Satiety Signalling in Obese Children and Adolescents

Physiological changes with weight loss in children

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The candidate confirms that the work submitted is her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

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Abstract

Background: Studies in adults have shown physiological protection of a 'set-point' for weight, explaining why obese adults who diet eventually regain weight.

Objective: We hypothesised that set-points for weight, and their physiological defence, are flexible in childhood but become fixed around puberty. We aimed to show that obese children who lost weight had less 'reflex' changes in satiety hormone profiles (that would drive weight regain), compared with adolescents who had experienced a similar degree of weight change.

Method: Prospective cohort study. 41 subjects; 21 obese pre-pubertal children (age 3-7 years; 11 male) and 20 obese adolescents (age 14-18 years; 10 male). Obesity defined as BMI >2.4 SDS. Subjects recruited as either 'reducers' (relative/absolute weight loss of ≥ 10% in the preceding 9-15 months) or 'maintainers' (controls). Measures: Resting Energy Expenditure (REE), bioelectrical impedance, and fasting and post-prandial (every 30 minutes for 3 hours) satiety hormone profiles.

Results: Post-pubertal adolescents had 31% lower Ghrelin concentrations (4%-51%, p=0.03) and 50% higher Amylin concentrations than pre-pubertal children (18%-91%, p=0.001). The association between Ghrelin, Amylin and GIP concentration and weight change was similar for both pre and post-pubertal children (p=0.79, p=0.39, p=0.79 respectively). No associations were found for Peptide YY, Pancreatic Polypeptide, or active GLP-1. Regarding satiety, post-pubertal reducers reported less hunger and higher satiety than pre-pubertal children (p<0.05). REE in pre-pubertal weight reducers and maintainers were similar (50kcal lower, -143 to 242, p=0.6) but post-pubertal reducers had 250kcal lower REE compared to post-pubertal maintainers (-68 to 572, p=0.1).

Conclusion: Satiety hormone profiles were similar between pre and post-pubertal subjects, and contrast with adult data where weight reduction leads to sustained increases in Ghrelin and reductions in the other hormones. These findings indicate that the physiological mechanisms which act to protect against weight change in adults develop later than in the adolescent years.

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Abbreviations

WHO	World Health Organisation
NHS	National Health Service
BMI	Body Mass Index
SDS	Standard Deviation Score
IOTF	International Obesity Task Force
USA	United States of America
CT	Computerised Tomography
MRI	Magnetic Resonance Imaging
DEXA	Dual Energy X-Ray Absorptiometry
BIA	Bioelectrical Impedance
FFM	Fat Free Mass
FM	Fat Mass
KOPS	Kiel Obesity Prevention Programme
HyOb	Hypothalamic Obesity
PWS	Prader-Willi-Syndrome
GWAS	Genome Wide Association Studies
LEP	Leptin
LEPR	Leptin Receptor
MC4R	Melanocortin-4-Receptor
POMC	Proopiomelanocortin
BDNF	Brain Derived Neurotrophic Factor
NTKR2	Neurotrophic Tyrosine Kinase 2 Receptor
PCSK1	Prohormone Convertase 1
SIM1	Single-Minded Homolog 1
INSIG2	Insulin Induced Gene 2
FTO	Fat mass and obesity association gene
GOOS	Genetics of Obesity Study
STILTS	Study Into Lean and Thin Subjects
CNS	Central Nervous System
HPG	Hypothalamic Pituitary Gonadal Axis
GnRH	Gonadotrophin Releasing Hormone
LH	Luteinizing Hormone
FSH	Follicle Stimulating Hormone
GIH	Gonadotrophin Inhibitory Hormone
ARC	Arcuate Nucleus
CART	Cocaine and Amphetamine-Regulated Transcript
NPY	Neuropeptide Y
AgRP	Agouti-Related Peptide
PVN	Paraventricular Nucleus
DMN	Dorsomedial Nucleus
LHA	Lateral Hypothalamic Area
VMN	Ventromedial Nucleus
GLP-1	Glucagon Like Peptide-1
NTS	Nucleus of the Tractus Solitarius
GHS-R1	Growth Hormone Secretagogue Receptor 1
STAT-3	Signal Tranducer Activation of Transcription-3
VTA	Ventral Tegmental Area
OXN	Oxyntomodulin
CCK	Cholecystokinin

