with HO and those HWS (or SO controls); however visual ascription showed some tendencies towards an unusual eating pattern in those with HO which might be statistically significant if an appropriately powered study was undertaken and this warrants further investigation in a larger group of participants.

There were also no significant differences in the macrostructure of eating behaviour as measured by TFEQ, or reported differences in dietary intake recorded in three-day food diaries between participants with HO and those HWS (or between those with HO and controls). The absence of greater disinhibition in those with HO may reflect the disruption in internal, homeostatic regulatory mechanisms leading to weight-gain, rather than externally-influenced, emotional triggers which can drive over-eating and weight-gain in simple obesity. The difficulties with food-diary accuracy and under-reporting are well described and participants may have benefitted from increased dietetic guidance to enable these to be more accurately completed. Finally, a larger cohort, as found in other studies utilising these tools in different populations, may have led to more statistically significant differences between the groups and the lack of power in the studies may have strongly influenced findings.

Chapter 7

Final Discussion

Hypothalamic obesity (HO) is characterised by an acute increase in body weight secondary to a clear hypothalamic insult [7] with weight gain occurring faster than any expected age-related increase [9] despite appropriate replacement of pituitary hormone deficiencies. Although well-described for over a century [7, 9, 10] its exact pathophysiology and optimal treatments remain uncertain. As all patients with suprasellar tumours have increased mortality (Standardised Mortality Rate 2.88-8.75 [19, 71, 72]) and simple, nutritional obesity alone is associated with co-morbidities such as cardiovascular, renal and liver disease and type 2 diabetes mellitus [67, 258], understanding the risk factors and pathophysiology leading to HO are vital to try and prevent additional morbidity and mortality in patients with hypothalamic damage. This knowledge can aid health-care professionals to better direct resources to prevent weight-gain and obesity following hypothalamic damage and can potentially open up future avenues for further research into treatments for HO.

This thesis describes investigations into the prevalence and underlying pathophysiology of HO through several linked clinical research studies.

The prevalence of HO was explored with a descriptive cohort study, undertaken through case-note review. The prevalence of additional risk factors contributing to increased morbidity and mortality, known to be present in both adult patients with suprasellar tumours and individuals with simple obesity, were also investigated. In addition, I was interested in studying these risk factors and metabolic outcomes in children and young people with pituitary adenomas (both with and without hypothalamic damage) in view of the limited literature available and to increase awareness of their prevalence in this patient group. Identification of increased cardiovascular risk in this age group would mean these patients would benefit from earlier targeted dietary, pharmacological and other interventions. Furthermore if this group of patients were shown to develop a higher prevalence of obesity even in the absence of hypothalamic damage (as many have microadenomas too small to extend into the hypothalamus) it may suggest that additional mechanisms contribute to increased obesity risk both in the presence and absence of hypothalamic damage.

The underlying pathophysiology of HO was investigated using complementary approaches to determine whether disturbances in neural pathways affect food intake and eating behaviour, leading to weight-gain.

Prevalence of obesity and cardiovascular risk factors in patients with sellar/suprasellar tumours

Review of a large cohort of 110 adults attending specialist neuroendocrine clinics with tumours causing hypothalamic damage (Chapter 3) demonstrated a significant increase in median body mass index (BMI) from 28.1 kg/m^2 at diagnosis to 32 kg/m^2 ($p<0.0001$) after a median follow-up of 9 years. Additionally, an increased prevalence of obesity (63%) and overweight/greater (total 91%) was found in patients with hypothalamic damage compared to that found in the general population of a similar age [256]. As previously described, weight increase was fastest in the first year following tumour diagnosis and treatment (mean BMI increased to 30.4 kg/m^2 at this time, $p<0.0001$), making this a key period in which to address this issue and to target those at increased risk. Disappointingly, in 35 patients diagnosed after 2005, despite increased awareness of obesity and strategies in place to identify and curtail weight gain, median BMI still increased significantly from 29.9 kg/m^2 to 31.7 kg/m^2 ($p<0.001$) after a median of 3 years. The need for desmopressin (odds ratio [OR] = 3.5, p=0.03), growth hormone [GH] (OR = 2.7, p=0.03) and thyroxine (OR = 3.0, p=0.03) were associated with increased risk of HO, but there was no correlation between neuroimaging features and weight gain. Patients needing supplementation with these pituitary hormones should be particularly targeted for weight and lifestyle advice during follow-up. As hypertension, type 2 diabetes and cardiovascular disease were also more prevalent in the cohort of 110 patients than in the general population of a similar age (55-64 years) [256], screening for these additional risk-factors should be included in best-practice guidelines for the follow-up of patients with suprasellar tumours and hypothalamic damage. Furthermore, the findings described in this cohort of patients provides evidence that multi-disciplinary neurosurgical, endocrine and dietetic follow-up is needed, with routine monitoring for obesity and cardiovascular risk

factors and a low threshold for treatment in order to prevent further morbidity and mortality in this patient group.

The retrospective review of children and adolescents with pituitary tumours (with and without hypothalamic damage) described in Chapter 4 also demonstrated a high prevalence of cardiovascular risk factors such as obesity (39%) and dyslipidaemia (73%), despite the young age of the participants (mean age at study 26.9 years). This was the case even in patients with tumours confined to the sella, without evidence of hypothalamic involvement. Given the increased prevalence of obesity found in this patient cohort (39%) compared to the general female population of a similar age (21%), despite the predominance of microadenomas and therefore a lack of hypothalamic damage within the cohort, there is some evidence that hypothalamic damage is not the only factor contributing to an increased prevalence of obesity in children and adolescents with pituitary tumours. This is important to bear in mind when considering the underlying pathophysiological mechanisms responsible for hypothalamic obesity as it suggests that there may be other contributing factors causing weight gain in addition to the hypothalamic damage, at least in patients with pituitary adenomas. It also may suggest the need for future studies to include cohorts of patients both with and without hypothalamic damage when investigating the mechanisms of weight gain in those with acquired pituitary/hypothalamic lesions, to allow for potential additional non-hypothalamic mechanisms to also be studied. The findings indicate the need to focus on early identification and management of risk factors not otherwise commonly found in individuals of this age and the necessity for long-term follow-up. The knowledge and expertise acquired whilst conducting this research within an adult research and clinical neuroendocrine service has enabled me to transfer and apply these skills and knowledge within the field of paediatric endocrinology, where I am currently practicing as a clinician, and to disseminate and apply these findings into everyday clinical practice.

Establishment of a UK or European database to allow the study of larger numbers of patients with suprasellar tumours would be a valuable resource and should be encouraged. Further studies are needed to help determine the optimal management of these patients in order to reduce the morbidity and mortality

associated with hypothalamic damage, without compromising long-term disease control.

Investigation of the pathophysiology of HO

The hypothalamus is the critical homeostatic centre controlling energy intake/output. Following damage to this area, the interaction between the remaining homeostatic regulators (the nucleus accumbens and brainstem) and the limbic, paralimbic and higher cortical regions (which process non-homeostatic factors such as emotion, cognition and reward secondary to environmental and hedonic cues and food reward) remains poorly understood. At the beginning of this research it was hypothesized that in patients with hypothalamic damage who became obese (HO) there would be evidence of anomalous neural processing of food stimuli, leading to abnormal appetite, excessive food consumption and hyperphagia, which has previously been described in HO [11, 23, 40, 41]. This was investigated using four tools: i) functional magnetic resonance imaging (fMRI), ii) the Universal Eating Monitor (UEM), iii) the Three Factor Eating Questionnaire (TFEQ) and iv) the 3-day MRC-Human Nutrition Research diary, each evaluating different aspects of eating and appetite.

Chapter 5 describes the use of fMRI to determine whether differential activation of various brain regions and neural pathways involved in appetite and eating could be responsible for the weight-gain observed in those developing HO. This was a cross-sectional study of nine patients with HO, seven patients with hypothalamic damage remaining weight-stable (HWS), 10 age-matched obese controls (OC) and 10 non-obese controls (NOC). Although it was hypothesized that in those who had developed HO there would be evidence of abnormal neural processing of food stimuli, in fact the difference between the groups was found in those who were HWS. This group had significantly lower mean BOLD signal ($p = 0.001$) in brain regions associated with food motivation and reward, namely the posterior insula and lingual gyrus, when viewing high-calorie food photographs, compared to the other three groups. Similar differences have been observed in lean compared to obese individuals without hypothalamic damage [164, 300]. Insula activation has also been

found to be significantly different in obese individuals who have lost weight (post-obese) compared to normal-weight/lean controls. Both obese and post-obese individuals have demonstrated increased mid-insula activation compared to lean individuals [166], however whether this is causal to or a result of weight gain is not clear. I therefore postulate that in some patients with hypothalamic damage (those who remain HWS) there may be greater preservation of functional and anatomical connectivity between the insula, extra-hypothalamic homeostatic areas (such as the midbrain VTA and NA) and areas processing food-reward, explaining the pattern of reduced insula activation when viewing high-calorie food stimuli and allowing a continuation of a more coordinated response between homeostatic and reward-related networks regulating appetite/eating and energy balance.

It was then hypothesized that lower insula activation in HWS may protect against weight-gain by decreasing hunger and desire to eat, and leading to reduced food intake compared to HO. This was not reflected in the UEM findings, food diaries, or TFEQ assessment of eating behaviour (Chapter 6). The UEM study showed that *controls* (*OC/NOC)* ate significantly more and for significantly longer, with higher initial ratings of hunger than participants with hypothalamic damage *(HO/HWS)*, but there was no difference between *obese (HO/OC)* and *non-obese (HWS/NOC)* participants, or in the interaction between *obese vs. non-obese* and *patients vs. controls*. There was therefore no evidence of difference in food consumption or hunger between those who developed HO and those remaining HWS. Eating rate, change in eating rate, VAS scores of fullness and pleasantness, change in fullness and the development of satiation were also no different between any of the groups. This study was not therefore able to replicate evidence of hyperphagia in HO which has been reported in other studies [11, 23, 40, 41]. The lack of difference, however, is a pertinent finding as it highlights the *lack* of increased hunger in those with HO (although caution must be applied due to the small number of participants studied, risking the possibility of a Type II error). The evidence from the studies presented in this thesis are in contrast to individuals with Prader-Willi syndrome (PWS), where extreme hyperphagia is evident and orbitofrontal/pre-frontal cortex differences have been demonstrated on functional neuroimaging [171, 189, 195, 319]. While it is possible that sensory specific satiety

[220] was greater in the HO group than the other groups and a varied, free-buffet lunch may have led to different findings, there was no evidence to suggest this. The absence of hyperphagia in HO *is* in agreement with some other studies [42, 43].

The possibility that whilst intake over a single meal is not increased in HO, but that food frequency across the course of a day is increased, or that food choices are more unhealthy (higher calorie/fat/sugar content) in those with HO were not evident either. In the three-day food diaries (Chapter 6) there were no statistically significant differences in the number of reported eating episodes, amount of fat, protein, carbohydrate or sugar consumed, or the mean total calorie consumption per day (even when corrected for fat-free mass and basal metabolic rate). There are, however inconsistencies inherent in this method of real-world reporting.

Finally, in considering more long-term eating behaviour, the TFEQ showed some evidence of increased disinhibition in OC, but no other significant differences between the groups in median disinhibition, hunger or restraint scores. Increased disinhibition is associated with over-eating and increased BMI and previous studies report increased disinhibition in simple nutritional obesity [222]. Again there was a notable difference between the obese groups, as those with HO did *not* have evidence of increased disinhibition as was reported by OC, perhaps reflecting a lack of an external drive to over-eat, although possibly due to the low numbers of participants studied leading to a lack of power to demonstrate any differences. The wide range of disinhibition scores in those with HO is noted. Relevant to the three-day food diaries undertaken, individuals with high restraint scores have been shown to under-report dietary intakes [320], which may have contributed to a lack of difference in reported intake between OC and NOC (although there was no significant difference between these groups on reported TFEQ restraint scores). There were not enough participants in this study to consider eating behaviour as a covariant, however if larger studies were undertaken, then scores for eating behaviour (disinhibition, hunger and restraint) could be entered as covariates in statistical models to help to correct for measurement error in dietary intake reporting. Once again, however, accuracy is dependent on individuals not only being aware of their eating behaviour, but also being prepared to report it.

Hormone analysis from the UEM study day measured leptin concentrations and found them to be significantly higher in *obese* (*HO/OC*) compared to *non-obese* (*HWS/NOC*) participants. Leptin resistance has previously been described in both simple obesity [35] and HO [51].Both fasting and AUC active ghrelin were lower in *patients (HO/HWS)* compared to *controls*, but there was no difference between *obese (HO/OC)* and *non-obese* (*HWS/NOC)* participants, which might have been expected as ghrelin is known to be lower in obesity [36]. This may have been due to the use of a "non-obese" group for comparison, rather than a "lean" group, due to the higher BMI in those who remained HWS. The use of a lean group (BMI <25 kg/m^2) in future studies would be beneficial, as would study of a larger number of participants, as the small number of participants studied may also have led to the lack of evidence of difference between the groups.

Multiple different mechanisms have been proposed as the cause of weight gain and obesity in those with acquired, structural hypothalamic damage. The studies undertaken for this thesis found no evidence of several of these proposed mechanisms, including hyperphagia and increased energy intake or hyperinsulinism due to autonomic nervous system dysregulation. Studies were not undertaken to investigate whether reduced physical activity, reduced basal metabolic rate, melatonin dysregulation or enhanced 11-β hydroxysteroid dehydrogenase-1 activity could be causal contributors [11, 260-262]. It is possible that several mechanisms may be responsible and also that this may vary between individual patients or underlying pathologies [5, 7, 11].

Study of a larger, more homogenous patient group (for example only including patients with craniopharyngioma as the cause of hypothalamic damage) would have strengthened findings, however due to the rarity of this condition it was accepted at the outset that this would not be possible. One of the strengths of the functional neuroimaging study however was the use of stringent statistical thresholds when analysing the fMRI results. To prevent false-positive findings through the use of multiple comparisons, initial whole brain analysis (used to identify areas of BOLD signal which differentiated between food images and objects, which were then analysed using a region of interest approach) were corrected using a false discovery rate (FDR) of $p \leq 0.05$ and a cluster size of $k \geq 3{,}000$. This high statistical threshold

may have reduced the extent of positive findings and rejected additional significant voxel clusters where a moderate effect existed but which disappeared after correction. The use of a voxel threshold above 3,000 also excluded certain sub-cortical areas, such as the nucleus accumbens and amygdala, which have been shown to differ between lean and simple obese individuals studied. These criteria did however reduce the chance of a Type I statistical error. This adds weight to the positive findings identified, as the small group sizes (in common with all neuroimaging studies) mean the study was only powered to reliably detect larger effect sizes.

Taken in their entirety, these studies do not demonstrate a correlation between aberrant neural processing of food intake/eating and clear, appetitive behavioural differences in those becoming HO. They do however demonstrate differential BOLD signal in food reward-related brain regions in those remaining HWS compared to those becoming HO, with lower activation in the lingual gyrus and posterior insula.

Future directions

Study of a larger, more homogenous patient group, preferably prospectively from the time of diagnosis of hypothalamic damage and repeated after one-, 5- and 10-year intervals, when obesity has developed or weight-stability has become evident, would further enhance and add to the findings in both the neuroimaging and eating behaviour aspects of this study. Large studies would necessitate a national/international collaborative approach, given the rarity of this condition, with consistent approaches to initial surgical management, testing and supplementation of pituitary hormone deficiencies, dietetic approaches and treatment of those in whom obesity and other cardiovascular risk-factors developed. Multi-centre fMRI studies are becoming more widely undertaken and have certain advantages, such as the ability to recruit more participants (especially in rare clinical conditions such as patients with acquired, structural hypothalamic damage) and to include a more varied and therefore representative sample from the general population, but do require careful planning in order to ensure consistency of data [321-323]. It is important to minimise variability between sites involved in multi-centre fMRI studies as much as possible by considering factors such as participant recruitment, cognitive paradigms

(including factors such as the presentation of visual cues, resting between scanning sessions and administration of questionnaires), participant population (homogeneity of the group vs. greater variability to allow the findings to be generalised to the more general population with hypothalamic damage and the need for a larger sample size where intra-group variability is increased) and scanner software and hardware (including scanner manufacturer and strength, type of head coil and the use of standard vs. special sequences) [321, 323]. Reassuringly, studies have shown that the use of different fMRI scanners in different centres and from different manufacturers might cause fewer inconsistencies than was feared [321, 323] although field inhomogeneities can exist even when the same make and model of scanner are used in different centres, leading to variances in data if not stringently addressed [322]. Consistent training of the staff involved in undertaking the study at each site is also important [323] as is statistical analysis. The involvement of different sites can be included as a covariate in statistical models, but consistent pre-processing and first-level analysis is also essential [321].

Power calculations were undertaken using the online programme https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html, based on a primary outcome of detecting a decrease in insula activation in the posterior insula of the HWS group compared to that of HO, studying only these two groups. Using the measured value from the study in this thesis of the mean BOLD signal in the posterior insula of the HO group (0.28) and pooled SD of 0.4 whilst viewing high-calorie food photographs compared to objects, a further study would require 129 participants per group to detect a 50% change in BOLD signal, at α=0.05 with 80% power and 33 participants per group to detect a 100% change. Secondary outcomes of lingual gyrus, NA and amygdala differences could also be studied, although these would have to be considered exploratory outcomes.

Whilst the study characterising the microstructure of eating behaviour (Chapter 6) found no differences in cumulative change in eating rate at the UEM in those with HO compared to HWS or simple OC using repeated-measures ANOVA, visual ascription showed some evidence of an unusual eating pattern in those with HO. This may have been statistically significant in an appropriately powered trial and therefore use of this technique is warranted in further study of a larger group of participants. The same is true for the other tools examining aspects of eating

behaviour, as although there was no significant difference between HO and HWS, it is possible that this may reflect the size of the study groups (which were powered to detect differences in cerebral activation on fMRI) and by studying larger groups differences may become evident. Given the temporal pattern of weight gain commonly observed in patients with HO (initially rapid, followed by a slower rate of weight gain thereafter), changes in eating behaviour may occur over time, therefore further studies of food intake and eating behaviour may be better undertaken during this critical early phase of rapid weight gain.

Power calculations were also undertaken for a further UEM study (https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html). Using the measured value from the study in this thesis of a mean intake of 430g in the HO group and pooled SD of 177 whilst eating until sated at the UEM, a further study would require 30 participants per group to detect a 30% difference in food intake between HO and HWS, at α=0.05 with 80% power. Using the measured mean overall eating rate in the HO group of 62.4g/second and pooled SD of 21.7 whilst eating until sated at the UEM, 22 participants would be required per group to detect a 30% difference in overall eating rate between HO and HWS, at α=0.05 with 80% power.

Finally, if further studies were to include the TFEQ, a total of 21 participants per group would be required to detect a 50% difference in restraint between HO and HWS based on a mean (pooled SD) of 13.0(7.5), 142 per group for a 50% change in disinhibition (mean in HO of 4.0, pooled SD 6) and 304 per group for a 50% change in hunger (mean in HO of 2.5, pooled SD 5.5) at α=0.05 with 80% power.

Another interesting avenue would be to study the effects of bariatric surgery or GLP-1 receptor agonists in the HO population, with fMRI and UEM studies before and after these treatments. A recent study of GLP-1 receptor agonists in simple obese individuals with and without type 2 diabetes showed a reduction in both their food intake and in previously increased insula and amygdala activation compared to lean controls when viewing food photographs [324]. Following bariatric surgery, studies of individuals with simple, nutritional obesity found lower motivation to eat and increased restraint [325, 326] as well as lower cerebral activation to food vs. non-food photographs in regions associated with food reward/hedonic responses and motivation to eat, including the insula [325, 327] and

lingual gyrus [326]. A difference also existed between types of bariatric procedure [327]. It would be particularly interesting to see whether the same insula and/or lingual gyrus reductions are seen in participants with HO undergoing bariatric procedures and whether this results in comparable insula/lingual gyral activation to that seen in HWS.

Chapter 8

Publications and abstracts

Peer Reviewed Publications

Work from chapters 3, 4 and 5 have been published. Full references for the relevant articles are shown.

Chapter 3: Hypothalamic obesity: prevalence, associations and longitudinal trends in weight in a specialist adult neuroendocrine clinic. ***Steele CA***, Cuthbertson DJ, MacFarlane IA, Javadpour M, Das KS, Gilkes C, Wilding JP, Daousi C. European Journal of Endocrinology. 2013 168(4):501-7.

Chapter 4: Pituitary adenomas in childhood, adolescence and young adulthood: presentation, management, endocrine and metabolic outcomes. ***Steele CA***, MacFarlane IA, Blair J, Cuthbertson DJ, Didi M, Mallucci C, Javadpour M, Daousi C. European Journal of Endocrinology. 2010 163(4):515-22.

Chapter 5: Cerebral Activations During Viewing of Food Stimuli in Adult Patients with Acquired Structural Hypothalamic Damage: a Functional Neuroimaging Study. ***Steele CA,*** Powell JL, Kemp GJ, Halford JCG, Wilding JPW, Harrold JA, Das KSV, Cuthbertson DJ, Cross AA, Javadpour M, MacFarlane IA, Stancak AA and Daousi C. Int J Obes (Lond). 2015 39(9):1376-82

Abstracts

1. Exploring the Pathogenesis of Hypothalamic Obesity: the Interaction of Hormonal, Neuronal and Psychological Factors. *Poster presentation.* European Society for Paediatric Endocrinology (ESPE). September 2014.
2. Importance of Brain Reward Circuitry in Adult Patients with Acquired Structural Hypothalamic Damage: A Functional Neuroimaging Study. *Poster presentation.* ENDO 2012: Endocrine Society's 94th Annual Meeting & Expo. June 2012.
3. Prolactinomas presenting in children and young people: a single service experience. Society for British Neurological Surgeons. *Oral presentation.* September 2010.
4. The prevalence and severity of hypothalamic obesity following acquired structural hypothalamic damage, in adult patients attending a single UK neuroendocrine service. *Oral presentation* September 2010.
5. Obesity and cardiovascular risk factors in adult patients with acquired structural hypothalamic damage. Society for Endocrinology BES 2010 Meeting. P*oster presentation.* March 2010. Abstract published in Endocrine Abstracts. March 2010, Vol 21. P272.
6. Pituitary adenomas presenting in children and adolescents: a single service experience. 37th meeting of the British Society for Paediatric Endocrinology and Diabetes. *Oral presentation.* November 2009.
8. Pituitary adenomas presenting in children and young adults: a single centre experience. *Poster presentation.* ESPE/LWPES 8th Joint Meeting on Pediatric Endocrinology. September 2009.
10. A clinical and functional neuro-imaging study of the mechanisms underlying abnormal eating in patients with hypothalamic damage. Rank Prize Fund Appetite meeting. *Oral presentation.* July 2009.