GI	Gastrointestinal
GIP	Gastroinhibitory Polypeptide
DDP-IV	Dipeptidyl Peptidase IV
JAK	Janus Kinases
RCT	Randomised Control Trial
REE	Resting Energy Expenditure
TEE	Total Energy Expenditure
AEE	Activity Energy Expenditure
TEF	Thermic Effect of Feeding
BMR	Basal Metabolic Rate
D_2O^{18}	Deutronium and Oxygen 18
MG	MedGem® hand-held indirect calorimetry device
RQ	Respiratory Quotient
LBM	Lean Body Mass
ATP	Adenosine Triphosphate
FMI	Fat Mass Index
FFMI	Fat Free Mass Index
TV	Television
NCMP	National Child Measurement Programme
HIV	Human Immunodeficiency Virus
NCEP	National Cholesterol Education Programme
HDL	High Density Lipoprotein
IDF	International Diabetes Federation
FBG	Fasting Blood Glucose
TG	Triglycerides
FFA	Free Fatty Acid
CRP	C-Reactive Protein
IL-6	Interleukin-6
LDL	Low Density Lipoprotein
PAI-1	Plasma Activator Inhibitor-1
TNK-α	Tumour Necrosis Factor-α
T2DM	Type 2 Diabetes Mellitus
OGTT	Oral Glucose Tolerance Test
NAFLD	Non Alcoholic Fatty Liver Disease
NASH	Non Alcoholic Steatohepatitis
AST	Aspartate Amino Transferase
ALT	Alanine Amino Transferase
BP	Blood Pressure
AMBP	Ambulatory Blood Pressure
OSA	Obstructive Sleep Apnoea
SCFE	Slipped Capital Femoral Epiphysis
AHI	Apneoic/Hypopnea Index
mTOR	Mammalian Target of Rapamycin
MEND	Mind Exercise Nutrition Do it!
HOMA	Homeostatic Model Assessment
PHE	Public Health England
FDA	Federal Drug Administration
GLP-1R	Glucagon-Like-1 Receptor
GABA	Gamma-Aminobutyric Acid
RYGB	Roux-en-Y Gastric Bypass
LAGB	Laparoscopic Adjustable Gastric Banding

SG	Sleeve Gastrectomy
SE	Standard Error
WMS	Weight Management Service
COBRA	Childhood Overweight BioRepository of Australia
RCH	Royal Children's Hospital
LMS	Lamda, Mu, Sigma
EER	Estimated Energy Requirements
VAS	Visual Analogue Scale
AUC	Area Under the Curve
OX-SR	Oxyntomodulin Sustained Release
FMT	Faecal Microbiota Transplant
HGC	High Gene Count
LGC	Low Gene Count

Chapter 1 Introduction

1.1 Summary of Thesis Chapters

In this chapter the definitions used in obesity and the different techniques available for measuring obesity are covered. Factors contributing to the aetiology of obesity are detailed such as dietary intake and food types, hypothalamic obesity and genetic elements. The theories underpinning body weight regulation and the evidence supporting them are explored, and the basic science of appetite regulation including the different satiety hormones involved, and factors influencing their production are discussed. The physiology behind pubertal development in males and females and the influence that obesity has on its progression is debated, and finally, different aspects of energy expenditure are discussed, including definitions, measurement with particular emphasis on the accuracy of the Medgem® Handheld indirect calorimeter, and the relationship of resting energy expenditure to obesity and weight loss.