Appendix 1

Tables from Chapter 1 (Introduction)

Table 1.2. Prevalence of obesity/weight gain in studies with mixed pathogenesis

First Author	Age (range) at diagnosis	Number of patients	Definition of weight gain/obesity	Follow-up (range)	Initially obese (%)	Obese at latest follow-up (%)
Daousi [6]	44 (17 – 78) years	52 (42 with follow-up); 24 PA, 22 CP, 6 hypothalamic tumour	BMI ≥ 30 kg/m^2 and increase ≥ 2 kg/m^2 since diagnosis	Median 5 (1 – 19) years	10/42 (24) Overweight 28/42 (67)	Obese: 22/42 (52) Overweight: 37/42 (88) BMI ≥ 35 kg/m^2: 10/42 (24)
Adachi [20]	0-15 years (at surgery)	23; 10 CP, 7 germinoma, 4 optic nerve glioma, 2 other	> 20% overweight or body fat > 25% (M), > 30% (F < 11 years), > 35% (F > 11)	2-13 years	-	12/23 (52%)
Rakhshani [81]	7.6 (2.2–15.9) years	39; 33 CP, 3 germinoma, 1 lipoma, 1 hamartoma, 1 glioma	Report BMI, BMI percentile and BMI SDS score	0.3–3.4 years	Median BMI SDS 1.48 (−0.6 to 3.6)	BMI >95th% 27 (69), 85th–95th% 6 (15), <85th% 6 (15) Median BMI SD 2.0 (−0.2 to 2.6)
Lek [21]	Median (IQR) 7.49 (3.47–11.59) years	46; 11 pilocytic astrocytoma, 10 other glioma, 13 CP, 4 prolactinoma, 8 other	IOTF age- and sex-adjusted BMI centile chart, intersects with BMI 30kg/m^2 at 18	Median 3.93 (1.7–7.3) years	3/46 (6%) Median BMI SDS 0.73 (0.03 to 1.40)	20/46 (43%) at last follow-up Median (IQR) BMI SDS 1.85 (1.11–2.72)
Crowley [19]	Adult	188 70 CP, 89 NFPA, 29 post- TBI	Obesity: BMI >30 kg/m^2 Overweight: BMI 25– 29.9 kg/m^2	Median 8 years (1-50) for CP	-	**CP** 66% obese, 26% overweight **NFPA** 47% obese **Post-TBI** 31% obese

PA = pituitary adenoma, CP = craniopharyngioma, M = male, F = female, NFPA = non-functioning pituitary adenoma

Table 1.3. Prevalence of obesity/weight gain in craniopharyngioma studies

First Author	Age (range) at diagnosis, years	Number patients	Definition of weight gain/obesity	Follow-up (range), yrs	Initially obese (%)	Obese at latest follow-up (%)
Duff [22]	32 ≤ 16 years, 89 >16 years	98	Morbid obesity: weight ≥ 95th %	Mean 10 (1-20)	10/92 (10.2)	35/92 (35.7)
Stahnke [62]	Mean±SD 8.4± 3.3	28	kg above normal weight for height	-	2.0kg ± SD 3.57 (12%)	Grp 1: +3.78 ± SD 1.39kg Grp 2: +8.38 ± 2.23kg
Hoffman [328]	1.8 – 17.6	50	-	1-14, 39% at least 5 years	18 at presentation, 30 pre-operatively	52
Pinto [29]	7.7 (2.8–14.5) at surgery	17	Overweight: BMI >2 SDS	≤2	Overweight 5/17 (30%) BMI SD 1.1±0.4	13/17 overweight (77%). BMI SD 4.6±1.5 at 2 years (9 patients)
Kalapurakal [329]	Median 6 (1–15)	25	-	Median 10 (3–16)	-	8/25 (32%)
Poretti [330]	Mean 9.2 (2.8-15.9)	25	Obesity: BMI > 97th centile	11.3 (SD 7.6)	3/25 (12)	14/23 (61)
Srinivasan [57]	Mean±SD 12.2 ±3.7	15	Overweight: BMI 85th-95th%, obese: BMI > 95th%	Median 5.1 (1.8–10.7)	-	Obese 8 (53), overweight 3 (20)
Caldarelli [331]	Mean 9 (1.7 - 15.8)	52	-	Median 9	3/52 (5.8%)	4/10 (40%)
Kendall-Taylor [25]	AO mean 34.3 (±12.0 SD) CO mean 11.8 (±4.2 SD)	241 AO; 152 CO	Obesity: BMI ≥30.0 for males and BMI ≥28.6 for females	-	-	CO 39.1%; females 42.9%; males 36%. AO 51.8%; females 56.9%; males 47%

First Author	Age (range) at diagnosis, years	Number patients	Definition of weight gain/obesity	Follow-up (range), yrs	Initially obese (%)	Obese at latest follow-up (%)
Pereira [72]	Median 29.	54	BMI > 30kg⁄m^2	49 (6–76)		21 (40%)
Pierre-Kahn [332]	-	14	BMI > 2 SD	-	42.8% obese BMI SD 0.2-9.5, mean 2.0	85.7% obese After 1yr mean BMI SD 6.3
Tomita [333]	Median 8.2 (0.92-16)	54	-	1 - 21	3/54 obese (5.6)	Obesity 15/54 (27.8) Severe obesity 5/54
Zuccaro [334]	Mean 10.5 (0.04 - 21)	153	-	1-16	22%	35%
Sainte-Rose [32]	Mean 7.4	66	Hypothalamic involvement on MRI	Mean 7	Mean BMI SD 1.15	Morbid obesity 18% BMI correlated with degree hypothalamic damage
Ahmet [33]	Mean age: 8.5 (2.8-16.1)	43	BMI ≥ 2 SD	4.7 (0.3-10)	8 (19) 7/8 had hydrocephalus/ hypothalamic damage	25 (58), obesity associated with post-op hypothalamic damage (MRI)
Balde [335]	44.7 (21-74)	35	-	7.4 (0.1-19.1)	-	63% weight-increase, average 17.5 ± 14.7 kg
Vinchon [34]	2.2 – 16.2	45	BMI > 2SD with hypothalamic involvement	Mean 11	7 (15.6)	31 obese (70.5), 11 morbidly obese (25) Mean BMI 30.3
Hamilton [121]	<19	46	BMI ≥ 95th%	-	-	50

First Author	Age (range) at diagnosis, years	Number patients	Definition of weight gain/obesity	Follow-up (range), yrs	Initially obese (%)	Obese at latest follow-up (%)
Muller [117]	Median 10 (1.2 – 18)	120	Measuring change in BMI SDS (Δ)	3 years	-	No lesion: BMI SDS Δ+0.45 Anterior only: BMI SDS Δ+0.74. Anterior and posterior: BMI SDS Δ+3.22
Gautier [24]	Median (IQR) <10: 5.5 (4-6) 10-18: 12.5 (11-15) AO: 38 (28-52)	171; 33 < 10 yrs, 32 10-18 yrs, 106 AO	-	-	-	BMI> 30 (%) <10 years: 19 (67.9) 10-18 years: 8 (24.2) >18 years: 39 (41.5)
Cohen [118]	Mean 10.7 (2–17.2)	33	Obesity: BMI SD >2. Severe obesity: BMI SD>4	Mean FU 4.0 (0.7–9.3)	18-21%	52-65% (last decade 52%)
Roth [336]	<21	10	BMI z -score >2	Minimum 2	-	5 (50)

% = percentile, CO = childhood-onset, AO = adult-onset,

Table 1.5. Studies of ghrelin, PYY and HO.

Number	**Age at study**	**BMI/BMI SDS**	**Fasting ghrelin**	**Fasting glucose**	**Fasting insulin**	**HOMA-IR**	**Fasting PYY**
Kanumakala [47]	*Median (years)*	*Median (IQR)*	*Median (IQR) pg/mL*	*Median mmol/L*	*Median pmol/L*	*Median*	
16 HO	11.9	5.86 (2.28) 6.59	1345 (437)	4.35	89.7	2.65	-
16 SO	13.8	(3.63)	1399 (354)	4.70	114.8	3.41	-
16NWCo	13.9	0.63 (0.85)	1759 (602)	4.70	32.8	0.95	-
p-value	-	-	0.01^{a}, 0.88^{b}	0.01^{a}, 0.005^{c}	$<0.001^{a}$, 0.78^{b}	$<0.001^{a}$, 0.96^{b}	
Goldstone [39]	*Mean ± SEM (years)*	*Mean ± SEM*	*Mean ± SEM pmol/L*	*Mean ± SEM mmol/L*	*Mean ± SEM pmol/L*	*Mean ± SEM*	*Mean ± SEM pmol/L*
9 HO	$48 \pm 5^{d,e}$	33.1±1.6	471 ± 76^{e}	4.9 ±0.1	$111.2 \pm 16.8^{d,e}$	$3.21 \pm 0.51^{d,e}$	22.5±1.9
15 NOC	31 ± 2^{f}	$23.5 \pm 0.8^{e,g}$	807 ± 82^{g}	4.8 ±0.1	$46.8 \pm 6.9^{f,g}$	$1.35 \pm 0.21^{f,g}$	20.6±1.6
26 PWS	28 ± 1^{f}	32.0 ± 2.0^{d}	$1009 \pm 104^{f,g}$	4.8 ± 0.1	$46.4 \pm 7.5^{f,g}$	$1.41 \pm 0.22^{f,g}$	23.0±1.7
16 SO	38 ± 3	38.9 ± 1.9^{d}	$462 \pm 55^{d,e}$	5.1 ± 0.1	$105.2 \pm 13.1^{d,e}$	$3.26 \pm 0.49^{d,e}$	23.8±1.8
Part 2							
10 PWS	28 ± 2^{f}	31.7 ± 1.6^{d}	979 ± 173	4.4 ± 0.1	$38 \pm 5^{f,g}$	1.02 ± 0.14^{g}	-
8 NOC	28 ± 1^{f}	$22.0 \pm 1.0^{e,f,g}$	$742 \pm 62^{f,g}$	4.7 ± 0.1	$46 \pm 9^{f,g}$	1.27 ± 0.25	-
9 SO	34 ± 3	37.3 ± 2.4^{d}	414 ± 38^{e}	4.8 ± 0.2	$97 \pm 13^{d,e}$	2.79 ± 0.37^{e}	-
6 HO	$43 \pm 5^{d,e}$	34.1 ± 2.3^{d}	430 ± 71^{e}	4.8 ± 0.2	$138 \pm 15^{d,e}$	$3.94 \pm 0.46^{d,e}$	-

Number studied	**Age at study (years)**	**BMI/BMI SDS**	**Fasting ghrelin**	**Fasting glucose**	**Fasting insulin per kg fat mass**	**HOMA-IR**	**Fasting PYY**
Daousi [2]	*Median (range)*	*Mean ± SEM (kg/m^2)*	*Median (IQR) pmol/L*	*Median (IQR) mmol/L*		*Median (IQR)*	Median (IQR) pmol/L
14 HO	48.4 (29-75)	37.8 ± 1.5	432.8 (306-569)	5.6 (4.8–6.2)	-	1.6 (1.3-3.7)	29.7 (22.4-36.4)
15 SO	48.7 (23-64)	37.5 ± 1.7	564.7 (383-825)	5.3 (5.1–5.8)	-	1.4 (0.9-2.0)	23.7 (16.9-28.1)
p-value	0.9[h]	0.9[h]	0.03[i]			0.1[h]	0.06[j]
Holmer [3]	*Median (range)*	*Median (kg/m^2)*	*Median (range) pg/mL*	*Median (range) mmol/L*	*Median (range) mIU/L/kg*		
42 CP	F 28 (18–57)	30 (19-41)	1013 (628-2532)	-	0.19 (0.05-0.59)	-	-
	M 29 (17–57)					-	-
42 controls	-	23 (18-36)	1344 (341-3455)	-	0.21 (0.05-0.33)	-	-
p-value	-	<0.001[k]	0.008[l]	-	>0.5[m]	-	-
With TVE	-	32 (21–41)	970 (328-1785)	-	0.21 (0.05-0.59)	-	-
Without TVE	-	25 (19–35)	1197 (764-2532)	-	0.15 (0.06-0.35)	-	-
p-value	-	0.001[n]	0.008[o]	-	0.05[p]	-	-

SO – simple obese; NWCo – normal-weight controls; HO – hypothalamic obese; NOC – non-obese controls; PWS – Prader-Willi syndrome; CP – craniopharyngioma. IQR – interquartile range; SDS – standard deviation score; SEM – standard error of the mean; AUC – area under the curve; HOMA-IR - homeostatic model assessment-insulin resistance. a: significant differences between the groups, b: no significant difference between HO and SO, c: statistically significantly lower fasting glucose in HO than in SO, d: p<0.001 vs. NOC, e: p<0.001 vs. PWS, f: p<0.001 vs. HO, g: p<0.001 vs. SO, h: no significant difference between HO and SO, i: significantly lower in HO vs. SO, j: significantly lower in SO vs.

(Continues over)

HO, k: significantly higher BMI in CP vs. controls, l: significantly lower ghrelin in CP vs. controls, m: no significant difference between CP and controls, n: significantly higher BMI in those with TVE than those without, o: significantly lower ghrelin in those with TVE than those without, p: borderline significantly higher fasting insulin per kg fat mass in those with TVE than those without.

Table 1.6. Studies of leptin and HO.

Number	Age at study, years	BMI SDS/ BMI	Leptin:BMI ratio	Free leptin index	Leptin	Leptin/kg fat mass
Holmer [3]	*Median*	*Median BMI (IQR) kg/m^2*			*Median (range) ng/mL*	*Median (range) ng/mL·kg*
42 CP	F 28, M 29	30 (19-41)			25 (3-215)	0.8 (0.2 - 4.5)
42 controls	-	23 (18-36)	-	-	8 (2-37)	0.4 (0.1-1.4)
p-value		<0.001[a]			<0.001[b]	<0.001[c]
With TVE	-	32 (21–41)			28 (4-215)	0.7 (0.3-4.5)
Without TVE	-	25 (19–35)	-	-	16 (3-67)	1.0 (0.2-1.6) 0.05[f]
p-value		0.001[d]			0.07[e]	
Guran [51]	*Median (IQR)*	*Median BMI SDS (IQR)*	*Median (IQR)*	*Median (IQR)*	*Median (IQR) ng/mL*	
23 HO	10.3 (8–14.6)	2.0 (1.5-2.1)	4.0 (1.6-5.2)	2.0 (0.8-3.5)	89.2 (41–144)	
16 HNO	11.4 (8.9–14.1)	0.18 (-0.5-0.56)	1.5 (0.8-3.1)	0.6 (0.3-1.2)	25.3 (13–53)	-
22 SO	10.8 (8.9–12.8)	2.1 (1.8-2.3)	2.5 (1.8-3.5)	1.5 (1-2.3)	66 (48–90)	
p-value	NS[g]	<0.001[h]	<0.05[i]	<0.05[i]	<0.05[i]	
Patel [53]		*Mean (SD) BMI SDS*			*Median (IQR) ng/mL*	*Median leptin/BMI (IQR) mcg/L:kg/m^2*
BMI < 2 SDS:						
17 patients	14.6 (10.9-17.2)	0.9 (0.9)			9.2 (4.4-17.6)	0.42 (0.27-0.72)
57 controls	13.4 (11.6-15.3)	0.4 (0.7)			4.5 (2.3-9.4)	0.23 (0.13-0.45)
p-value	0.3[j]	0.02[k]			-	0.02[k]
BMI ≥ 2 SDS:						
17 patients	13.8 (9.2-15.6)	2.6 (0.6)			26.9 (18.4-47.6)	1.08 (0.72-1.57)
28 controls	12.6 (9.6-13.7)	3.1 (0.7)			26.8 (16-53.4)	0.95 (0.63-1.35)
p-value	0.1[j]	0.03[l]			-	0.6[j]

HNO – hypothalamic non-obese; TVE – third ventricular extension. IQR – interquartile range; SDS – standard deviation score. (Continues over)

a: significantly higher BMI in CP vs. controls, b: significantly higher leptin in CP vs. controls, c: significantly higher leptin/kg fat mass in CP vs. controls, d: significantly higher BMI in those with TVE than without, e: significantly higher leptin in those with TVE than without, f: significantly lower leptin/kg fat mass in those with TVE than without, g: no significant differences between the groups, h: significantly lower leptin in HNO vs. HO and SO, i: significantly higher in HO and SO vs. HNO, j: no significant difference between patients and controls, k: significantly greater in patients than controls, l: significantly lower in patients than controls.

Table 1.7. Fasting leptin, adiponectin and resistin concentrations (adjusted for fat mass) in patients with HO, CH and SO controls [52]

	Mean leptin concentration (pg/mL)	**Mean adiponectin concentration (ng/mL)**	**Mean resistin concentration (pg/mL)**	**Number (%) with fasting insulin >132 pmol/L**	**Number (%) with HOMA-IR > 2.5**
HO	3420	277	7560	7/28 (26)	6/28 (22)
CH	2795	219	4590	5/18 (28)	5/18 (27)
SO	2240	225	4260	8/23 (35)	8/23 (34)
p	$< 0.01^{a}$	$< 0.05^{b}$	$<0.01^{a,c}$, $< 0.05^{a,d}$	$= 0.680^{e}$	

HOMA-IR > 2.5 (adult cut-off for insulin resistance). All biochemical parameters were adjusted for fat-mass; adiponectin and resistin also adjusted for gender and age; fasting insulin and HOMA-IR also adjusted for gender, age and pubertal status. a: significant difference between all three groups, b: significant difference between HO and both CH and SO, c: significant difference between HO and CH, d: significant difference between HO and SO, e: no significant difference between the groups

Table 1.8. Studies of insulin and HO.

Number	**BMI (kg/m^2)**	**BMI SDS**	**Fasting insulin**	**Peak insulin**	**AUC insulin**	**HOMA-IR**	**Insulin sensitivity**	
Simoneau-Roy [54]	*Mean ± SD*	*Mean ± SD*	*Mean ± SD pmol/L*	*Mean ± SD pmol/L*	*Mean ± SD pmol/L*	-	*WBISI Mean*	*IS Mean*
15 CP	35.2 ±8.0	2.2 ±14.6	242.2 ± 160	4456 ± 2084	4443 ±2750	-	5 ± 5	0.96± 0.3
15 SO	33.5 ± 4.9	2.1 ± 0.3	161.3 ±84.3	3028 ± 1335	2534 ± 1113	-	7 ± 6	1.7±0.7
p-value	0.49[a]	0.53[a]	0.11[a]	0.03[a]	0.03[a]		0.11[a]	0.01[a]
Roth [4]	*Median (IQR)*	*Mean ± SEM*	*Median (IQR) mU/L*	-	-	*Median (IQR)*	*QUICKI*	
15 CrO	32.9 (30.2 - 36.8)[b]	2.9 (2.5 to 3.4)[b]	28 (12-58)[c,d]	-	-	6.0 (2.9-11.1) [c,d]	0.29 (0.27-0.32) [c,d]	
12 CrNW	22.8 (20.1 - 26.6)[b]	1.3 (0.8 to	10.5 (6.7-21.5)[e]			2.4 (1.3 - 6.3)[e,g]	0.34 (0.30 - 0.36)[e]	
15 SO	31.8 (29.5 - 35.3)[b]	1.8)[b]2.6 (2.4 to	15.2 (7.9-23.3)[d,f]			3.6 (1.9 - 5.4)[g]	0.32 (0.3 - 0.35)[f]	
12 NOC	20.2 (16.8 - 24.2)[b]	2.8)[b]	7.4 (2.5-11.5)[c,e,f]			1.83 (0.6 - 2.4)[e]	0.35 (0.33 - 0.42)[e]	
		1.0 (−0.1 to1.7)[b]						
Goldstone [39]	*Mean ± SEM*	-	*Mean ± SEM pmol/L*	*Mean ± SEM pmol/L*	*Mean ± SEM min·nmol/L*	*Mean ± SEM pmol/L*	-	
10 PWS	31.7 ± 1.6[h]	-	38 ± 5[k,l]	498 ± 77	49.3 ± 5.8[k]	306 ± 38[k]	-	
8 NOC	22.0 ± 1.0[h,i,j]		46 ± 9[i,j]	415 ± 96[j]	37.4 ± 5.7[j]	222± 35[j]		
9 SO	37.3 ± 2.4[i]		97 ± 13[j,l]	583 ± 64	68.2 ± 8.0	431 ± 53		
6 HO	34.1 ± 2.3[j]		138 ± 15[j,k]	1016 ± 133[j]	113.2±13.7[j,k]	733 ± 83 [j,k]		

Number	BMI (kg/m^2)	BMI SDS	Fasting insulin	Peak insulin	AUC insulin	HOMA-IR	Insulin sensitivity
Lustig 1999 [56]	Mean ± SEM	-	Mean ± SEM μU/mL	Mean ± SEM μU/mL	-	-	-
8 HO	36.0 ± 2.5	-	23 ± 5	281 ± 47[m]	-	-	-
NOC	Not stated		-	92			
Lustig 2003 [55]	Mean ± SEM	-	Mean ± SEM μU/mL	Mean ± SEM μU/mL	-	-	-
9 on Octreotide	37.4 ± 2.5[m]	-	29.2 ± 4.9[m]	205.2 ± 22.2[m]	-	-	-
9 on placebo	36.8 ± 1.2[m]		36.9 ± 6.8[m]	308.8 ± 49.3[m]			
Srinivasan [57]	-	Mean (± SD)	Median (range) pmol/L	-	-	-	Min/mUL/min
9 patients	-	2.24 ± 1.0	91 (30-192)	-	-	-	0.95 (0.43-2.48)
9 controls		2.59 ± 1.3	82 (51-208)				1.17 (0.39-1.74)
p-value		0.26[n]	0.68[n]				0.86[n]

CP – craniopharyngioma; SO – simple obese; CrO – craniopharyngioma with obesity; CrNW - craniopharyngioma normal weight; NOC – non-obese controls; PWS – Prader-Willi syndrome; HO – hypothalamic obese; CH – congenital hypopituitarism; HNO – hypothalamic non-obese. IQR – interquartile range; SD – standard deviation; SEM – standard error of the mean; AUC – area under the curve; HOMA-IR - homeostatic model assessment-insulin resistance; QUICKI - quantitative insulin sensitivity check index. a: difference between patients with CP and SO, b: no significant difference between NOC and CrNW or SO and CrO, but significant difference between lean and obese groups, c: $p<0.01$ in CrO vs. NOC, d: $p<0.05$ in CrO vs. SO, e: no significant difference between CrNW and NOC, f: $p<0.05$ in SO vs. NOC, g: $p<0.05$ in SO vs. CrNW, h: $p<0.001$ in PWS vs. NOC, i: $p<0.001$ in NOC vs. SO, j: $p<0.001$ in NOC vs. HO, k: $p<0.001$ in PWS vs. HO, l; $p<0.001$ in PWS vs. SO, m: no statistical comparison undertaken, n: no significant difference between patients and controls

Table 1.9. Holmer et al [3] Insulin Table

	Number studied	BMI (kg/m^2) Median (range)	Fasting insulin Median (range) mIU/L	Insulin/kg fat mass Median (range)	Fat mass Median (range)	Fat-free mass Median (range)
Patients	42	30 (19-41)	6 (1-38)	0.19 (0.05-0.59)	32 (9-64)	55 (40-86)
Controls	42	23 (18-36)	3 (1-13)	0.21 (0.05-0.33)	16 (8-46)	55 (37-73)
p value	-	<0.001[a]	<0.001[a]	>0.5[b]	<0.001[a]	0.01[a]
Patients with TVE	25	32 (21-41)	7 (2-38)	0.21 (0.05-0.59)	40 (9-64)	57 (41-86)
Patients without TVE	17	25 (19-35)	4 (1-8)	0.15 (0.06-0.35)	20 (12-56)	51 (40-68)
p value	-	0.001[c]	<0.001[c]	0.05[d]	0.003[c]	0.02[c]

TVE = third ventricular extension. a: significantly greater in patients vs. controls, b: no significant difference between patients and controls, c: significantly greater in patients with TVE than those without, d: no significant difference between patients with TVE than those without

Table 1.10. Studies of octreotide [56].

Study type	**Open-label Octreotide (max 15 mcg/kg/day)** [56]	**Double-blind placebo-controlled (octreotide max 15 mcg/kg/day)** [55]
Participants	9 pre-pubertal children (4 M, 5 F; aged 10 - 18 years) with HO secondary to leukaemia (2 patients) or brain tumours where hypothalamic damage expected (7) and weight gain > 2 SDS. ≥2 years post-tumour diagnosis	20 children (11 M, 9 F; age 14.2 ± 0.7 year) with HO (weight gain more than + 2 SDS) secondary to intracranial tumour (13 CP, 4 hypothalamic astrocytoma, one pineal germinoma) or cranial irradiation for ALL (2 patients). ≥1 year post-tumour diagnosis
Before treatment	Mean weight gain previous 6 months: 6.0 ± 0.7 kg (range 4.1-9.2) Mean increase in BMI +2.1 ± 0.3 kg/m^2 (range 1.0-3.2)	Mean BMI (± SEM) 36.3 ± 1.3 kg/m^2 Annualized weight gain 17.1 ± 3.0 kg/year
Latest BMI		9 octreotide treated: Δ - 0.2 ± 0.2 (range - 0.7 to + 0.9) kg/m^2 9 placebo treated: Δ + 2.2 ± 0.5(range + 0.1 to + 4.4) kg/m^2
Follow up	1 patient 5 months, 7 patients 6 months	6 months
Side effects	7 had self-resolving abdominal symptoms within the first month; 4 had gallstones/gallbladder sludging (self-resolved by 6 months after discontinuation of octreotide)	Octreotide-treated: all had self-resolving abdominal symptoms, gallstones or biliary sludging (4 participants, treated with ursodeoxycholic acid at 6 months) which resolved by 12 months despite continuation of octreotide. 1 had poor compliance. 3 placebo-treated patients suffered diarrhoea

Study	Open-label Octreotide (max 15 mcg/kg/day)	Double-blind placebo-controlled
Treatment prematurely stopped	1 participant discontinued octreotide within first month due to severe peripheral oedema, 1 was non-compliant, 1 dropped out after 5 months due to failure to lose weight	2 additional patients were excluded (1 octreotide-treated, 1 placebo-treated) for craniopharyngioma recurrence and hyperosmolar non-ketotic coma respectively
Biochemical assessment	All had lower IGF-1 concentrations after six months of octreotide; five with measureable GH on stimulation testing at baseline had lower peak GH	IGF-1 essentially unchanged.
Outcomes	Mean weight loss 4.8 ± 1.8 kg at study end (range-12.8 to +1.8kg, p=0.0003 on paired *t* test); mean change in BMI -2.0 ± 0.7 (range -4.7 to +0.8, p=0.0004 on paired *t* test) kg/m^2	Ongoing weight gain on placebo (mean weight gain + 9.2 ± 1.7 kg, range 3.8 to 19.8; mean BMI increase + 2.2 ± 0.5 kg/m^2, range 0.1 to 4.4) compared to weight stability on octreotide (mean weight increase 1.6 ± 0.6 kg, range -0.9 to 5.3, $p<0.001$; mean change in BMI -0.2 ± 0.2 kg/m^2, range -0.7 to 0.9, $p<0.001$). Weight loss in months 4-6 of octreotide treatment. Parent-reported improvement in QoL in physical functioning in those on octreotide therapy compared to controls ($p = 0.03$), however child-reported QoL remained unaltered

Table 1.11. Studies of energy expenditure (EE) and energy intake (EI).

Study and number	**BMI SDS**	**BMR**	**Adjusted BMR**	**Activity**	**Energy intake**
Harz [43]	*Mean SD ±SEM*	-	-	Movement (cpm)	
9 CP home	2.9±3.0	-	-	228	
11 controls home	2.7±2.7			282	
p-value	-			0.08[a]	
10 CP hospital	4.2±2.1	-	-	228	
15 controls hospital	5.2±1.9			298	
p-value	-			0.01[a]	
Shaikh [65]	*Median SD*	*Mean±SE kcal/day*	*Mean kcal/day*	Total active minutes *Median(range)*	*Mean±SE kcal/day*
18 HO	2.74	1667 ± 108	1619	4,607 (863-6,027)	1980 ± 204
13 CH	2.44	1535 ± 94	1789	4,227 (2,594-5,003)	1569 ± 103
16 SO	3.21	2150 ± 110	2028	4,560 (2,887-5,013)	1782 ± 141
p-value	NS[b]	p<0.01[c]	p<0.001[d]	NS[b]	NS[b]
Holmer [3]	**BMI (kg/m^2)**	Median % of expected			Median (range)
42 CP	F: 31 (19-41) M: 28 (21-41)	85 (38-126)	-	-	1778 (970-3230)
42 controls	F: 21 (18-36) M: 24 (20-34)	90 (78-101)	-	-	2094 (1128-5449)
p-value	-	0.003	-	-	0.003

CP = craniopharyngioma, cpm = counts per minute, BMR = basal metabolic rate. a: significantly lower in CP than controls, b: no significant differences between the groups, c: significantly lower in HO and CH vs. SO, d: significantly lower in HO vs. SO, e: significantly lower in CP than controls.

Table 1.12 Mortality rates

Study	Number studied	Lesion	Deaths	SMR (95% CI)	SMR (95% CI) in selected patients	Median follow-up (range)	Cause of death
Bulow [71]	60 children and adults	CP	27 (45%)	5.55 (3.68-8.22)	F 11.4 (4.9-22.5) M 4.79 (2.9-7.8)	12.5 years (1 day to 40 years)	
Pereira [72]	54 children and adults	CP	10 (18.5%), 7 female.	2.88 (1.35-4.99)	F 3.8 (1.5-7.2) M 1.8 (0.3-4.6)	10 years (0.5-37)	3 infection/sepsis, 1 Addisonian crisis, 2 carcinoma, 4 CV events
Crowley [19]	70 children and adults	CP	21 deaths (30%)	8.75 (5.4-13.3)	F 10.5 (5.0-19.3) M 7.6 (3.8-13.5)	8 years (1-50)	3 CP, 4 other tumours, 3 CV, 2 CVD, 5 respiratory, 4 other causes
Tomlinson [73]	1014	NFPA 57% CP 12% PRLma 9% Idiopathic HP 9%		Operated: 1.86. Non-operated: 1.23	RTx 2.1, without RTx 1.5 CP 8.7, other 1.5 Untreated Gn deficiency 2.7, treated 1.3		CP patients: CVD SMR 19.4, respiratory SMR 22.1 Post-RTx: SMR 4.36 vs. 1.64 without (p=0.001)
Neilsen [74]	5412	HP		1.21-3.80	F weighted 2.8 (2.6-3.0) M weighted 2.1 (1.9-2.2)		
Muller [28]	183 children and adults	CP	10 deaths (5.5%)		**BMI SDS ≥ 7** 20 year survival 0.76 (± 0.15) **BMI SDS 2-7** 20 year survival 0.96 (± 0.03) **BMI SDS < 2**, 20 year survival 0.94 (± 0.04)	10.8 years (2.2-25.9) 2 peri-operative deaths excluded	1 MI, 2 ICH, 1 adrenal insufficiency. 3 “hypothalamic dysregulation”

SMR = standardised mortality rate, CP = craniopharyngioma, NFPA = non-functioning pituitary adenoma, PRLma = prolactinoma, HP = hypopituitarism, CV = cardiovascular, CVD = cerebrovascular events, RR = relative risk, SAH = subarachnoid haemorrhage, ICH = intracerebral haemorrhage, HP = hypopituitarism, RTx = radiotherapy

Table 1.13 Cardiovascular mortality rates

Study	Number of patients	Lesion	SMR or RR (95% CI)	Risks/cause of death
Bulow [77]	344	CP or PA	CVD SMR 3.39 (2.3-4.99) F 4.9 (2.6-8.4) M 2.6 (1.4-4.4) CV SMR 1.75 (1.4-2.19)	Under 55 years: SMR 6.7 (3.4-12.1) Over 55 years: SMR 2.5, 95% CI 1.5-4.3
Brada [78]	334	PA: 31% hormone secreting, 63% non-secreting, 6% unknown	RR death 1.58 (1.3-1.9) Surgically debulked: RR 5.19 (3.5-7.4) compared to biopsy only/ no surgical intervention	Pituitary or hypothalamic disease RR 600 (120-1080) Other brain tumours RR 8.3 (1.0-15.6) CVD RR 4.11 (2.7-5.5) - 33 deaths compared to 8 expected, including 3 SAH

SMR = standardised mortality rate, RR = relative risk, CP = craniopharyngioma, PA = pituitary adenoma, CVD = cerebrovascular events, CV = cardiovascular, SAH = subarachnoid haemorrhage

Table 1.14. Hamilton [121] use of diazoxide and metformin

Study type	**Open-label, pilot study**
Treatment	Diazoxide 2mg/kg/day (max 200 mg/day) and metformin 1g bd
Participants	7 patients (5 M, 2 F; mean age 15.4 years, range 9.2-18.3) with HO secondary to CP, ≥ 1year post-resection with panhypopituitarism. All had received at least 6 months of diet and exercise advice
Pituitary disease	Mean time since diagnosis 5.9 (2.8-8.7) years
BMI before treatment	Mean BMI 35.5 ± 5.6 kg/m^2, mean BMI SDS +2.3 (1.8-2.96)
Latest BMI	Mean ΔBMI: −0.3 ± 2.3 (prev 6 months +2.2 ± 1.5 kg/m2, p = 0.02) Mean ΔBMI SDS: −0.04 ±0.15 (prev 6 months +0.11 ± 0.08, p=0.02)
Follow up	6 months
Side effects	Pedal oedema, vomiting, mildly elevated liver enzymes
Treatment prematurely stopped	9 patients originally recruited, 2 withdrew due to side-effects: 1 had pedal oedema after 2 months treatment; 1 experienced vomiting with mildly elevated liver enzymes
Biochemical assessment	All patients had normal fasting and stimulated glucose levels on OGTT at baseline and latest follow-up. Insulin sensitivity improved from mean±SD 2.2±1.1 to 5.7±8.1. AUC insulin dropped from mean 2078.8 ± 668.8 pmol/L to 1438.3 ±988.5 pmol/L
Outcomes	3 patients lost weight, all gained less weight than previously. Mean BMI± SD changed by -0.6 ± 2.3 kg/m^2 after treatment compared to +2.2 ± 1.5 kg/m^2 before (p = 0.02) and mean BMI SDS by -0.04 ± 0.15 compared to +0.11 ± 0.08 (p = 0.021) the preceding six months. AUC insulin at baseline was significantly correlated with a decrease in BMI (r = 0.096, p = 0.009). There was no correlation for either leptin or adiponectin and no difference in baseline and study-end WBISI, AUC insulin, adiponectin, leptin, liver enzymes or fasting lipids

Table 1.15. Studies of Dextroamphetamine

Study type	**Mason** [41] **Open-label**	**Ismail** [122] **Retrospective case-note review**
Treatment	Dextroamphetamine 12.5-20 mg/day	5 mg dextroamphetamine bd
Participants	5 children (3 M, 2 F; mean age 8.3 years, range 6-9). HO secondary to CP. All had post-operative hypothalamic damage with absent 3rd ventricular floor. All served as their own control	12 patients (7 F, 5 M; median age 15.1 years in F, 19.6 years in M). 9/12 CP. All had surgery (6 total resection); 7 received > 51 Gy radiotherapy. All had ≥ 1 pituitary hormone deficit
Pituitary disease	Mean duration 10.4 months	Median 7.3 years (range 3.3 – 20.5) after tumour diagnosis
BMI before treatment	Mean BMI at study 32 ± 2.8 kg/m^2, mean weight gain 2 ± 0.3 kg/m^2/month	Not reported
Latest BMI	Mean BMI 31±3.3 kg/m^2	Not reported
Follow up	24-months	Median 15 months (6 – 48) in F and 13 months (7 – 63) in M
Side effects	Headache (1 participant), resolved with dose decrease. Cyst enlargement (1 patient) treated with shunt then radiotherapy	Insomnia (1 patient), resolved when evening dose omitted. Treatment discontinued (1 patient) due to tumour recurrence
Outcomes	Weight gain significantly decreased (mean increase 0.4 ± 0.2 kg/month, p = 0.009). Decreased mean BMI (31 ± 3.3 kg/m^2) No difference in calorie intake (mean daily calories pre-treatment 1189 ± 69, post-treatment 1335 ± 137). Increase in physical activity from < 1 hour/day to >2 hours.	Weight stabilisation/loss in 10. Median reduction in SDS: M -0.7 after median 13 months; F -0.44 after 15 months. Concentration and exercise tolerance were improved, with/without improvement in daytime somnolence

Table 1.16. Molloy [123]. Effects of phentermine and *dl*-fenfluramine or fluoxetine

Study type	**Conference abstract**
Treatment	Phentermine with either *dl*-fenfluramine or fluoxetine with behaviour modification
Participants	10 patients. HO (ideal body weight > 130%) secondary to CP, hypothalamic glioma or optic chiasm glioma. No details of age, gender, or duration of pituitary disease. One had pre-morbid obesity
BMI	Weight 165-243% of ideal body weight. Latest BMI - not reported
Follow up	5 treated for > 3 months
Co-morbidities	5 patients had depression/mood disorder, 4 ADHD and 3 general anxiety
Side effects	No "significant toxicity" or echocardiographic changes
Outcomes	Weight loss 2.9-13.2 kg (mean 7.5kg). No further details given

Table 1.17 Greenway [124] Effects of caffeine and ephedrine

Study type	**Observational case studies**
Treatment	Caffeine and ephedrine
Participants	3 F (15, 16 and 25 years). HO secondary to CP (2 patients), pituitary adenoma (1 patient). Panhypopituitarism All had surgical resection.
BMI	27.7 – 41.4 kg/m^2 Latest BMI - not reported
Follow up	2-6 years
Co-morbidities	Hemiparesis and visual field loss due to intra-operative carotid artery tear and optic nerve damage (1 patient). Cholecystectomy (1 patient)
Side effects	Shaking (1 patient, resolved)
Outcomes	2 lost weight: 8-9%, sustained for 2 years. 1 patient initially lost weight (14% in six months) but weight loss stabilised and metformin, propantheline bromide, cimetidine, growth hormone and Orlistat were added then discontinued (chronologically). Bariatric surgery after 5 years as weight returned to baseline

Table 1.18. Fernandes [125] Treatment with triiodothyronine (T_3)

Study type	**Observational**
Treatment	Supraphysiological doses of T_3 25 – 60 micrograms/day
Participants	3 children (2 F, 1 M). HO secondary to pineal germ call tumour and anaplastic astrocytoma, optic glioma and suprasellar mixed germ-cell tumour. All treated with surgery, chemotherapy and radiotherapy. Panhypopituitarism including DI
Pituitary disease	3.5 – 14 years after diagnosis
BMI	28.1 kg/m^2 fell to 21.6 kg/m^2 25.7 kg/m^2 (BMI SDS 2.7) fell to 20.64 kg/m^2 25.2 kg/m^2 (BMI SDS 2.1), latest not reported
Follow up	≤ 27 months
Initial investigations	All had T4 in normal range, 2 had T3 in normal range. Patient 1 - BMD: lumbar spine T-score of -3.02 and total hip T-score of -3.4 (right) and -3.1 (left)
Investigations on treatment	All had raised T_3 levels (242-490 ng/dL). Patient 1 - BMD: lumbar spine T-score -1.2, total hip T-score -2.5 (right) and -2.0 (left) 27 months after starting T_3
Side effects	None
Outcomes	Weight loss 4.3-14kg. Patient 2: Height velocity improved to 8.6 cm in the 11 months following T_3 administration (previously 4.7 cm/year, despite GH treatment) Patient 3: Improvement in school performance reported. All reported improvement in energy levels and decrease in appetite

Table 1.19. Treatment with incretin mimetics.

Study	Simmons [128].	Thondam [127]	Zoicas [129]
Treatment	Exenatide 5 micrograms twice daily	Exenatide then liraglutide	8 exenatide (mean TDD 11.4 μg), 1 liraglutide (0.6 mg)
Participants	M, 17 years, hypothalamic germ cell tumour. Received chemotherapy and 36 Gy craniospinal RTx, with 18 Gy cranial boost. Panhypopituitarism (replaced except GH) 4 years	F, 42 years, hypothalamic tumour 33 years previously. Received cranial RTx and intracranial shunt. GHD, partially sighted, self-reported hyperphagia	9 patients - 8 M, 1 unknown; mean age 35.1 years (range 17 – 49). 6 Cr, 1 pilocytic astrocytoma, 1 hypothalamic hamartoma (all surgically resected, 1 RTx), 1 germinoma (RTx alone). One untreated GHD, 5 required DDAVP and testosterone supplementation. Mean duration pituitary disease 9.5 years (range 3 – 36)
Initial BMI	39.3 kg/m^2	65.1 kg/m^2	Mean BMI 37.6 ± 7.2 kg/m^2
Latest BMI	29.1 kg/m^2 at 32 months. 1 yr after discontinuation: BMI 33.7 kg/m^2	61.5 kg/m^2 after 4 years follow-up	Mean BMI 33.4 kg/m^2 at last follow-up, $p<0.01$ Treatment duration 6-51 months (mean 24.3 months)
Co-morbidities	T2DM, NASH, borderline hypertension, osteopenia.	T2DM (poorly controlled), OSA, hyperlipidaemia.	8 had T2DM
Side-effects		Occasional nausea, improved with liraglutide	Nausea and vomiting (1 discontinued Exenatide after 2 weeks, 1 after 6 months, 1 continued despite vomiting)
Outcomes	Weight loss 29 kg after 2.5 years. BP and LDL cholesterol improved	Reduced food cravings, increased satiety.	8 treated and followed-up for at least 6 months lost weight. 6 improved appetite/satiety. Significant improvements in mean HbA1c ($p<0.05$), HOMA-IR ($p<0.05$) and fasting triglycerides ($p<0.05$)

RTx = radiotherapy, GH – growth hormone, T2DM = Type 2 diabetes, NASH = non-alcoholic steatohepatitis, OSA = obstructive sleep apnoea

Table 1.20. Bariatric surgery case reports

Case report	Participant	Initial management	Pituitary disease	Co-morbidities	Surgery	Pre-op BMI	Last BMI	Follow-up	Outcomes
Inge [130]	14 year-old M, CP. Subtotal resection, RTx. Post-operative hyperphagia	Diet and exercise, octreotide - ameliorated weight gain	Fully replaced (inc GH). Duration 4 years	LVH, OSA. Raised insulin and triglycerides	RGB with TV aged 18 years	67	52.7	30 months	Co-morbidities improved
Schultes [131]	8 year-old M, CP, surgical resection. Absent hypothalamus on MRI brain	Diet and exercise, Sibutramine	Fully replaced (inc GH). Duration 22 years	T2DM treated with metformin and nateglinide; nasal CPAP for OSA; mildly deranged lipids	DGB	48.5	31.9	18 months	Metformin and nasal CPAP discontinued. Reduced hunger and disinhibition
Bender [133]	7 year-old F, CP. Hyperphagia	Liraglutide and selective proximal vagotomy	Replaced (not specified). Duration 5 years	Arthralgia resulting in an inability to walk. Aggression with meal restriction	LSG aged 12 years	57.1	48.7	6 months	Reduced hyperphagia and aggression. Arthralgia resolved, mobility increased, school re-attendance

Case report	Participant	Initial management	Pituitary disease	Co-morbidities	Surgery	Pre-op BMI	Last BMI	Follow-up	Outcomes
Page-Wilson [134]	16.5 years F, CP. 2 resections. Hyperphagia. MRI brain: hypothalamic damage	Diet and exercise advice, sibutramine, strict exercise regimen	Fully replaced (inc GH). 4.5 years	OA knees. Required cholecystectomy	RGB aged 21	51.6	39	19 months	BMI reduced after 9 months, remained stable. Fasting leptin and insulin fell, fasting ghrelin increased
Rottembourg [132]	P1: 6 years, F, CP. Gross total resection. P2: 4 years, M, CP. Gross total resection, RTx	P1: Diet and exercise advice. P2: Diet and exercise advice, octreotide	P1 & P2: Fully replaced. P1: 6 years duration	Both: raised lipids, NAFLD P1: depression, respiratory complications P2: Raised insulin, abnormal liver function	P1: RBG, aged 12.7 years P2: BPD, aged 15 years	P1: 65 P2: 42	P1: 43 P2: 32	P1: 6 years P2: 2 years	Both: Lipids improved. P1: Diarrhoea, dumping , acute pancreatitis. P2: Intestinal stenosis and unremitting bradycardia required further surgery

M = male, F = female, P1 = patient 1, P2 = patient 2, CP = craniopharyngioma, RTx = radiotherapy, LVH = Left ventricular hypertrophy, OSA = obstructive sleep apnoea, OA = osteoarthritis, RGB = Roux-en-Y gastric bypass, DGB = Distal gastric bypass, LSG = Laparoscopic sleeve gastrectomy, TV = truncal vagotomy, BPD = bilio-pancreatic diversion with duodenal switch, LFTs = liver function tests.