Chapter 2 covers more specific topics pertaining to paediatric obesity and the diagnosis, treatment and management of the condition and its associated co-morbidities are discussed, with special significance given to the metabolic syndrome in children and adolescents. Areas covered include the prevalence of paediatric obesity, the impact of diet and lifestyle choices, the influence of socioeconomic factors and the effectiveness of obesity treatment programmes. Current pharmacological treatments and potential future therapies are discussed, and also the different surgical options available in adolescents, including how satiety hormone responses and weight regulation are affected by the different surgical procedures.

The clinical implications of the set point theory and how it differs in children and adults are discussed in Chapter 3. The paper published in the New England Journal of Medicine by *Sumithran et a*l 2011, detailing the long-term persistence of hormonal adaptations to weight loss in obese adults is explored, and how the findings from this paper formed the concept behind this research study.

There were 3 distinct parts to the research study, the first of which is included in chapter 3, and was a scoping project looking at auxological data collected from all male patients attending the Weight Management Service from 2008

to 2014 at The Royal Children's Hospital in Melbourne. The scoping project highlighted that there were insufficient post-pubertal obese male patients, which is why the decision was made to recruit female participants as well. The conclusions from the scoping project helped to inform the aims and outcomes of the thesis.

The aim of the research was to carry out a pilot study to observe how satiety signalling varies in obese children and adolescents for a given level of preceding BMI SDS change, and to compare future weight trajectories and associated metabolic adaptations.

Chapter 4 details all aspects of the empirical research study. Firstly the methodology, with particular emphasis on how the 41 participants were recruited and how the study groups were chosen, and then information regarding data collection, coupled with the measures used to collect the data. This is followed by a comprehensive section on the statistics used to analyse the data and why certain statistical models were chosen, namely linear mixed-effect models.

The results, in particular with respect to the satiety hormone responses recorded in the 4 different groups are presented, and the discussion concludes that there were no significant differences found in satiety hormone profiles between the pre and post-pubertal groups, so the findings did not support the study hypothesis.

The third part of the empirical study is in chapter 5 and involves the follow-up period, where 23 of the original 41 participants had their resting energy expenditure and auxological measures repeated in clinic, on average 11 months after they had their satiety hormone profiles carried out. The methodology is listed, but owing to the smaller participant numbers, the statistical analysis of the data was limited. The influence of fat free mass on resting energy expenditure is discussed, and how it changes with increasing age and puberty.

Study results showed that pre-pubertal children had a higher resting energy expenditure adjusted for weight and fat free mass compared to post-pubertal children, which would help them to maintain a lower weight trajectory and prevent weight regain. In obese adolescents, physical activity, particularly if it promotes substantial fat oxidation, is important for weight maintenance after diet induced weight loss, and larger studies are still needed to explore the relationship between weight loss and resting energy expenditure in pre and post-pubertal adolescents.

The final chapter starts by revisiting the hypothesis for the study, namely that set-points for weight and their physiological defence are flexible in childhood but become fixed around puberty. This is followed by a summary of the research findings and how they relate to the hypothesis. While distinct variations were found in several specific hormones between the pre and post-pubertal groups, there were no demonstrable differences in satiety hormone profiles between the two groups. These findings did not support our hypothesis and suggested that the physiological mechanisms involving satiety hormone responses which act to protect against weight change develop later than in the adolescent years.

The resting energy expenditure results however found that the obese adolescents who lost weight experienced a similar reduction in resting energy expenditure to obese adults who lose weight, which would act to promote weight regain, and these findings did support the hypothesis, and is an area for further research development.

The future direction of obesity research is then discussed, including new and exciting pharmacotherapy developments with satiety hormones, successful public health initiatives from other countries, electronic implants to supress appetite, and new and interesting theories regarding body weight regulation.

The thesis concludes by looking at the overall picture of paediatric obesity and describes all of the factors that would need to be addressed in order to cure the condition.