Table 1.21. Bariatric surgery case series

Series	Participants	Tumour treatment	Initial obesity management	Pituitary disease	Obesity surgery	Pre-op BMI	Latest BMI	Follow-up	Outcomes
Müller [135]	3 F, 1 M Diagnosed CP aged 2-21 years	Relapses 0-2, surgical resection 1-3 occasions, RTx (1 patient)	None (2), orlistat and sibutramine (2)		LAGB at 14-24 years	+7.3 to +13.9 SDS	+5.9 to +9.9 SDS	1.5-3.5 years	Dislocation of LAGB required revision (1 patient)
Gatta [136]	2F, 2 M 3 CP, 1 choristoma	All surgically resected, 1 RTx (CP)	Orlistat (1 patient)	Duration 1.5 – 12 years	Sleeve gastrectomy (2 M aged 24 and 43), gastric bypass (2 F aged 30 and 51)	37.6-51 kg/m^2	34-49 kg/m^2	30-64 months	Persisting T2DM (2 patients). P3: resolution of T2DM, NAFLD improved

M = male, F = female, CP = craniopharyngioma, RTx = radiotherapy, T2DM = type 2 diabetes, NAFLD = non-alcoholic fatty liver, P3 = patient 3

Table 1.22. Weismann [137] case series.

Participants	9 children/adolescents (7 F, 2 M). HO secondary to CP. Pituitary disease 2-22 years. Bariatric surgery undertaken post-pubertally (median age 17 years, range 12-30) in all except one case. 154 individuals with simple obesity (SO)
Treatment	Sleeve gastrectomy, gastric bypass or LAGB
BMI pre-treatment	HO: median 44.7 kg/m^2 (range 40.2 – 61.6)
Follow up	HO: median 5.5 years, range 1-9. SO: 5 years
Outcomes	**LAGB**: 6 HO, 3 had second surgery due to lack of weight loss. Significantly less weight loss in HO than in SO ($p < 0.01$). **Sleeve gastrectomy**: 4 HO (2 after LAGB). After mean follow-up of 2 years (range 0.5-4) patients had regained weight lost in the first 6 months post-gastrectomy. SO: ongoing weight loss (percentage weight loss significantly lower in HO than in SO at 1 and 2 years, $p<0.05$). **Gastric bypass**: 2 HO (one following LAGB). Significant weight loss after mean follow-up of three years (range 2-4); at 2, 3 and 4 years (1 patient only for last 2 time-points) no significant difference in percentage weight loss between HO patients and 54 controls (median follow-up two years, range 0.5-5)

Table 1.23. Results of meta-analysis of patients with CP undergoing bariatric surgery [138].

	All	LAGB	SG	RYGB	BPD
Number of patients	21	6	8	6	1
Mean BMI pre-surgery (kg/m^2)	49.6	45.6	48.9	45.6	45
	6 months after bariatric surgery				
Mean weight loss (kg) (95% CI)	-20.9 (-35.4 to -6.3)	-12.9 (-33.5 to +7.7)	-28.3 (-51.3 to -5.3)	-31.0 (-77.5 to +15.5)	BMI 40.4 kg/m^2
Net weight loss (%)	7	10.5	20.7	18.6	11.3
	12 months after bariatric surgery				
Mean weight loss (95% CI)	-15.1 (-31.7 to +1.4)	-7.5 (-28.2 to +13.2)	-25.9 (-59.7 to + 7.9)	-33.7 (-80.7 to + 13.3)	BMI 34 kg/m^2
Net weight loss (%)	-	6.1	19.6	20.2	24.8

Table 1.24. Areas of cerebral activation identified in studies comparing fasted and fed states.

Study	Sex	Weight	Comparison	Findings (rCBF/activation)
Tataranni [149]	M	11, normal	Fasted	Increase in hypothalamus, limbic/paralimbic areas (ACC, hippocampus, parahippocampus and insula, ant temporal and post OFC), temporal cortex, thalamus, precuneus, caudate, putamen and cerebellum ($p<0.005$, uncorrected for multiple comparisons on ^{15}O-PET scanning)
Tataranni [149]	M	11, normal	Fed (liquid meal)	Increase in inferior parietal lobe and vmPFC and dlPFC ($p<0.005$, uncorrected for multiple comparisons on ^{15}O-PET scanning) Negative correlation with insulin in insula (left: r= -0.69, p=0.02; right: r= -0.57, p=0.06) and OFC (left: r= -0.72, p=0.01; right: r= -0.59, p=0.05) and FFAs in ACC (r= -0.76, p=0.007) Positive correlation with FFAs in dlPFC (left: r=0.62, p=0.04; right: r=0.28, p=0.04)

Table 1.26a. Holsen et al [195]. Comparison of brain activation in PWS and lean controls using fMRI scanning. All findings used the contrast food > non-food.

	Meal	Area of increased activation
LCo	Pre	Right lateral OFC and medial PFC, left posterior OFC, bilateral fusiform gyrus
	Post	Left posterior OFC, bilateral fusiform gyrus
	Pre > post	Right amygdala, bilateral OFC, left frontal operculum and fusiform gyrus
	Post > pre	Medial PFC
PWS	Pre	None in *a priori* regions
	Post	Bilateral medial PFC and insula, left hippocampus and parahippocampal gyrus, right fusiform gyrus
	Pre > post	None
	Post > pre	Bilateral OFC, medial PFC and insula, left fusiform gyrus
PWS > LCo	Pre	None
	Post	Right amygdala, OFC and fusiform gyrus, bilateral PFC and insula, left parahippocampus
LCo > PWS	Pre	Bilateral OFC, medial PFC, insula and parahippocampal gyrus, right fusiform gyrus
	Post	None

LCo: lean controls. PWS: Prader-Willi Syndrome. Pre: pre-meal findings. Post: post-prandial. $p<0.01$, corrected for whole brain analysis.

Table 1.26b. Holsen et al [171]. Comparison of brain activation in PWS, SO and lean controls using fMRI scanning. All findings used the contrast food > non-food.

	Meal	Area of increased activation
PWS > SO	Pre	Right NA[a] and amygdala[b]
	Post	Right hypothalamus[c] and amygdala[d], left hippocampus[c]
SO > PWS	Pre	Left hypothalamus[e] and hippocampus[f]
	Post	Left OFC[g] and dlPFC[c]
SO > LCo	Pre	Bilateral hypothalamus[h], left amygdala[i] and OFC[g], right mPFC[j]
	Post	Right hypothalamus[k] and dlPFC[i]
LCo > SO	Pre	None
	Post	Right OFC[j]
PWS > LCo	Pre	Left hypothalamus[g], mPFC[h] and hippocampus[j], bilateral amygdala[g]
	Post	Right hypothalamus[j], amygdala[d] and hippocampus[g]

PWS: Prader-Willi Syndrome. SO: simple obese. LCo: lean controls. Pre: pre-meal findings. Post: post-prandial.

p-values (all uncorrected for multiple comparisons) – a: $p=0.008$, b: $p=0.014$, c: $p=0.003$, d: $p=0.005$, e: $p=0.016$, f: $p=0.006$, g: $p<0.001$, h: $p\leq 0.003$, i: $p=0.007$, j: $p=0.001$, k: $p=0.004$

Appendix 2

Visual Analogue Scale (VAS)

OFFICE USE ONLY:
PARTICIPANT: DATE:

TEST DAY: BASELINE / DAY 1 / DAY 2 / DAY 3

TIME:

INSTRUCTIONS FOR PARTICIPANTS:
Please read each question and then put a mark through the line that best represents how you are feeling in relation to that particular sensation at this moment.

EXAMPLE:

How **TIRED** do you feel **at this moment**?

Not at all tired ——/—— Extremely tired

PLEASE ANSWER THE FOLLOWING QUESTIONS:

How **HUNGRY** do you feel **at this moment**?

Not at all hungry _____ Extremely hungry

How **FULL** do you feel **at this moment**?

Not at all full _____ Extremely full

How **STRONG** is your desire to **eat at this moment**?

Not at all strong _____ Extremely strong

THANK YOU

Three Factor Eating Questionnaire (TFEQ)

Patient code:

Directions: This booklet contains a number of statements. Each statement should be answered either TRUE or FALSE. Read each statement and decide how you feel about it, in part I.
If you agree with the statement, or if you feel that it is true about you, circle the T next to the statement.
If you disagree with a statement, or if you feel that it is false as applied to you, circle the F next to the statement.
Please put down the first answer that comes into your head.

Part I.

1. When I smell some fish & chips or see a juicy piece of meat I find it very difficult to keep from eating, even if I have just finished a meal.	T	F
2. I usually eat too much at social occasions, like parties and picnics.	T	F
3. I am usually so hungry that I eat more than 3 times a day.	T	F
4. When I have eaten my quota of calories I am usually very good about not eating anymore.	T	F
5. Dieting is so hard for me because I just get too hungry.	T	F
6. I deliberately take small helpings as a means of controlling my weight.	T	F
7. Sometimes things just taste so good that I keep on eating, even when I am no longer hungry.	T	F
8. Since I am often hungry, I sometimes wish that while I am eating an expert would tell me that I have had enough or that I can have something more to eat.	T	F
9. When I feel anxious I find myself eating.	T	F
10. Life is too short to worry about dieting.	T	F
11. Since my weight goes up and down, I have gone on reducing diets more than once.	T	F
12. I often feel so hungry I just have to eat something.	T	F
13. When I am with someone who is overeating, I usually overeat too.	T	F
14. I have a pretty good idea of the number of calories in common foods.	T	F
15. Sometimes when I start eating, I just can't seem to stop.	T	F
16. It is not difficult for me to leave something on my plate.	T	F
17. At certain times of the day I get hungry because I have gotten used to eating then.	T	F
18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.	T	F
19. Being with someone who is overeating often makes me hungry enough to eat also.	T	F
20. When I feel blue I often overeat.	T	F
21. I enjoy eating too much, to spoil it by counting calories or watching my weight.	T	F

22. When I see a real delicacy I often get so hungry that I have to eat right away. T F

23. I often stop eating when I am not really full as a conscious means of limiting the amount I eat. T F

24. I get so hungry my stomach often feels like a bottomless pit. T F

25. My weight has hardly changed at all in the last ten years. T F

26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate. T F

27. When I feel lonely, I console myself by eating. T F

28. I consciously hold back at meals in order not to gain weight. T F

29. I sometimes get very hungry late in the evening or at night. T F

30. I eat anything I want, anytime I want. T F

31. Without even thinking about it I take a long time to eat. T F

32. I count calories as a conscious means of controlling my weight. T F

33. I do not eat some foods because they make me fat. T F

34. I am always hungry enough to eat at anytime. T F

35. I pay a great deal of attention to changes in my figure. T F

36. While on a diet, if I eat food that is not allowed, I often then splurge and eat other high calorie foods. T F

Part II.

Please answer the following questions by circling the number above the response that is appropriate for you.

37. How often are you dieting in a conscious effort to control your weight?

1	2	3	4
rarely	sometimes	usually	always

38. Would a weight fluctuation of 5lb affect the way you live your life?

1	2	3	4
not at all	slightly	moderately	very much

39. How often do you feel hungry?

1	2	3	4
only at mealtimes	sometimes between meals	often between meals	almost always

40. Do your feelings of guilt about overeating help you to control your food intake?

1	2	3	4
never	rarely	often	always

41. How difficult would it be for you to stop eating half way through dinner and not eat for the next four hours?

1	2	3	4
easy	slightly difficult	moderately difficult	very difficult

42. How conscious are you of what you are eating?

1	2	3	4
not at all	slightly	moderately	extremely

43. How frequently do you avoid 'stocking up' on tempting foods?

1	2	3	4
almost never	seldom	usually	almost always

44. How likely are you to shop for low calorie foods?

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

45. Do you eat sensibly in front of others and splurge alone?

1	2	3	4
never	rarely	often	always

46. How likely are you to consciously eat slowly in order to cut down on how much you eat?

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

47. How frequently do you skip dessert because you are no longer hungry?

1	2	3	4
almost never	seldom	at least once a week	everyday

48. How likely are you to consciously eat less than you want?

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

49. Do you go on eating binges even though you are not hungry?

1	2	3	4
never	rarely	sometimes	once a week

50. On a scale of 0 to 10, where 0 means no restraint in eating (eat whatever you want, whenever you want it) and 10 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself. (Choose one number only).

eat whatever you want, whenever you want it

usually eat whatever you want, whenever you want it

often eat whatever you want, whenever you want it

often limit food intake, but often 'give in'

usually limit food intake, but rarely 'give in'

constantly limiting food intake, never 'giving in'

51. To what extent does this statement describe your eating behaviour ?

'I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'

1	2	3	4
not like me	a little like me	pretty good description	describes me

Appendix 2:

Please answer the following questions as carefully and honestly as possible. Read each question and simply fill in the column which best applies to you.

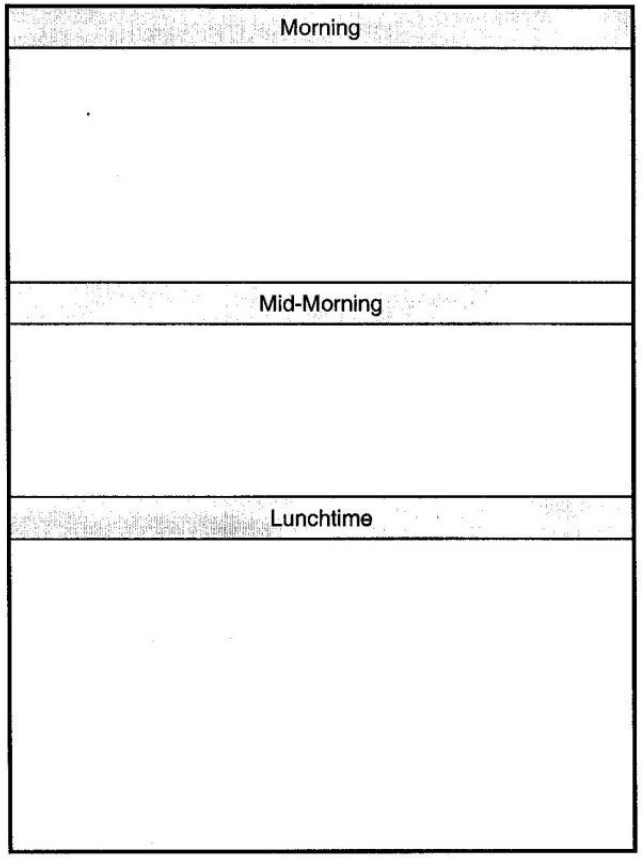
Never
Seldom
Sometimes
Often
Very often

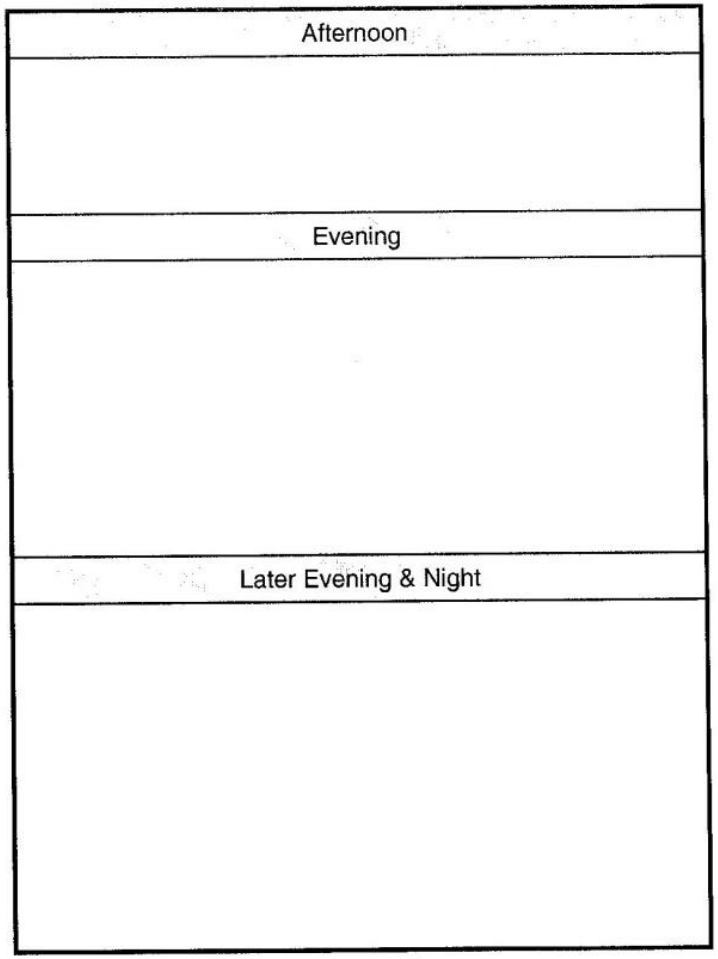
1. If you have put on weight, do you eat less than you usually do?
2. Do you have a desire to eat when you are irritated?
3. If food tastes good to you, do you eat more than you usually do?
4. Do you try to eat less at meals times than you would like to eat?
5. Do you have a desire to eat when you have nothing to do?
6. Do you have a desire to eat when you are fed-up?
7. If food smells good and looks good, do you eat more than you usually do?

8. How often do you refuse food and drink offered because you are concerned about your weight?
9. Do you have a desire to eat when you are feeling lonely?
10. If you see or smell something delicious do you have a desire to eat it?
11. . Do you watch exactly what you eat?
12. Do you have a desire to eat when someone disappoints you?
13. If you have something delicious to eat, do you eat it straight away?
14. Do you deliberately eat foods that are slimming?
15. Do you have a desire to eat when you are cross?
16. . Do you have a desire to eat when you are expecting something to happen?
17. If you walk past the baker do you have a desire to buy something delicious?
18. When you have eaten too much, do you eat less than usual on the following days?
19. Do you get a desire to eat when you are anxious, worried or tense?
20. If you walk past a snack bar or cafe, do you have a desire to buy something delicious?
21. Do you deliberately eat less in order not to become heavier?
22. Do you have a desire to eat when things go against you or when things have gone wrong?
23. If you see others eating, do you also have a desire to eat?
24. How often do you try not to eat between meals because you are watching your weight?
25. Do you have a desire to eat when you are frightened?
26. Can you resist eating delicious foods?
27. How often in the evening do you try not to eat because you are watching your weight?
28. Do you have a desire to eat when you are disappointed?
29. Do you eat more than usual when you see others eating?
30. Do you think about how much you weigh before deciding how much to eat?
31. Do you have a desire to eat when you are upset?
32. When you see someone preparing a meal, does it make you want to eat something?
33. Do you have a desire to eat when you are bored or restless?

Three-day MRC food diary – example page

Day: Date:





Appendix 3

Food photographs

Examples of low calorie food photographs

Examples of high calorie food photographs

Appendix 4

Timeline of study days

Study day 1: screening visit

1. Informed consent undertaken

2. All participants (patients and controls) medically screened and adequacy of vision assessed in the patient group.

3. Body composition data collected (height and weight; estimation of percentage body fat by whole-body bioelectrical impedance analysis [Tanita Systems, Tanita Corp, Tokyo, Japan]).

4. Estimate of BMR using the Harris-Benedict formula - For men: 66.5 + (13.75 x weight) + (500.3 x height) - (6.775 x age) and for women: 655.1 + (9.563 x weight) + (185 x height) - (4.676 x age).

5. Trial session at the UEM with participants given 25% of their calculated daily energy requirement as porridge and orange juice to be eaten to assess whether they could consume all of the food and drink provided and to enable familiarisation with the UEM.