1.2 Aetiology of Obesity

The World Health Organisation (WHO) defines obesity as “abnormal or excessive fat accumulation that may impair health” (1). However, the National Health Service (NHS) defines obesity as “when a person is carrying too much body fat for their height and sex” (2), and uses Body Mass Index (BMI) to categorise individuals into overweight, obese and morbidly obese groups. Currently there is no statutory definition of obesity, and the aetiology of obesity is complex and has been attributed to several different factors.

1.2.1 Definition of Obesity

Unlike in adults, where there are universally accepted parameters of obesity, there is greater variation in defining “overweight” and “obesity” in children, owing to the fact that childhood is a time of growth and development (3).

BMI, which is the weight in kilograms divided by the square of the height in metres, is expressed as a Standard Deviation Score (SDS) or Z score, which allows for comparisons between children of different ages and sex.

An adult aged 18 years or over, would be classified as obese if their BMI exceeded 30kg/m^2, and overweight if their BMI was greater than 25kg/m^2. As a child’s BMI will change considerably from birth to adulthood, fixed cut-offs, such as those used in adults, cannot be applied in children. Instead thresholds are used, which are derived from a reference population, and they take into account the child’s age and sex. They are calculated by weighing and measuring a large sample of children in order to provide average BMI values for male and female children at different ages. An individual child’s measurements can then be compared to the reference population, and the degree of variation from the expected value determined. A child’s BMI Z score or SDS therefore indicates how many units (of the standard deviation) a child’s BMI is above or below the average BMI value for their age group and sex (3).

Many countries have their own population specific thresholds for assessing BMI in children, so there are a number of child growth references available. There are however certain child growth references that are more commonly used in the UK and internationally.

In the UK, the British 1990 growth reference (UK90) is used for population monitoring and clinical assessment in children aged ≥ 4 years, with the WHO (World Health Organisation) child growth standard used for children from 0-4

years. The UK90 BMI reference provides centile curves for BMI for British children from 0-23 years, based on 32,222 measurements taken between 1978 and 1994. The BMI cut-offs used are:

- 2nd centile for underweight,
- 85th and 91st centile for overweight for population monitoring and clinical assessment respectively, and
- 95th and 98th centile for obese for population monitoring and clinical assessment respectively.

Most published prevalence figures in the UK are based on the population surveillance thresholds of 85th and 95th centiles of the UK90 growth reference, while the 91st and 98th centiles of the UK90 reference are generally used to classify children as overweight and obese and in need of clinically intervention, although a diagnosis of obesity or overweight is rarely made on BMI measurements alone. The UK90 thresholds are rarely used outside of the UK(4, 5).

In contrast, the International Obesity Task Force thresholds (IOTF) are widely used internationally. The IOTF thresholds are taken from BMI data from 6 large cross-sectional surveys from Brazil, U.K, Hong Kong, the Netherlands, Singapore and the United States, and includes samples taken from 192,727 children aged 0-25 years. Three grades of thinness are defined from equivalent adult BMIs of 16,17 and 18.5, having been derived by extrapolating from the adult BMI cut-offs of 25kg/m^2 and 30Kg/m^2 for overweight and obese respectively, and taking into account age and sex (6, 7). While the advantage of using the IOTF thresholds are that it allows international comparisons of levels of obesity in children to be made, there are disadvantages to averaging the centiles of several countries. It has been highlighted that the international definitions may not be appropriate to use with national data as they exaggerate the difference in prevalence between girls and boys, especially in under 5 year olds (8). Concerns have also been raised about the sensitivity of the IOTF values which are adequate at the lower overweight cut-off but are less robust at the obesity cut-off values (9). Consequently it has been recommended that for single country studies, definitions compatible with national reference curves would be more appropriate (8).