5. Participants given TFEQ and 3-day food diaries for completion.

Study day 2: fMRI study day timeline

72 hours before fMRI	Participants asked to refrain from alcohol, strenuous physical activity, cigarettes and caffeine-containing drinks
22.00 previous day	Start fasting

Study day: Timepoint	**Task**
Baseline	Cannulate. Blood samples
	Anatomical then functional imaging
After 1st scan	1st VAS ratings and 1st photo ratings
	Test meal (porridge and OJ)
Immediately after test meal	2nd VAS ratings
15 mins after meal	Blood sample
30 mins after meal	Blood sample
60 mins after meal	Blood sample
	Functional imaging
After 2nd scan	3rd VAS ratings and 2nd photo ratings
90 mins after meal	Blood sample
120 mins after meal	Blood sample
	4th VAS ratings
150 mins after meal	Blood sample
180 mins after meal	Blood sample
	Functional imaging
	5th VAS ratings and 3rd photo ratings
End	Blood sample

Study day 3: UEM study day timeline

24 hours before UEM	Participants asked to refrain from alcohol, strenuous physical activity, cigarettes and caffeine-containing drinks
22.00 previous day	Start fasting
Study day: Timepoint	**Task**
Baseline	**Breakfast at UEM (porridge and OJ)**
3 hours after breakfast	Cannulate. Blood samples
Lunch	**Lunch meal UEM (pasta) and completion of intra-meal VAS scores**
15 mins after end of meal	Blood sample
30 mins after end of meal	Blood sample
60 mins after end of meal	Blood sample
90 mins after end of meal	Blood sample
120 mins after end of meal	Blood sample
150 mins after end of meal	Blood sample
180 mins after end of meal	Blood sample

Appendix 5

PARTICIPANTS' INFORMATION SHEET B

Abnormal Eating Behaviour and Brain Areas Associated with Hunger in Adult Patients with Damage to the Hypothalamus (August 2010 - Version 3).

Part 1

We would like to invite you to take part in a research study. Before you decide if you would like to take part you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully and talk to friends, relatives or your GP about the study if you wish. Ask us if there is anything that is not clear or if you would like more information and take time to decide whether or not you wish to take part.

Part 1 tells you the purpose of this study and what will happen to you if you take part.
Part 2 gives you more detailed information about the conduct of the study.

What is the purpose of the study?

The hypothalamus is an area of the brain, which is important in controlling energy balance. Obesity is a common problem in people who have had disorders affecting this area. Obesity is associated with an increased risk of other health problems. The purpose of this study is to help us understand what makes some people with damage to the hypothalamus more likely to gain weight. If we can identify individuals at risk of obesity from hypothalamic damage early, we can try to prevent them from becoming obese.

Why have I been asked to take part?

The study will compare people in 4 categories:-

1. People who are obese with damage to the hypothalamus
2. People who are weight stable with damage to the hypothalamus
3. People who are obese without damage to the hypothalamus

4. People who are weight stable without damage to the hypothalamus.

You have been asked to take part in one of the control groups *(i.e./ one of the groups **without damage to the hypothalamus**)*. We wish to include participants without damage to the hypothalamus so that we can assess what (if any) are the differences are seen between the groups and therefore determine which areas of the brain help in controlling energy balance.

Do I have to take part?

It is up to you – you do not have to take part.

We will describe the study to you in this information sheet, which you can keep. If you decide to take part, we will ask you to sign a consent form. If you change your mind later on, you are free to withdraw from the study at any time without giving a reason. If you decide not to take part, or change your mind, this will not affect any medical care that you receive currently or in the future.

What will happen to me if I take part?

If you decide to take part we will ask you to:-

1. Fill in a questionnaire about any attempts you have made to diet to control your weight, if you feel you have loss of control over food intake and whether you feel able to resist hunger. You will be asked to fill out the questionnaire at home and return it during the next study visit.
2. You will also be given a nutrition diary and asked to fill this out over 3 days at home.

You will also be asked to attend for 3 visits, which will be in addition to any normal clinic visits.

1ST VISIT

We will ask you to attend our investigational unit at the Clinical Sciences Centre, University Hospital Aintree on a day suitable for you. We will answer any questions or concerns that you have and if you decide to participate in the study we will ask you to sign a consent form agreeing to take part. Your height, weight, waist and hip circumference will be recorded and an estimation of body fat made by standing on scales with special sensors. You will then be given breakfast and asked to complete

ratings of hunger and fullness on a computer screen in front of you as you eat. This visit will last about 2 hours.

2ND VISIT

You will be asked to attend the MARIARC (Magnetic Resonance And Image Analysis Research Centre) Centre on Pembroke Place in Liverpool at 0800 in the morning after fasting from 22:00 the night before. We will also ask you to refrain from alcohol, strenuous physical activity, cigarettes and caffeine containing drinks for at least 72 hours before the study day. We will insert a cannula to take blood that will be left in your vein for 3 hours. You will undergo 4 magnetic resonance imaging (MRI) scans in total. You will be asked to wear a gown (changing rooms are provided) and remove items that are affected by the magnetic field (e.g./ hearing aids, mobile phones, keys, coin, pens, credit cards (secure lockers are provided)). While inside the scanner you will be asked to lie as still as possible on your back, with your head immobilized with head cushions.

You'll have the first 2 MRI scans before being given a breakfast meal. During the 2nd scan, you'll be shown 4 sets of 10 photographs in a mirror inside the scanner. Each photograph will be shown for 5 seconds with 1 second in between. You'll be asked to try and remember each photograph for a recognition test at the end of each scan and to rate your feelings of hunger, fullness, urge to eat, how much food you think you will eat, preoccupation with food, and non-food-related feelings of mood on a scale of 0 (low) to 100 (high) before the first scan and after the second scan and to rate whether you think the food in the photographs is low or high calorie and how appealing you find it.

You will then be given an identical breakfast to that eaten in the laboratory at the Clinical Sciences Centre, University Hospital Aintree. The 3rd and 4th scans will be done one hour and three hours after eating breakfast. You will again be shown the 4 sets of 10 photographs and asked to remember them. You will be asked to rate your feelings (as previous) before and after both scans. The total scanning time will be about 1 hour.

Blood will be taken at the start and end of each of the three scanning sessions and at 15, 30 and 60 minutes after eating the breakfast meal (9 samples of 10 ml over 3

hours = total 90 ml = 18 teaspoons) for glucose, insulin, free fatty acids, incretins and gut hormones. This visit will last about 4 hours.

3RD VISIT

We will ask you to attend our investigational unit at the Clinical Sciences Centre, University Hospital Aintree or the Kissileff Laboratory, in the Eleanor Rathbone Building at the University of Liverpool, at around 8:30 am on a day suitable for you. We will ask you to fast from 22:00 the night before. We will also ask you to refrain from alcohol, strenuous physical activity and cigarettes for at least 24 hours and caffeine containing drinks for at least 12 hours before the study day. Bloods will be taken from a cannula inserted into a hand or arm vein and will be left there for 6 hours, to allow further blood samples to be taken. You will be given a standard breakfast meal and asked to complete ratings of hunger and fullness on a computer screen in front of you as you eat. Three hours after breakfast you will be given a standard lunch meal and again asked to complete ratings of hunger and fullness as you eat. Blood samples will be taken immediately after the lunch meal and at 15, 30, 60, 90, 120, 150 and 180 minutes after this (9 samples of 10 ml over 3 hours = total of 90 ml = 18 teaspoons) for measurement of glucose and hormones - insulin and C-peptide, incretins and hormones made in the gut and by fat cells. You will also be asked to continue rating how hungry and full you feel, every hour for three hours after finishing the lunch meal. This visit will last about 7 hours.

All venues have bathroom facilities and comfortable seating. The Clinical Sciences Centre and the Kissileff Laboratory have a television and magazines available.

Expenses

For all visits, travel expenses and arrangements to help with transport are available. Reimbursement will also be offered in recognition of the time involved in taking part in the study – up to £40 for visit 2 and up to £60 for visit 3, up to a total of £100.

What will I have to do?

We will ask you to fill in the food diary and questionnaire at home and return them at the 1st study visit.

You will be asked to attend for 3 visits, which will be extra to your normal clinic visits and to fast from about ten o'clock the night before each visit. We will ask you to refrain from alcohol or strenuous physical activity for at least 24 hours and from cigarettes and caffeine containing drinks for at least 12 hours before the visit to the Clinical Sciences Centre, University Hospital Aintree. We will also ask you to refrain from alcohol, strenuous physical activity, cigarettes and caffeine containing drinks for at least 72 hours before the visit to the MARIARC centre in Liverpool

What are the possible disadvantages and risks of taking part?

There will be the inconvenience of the time taken for three visits to the research centres.

Inserting drips ("cannulae") may cause slight discomfort and occasionally bruising at the site of insertion.

Some people may find the scanner claustrophobic, or uncomfortable and we will check this with you. There are no known risks in properly conducted MRI scanning. Certain precautions need to be observed as it involves a strong magnet. ***Most importantly, you cannot have an MRI if you are fitted with a heart pacemaker, mini-defibrillator or neurostimulator or have an artificial heart valve; if you have surgical clips in your head; if you have ever had an injury which may have left metal particles in your eye, head, or elsewhere in your body.***

Occasionally research studies using MRI scans reveal unexpected abnormalities, which require medical follow-up, either for further investigation or (more rarely) treatment. The scans we do are for research purposes, but they are reviewed carefully to avoid missing any abnormality. If any significant abnormality is found, we will send the report to your GP who will be able to take it further.

Most of the blood tests that will be undertaken are currently only used in a research setting, however, all blood results will be reviewed carefully to avoid missing any abnormality. If any significant abnormality is found, we will send the report to your GP who will be able to take it further.

There are no interventions involved that should interfere with your usual treatment or normal daily activities.

What are the possible benefits of taking part?

You will not directly benefit from participation in this study. Knowledge gained may help to provide important insights into the development of obesity in people who have acquired hypothalamic damage.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. More detailed information on this and contact details are given in Part 2.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

This completes part I. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2 of the information sheet

What will happen if I don't want to carry on with the study?

Nothing! You are free to withdraw at any time and without giving a reason. In case of withdrawal, you have the right to make a decision about any information, data and samples we have collected. All your data and samples will be destroyed if you wish so, and no copy of the data will be retained in any database.

What if there is a problem?

If you have concerns about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions.

Please contact Dr Caroline Steele on: (**0151) 529 6146**.

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure (Details can be obtained from the PALS team, Ground Floor, University Hospital Aintree, phone: (0151) 5293287) or you can use the complaints procedure at the University of Liverpool, addressed to the Research Governance Officer in Legal Services (ethics@liv.ac.uk, 0151 794 8920).

The study does not involve any therapeutic interventions or potentially hazardous procedures. In the unlikely event that you become ill or suffer any injury as a direct result of a procedure of the study, the study doctor will arrange for the correct treatment. In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against (Aintree Hospital NHS Trust) but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

All information collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will be coded (have your name and address removed so that you cannot be recognised). Data will not be retained for use in future studies and will be disposed of securely after completion of the study.

All the test results will be fed into a secure computer kept in the research centre (3rd Floor of Clinical Sciences Centre, University Hospital Aintree) and all your data will be coded and stored anonymously. Complete data will remain known to members of the research team (Dr Caroline Steele and Dr Christina Daousi).

Your GP will be informed of your participation in our study.
You have the right to check the accuracy of data held about you and correct any errors.

What will happen to any samples I give?

The blood samples are taken for research purposes and will be stored securely in freezers at the Clinical Sciences Centre, University Hospital Aintree. They will not

be retained after completion of the study. The results of the blood samples and the MRI scans will be fed into a secure computer kept in the research centre (3rd Floor of Clinical Sciences Centre, University Hospital Aintree) and all your data will be coded and stored anonymously. Complete data and access to the samples will only be available to the research team (Dr Caroline Steele and Dr Christina Daousi).

It is unlikely that the study will produce any individually significant information because the factors we propose to study are not currently part of routine clinical care.

What will happen to the results of the study?

The overall collective results of the study will be presented and published in medical journals in the future after analysis of the complete data. We will not identify you in any way when the results are presented or published.

It is unlikely that the study will produce any individually significant information because the factors we propose to study are not currently part of routine clinical care.

Who is organising and funding the research?

The principal researcher is employed by the University of Liverpool, with funding from Eli Lilly(a pharmaceutical company), but no additional money will be received based on your participation.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable ethical opinion for conduct in the NHS by Sefton Research Ethics Committee. If you decide to take part in the study, you will be given a copy of this information leaflet and a copy of your signed consent form to keep at home.

Further information and contact details

If you have any concerns, or would like to discuss further any aspects of the study, if you have any questions and finally if you have any complaints you can contact **Dr**

Caroline Steele, Clinical Research Fellow in Diabetes & Endocrinology on**: 0151 529 6146.**

My postal address is:
Diabetes and Endocrinology Clinical Research Group,
3rd floor Clinical Sciences Centre,
University Hospital Aintree,
Longmoor Lane,
Liverpool,
L9 7AL.

You can also contact the PALS team located on the ground floor, University Hospital Aintree for further information.

Thank you for taking the time to read this information sheet. If you have any comments or queries please contact Dr Caroline Steele by phone (telephone number (0151) 529 6146) or by e-mail (e-mail address C.A.Steele@liverpool.ac.uk).

References

1. Aurelie L, Gilles F, Jean-Jacques D, Agathe A, Sophie V, Daniel T, et al. Characterization of the Three-Factor Eating Questionnaire scores of a young French cohort. Appetite. 2012;59(2):385-90.

2. Daousi C, MacFarlane IA, English PJ, Wilding JP, Patterson M, Dovey TM, et al. Is there a role for ghrelin and peptide-YY in the pathogenesis of obesity in adults with acquired structural hypothalamic damage? J Clin Endocrinol Metab. 2005 Sep;90(9):5025-30.

3. Holmer H, Pozarek G, Wirfalt E, Popovic V, Ekman B, Bjork J, et al. Reduced Energy Expenditure and Impaired Feeding-Related Signals But Not High Energy Intake Reinforces Hypothalamic Obesity in Adults with Childhood Onset Craniopharyngioma. J Clin Endocrinol Metab. 2010 Sep 8.

4. Roth CL, Gebhardt U, Muller HL. Appetite-Regulating Hormone Changes in Patients With Craniopharyngioma. Obesity (Silver Spring). 2010 Apr 8.

5. Pinkney J, Wilding J, Williams G, MacFarlane I. Hypothalamic obesity in humans: what do we know and what can be done? Obes Rev. 2002 Feb;3(1):27-34.

6. Daousi C, Dunn AJ, Foy PM, MacFarlane IA, Pinkney JH. Endocrine and neuroanatomic features associated with weight gain and obesity in adult patients with hypothalamic damage. Am J Med. 2005 Jan;118(1):45-50.

7. Hochberg I, Hochberg Z. Expanding the definition of hypothalamic obesity. Obes Rev. 2010 Oct;11(10):709-21.

8. Bereket A, Kiess W, Lustig RH, Muller HL, Goldstone AP, Weiss R, et al. Hypothalamic obesity in children. Obes Rev. 2012 Sep;13(9):780-98.

9. Bray GA, Gallagher TF,Jr. Manifestations of hypothalamic obesity in man: a comprehensive investigation of eight patients and a reveiw of the literature. Medicine (Baltimore). 1975 Jul;54(4):301-30.

10. Pinkney JH, Daousi C, MacFarlane IA. Recent Advances in the Understanding and Treatment of Hypothalamic Obesity in Humans. In: Ling PR, editor. Trends In Obesity Research. Nova Science Publishers, Inc.; 2005. p. 41.

11. Lee M, Korner J. Review of physiology, clinical manifestations, and management of hypothalamic obesity in humans. Pituitary. 2009;12(2):87-95.

12. May JA, Krieger MD, Bowen I, Geffner ME. Craniopharyngioma in childhood. Adv Pediatr. 2006;53:183-209.

13. King BM. The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. Physiol Behav. 2006 Feb 28;87(2):221-44.

14. Bray GA, York DA. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol Rev. 1979 Jul;59(3):719-809.

15. Heymsfield SB, Avena NM, Baier L, Brantley P, Bray GA, Burnett LC, et al. Hyperphagia: current concepts and future directions proceedings of the 2nd international conference on hyperphagia. Obesity (Silver Spring). 2014 Feb;22 Suppl 1:S1-S17.

16. Frohman L, Bernardis L, Schnatz J, Burek L. Plasma insulin and triglyceride levels after hypothalamic lesions in weanling rats. American Journal of Physiology -- Legacy Content. 1969 June 01;216(6):1496-501.

17. A midsagittal view showing the inner boundaries of the lobes of the cerebral cortex. [Internet].; 2011 []. Available from: highlands.edu/academics/divisions/scipe/biology/faculty/harnden/2121/notes/cns.htm .

18. Netter F. Atlas of Human Anatomy, 6th Edition. 6th ed. Philidelphia: Saunders; 2014.

19. Crowley RK, Hamnvik OP, O'Sullivan EP, Behan LA, Smith D, Agha A, et al. Morbidity and mortality in patients with craniopharyngioma after surgery. Clin Endocrinol (Oxf). 2010 Oct;73(4):516-21.

20. Adachi M, Tsuchiya T, Muroya K, Asakura Y, Sekido K, Sato H. Prevalence of obesity, hyperlipidemia and insulin resistance
in children with suprasellar brain tumours. Clin Pediatr Endocrinol. 2007;16:1--9.

21. Lek N, Prentice P, Williams RM, Ong KK, Burke GA, Acerini CL. Risk factors for obesity in childhood survivors of suprasellar brain tumours: a retrospective study. Acta Paediatr. 2010 Oct;99(10):1522-6.

22. Duff J, Meyer FB, Ilstrup DM, Laws ER,Jr, Schleck CD, Scheithauer BW. Long-term outcomes for surgically resected craniopharyngiomas. Neurosurgery. 2000 Feb;46(2):291,302; discussion 302-5.

23. Karavitaki N, Brufani C, Warner JT, Adams CB, Richards P, Ansorge O, et al. Craniopharyngiomas in children and adults: systematic analysis of 121 cases with long-term follow-up. Clin Endocrinol (Oxf). 2005 Apr;62(4):397-409.

24. Gautier A, Godbout A, Grosheny C, Tejedor I, Coudert M, Courtillot C, et al. Markers of recurrence and long-term morbidity in craniopharyngioma: a systematic analysis of 171 patients. J Clin Endocrinol Metab. 2012 Apr;97(4):1258-67.

25. Kendall-Taylor P, Jonsson PJ, Abs R, Erfurth EM, Koltowska-Haggstrom M, Price DA, et al. The clinical, metabolic and endocrine features and the quality of life in adults with childhood-onset craniopharyngioma compared with adult-onset craniopharyngioma. Eur J Endocrinol. 2005 Apr;152(4):557-67.

26. Sorva R. Children with craniopharyngioma. Early growth failure and rapid postoperative weight gain. Acta Paediatr Scand. 1988 Jul;77(4):587-92.

27. Muller HL, Bueb K, Bartels U, Roth C, Harz K, Graf N, et al. Obesity after childhood craniopharyngioma--German multicenter study on pre-operative risk factors and quality of life. Klin Padiatr. 2001 Jul-Aug;213(4):244-9.

28. Muller HL, Gebhardt U, Etavard-Gorris N, Korenke E, Warmuth-Metz M, Kolb R, et al. Prognosis and sequela in patients with childhood craniopharyngioma -- results of HIT-ENDO and update on KRANIOPHARYNGEOM 2000. Klin Padiatr. 2004 Nov-Dec;216(6):343-8.

29. Pinto G, Bussieres L, Recasens C, Souberbielle JC, Zerah M, Brauner R. Hormonal factors influencing weight and growth pattern in craniopharyngioma. Horm Res. 2000;53(4):163-9.

30. Cohen M, Guger S, Hamilton J. Long term sequelae of pediatric craniopharyngioma - literature review and 20 years of experience. Front Endocrinol (Lausanne). 2011;2:81.

31. Crom DB, Smith D, Xiong Z, Onar A, Hudson MM, Merchant TE, et al. Health status in long-term survivors of pediatric craniopharyngiomas. J Neurosci Nurs. 2010 Dec;42(6):323,8; quiz 329-30.

32. Sainte-Rose C, Puget S, Wray A, Zerah M, Grill J, Brauner R, et al. Craniopharyngioma: the pendulum of surgical management. Childs Nerv Syst. 2005 Aug;21(8-9):691-5.

33. Ahmet A, Blaser S, Stephens D, Guger S, Rutkas JT, Hamilton J. Weight gain in craniopharyngioma--a model for hypothalamic obesity. J Pediatr Endocrinol Metab. 2006 Feb;19(2):121-7.

34. Vinchon M, Weill J, Delestret I, Dhellemmes P. Craniopharyngioma and hypothalamic obesity in children. Childs Nerv Syst. 2009 Mar;25(3):347-52.

35. Walley AJ, Asher JE, Froguel P. The genetic contribution to non-syndromic human obesity. Nat Rev Genet. 2009 Jul;10(7):431-42.

36. Huda MS, Wilding JP, Pinkney JH. Gut peptides and the regulation of appetite. Obes Rev. 2006 May;7(2):163-82.

37. Roth CL. Hypothalamic obesity in patients with craniopharyngioma: profound changes of several weight regulatory circuits. Front Endocrinol (Lausanne). 2011;2:49.

38. Lustig RH. Hypothalamic obesity after craniopharyngioma: mechanisms, diagnosis, and treatment. Front Endocrinol (Lausanne). 2011;2:60.

39. Goldstone AP, Patterson M, Kalingag N, Ghatei MA, Brynes AE, Bloom SR, et al. Fasting and postprandial hyperghrelinemia in Prader-Willi syndrome is partially explained by hypoinsulinemia, and is not due to peptide YY3-36 deficiency or seen in hypothalamic obesity due to craniopharyngioma. J Clin Endocrinol Metab. 2005 May;90(5):2681-90.

40. Roth C, Wilken B, Hanefeld F, Schroter W, Leonhardt U. Hyperphagia in children with craniopharyngioma is associated with hyperleptinaemia and a failure in the downregulation of appetite. Eur J Endocrinol. 1998 Jan;138(1):89-91.

41. Mason PW, Krawiecki N, Meacham LR. The use of dextroamphetamine to treat obesity and hyperphagia in children treated for craniopharyngioma. Arch Pediatr Adolesc Med. 2002 Sep;156(9):887-92.

42. Tiosano D, Eisentein I, Militianu D, Chrousos GP, Hochberg Z. 11 beta-Hydroxysteroid dehydrogenase activity in hypothalamic obesity. J Clin Endocrinol Metab. 2003 Jan;88(1):379-84.

43. Harz KJ, Muller HL, Waldeck E, Pudel V, Roth C. Obesity in patients with craniopharyngioma: assessment of food intake and movement counts indicating physical activity. J Clin Endocrinol Metab. 2003 Nov;88(11):5227-31.

44. Seim I, Amorim L, Walpole C, Carter S, Chopin LK, Herington AC. Ghrelin gene-related peptides: Multifunctional endocrine / autocrine modulators in health and disease. Clinical & Experimental Pharmacology & Physiology. 2010 01;37(1):125-31.

45. Jameson JL, DeGroot LJ, De Kretser DM, Giudice L, Grossman A, Melmed S, et al. Endocrinology. [electronic book] : adult & pediatric. 7th edition.

46. Haqq AM, Stadler DD, Rosenfeld RG, Pratt KL, Weigle DS, Frayo RS, et al. Circulating ghrelin levels are suppressed by meals and octreotide therapy in children with Prader-Willi syndrome. J Clin Endocrinol Metab. 2003 Aug;88(8):3573-6.

47. Kanumakala S, Greaves R, Pedreira CC, Donath S, Warne GL, Zacharin MR, et al. Fasting ghrelin levels are not elevated in children with hypothalamic obesity. J Clin Endocrinol Metab. 2005 May;90(5):2691-5.

48. Karra E, Chandarana K, Batterham RL. The role of peptide YY in appetite regulation and obesity. J Physiol. 2009 Jan 15;587(Pt 1):19-25.

49. Ballantyne, G.H. (1,2). Peptide YY(1-36) and peptide YY(3-36): Part II. Changes after gastrointestinal surgery and bariatric surgery - Part. I. Distribution, release and actions. Obesity Surg. 2006 / 06 / 01 /;16(6):795-803.

50. Allison MB, Myers,Martin G.,,Jr. 20 Years of Leptin: Connecting Leptin Signaling to Biological Function. J Endocrinol. 2014 10;223(1):T25-35.

51. Guran T, Turan S, Bereket A, Akcay T, Unluguzel G, Bas F, et al. The role of leptin, soluble leptin receptor, resistin, and insulin secretory dynamics in the pathogenesis of hypothalamic obesity in children. Eur J Pediatr. 2009 Sep;168(9):1043-8.

52. Shaikh MG, Grundy RG, Kirk JM. Hyperleptinaemia rather than fasting hyperinsulinaemia is associated with obesity following hypothalamic damage in children. Eur J Endocrinol. 2008 Dec;159(6):791-7.

53. Patel L, Cooper CD, Quinton ND, Butler GE, Gill MS, Jefferson IG, et al. Serum leptin and leptin binding activity in children and adolescents with hypothalamic dysfunction. J Pediatr Endocrinol Metab. 2002 Jul-Aug;15(7):963-71.

54. Simoneau-Roy J, O'Gorman C, Pencharz P, Adeli K, Daneman D, Hamilton J. Insulin sensitivity and secretion in children and adolescents with hypothalamic obesity following treatment for craniopharyngioma. Clin Endocrinol (Oxf). 2009 May 25.

55. Lustig RH, Hinds PS, Ringwald-Smith K, Christensen RK, Kaste SC, Schreiber RE, et al. Octreotide therapy of pediatric hypothalamic obesity: a double-blind, placebo-controlled trial. J Clin Endocrinol Metab. 2003 Jun;88(6):2586-92.

56. Lustig RH, Rose SR, Burghen GA, Velasquez-Mieyer P, Broome DC, Smith K, et al. Hypothalamic obesity caused by cranial insult in children: altered glucose and insulin dynamics and reversal by a somatostatin agonist. J Pediatr. 1999 Aug;135(2 Pt 1):162-8.

57. Srinivasan S, Ogle GD, Garnett SP, Briody JN, Lee JW, Cowell CT. Features of the metabolic syndrome after childhood craniopharyngioma. J Clin Endocrinol Metab. 2004 Jan;89(1):81-6.

58. Sahakitrungruang T, Klomchan T, Supornsilchai V, Wacharasindhu S. Obesity, metabolic syndrome, and insulin dynamics in children after craniopharyngioma surgery. Eur J Pediatr. 2011 Jun;170(6):763-9.

59. Holmes LB, Frantz AG, Rabkin MT, Soeldner JS, Crawford JD. Normal growth with subnormal growth-hormone levels. N Engl J Med. 1968 Sep 12;279(11):559-66.

60. Bucher H, Zapf J, Torresani T, Prader A, Froesch ER, Illig R. Insulin-like growth factors I and II, prolactin, and insulin in 19 growth hormone-deficient children with excessive, normal, or decreased longitudinal growth after operation for craniopharyngioma. N Engl J Med. 1983 Nov 10;309(19):1142-6.

61. Costin G, Kogut MD, Phillips LS, Daughaday WH. Craniopharyngioma: the role of insulin in promoting postoperative growth. J Clin Endocrinol Metab. 1976 Feb;42(2):370-9.

62. Stahnke N, Grubel G, Lagenstein I, Willig RP. Long-term follow-up of children with craniopharyngioma. Eur J Pediatr. 1984 Aug;142(3):179-85.

63. Schofl C, Schleth A, Berger D, Terkamp C, von zur Muhlen A, Brabant G. Sympathoadrenal counterregulation in patients with hypothalamic craniopharyngioma. J Clin Endocrinol Metab. 2002 Feb;87(2):624-9.

64. Roth CL, Hunneman DH, Gebhardt U, Stoffel-Wagner B, Reinehr T, Muller HL. Reduced sympathetic metabolites in urine of obese patients with craniopharyngioma. Pediatr Res. 2007 Apr;61(4):496-501.

65. Shaikh MG, Grundy RG, Kirk JM. Reductions in basal metabolic rate and physical activity contribute to hypothalamic obesity. J Clin Endocrinol Metab. 2008 Jul;93(7):2588-93.

66. Muller HL, Handwerker G, Wollny B, Faldum A, Sorensen N. Melatonin secretion and increased daytime sleepiness in childhood craniopharyngioma patients. J Clin Endocrinol Metab. 2002 Aug;87(8):3993-6.

67. SINCLAIR HM. Assessment and results of obesity. Br Med J. 1953 Dec 26;2(4851):1404-7.

68. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:i,xii, 1-253.

69. Adams LA, Feldstein A, Lindor KD, Angulo P. Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. Hepatology. 2004 Apr;39(4):909-14.

70. van der Klaauw AA, Biermasz NR, Pereira AM, van Kralingen KW, Dekkers OM, Rabe KF, et al. Patients cured from craniopharyngioma or nonfunctioning pituitary macroadenoma (NFMA) suffer similarly from increased daytime somnolence despite normal sleep patterns compared to healthy controls. Clin Endocrinol (Oxf). 2008 Nov;69(5):769-74.

71. Bulow B, Attewell R, Hagmar L, Malmstrom P, Nordstrom CH, Erfurth EM. Postoperative prognosis in craniopharyngioma with respect to cardiovascular mortality, survival, and tumor recurrence. J Clin Endocrinol Metab. 1998 Nov;83(11):3897-904.

72. Pereira AM, Schmid EM, Schutte PJ, Voormolen JH, Biermasz NR, van Thiel SW, et al. High prevalence of long-term cardiovascular, neurological and psychosocial morbidity after treatment for craniopharyngioma. Clin Endocrinol (Oxf). 2005 Feb;62(2):197-204.

73. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, et al. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. Lancet. 2001 Feb 10;357(9254):425-31.

74. Nielsen EH, Lindholm J, Laurberg P. Excess mortality in women with pituitary disease: a meta-analysis. Clin Endocrinol (Oxf). 2007 Nov;67(5):693-7.

75. Lindholm J, Nielsen EH, Bjerre P, Christiansen JS, Hagen C, Juul S, et al. Hypopituitarism and mortality in pituitary adenoma. Clin Endocrinol (Oxf). 2006 Jul;65(1):51-8.

76. Erfurth EM, Siesjo P, Bjork-Eriksson T. Pituitary disease mortality: is it fiction? Pituitary. 2013 Sep;16(3):402-12.

77. Bulow B, Hagmar L, Mikoczy Z, Nordstrom CH, Erfurth EM. Increased cerebrovascular mortality in patients with hypopituitarism. Clin Endocrinol (Oxf). 1997 Jan;46(1):75-81.

78. Brada M, Ashley S, Ford D, Traish D, Burchell L, Rajan B. Cerebrovascular mortality in patients with pituitary adenoma. Clin Endocrinol (Oxf). 2002 Dec;57(6):713-7.

79. Deepak D, Furlong NJ, Wilding JP, MacFarlane IA. Cardiovascular disease, hypertension, dyslipidaemia and obesity in patients with hypothalamic-pituitary disease. Postgrad Med J. 2007 Apr;83(978):277-80.

80. Adachi M, Muroya K, Asakura Y. Unfavorable lipoprotein profile in childhood cancer survivors with suprasellar brain tumors--a high Apo B level and increased small dense LDL-cholesterol. Childs Nerv Syst. 2009 Jun;25(6):669-75.

81. Rakhshani N, Jeffery AS, Schulte F, Barrera M, Atenafu EG, Hamilton JK. Evaluation of a comprehensive care clinic model for children with brain tumor and risk for hypothalamic obesity. Obesity (Silver Spring). 2010 Sep;18(9):1768-74.

82. Nakajima K, Hashimoto E, Kaneda H, Tokushige K, Shiratori K, Hizuka N, et al. Pediatric nonalcoholic steatohepatitis associated with hypopituitarism. J Gastroenterol. 2005 Mar;40(3):312-5.

83. Basenau D, Stehphani U, Fischer G. Development of complete liver cirrhosis in hyperphagia-induced fatty liver. Klin Padiatr. 1994 Jan-Feb;206(1):62-4.

84. Nishizawa H, Iguchi G, Murawaki A, Fukuoka H, Hayashi Y, Kaji H, et al. Nonalcoholic fatty liver disease in adult hypopituitary patients with GH deficiency and the impact of GH replacement therapy. Eur J Endocrinol. 2012 Jul;167(1):67-74.

85. Hong JW, Kim JY, Kim YE, Lee EJ. Metabolic parameters and nonalcoholic fatty liver disease in hypopituitary men. Horm Metab Res. 2011 Jan;43(1):48-54.

86. Honegger J, Barocka A, Sadri B, Fahlbusch R. Neuropsychological results of craniopharyngioma surgery in adults: a prospective study. Surg Neurol. 1998 Jul;50(1):19,28; discussion 28-9.

87. Faglia G. Epidemiology and pathogenesis of pituitary adenomas. Acta Endocrinol (Copenh). 1993 Jul;129 Suppl 1:1-5.

88. Webb C, Prayson RA. Pediatric pituitary adenomas. Arch Pathol Lab Med. 2008 Jan;132(1):77-80.

89. Lafferty AR, Chrousos GP. Pituitary tumors in children and adolescents. J Clin Endocrinol Metab. 1999 Dec;84(12):4317-23.

90. Mukai K, Seljeskog EL, Dehner LP. Pituitary adenomas in patients under 20 years old. A clinicopathological study of 12 cases. J Neurooncol. 1986;4(1):79-89.

91. Lee AG, Sforza PD, Fard AK, Repka MX, Baskin DS, Dauser RC. Pituitary adenoma in children. J Neuroophthalmol. 1998 Jun;18(2):102-5.

92. Mehrazin M. Pituitary tumors in children: clinical analysis of 21 cases. Childs Nerv Syst. 2007 Apr;23(4):391-8.

93. Colao A, Loche S, Cappa M, Di Sarno A, Landi ML, Sarnacchiaro F, et al. Prolactinomas in children and adolescents. Clinical presentation and long-term follow-up. J Clin Endocrinol Metab. 1998 Aug;83(8):2777-80.

94. Tamura T, Tanaka R, Korii K, Okazaki H. Pediatric pituitary adenoma. Endocr J. 2000 Mar;47 Suppl:S95-9.

95. Cannavo S, Venturino M, Curto L, De Menis E, D'Arrigo C, Tita P, et al. Clinical presentation and outcome of pituitary adenomas in teenagers. Clin Endocrinol (Oxf). 2003 Apr;58(4):519-27.

96. Pandey P, Ojha BK, Mahapatra AK. Pediatric pituitary adenoma: a series of 42 patients. J Clin Neurosci. 2005 Feb;12(2):124-7.

97. Mindermann T, Wilson CB. Pediatric pituitary adenomas. Neurosurgery. 1995 Feb;36(2):259,68; discussion 269.

98. Mindermann T, Wilson CB. Pituitary adenomas in childhood and adolescence. J Pediatr Endocrinol Metab. 1995 Apr-Jun;8(2):79-83.

99. Kunwar S, Wilson CB. Pediatric pituitary adenomas. J Clin Endocrinol Metab. 1999 Dec;84(12):4385-9.

100. Dyer EH, Civit T, Visot A, Delalande O, Derome P. Transsphenoidal surgery for pituitary adenomas in children. Neurosurgery. 1994 Feb;34(2):207,12; discussion 212.

101. Hirshfeld-Cytron J, Kim HH. Treatment of infertility in women with pituitary tumors. Expert Rev Anticancer Ther. 2006 Sep;6 Suppl 9:S55-62.

102. Hall R, Manski-Nankervis J, Goni N, Davies MC, Conway GS. Fertility outcomes in women with hypopituitarism. Clin Endocrinol (Oxf). 2006 Jul;65(1):71-4.

103. Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC, et al. Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol. 2009 Jun 1;27(16):2677-85.

104. Lustig RH, Post SR, Srivannaboon K, Rose SR, Danish RK, Burghen GA, et al. Risk factors for the development of obesity in children surviving brain tumors. J Clin Endocrinol Metab. 2003 Feb;88(2):611-6.

105. Park SW, Jung HW, Lee YA, Shin CH, Yang SW, Cheon JE, et al. Tumor origin and growth pattern at diagnosis and surgical hypothalamic damage predict obesity in pediatric craniopharyngioma. J Neurooncol. 2013 Apr 12.

106. Muller HL, Emser A, Faldum A, Bruhnken G, Etavard-Gorris N, Gebhardt U, et al. Longitudinal study on growth and body mass index before and after diagnosis of childhood craniopharyngioma. J Clin Endocrinol Metab. 2004 Jul;89(7):3298-305.

107. Tzanela M, Zianni D, Bilariki K, Vezalis A, Gavalas N, Szabo A, et al. The effect of body mass index on the diagnosis of GH deficiency in patients at risk due to a pituitary insult. Eur J Endocrinol. 2010 Jan;162(1):29-35.

108. National Institute of Clinical Excellence. Full guidelines for: Growth hormone deficiency (adults) - human growth hormone (TA64) [Internet].; 2003 []. Available from: http://www.nice.org.uk.

109. Geffner M, Lundberg M, Koltowska-Haggstrom M, Abs R, Verhelst J, Erfurth EM, et al. Changes in height, weight, and body mass index in children with craniopharyngioma after three years of growth hormone therapy: analysis of KIGS (Pfizer International Growth Database). J Clin Endocrinol Metab. 2004 Nov;89(11):5435-40.

110. DeVile CJ, Grant DB, Hayward RD, Stanhope R. Growth and endocrine sequelae of craniopharyngioma. Arch Dis Child. 1996 Aug;75(2):108-14.

111. Soran H, Wilding J, MacFarlane I. Body weight and prolactinoma: a retrospective study. Int J Obes Relat Metab Disord. 2004 Jan;28(1):183.

112. Greenman Y, Tordjman K, Stern N. Increased body weight associated with prolactin secreting pituitary adenomas: weight loss with normalization of prolactin levels. Clin Endocrinol (Oxf). 1998 May;48(5):547-53.

113. de Vile CJ, Grant DB, Hayward RD, Kendall BE, Neville BG, Stanhope R. Obesity in childhood craniopharyngioma: relation to post-operative hypothalamic damage shown by magnetic resonance imaging. J Clin Endocrinol Metab. 1996 Jul;81(7):2734-7.

114. Trivin C, Busiah K, Mahlaoui N, Recasens C, Souberbielle JC, Zerah M, et al. Childhood craniopharyngioma: greater hypothalamic involvement before surgery is

associated with higher homeostasis model insulin resistance index. BMC Pediatr. 2009 Apr 2;9:24.

115. Meuric S, Brauner R, Trivin C, Souberbielle JC, Zerah M, Sainte-Rose C. Influence of tumor location on the presentation and evolution of craniopharyngiomas. J Neurosurg. 2005 Nov;103(5 Suppl):421-6.

116. Puget S, Garnett M, Wray A, Grill J, Habrand JL, Bodaert N, et al. Pediatric craniopharyngiomas: classification and treatment according to the degree of hypothalamic involvement. J Neurosurg. 2007 Jan;106(1 Suppl):3-12.

117. Muller HL, Gebhardt U, Teske C, Faldum A, Zwiener I, Warmuth-Metz M, et al. Post-operative hypothalamic lesions and obesity in childhood craniopharyngioma: results of the multinational prospective trial KRANIOPHARYNGEOM 2000 after 3-year follow-up. Eur J Endocrinol. 2011 Jul;165(1):17-24.

118. Cohen M, Bartels U, Branson H, Kulkarni AV, Hamilton J. Trends in treatment and outcomes of pediatric craniopharyngioma, 1975-2011. Neuro Oncol. 2013 Mar 13.

119. Van Gompel JJ, Nippoldt TB, Higgins DM, Meyer FB. Magnetic resonance imaging-graded hypothalamic compression in surgically treated adult craniopharyngiomas determining postoperative obesity. Neurosurg Focus. 2010 Apr;28(4):E3.

120. Araujo JR, Martel F. Sibutramine effects on central mechanisms regulating energy homeostasis. Curr Neuropharmacol. 2012 Mar;10(1):49-52.

121. Hamilton JK, Conwell LS, Syme C, Ahmet A, Jeffery A, Daneman D. Hypothalamic Obesity following Craniopharyngioma Surgery: Results of a Pilot Trial of Combined Diazoxide and Metformin Therapy. Int J Pediatr Endocrinol. 2011;2011:417949.

122. Ismail D, O'Connell MA, Zacharin MR. Dexamphetamine use for management of obesity and hypersomnolence following hypothalamic injury. J Pediatr Endocrinol Metab. 2006 Feb;19(2):129-34.

123. 8th International Symposium on Pediatric Neuro-oncology, Rome, Italy, 6-9 May 1998 - Abstracts. CHILDS NERVOUS SYSTEM. 1998;14(9):470-528.

124. Greenway FL, Bray GA. Treatment of hypothalamic obesity with caffeine and ephedrine. Endocr Pract. 2008 Sep;14(6):697-703.

125. Fernandes JK, Klein MJ, Ater JL, Kuttesch JF, Vassilopoulou-Sellin R. Triiodothyronine supplementation for hypothalamic obesity. Metabolism. 2002 Nov;51(11):1381-3.

126. Barber TM, Begbie H, Levy J. The incretin pathway as a new therapeutic target for obesity. Maturitas. 2010 Nov;67(3):197-202.

127. Thondam SK, Cuthbertson DJ, Aditya BS, Macfarlane IA, Wilding JP, Daousi C. A glucagon-like peptide-1 (GLP-1) receptor agonist in the treatment for hypothalamic obesity complicated by type 2 diabetes mellitus. Clin Endocrinol (Oxf). 2012 Oct;77(4):635-7.

128. Simmons JH, Shoemaker AH, Roth CL. Treatment with glucagon-like Peptide-1 agonist exendin-4 in a patient with hypothalamic obesity secondary to intracranial tumor. Horm Res Paediatr. 2012;78(1):54-8.

129. Zoicas F, Droste M, Mayr B, Buchfelder M, Schofl C. GLP-1 analogues as a new treatment option for hypothalamic obesity in adults: report of nine cases. Eur J Endocrinol. 2013 Apr 15;168(5):699-706.

130. Inge TH, Pfluger P, Zeller M, Rose SR, Burget L, Sundararajan S, et al. Gastric bypass surgery for treatment of hypothalamic obesity after craniopharyngioma therapy. Nat Clin Pract Endocrinol Metab. 2007 Aug;3(8):606-9.

131. Schultes B, Ernst B, Schmid F, Thurnheer M. Distal gastric bypass surgery for the treatment of hypothalamic obesity after childhood craniopharyngioma. Eur J Endocrinol. 2009 Jul;161(1):201-6.

132. Rottembourg D, O'Gorman CS, Urbach S, Garneau PY, Langer JC, Van Vliet G, et al. Outcome after bariatric surgery in two adolescents with hypothalamic

obesity following treatment of craniopharyngioma. J Pediatr Endocrinol Metab. 2009 Sep;22(9):867-72.

133. Bender G, Fassnacht M, Jurowich C, Renner T, Thalheimer A, Allolio B. Beneficial results of sleeve gastrectomy in a 12-year old girl with massive hyperphagia after surgery for craniopharyngioma. Obesity Surgery. 2011;21:956.

134. Page-Wilson G, Wardlaw SL, Khandji AG, Korner J. Hypothalamic obesity in patients with craniopharyngioma: treatment approaches and the emerging role of gastric bypass surgery. Pituitary. 2012 Mar;15(1):84-92.

135. Muller HL, Gebhardt U, Wessel V, Schroder S, Kolb R, Sorensen N, et al. First experiences with laparoscopic adjustable gastric banding (LAGB) in the treatment of patients with childhood craniopharyngioma and morbid obesity. Klin Padiatr. 2007 Nov-Dec;219(6):323-5.