The WHO 2007 growth reference was derived from a combination of the Multicentre Growth Reference Study (1997-2003) with samples from countries including Brazil, Ghana, India, Norway, Oman and the United States, in children aged 0-5 years, and pooled growth data from the USA National Centre for Health Statistics (1977) in 1 – 24 years olds. The WHO BMI

thresholds used are based on a single standard deviation spacing, with thinness < -2 SDS, Overweight between +1 SDS and < +2 SDS and Obese > +2 SDS. Although the WHO 2007 growth reference is used internationally it is not used as widely as the IOTF thresholds.

The relevance of which growth reference data are used becomes important when comparing results from different studies, as it is vital to compare like with like. In this study, the UK90 growth reference were used, as this was felt to be the most appropriate for the cohort being studied.

However, there is a lack of consensus regarding the definition of BMI in children, with various cut-off criteria based on difference reference populations.

1.2.2 Measures of Obesity

Obesity refers to a state where excess fat is stored in adipose tissue (10). BMI is a good measure of adiposity but it is limited by the fact that it cannot distinguish between fat and fat-free components of body weight, and can only give an indirect estimate of total body fat. In addition, as the distribution and percentage of fat varies depending on an individual's sex and ethnicity, the significance of a particular BMI will also vary. Another disadvantage of BMI, is that height, which is one of the components in calculating BMI, is dependent on sex, age and relative leg length, and this is particularly relevant in growing children (11).

However, BMI measurements remain a useful first point of problem identification, are easy to calculate and provide a useful routine screening tool. Current BMI cut-offs have relatively high specificity, but lower sensitivity, which means that non-obese children are unlikely to be wrongly diagnosed, whereas obese children may be missed (11).

Different techniques have been adopted to measure fat in people, and they can be categorised depending on the general principle on which they are based into anthropometric, density-based, diagnostic imaging and bioelectrical impedance.

The optimal technique to measure fat would be accurate, precise, accessible, inexpensive, acceptable, and well documented. While many of the commonly used reference methods such as Computerised Tomography (CT), Magnetic Resonance Imaging (MRI), densitometry and Dual Energy X-ray Absorptiometry (DEXA) fulfil the accuracy criterion, commonly encountered

problems include the cost, radiation, training of operators and accessibility to equipment, such that the optimal measurement technique, which is able to satisfy all the above criteria, does not exist (12).

1.2.2.1 Anthropometric measures

These are simple and inexpensive proxy measures of overall and central fatness, which do not have optimal accuracy and are unable to distinguish between fat mass and fat-free mass (12).

1.2.2.1.1 Waist circumference

Central fat, which is also described as intra-abdominal or visceral fat, is more pathogenic i.e. associated with more co-morbidities, and there is evidence that excess fat in children and adolescents is more likely to accumulate in the abdominal region (13) .

Waist circumference measurement is a useful tool in the assessment of central obesity in overweight and obese individuals, as it is more closely correlated with intra-abdominal fat content and cardiovascular risk factors (14). However, training is required to perform waist circumference measurement accurately and different techniques exist. A common technique is to use a non-elastic measuring tape, applied horizontally to the ground and under tension, between the iliac crest and costal arch, following expiration by the patient (figure 1.1). The mean value from two measurements should be used to improve accuracy (15).

Waist circumference is also subject to age, sex and ethnic specific alterations. While in adults, abdominal obesity is defined by waist circumferences of ≥80cm in females and ≥94cm in males (16), there is no uniform cut-off value in children and adolescents, and many countries have their own specific percentiles for waist circumferences. In Australia, published standardised techniques exist for waist circumference measurements comparing them to reference data generated specifically from Australian children (17).

Standard waist circumference measures may however miss an apron of fat termed panniculus, and it has been shown that pannicular rather than standard waist circumference measurements better correlate with absolute measures of fat mass, and their change over time in clinically overweight/obese youth (figure 1.1) (18).