136. Gatta B, Nunes ML, Bailacq-Auder C, Etchechoury L, Collet D, Tabarin A. Is bariatric surgery really inefficient in hypothalamic obesity? Clin Endocrinol (Oxf). 2013 Apr;78(4):636-8.

137. Weismann D, Pelka T, Bender G, Jurowich C, Fassnacht M, Thalheimer A, et al. Bariatric surgery for morbid obesity in craniopharyngioma. Clin Endocrinol (Oxf). 2013 Mar;78(3):385-90.

138. Bretault M, Boillot A, Muzard L, Poitou C, Oppert JM, Barsamian C, et al. Bariatric surgery following treatment for craniopharyngioma: a systematic review and individual-level data meta-analysis. J Clin Endocrinol Metab. 2013 Mar 26.

139. Berthoud HR, Lenard NR, Shin AC. Food reward, hyperphagia, and obesity. Am J Physiol Regul Integr Comp Physiol. 2011 Jun;300(6):R1266-77.

140. Berthoud H, Morrison C. The brain, appetite, and obesity. Annu Rev Psychol. 2008;59:55-92.

141. Berthoud H. Metabolic and hedonic drives in the neural control of appetite: who is the boss? Curr Opin Neurobiol. 2011;21:888-96.

142. Berthoud H-, Münzberg H, Morrison CD. Blaming the Brain for Obesity: Integration of Hedonic and Homeostatic Mechanisms. Gastroenterology. 2017 / 05 / 01 /;152(7):1728-38.

143. Berthoud HR. Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. Obesity (Silver Spring). 2006 Aug;14 Suppl 5:197S-200S.

144. Berthoud H, Lenard NR, Shin AC. Food reward, hyperphagia, and obesity. Am J Physiol Regul Integr Comp Physiol. 2011 06;300(6):R1266-77.

145. Neary MT, Batterham RL. Gaining new insights into food reward with functional neuroimaging. Forum Nutr. 2010;63:152-63.

146. Le DS, Chen K, Pannacciulli N, Gluck M, Reiman EM, Krakoff J. Reanalysis of the obesity-related attenuation in the left dorsolateral prefrontal cortex response to a satiating meal using gyral regions-of-interest. J Am Coll Nutr. 2009 Dec;28(6):667-73.

147. Smeets PA, de Graaf C, Stafleu A, van Osch MJ, Nievelstein RA, van der Grond J. Effect of satiety on brain activation during chocolate tasting in men and women. Am J Clin Nutr. 2006 Jun;83(6):1297-305.

148. Page KA, Chan O, Arora J, Belfort-Deaguiar R, Dzuira J, Roehmholdt B, et al. Effects of fructose vs glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways. JAMA. 2013 Jan 2;309(1):63-70.

149. Tataranni PA, Gautier JF, Chen K, Uecker A, Bandy D, Salbe AD, et al. Neuroanatomical correlates of hunger and satiation in humans using positron emission tomography. Proc Natl Acad Sci U S A. 1999 Apr 13;96(8):4569-74.

150. Carnell S, Gibson C, Benson L, Ochner CN, Geliebter A. Neuroimaging and obesity: current knowledge and future directions. Obes Rev. 2012 Jan;13(1):43-56.

151. Lam CK, Chari M, Lam TK. CNS regulation of glucose homeostasis. Physiology (Bethesda). 2009 Jun;24:159-70.

152. Berridge KC, Ho C, Richard JM, DiFeliceantonio AG. Review: The tempted brain eats: Pleasure and desire circuits in obesity and eating disorders. Brain Res. 2010;1350:43-64.

153. De Silva A, Salem V, Matthews PM, Dhillo WS. The Use of Functional MRI to Study Appetite Control in the CNS. EXPERIMENTAL DIABETES RESEARCH. 2012.

154. Burger KS, Berner LA. A functional neuroimaging review of obesity, appetitive hormones and ingestive behavior. Physiol Behav. 2014;136:121-7.

155. Burger KS, Shearrer GE, Sanders AJ. Brain-Based Etiology of Weight Regulation. CURRENT DIABETES REPORTS. 2015;15(11).

156. Francis ST, Eldeghaidy S. Imaging methodologies and applications for nutrition research: what can functional MRI offer? Proc Nutr Soc. 2015 05;74(2):89-98.

157. Farr OM, Tsoukas MA, Triantafyllou G, Dincer F, Filippaios A, Ko B-, et al. Short-term administration of the GLP-1 analog liraglutide decreases circulating leptin and increases GIP levels and these changes are associated with alterations in CNS responses to food cues: A randomized, placebo-controlled, crossover study. Metabolism: Clinical and Experimental. 2016 / 07 / 01 /;65(7):945-53.

158. Val-Laillet D, Aarts E, Weber B, Ferrari M, Quaresima V, L.E. Stoeckel, et al. Neuroimaging and neuromodulation approaches to study eating behavior and prevent and treat eating disorders and obesity. NeuroImage: Clinical, Vol 8, Iss C, Pp 1-31 (2015). 2015:1.

159. Farr OM, Li CR, Mantzoros CS. Central nervous system regulation of eating: Insights from human brain imaging. Metabolism. 2016 05;65(5):699-713.

160. Patriarca L, Magerowski G, Alonso-Alonso M. Functional neuroimaging in obesity. CURR OPIN ENDOCRINOL DIABETES OBESITY. 2017 04;24(2):154-9.

161. Small DM, Zatorre RJ, Dagher A, Evans AC, Jones-Gotman M. Changes in brain activity related to eating chocolate: from pleasure to aversion. Brain. 2001 Sep;124(Pt 9):1720-33.

162. Porubska K, Veit R, Preissl H, Fritsche A, Birbaumer N. Subjective feeling of appetite modulates brain activity: an fMRI study. Neuroimage. 2006 Sep;32(3):1273-80.

163. Cauda F, D'Agata F, Sacco K, Duca S, Geminiani G, Vercelli A. Functional connectivity of the insula in the resting brain. Neuroimage. 2011 Mar 1;55(1):8-23.

164. DelParigi A, Chen K, Salbe AD, Reiman EM, Tataranni PA. Sensory experience of food and obesity: a positron emission tomography study of the brain regions affected by tasting a liquid meal after a prolonged fast. Neuroimage. 2005 Jan 15;24(2):436-43.

165. Ibanez A, Gleichgerrcht E, Manes F. Clinical effects of insular damage in humans. Brain Struct Funct. 2010 Jun;214(5-6):397-410.

166. DelParigi A, Chen K, Salbe AD, Hill JO, Wing RR, Reiman EM, et al. Persistence of abnormal neural responses to a meal in postobese individuals. Int J Obes Relat Metab Disord. 2004 Mar;28(3):370-7.

167. Hinton EC, Parkinson JA, Holland AJ, Arana FS, Roberts AC, Owen AM. Neural contributions to the motivational control of appetite in humans. Eur J Neurosci. 2004 Sep;20(5):1411-8.

168. Gautier JF, Chen K, Salbe AD, Bandy D, Pratley RE, Heiman M, et al. Differential brain responses to satiation in obese and lean men. Diabetes. 2000 May;49(5):838-46.

169. Gautier JF, Del Parigi A, Chen K, Salbe AD, Bandy D, Pratley RE, et al. Effect of satiation on brain activity in obese and lean women. Obes Res. 2001 Nov;9(11):676-84.

170. St-Onge MP, Sy M, Heymsfield SB, Hirsch J. Human cortical specialization for food: a functional magnetic resonance imaging investigation. J Nutr. 2005 May;135(5):1014-8.

171. Holsen LM, Savage CR, Martin LE, Bruce AS, Lepping RJ, Ko E, et al. Importance of reward and prefrontal circuitry in hunger and satiety: Prader-Willi syndrome vs simple obesity. Int J Obes (Lond). 2011 Oct 25.

172. Fuster JM. The prefrontal cortex. [electronic book]. Amsterdam ; Academic Press/Elsevier, 2008; 4th ed; 2008.

173. Le DS, Pannacciulli N, Chen K, Del Parigi A, Salbe AD, Reiman EM, et al. Less activation of the left dorsolateral prefrontal cortex in response to a meal: a feature of obesity. Am J Clin Nutr. 2006 Oct;84(4):725-31.

174. Rolls ET. The functions of the orbitofrontal cortex. Brain Cogn. 2004 Jun;55(1):11-29.

175. Killgore WD, Young AD, Femia LA, Bogorodzki P, Rogowska J, Yurgelun-Todd DA. Cortical and limbic activation during viewing of high- versus low-calorie foods. Neuroimage. 2003 Aug;19(4):1381-94.

176. Simmons WK, Martin A, Barsalou LW. Pictures of appetizing foods activate gustatory cortices for taste and reward. Cereb Cortex. 2005 Oct;15(10):1602-8.

177. Rothemund Y, Preuschhof C, Bohner G, Bauknecht HC, Klingebiel R, Flor H, et al. Differential activation of the dorsal striatum by high-calorie visual food stimuli in obese individuals. Neuroimage. 2007 Aug 15;37(2):410-21.

178. Cornier MA, Von Kaenel SS, Bessesen DH, Tregellas JR. Effects of overfeeding on the neuronal response to visual food cues. Am J Clin Nutr. 2007 Oct;86(4):965-71.

179. Stoeckel LE, Weller RE, Cook EW,3rd, Twieg DB, Knowlton RC, Cox JE. Widespread reward-system activation in obese women in response to pictures of high-calorie foods. Neuroimage. 2008 Jun;41(2):636-47.

180. Schur EA, Kleinhans NM, Goldberg J, Buchwald D, Schwartz MW, Maravilla K. Activation in brain energy regulation and reward centers by food cues varies with choice of visual stimulus. Int J Obes (Lond). 2009 Jun;33(6):653-61.

181. Rosenbaum M, Sy M, Pavlovich K, Leibel RL, Hirsch J. Leptin reverses weight loss-induced changes in regional neural activity responses to visual food stimuli. J Clin Invest. 2008 Jul;118(7):2583-91.

182. Pliquett RU, Fuhrer D, Falk S, Zysset S, von Cramon DY, Stumvoll M. The effects of insulin on the central nervous system--focus on appetite regulation. Horm Metab Res. 2006 Jul;38(7):442-6.

183. Holsen LM, Lawson EA, Blum J, Ko E, Makris N, Fazeli PK, et al. Food motivation circuitry hypoactivation related to hedonic and nonhedonic aspects of hunger and satiety in women with active anorexia nervosa and weight-restored women with anorexia nervosa. J Psychiatry Neurosci. 2012 Sep;37(5):322-32.

184. Ahima RS, Antwi DA. Brain regulation of appetite and satiety. Endocrinol Metab Clin North Am. 2008 Dec;37(4):811-23.

185. Kroemer, N.B. (1,2), Kobiella, A. (1,2), Pilhatsch, M. (1,2), Zimmermann US(1), Smolka, M.N. (1,2), Krebs L(3), et al. Fasting levels of ghrelin covary with the brain response to food pictures. Addict Biol. 2013 / 09 / 01 /;18(5):855-62.

186. Malik S, McGlone F, Bedrossian D, Dagher A. Ghrelin modulates brain activity in areas that control appetitive behavior. Cell Metab. 2008 May;7(5):400-9.

187. Jones RB(1), Astbury N(1), Little TJ(1), Tivey S(1), Lassman DJ(1), McLaughlin J(1), et al. Functional neuroimaging demonstrates that ghrelin inhibits the central nervous system response to ingested lipid. Gut. 2012 / 11 / 01 /;61(11):1543-51.

188. Goldstone AP, Prechtl CG, Scholtz S, Miras AD, Chhina N, Durighel G, et al. Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex, and hippocampal responses to food. Am J Clin Nutr. 2014;99(6):1319-30.

189. Hinton EC, Holland AJ, Gellatly MS, Soni S, Patterson M, Ghatei MA, et al. Neural representations of hunger and satiety in Prader-Willi syndrome. Int J Obes (Lond). 2006 Feb;30(2):313-21.

190. Cornier MA, Salzberg AK, Endly DC, Bessesen DH, Rojas DC, Tregellas JR. The effects of overfeeding on the neuronal response to visual food cues in thin and reduced-obese individuals. PLoS One. 2009 Jul 28;4(7):e6310.

191. Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res. 1985;29(1):71-83.

192. Santel S, Baving L, Krauel K, Munte TF, Rotte M. Hunger and satiety in anorexia nervosa: fMRI during cognitive processing of food pictures. Brain Res. 2006 Oct 9;1114(1):138-48.

193. Baicy K, London ED, Monterosso J, Wong ML, Delibasi T, Sharma A, et al. Leptin replacement alters brain response to food cues in genetically leptin-deficient adults. Proc Natl Acad Sci U S A. 2007 Nov 13;104(46):18276-9.

194. Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC. Leptin regulates striatal regions and human eating behavior. Science. 2007 Sep 7;317(5843):1355.

195. Holsen LM, Zarcone JR, Brooks WM, Butler MG, Thompson TI, Ahluwalia JS, et al. Neural mechanisms underlying hyperphagia in Prader-Willi syndrome. Obesity (Silver Spring). 2006 Jun;14(6):1028-37.

196. van dK, von dH, Keogh JM, Henning E, O'Rahilly S, Lawrence AD, et al. Obesity-Associated Melanocortin-4 Receptor Mutations Are Associated With Changes in the Brain Response to Food Cues. JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM. 2014;99(10):E2101-6.

197. Halford JC, Boyland EJ, Cooper SJ, Dovey TM, Huda MS, Dourish CT, et al. The effects of sibutramine on the microstructure of eating behaviour and energy expenditure in obese women. J Psychopharmacol. 2008 Aug 28.

198. Blundell JE, De Graaf K, Finlayson G, Halford JC, Hetherington M, King N, et al. Measuring food intake, hunger, satiety and satiation in the laboratory. Handbook of assessment methods for eating behaviours and weight-related problems: Measures, theory and research.2nd ed.Newbury Park, CA: Sage. 2009:283-325.

199. Collins CE, Watson J, Burrows T. Measuring dietary intake in children and adolescents in the context of overweight and obesity. Int J Obes. 2010 07;34(7):1103-15.

200. Beaton GH. Approaches to analysis of dietary data: relationship between planned analyses and choice of methodology. Am J Clin Nutr. 1994 01;59(1):253s-61s.

201. French SA, Mitchell NR, Wolfson J, Finlayson G, Blundell JE, Jeffery RW. Questionnaire and laboratory measures of eating behavior. Associations with energy intake and BMI in a community sample of working adults. Appetite. 2014 Jan;72:50-8.

202. Westerterp-Plantenga M, Wouters L, Ten Hoor F. Restrained eating, obesity, and cumulative food intake curves during four-course meals. Appetite. 1991 / 01 / 01 /;16(2):149-58.

203. Kissileff HR, Wentzlaff TH, Guss JL, Walsh BT, Devlin MJ, Thornton JC. A direct measure of satiety disturbance in patients with bulimia nervosa. Physiol Behav. 1996 Oct;60(4):1077-85.

204. Jordan HA. Direct Measurement of Food Intake in Man: a Method for the Objective Study of Eating Behavior. Psychosom Med. 1966;28(6):836-41.

205. Meyer JE, Pudel V. Experimental studies on food-intake in obese and normal weight subjects. J Psychosom Res. 1972 Aug;16(4):305-8.

206. Kissileff HR, Klingsberg G, Van Itallie TB. Universal eating monitor for continuous recording of solid or liquid consumption in man. Am J Physiol. 1980 Jan;238(1):R14-22.

207. Dovey TM, Clark-Carter D, Boyland EJ, Halford JC. A guide to analysing Universal Eating Monitor data: assessing the impact of different analysis techniques. Physiol Behav. 2009 Jan 8;96(1):78-84.

208. Yeomans MR, Lee MD, Gray RW, French SJ. Effects of test-meal palatability on compensatory eating following disguised fat and carbohydrate preloads. Int J Obes Relat Metab Disord. 2001 Aug;25(8):1215-24.

209. Yeomans MR, Lartamo S, Procter EL, Lee MD, Gray RW. The actual, but not labelled, fat content of a soup preload alters short-term appetite in healthy men. Physiol Behav. 2001 Jul;73(4):533-40.

210. Kissileff HR, Pi-Sunyer F, Thornton J, Smith GP. C-terminal octapeptide of cholecystokinin decreases food intake in man. Am J Clin Nutr. 1981 / 01 / 01 /;34(2):154-60.

211. Pi-Sunyer X, Kissileff HR, Thornton J, Smith GP. C-terminal octapeptide of cholecystokinin decreases food intake in obese men. Physiology and Behavior. 1982 / 01 / 01 /;29(4):627-30.

212. Hubel R, Laessle RG, Lehrke S, Jass J. Laboratory measurement of cumulative food intake in humans: results on reliability. Appetite. 2006 Jan;46(1):57-62.

213. Rossner S, Barkeling B, Erlanson-Albertsson C, Larsson P, Wahlin-Boll E. Intravenous enterostatin does not affect single meal food intake in man. Appetite. 1995 Feb;24(1):37-42.

214. Barkeling B, Elfhag K, Rooth P, Rossner S. Short-term effects of sibutramine (Reductil) on appetite and eating behaviour and the long-term therapeutic outcome. Int J Obes Relat Metab Disord. 2003 Jun;27(6):693-700.

215. Linne Y, Barkeling B, Rossner S, Rooth P. Vision and eating behavior. Obes Res. 2002 Feb;10(2):92-5.

216. Lindgren AC, Barkeling B, Hägg A, Ritzén EM, Marcus C, Rössner S. Eating behavior in Prader-Willi syndrome, normal weight, and obese control groups. J Pediatr. 2000 / 07 / 01 /;137(1):50-5.

217. Laessle RG, Lehrke S, Duckers S. Laboratory eating behavior in obesity. Appetite. 2007 Sep;49(2):399-404.

218. Schulz S, Laessle RG. Stress-induced laboratory eating behavior in obese women with binge eating disorder. Appetite. 2012 / 04 / 01 /;58(2):457-61.

219. Yeomans MR. Rating changes over the course of meals: what do they tell us about motivation to eat? Neurosci Biobehav Rev. 2000;24(2):249-59.

220. Kissileff HR, Thornton J, Becker E. A quadratic equation adequately describes the cumulative food intake curve in man. Appetite. 1982 Sep;3(3):255-72.

221. Westerterp-Plantenga M. Eating behavior in humans, characterized by cumulative food intake curves - a review. Neurosci Biobehav Rev. 2000;24(2):239-48.

222. Westerterp KR(1), Nicolson NA(1), Boots JMJ(1), Mordant A(1), Westerterp MS(2). Obesity, restrained eating and the cumulative intake curve. Appetite. 1988 / 10 / 01 /;11(2):119-28.

223. Westerterp-Platenga M, Westerterp KR, Nicolson NA, Mordant A, Schoffelen PFM, Ten Hoor F. The shape of the cumulative food intake curve in humans, during basic and manipulated meals. Physiology and Behavior. 1990 / 01 / 01 /;47(3):569-76.

224. Kissileff HR. Is there an eating disorder in the obese? Ann N Y Acad Sci. 1989;575:410-9.

225. Laessle RG, Uhl H, Lindel B, Müller A. Parental influences on laboratory eating behavior in obese and non-obese children. Int J Obes. 2001 / 01 / 01 /;25:S60-2.

226. Laessle RG, Uhl H, Lindel B. Parental influences on eating behavior in obese and nonobese preadolescents. Int J Eat Disord. 2001 Dec;30(4):447-53.

227. Guss JL, Kissileff HR. Microstructural analyses of human ingestive patterns: from description to mechanistic hypotheses. Neurosci Biobehav Rev. 2000 Mar;24(2):261-8.

228. Kissileff HR, Guss JL. Microstructure of eating behavior in humans. Appetite. 2001 Feb;36(1):70-8.

229. Kral JG, Buckley MC, Kissileff HR, Schaffner F. Metabolic correlates of eating behavior in severe obesity. Int J Obes Relat Metab Disord. 2001 Feb;25(2):258-64.

230. Bobroff EM, Kissileff HR. Effects of changes in palatability on food intake and the cumulative food intake curve in man. Appetite. 1986 / 03 / 01 /;7(1):85-96.

231. Yeomans MR. Palatability and the micro-structure of feeding in humans: the appetizer effect. Appetite. 1996 Oct;27(2):119-33.

232. Robinson, T.M. (1,4), Gray RW(2), Yeomans MR(2), French SJ(3). Test-meal palatability alters the effects of intragastric fat but not carbohydrate preloads on intake and rated appetite in healthy volunteers. Physiology and Behavior. 2005 / 02 / 15 /;84(2):193-203.

233. Yeomans MR(1), Gray RW, Mitchell CJ, True S. Independent effects of palatability and within-meal pauses on intake and appetite ratings in human volunteers. Appetite. 1997 / 08 / 01 /;29(1):61-76.

234. Zandian M, Ioakimidis L, Bergh C, Brodin U, Sodersten P. Decelerated and linear eaters: Effect of eating rate on food intake and satiety. Physiol Behav. 2009;96(2):270-5.

235. Barkeling B, Granfelt Y, Björck I, Rössner S. Effects of carbohydrates in the form of pasta and bread on food intake and satiety in man. Nutr Res. 1995;15(4):467-76.

236. Gray RW, French SJ, Robinson TM, Yeomans MR. Dissociation of the effects of preload volume and energy content on subjective appetite and food intake. Physiol Behav. 2002;76(1):57-64.

237. Thomas JM(1), Higgs S(1), Dourish CT(2). Effects of awareness that food intake is being measured by a universal eating monitor on the consumption of a pasta lunch and a cookie snack in healthy female volunteers. Appetite. 2015 / 09 / 01 /;92:247-51.

238. Burrows T, Golley RK, Khambalia A, McNaughton SA, Magarey A, Rosenkranz RR, et al. The quality of dietary intake methodology and reporting in child and adolescent obesity intervention trials: a systematic review. Obesity Reviews. 2012 12;13(12):1125-38.

239. Magarey A, Watson J, Golley RK, Burrows T, Sutherland R, McNaughton SA, et al. Assessing dietary intake in children and adolescents: Considerations and recommendations for obesity research. International Journal of Pediatric Obesity. 2011 02;6(1):2-11.

240. Crawford PB, Obarzanek E, Morrison J, Sabry ZI. Comparative advantage of 3-day food records over 24-hour recall and 5-day food frequency validated by observation of 9- and 10-year-old girls. J Am Diet Assoc. 1994 Jun;94(6):626-30.

241. White J, Jago R, Thompson JL. Dietary risk factors for the development of insulin resistance in adolescent girls: a 3-year prospective study. Public Health Nutr. 2014 Feb;17(2):361-8.

242. Blundell JE, Stubbs RJ, Golding C, Croden F, Alam R, Whybrow S, et al. Resistance and susceptibility to weight gain: Individual variability in response to a high-fat diet. Physiol Behav. 2005;86:614-22.

243. Sandberg KM, Erford BT, Richards TE. Assessing Common Mental Health and Addiction Issues with Free-access Instruments. Routledge; 2013.

244. Zheng H, Lenard NR, Shin AC, Berthoud HR. Appetite control and energy balance regulation in the modern world: reward-driven brain overrides repletion signals. Int J Obes (Lond). 2009 Jun;33 Suppl 2:S8-13.

245. Steele CA, Cuthbertson DJ, Macfarlane IA, Javadpour M, Das KS, Gilkes C, et al. Hypothalamic obesity: prevalence, associations and longitudinal trends in weight in a specialist adult neuroendocrine clinic. Eur J Endocrinol. 2013 Mar 15;168(4):501-7.

246. Leong KS, Walker AB, Martin I, Wile D, Wilding J, MacFarlane IA. An audit of 500 subcutaneous glucagon stimulation tests to assess growth hormone and ACTH

secretion in patients with hypothalamic–pituitary disease. Clin Endocrinol (Oxf). 2001 04;54(4):463-8.

247. Human growth hormone (somatropin) in adults with growth hormone deficiency [Internet].; 2003 []. Available from: https://www.nice.org.uk/guidance/TA64.

248. Deichmann R, Schwarzbauer C, Turner R. Optimisation of the 3D MDEFT sequence for anatomical brain imaging: technical implications at 1.5 and 3 T. Neuroimage. 2004 Feb;21(2):757-67.

249. Brett M, Anton J, Valabregue R, Poline J. Region of interest analysis using an SPM toolbox [abstract]: 8th International Conference on Functional Mapping of the Human Brain [on CD-ROM]. Neuroimage. 2002;16(2).

250. Hill AJ, Rogers PJ, Blundell JE. Techniques for the experimental measurement of human eating behaviour and food intake: a practical guide. Int J Obes Relat Metab Disord. 1995 Jun;19(6):361-75.

251. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. International Journal of Obesity & Related Metabolic Disorders. 2000 01;24(1):38.

252. Karlsson J, Persson LO, Sjostrom L, Sullivan M. Psychometric properties and factor structure of the Three-Factor Eating Questionnaire (TFEQ) in obese men and women. Results from the Swedish Obese Subjects (SOS) study. Int J Obes Relat Metab Disord. 2000 Dec;24(12):1715-25.

253. Schembre SM. Weight-Related Eating Behavior Questionnaires: Applying Theory to Measurement.
. In: Preedy VR, Watson RR, Martin CR, editors. Handbook of behavior, food and nutrition. New York.: Springer; 2011. p. 3487-506.

254. Bond MJ, McDowell AJ, Wilkinson JY. The measurement of dietary restraint, disinhibition and hunger: an examination of the factor structure of the Three Factor Eating Questionnaire (TFEQ). International Journal of Obesity & Related Metabolic Disorders. 2001 06;25(6):900.

255. Harden CJ, Corfe BM, Richardson JC, Dettmar PW, Paxman JR. Body mass index and age affect Three-Factor Eating Questionnaire scores in male subjects. Nutr Res. 2009;29:379-82.

256. Health Survey for England - 2012, Trend tables [Internet].: Health and Social Care Information Centre.; 2014 []. Available from: http://www.hscic.gov.uk/searchcatalogue?productid=13888&q=hypertension&infotype=0%2fOfficial+statistics&sort=Most+recent&size=10&page=1#top.

257. Holmer H, Ekman B, Bjork J, Nordstom CH, Popovic V, Siversson A, et al. Hypothalamic involvement predicts cardiovascular risk in adults with childhood onset craniopharyngioma on long-term GH therapy. Eur J Endocrinol. 2009 Nov;161(5):671-9.

258. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation. 2006 Feb 14;113(6):898-918.

259. Mersebach H, Klose M, Svendsen OL, Astrup A, Feldt-Rasmussen U. Combined dietary and pharmacological weight management in obese hypopituitary patients. Obes Res. 2004 Nov;12(11):1835-43.

260. Lustig RH. Hypothalamic obesity: causes, consequences, treatment. Pediatr Endocrinol Rev. 2008 Dec;6(2):220-7.

261. Muller HL. Consequences of craniopharyngioma surgery in children. J Clin Endocrinol Metab. 2011 Jul;96(7):1981-91.

262. Roth CL, Gebhardt U, Muller HL. Appetite-regulating hormone changes in patients with craniopharyngioma. Obesity (Silver Spring). 2011 Jan;19(1):36-42.

263. Wang GJ, Volkow ND, Telang F, Jayne M, Ma J, Rao M, et al. Exposure to appetitive food stimuli markedly activates the human brain. Neuroimage. 2004 Apr;21(4):1790-7.

264. Suh DY, Mapstone T. Pediatric supratentorial intraventricular tumors. Neurosurg Focus. 2001 06/15;10(6):E4-.

265. Mindermann T, Wilson CB. Age-related and gender-related occurrence of pituitary adenomas. Clin Endocrinol (Oxf). 1994 Sep;41(3):359-64.

266. Abe T, Ludecke DK, Saeger W. Clinically nonsecreting pituitary adenomas in childhood and adolescence. Neurosurgery. 1998 Apr;42(4):744,50; discussion 750-1.

267. Fertility treatment in 2012: trends and figures [Internet].; 2014 []. Available from: http://www.hfea.gov.uk/104.html.

268. 2011 Census: Population Estimates for the United Kingdom, 27 March 2011 [Internet].; 2012 []. Available from: http://www.ons.gov.uk/ons/rel/census/2011-census/population-and-household-estimates-for-the-united-kingdom/stb-2011-census--population-estimates-for-the-united-kingdom.html.

269. Health Survey for England 2003 [Internet]. []. Available from: http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsStatistics/DH_4098712.

270. Magnussen CG, Venn A, Thomson R, Juonala M, Srinivasan SR, Viikari JS, et al. The association of pediatric low- and high-density lipoprotein cholesterol dyslipidemia classifications and change in dyslipidemia status with carotid intima-media thickness in adulthood evidence from the cardiovascular risk in Young Finns study, the Bogalusa Heart study, and the CDAH (Childhood Determinants of Adult Health) study. J Am Coll Cardiol. 2009 Mar 10;53(10):860-9.

271. McCrindle BW, Urbina EM, Dennison BA, Jacobson MS, Steinberger J, Rocchini AP, et al. Drug therapy of high-risk lipid abnormalities in children and adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee, Council of Cardiovascular Disease in the Young, with the Council on Cardiovascular Nursing. Circulation. 2007 Apr 10;115(14):1948-67.

272. Casanueva FF, Molitch ME, Schlechte JA, Abs R, Bonert V, Bronstein MD, et al. Guidelines of the Pituitary Society for the diagnosis and management of prolactinomas. Clin Endocrinol (Oxf). 2006 Aug;65(2):265-73.

273. Leinung MC, Kane LA, Scheithauer BW, Carpenter PC, Laws ER,Jr, Zimmerman D. Long term follow-up of transsphenoidal surgery for the treatment of Cushing's disease in childhood. J Clin Endocrinol Metab. 1995 Aug;80(8):2475-9.

274. Linglart A, Visot A. Cushing's disease in children and adolescents. Neurochirurgie. 2002 May;48(2-3 Pt 2):271-80.

275. Magiakou MA, Mastorakos G, Oldfield EH, Gomez MT, Doppman JL, Cutler GB,Jr, et al. Cushing's syndrome in children and adolescents. Presentation, diagnosis, and therapy. N Engl J Med. 1994 Sep 8;331(10):629-36.

276. Massoud AF, Powell M, Williams RA, Hindmarsh PC, Brook CG. Transsphenoidal surgery for pituitary tumours. Arch Dis Child. 1997 May;76(5):398-404.

277. Styne DM, Grumbach MM, Kaplan SL, Wilson CB, Conte FA. Treatment of Cushing's disease in childhood and adolescence by transsphenoidal microadenomectomy. N Engl J Med. 1984 Apr 5;310(14):889-93.

278. Partington MD, Davis DH, Laws ER,Jr, Scheithauer BW. Pituitary adenomas in childhood and adolescence. Results of transsphenoidal surgery. J Neurosurg. 1994 Feb;80(2):209-16.

279. Daly AF, Tichomirowa MA, Beckers A. Update on familial pituitary tumors: from multiple endocrine neoplasia type 1 to familial isolated pituitary adenoma. Horm Res. 2009 Jan;71 Suppl 1:105-11.

280. Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, et al. The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas. J Clin Endocrinol Metab. 2008 Jun;93(6):2390-401.

281. Naves LA, Daly AF, Vanbellinghen JF, Casulari LA, Spilioti C, Magalhaes AV, et al. Variable pathological and clinical features of a large Brazilian family harboring

a mutation in the aryl hydrocarbon receptor-interacting protein gene. Eur J Endocrinol. 2007 Oct;157(4):383-91.

282. Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, et al. Pituitary adenoma predisposition caused by germline mutations in the AIP gene. Science. 2006 May 26;312(5777):1228-30.

283. Toledo RA, Lourenco DM,Jr, Liberman B, Cunha-Neto MB, Cavalcanti MG, Moyses CB, et al. Germline mutation in the aryl hydrocarbon receptor interacting protein gene in familial somatotropinoma. J Clin Endocrinol Metab. 2007 May;92(5):1934-7.

284. Cazabat L, Libe R, Perlemoine K, Rene-Corail F, Burnichon N, Gimenez-Roqueplo AP, et al. Germline inactivating mutations of the aryl hydrocarbon receptor-interacting protein gene in a large cohort of sporadic acromegaly: mutations are found in a subset of young patients with macroadenomas. Eur J Endocrinol. 2007 Jul;157(1):1-8.

285. Georgitsi M, De Menis E, Cannavo S, Makinen MJ, Tuppurainen K, Pauletto P, et al. Aryl hydrocarbon receptor interacting protein (AIP) gene mutation analysis in children and adolescents with sporadic pituitary adenomas. Clin Endocrinol (Oxf). 2008 Oct;69(4):621-7.

286. Daly AF, Tichomirowa MA, Beckers A. The epidemiology and genetics of pituitary adenomas. Best Pract Res Clin Endocrinol Metab. 2009 Oct;23(5):543-54.

287. Korbonits M, Storr H, Kumar AV. Familial pituitary adenomas - who should be tested for AIP mutations? Clin Endocrinol (Oxf). 2012 Sep;77(3):351-6.

288. Poldrack RA, Mumford JA, Nichols TE. Preprocessing fMRI data. In: Handbook of Functional MRI Data Analysis. Cambridge: Cambridge University Press; 2011. p. 34-52.

289. Ulmer S, Jansen O. fMRI. [electronic book] : basics and clinical applications. Berlin : Springer, 2013; 2nd ed; 2013.

290. TUKEY JW. Exploratory data analysis. Reading (Mass.): Addison-Wesley, 1977; 1977.

291. Fuhrer D, Zysset S, Stumvoll M. Brain activity in hunger and satiety: an exploratory visually stimulated FMRI study. Obesity (Silver Spring). 2008 May;16(5):945-50.

292. Haase L, Green E, Murphy C. Males and females show differential brain activation to taste when hungry and sated in gustatory and reward areas. Appetite. 2011 Oct;57(2):421-34.

293. Killgore WD, Yurgelun-Todd DA. Body mass predicts orbitofrontal activity during visual presentations of high-calorie foods. Neuroreport. 2005 May 31;16(8):859-63.

294. Alkan A, Sahin I, Keskin L, Cikim AS, Karakas HM, Sigirci A, et al. Diffusion-weighted imaging features of brain in obesity. Magn Reson Imaging. 2008 May;26(4):446-50.

295. Small DM, Zald DH, Jones-Gotman M, Zatorre RJ, Pardo JV, Frey S, et al. Human cortical gustatory areas: A review of functional neuroimaging data. Neuroreport. 1999;10(1):7-14.

296. Stephani C, Vaca G, Maciunas R, Koubeissi M, Luders HO. Functional neuroanatomy of the insular lobe. BRAIN STRUCTURE & FUNCTION. 2011;216(2):137-49.

297. Killgore WD, Yurgelun-Todd DA. Positive affect modulates activity in the visual cortex to images of high calorie foods. Int J Neurosci. 2007 May;117(5):643-53.

298. Kurth F, Zilles K, Fox PT, Laird AR, Eickhoff SB. A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. Brain Struct Funct. 2010 Jun;214(5-6):519-34.

299. Small DM. Taste representation in the human insula. BRAIN STRUCTURE & FUNCTION. 2010;214(5-6):551-61.

300. Tomasi D, Wang GJ, Wang RL, Caparelli EC, Logan J, Volkow ND. Overlapping Patterns of Brain Activation to Food and Cocaine Cues in Cocaine Abusers: Association to Striatal D2/D3 Receptors. Hum Brain Mapp. 2015;36(1):120-36.

301. Burger KS, Stice E. Neural Responsivity During Soft Drink Intake, Anticipation, and Advertisement Exposure in Habitually Consuming Youth. OBESITY. 2014;22(2):441-50.

302. Tomasi D, Gene-Jack Wang, Wang R, Backus W, Geliebter A, Telang F, et al. Association of Body Mass and Brain Activation during Gastric Distention: Implications for Obesity. PLoS ONE. 2009 08;4(8):1-11.

303. Bragulat V, Dzemidzic M, Bruno C, Cox CA, Talavage T, Considine RV, et al. Food-related odor probes of brain reward circuits during hunger: a pilot FMRI study. OBESITY (19307381). 2010 08;18(8):1566-71.

304. Small DM, Gregory MD, Mak YE, Gitelman D, Mesulam MM, Parrish T. Article: Dissociation of Neural Representation of Intensity and Affective Valuation in Human Gustation. Neuron. 2003;39:701-11.

305. Rolls ET. Sensory processing in the brain related to the control of food intake. Proc Nutr Soc. 2007 Feb;66(1):96-112.

306. Roth CL, Aylward E, Liang O, Kleinhans NM, Pauley G, Schur EA. Functional Neuroimaging in Craniopharyngioma: A Useful Tool to Better Understand Hypothalamic Obesity? Obes Facts. 2012 Apr 20;5(2):243-53.

307. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, et al. Gut hormone PYY[sub3-36] physiologically inhibits food intake. Nature. 2002 08/08;418(6898):650.

308. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, et al. Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med. 2003 09/04;349(10):941,948 8p.

309. Frank S, Heni M, Moss A, von Schnurbein J, Fritsche A, Haring HU, et al. Leptin Therapy in a Congenital Leptin-Deficient Patient Leads to Acute and Long-Term Changes in Homeostatic, Reward, and Food-Related Brain Areas. JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM. 2011;96(8):E1283-7.

310. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. J Clin Epidemiol. 1995 12;48(12):1503-10.

311. Peduzzi, P. (1,4,5), Kemper, E. (1,4), Concato, J. (2,3), Feinstem, A.R. (2,3,4), Holford TR(4). A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol. 1996 / 12 / 01 /;49(12):1373-9.

312. Simoneau-Roy J, O'Gorman C, Pencharz P, Adeli K, Daneman D, Hamilton J. Insulin sensitivity and secretion in children and adolescents with hypothalamic obesity following treatment for craniopharyngioma. Clin Endocrinol (Oxf). 2010 Mar;72(3):364-70.

313. Preedy VR, Watson RR, Martin CR. Handbook of behavior, food and nutrition. [electronic book]. New York : Springer, c2011; 2011.

314. Valette M, Bellisle F, Carette C, Poitou C, Dubern B, Paradis G, et al. Eating behaviour in obese patients with melanocortin-4 receptor mutations: a literature review. INT J OBESITY. 2013 08;37(8):1027,1035 9p.

315. Stutzmann F, Tan K, Vatin V, Dina C, Jouret B, Tichet J, et al. Prevalence of melanocortin-4 receptor deficiency in Europeans and their age-dependent penetrance in multigenerational pedigrees. Diabetes. 2008 09;57(9):2511,2518 8p.

316. Stutzmann F, Cauchi S, Durand E, Calvacanti-Proença C, Pigeyre M, Hartikainen AL, et al. Common genetic variation near MC4R is associated with eating behaviour patterns in European populations. INT J OBESITY. 2009 03;33(3):373,378 6p.

317. Asbeck I, Mast M, Bierwag A, Westenhöfer J, Acheson KJ, Müller MJ. Severe underreporting of energy intake in normal weight subjects: use of an appropriate

standard and relation to restrained eating. Public Health Nutr. 2002 10;5(5):683,690 8p.

318. Abbot JM, Thomson CA, Ranger-Moore J, Teixeira PJ, Lohman TG, Taren DL, et al. Psychosocial and behavioral profile and predictors of self-reported energy underreporting in obese middle-aged women. J Am Diet Assoc. 2008 01;108(1):114,119 6p.

319. Miller JL, James GA, Goldstone AP, Couch JA, He G, Driscoll DJ, et al. Enhanced activation of reward mediating prefrontal regions in response to food stimuli in Prader-Willi syndrome. J Neurol Neurosurg Psychiatry. 2007 Jun;78(6):615-9.

320. Bothwell EKG, Ayala GX, Conway TL, Rock CL, Gallo LC, Elder JP. Research: Underreporting of Food Intake among Mexican/Mexican-American Women: Rates and Correlates. J Am Diet Assoc. 2009;109:624-32.

321. Sergi GC. Pooling fMRI data: meta-analysis, mega-analysis and multi-center studies. Frontiers in Neuroinformatics, Vol 3 (2009). 2009.

322. Van Horn JD, Toga AW. Multisite neuroimaging trials. Curr Opin Neurol. 2009 Aug;22(4):370-8.

323. Mueller B. How to do a functional multicenter neuroimaging study, *Proc. 18th Scientif. Mtg. Int. Soc. Magn. Reson. Med.*, 2010-May. Proceedings of the 18th Scientific Meeting of the International Society of Magnetic Resonance in Medicine. 2010.

324. van Bloemendaal L, IJzerman RG, Ten Kulve JS, Barkhof F, Konrad RJ, Drent ML, et al. GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. Diabetes. 2014 Dec;63(12):4186-96.

325. Bruce JM(1), Hancock L(1), Bruce A(1), Lundgren JD(1), Lepping RJ(2), Savage CR(2), et al. Changes in brain activation to food pictures after adjustable gastric banding. Surgery for Obesity and Related Diseases. 2012 / 09 / 01 /;8(5):602-8.

326. Ochner CN, Kwok Y, Conceicao E, Pantazatos SP, Puma LM, Carnell S, et al. Selective reduction in neural responses to high calorie foods following gastric bypass surgery. Ann Surg. 2011 Mar;253(3):502-7.

327. Scholtz S, Miras AD, Chhina N, Prechtl CG, Sleeth ML, Daud NM, et al. Obese patients after gastric bypass surgery have lower brain-hedonic responses to food than after gastric banding. Gut. 2014 Jun;63(6):891-902.

328. Hoffman HJ, De Silva M, Humphreys RP, Drake JM, Smith ML, Blaser SI. Aggressive surgical management of craniopharyngiomas in children. J Neurosurg. 1992 Jan;76(1):47-52.

329. Kalapurakal JA, Goldman S, Hsieh YC, Tomita T, Marymont MH. Clinical outcome in children with craniopharyngioma treated with primary surgery and radiotherapy deferred until relapse. Med Pediatr Oncol. 2003 Apr;40(4):214-8.

330. Poretti A, Grotzer MA, Ribi K, Schonle E, Boltshauser E. Outcome of craniopharyngioma in children: long-term complications and quality of life. Dev Med Child Neurol. 2004 Apr;46(4):220-9.

331. Caldarelli M, Massimi L, Tamburrini G, Cappa M, Di Rocco C. Long-term results of the surgical treatment of craniopharyngioma: the experience at the Policlinico Gemelli, Catholic University, Rome. Childs Nerv Syst. 2005 Aug;21(8-9):747-57.

332. Pierre-Kahn A, Recassens C, Pinto G, Thalassinos C, Chokron S, Soubervielle JC, et al. Social and psycho-intellectual outcome following radical removal of craniopharyngiomas in childhood. A prospective series. Childs Nerv Syst. 2005 Aug;21(8-9):817-24.

333. Tomita T, Bowman RM. Craniopharyngiomas in children: surgical experience at Children's Memorial Hospital. Childs Nerv Syst. 2005 Aug;21(8-9):729-46.

334. Zuccaro G. Radical resection of craniopharyngioma. Childs Nerv Syst. 2005 Aug;21(8-9):679-90.

335. Balde NM, Diallo MM, Poirier JY, Sow MS, Brassier G, Lorcy Y. Long-term outcome of the adult onset craniopharyngiomas. Ann Endocrinol (Paris). 2007 Jun;68(2-3):186-90.

336. Rath SR, Lee S, Kotecha RS, Taylor M, Junckerstorff RC, Choong CS. Childhood craniopharyngioma: 20-year institutional experience in Western Australia. J Paediatr Child Health. 2013 May;49(5):403-8.