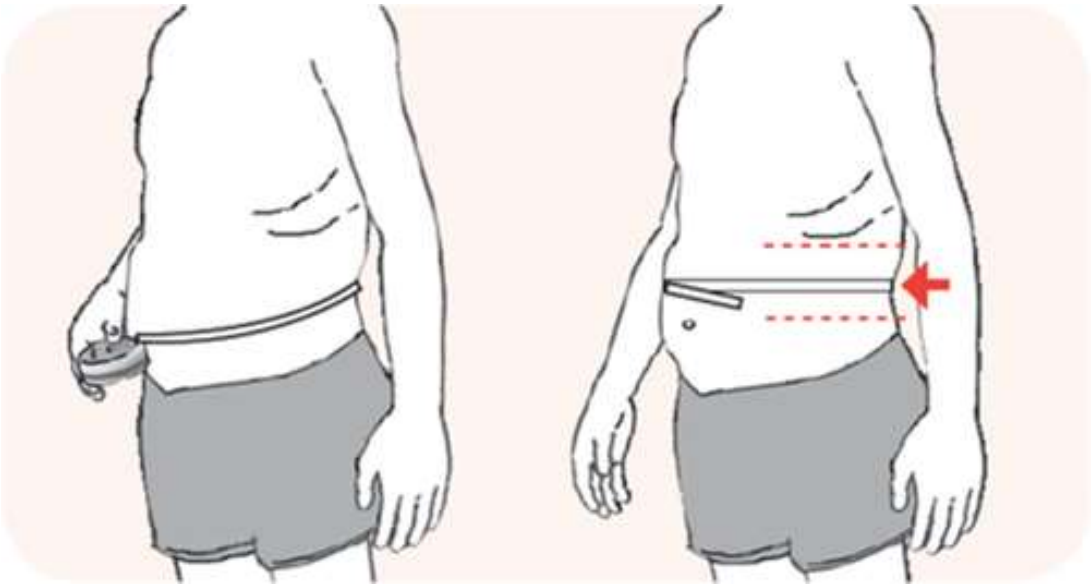


Figure 1.1. Pannicular (left) versus standard (right) measurements of waist circumference. (18)

As indicated by the red arrow, the tape position remains between the lower costal border and the iliac crest (dotted lines) in both measurements of waist circumference, but pannicular waist measurements include any apron of fat, whereas the tape is kept horizontal in the standard measurement.

1.2.2.1.2 Waist to hip and waist to height ratios

In adults, a greater waist to hip ratio indicates a relatively larger amount of abdominal fat, although it is influenced by several other factors, and is a poorer measure of body fat distribution in children (11). However, waist to height ratios are now being used more frequently in children to assess fat distribution as they have the advantage of being more age dependent, and a uniform cut-off of 0.5 can be applied i.e. cardiometabolic risk factors are increased if the weight to height ration is > 0.5 (19, 20).

1.2.2.1.3 Skin-fold thickness

Subcutaneous fat can be used as an indicator of total fat, and can be measured by firmly grasping a fold of skin with callipers, such as the triceps skinfold, and raising it with no muscle included (figure 1.2). Skinfolds from a variety of sites can be included, representing both peripheral and trunk area, and equations can be applied to predict percentage body fat. While this is a cheap and relatively simple measurement to perform, some patients may be reluctant to undress to have it carried out, and measure reproducibility can be difficult, particularly if the patient is very obese (11).

Figure 1.2. Measurement of Triceps skinfold

1.2.2.2 Body composition

1.2.2.2.1 Hydrodensitometry

If the density (weight per unit volume) of a human body is known, then the relative proportions of fat and fat-free mass can be estimated by using specific equations (21). Hydrodensitometry involves weighing a patient both inside and outside of a large tank of water, and is based on Archimedes' principle, that if the density of an object exceeds that of water then it will sink. Fat is less dense than water, whereas fat-free tissue (bone and muscle) is more dense than water. While this is often considered to be the gold standard for body composition assessment, it is time consuming, difficult to carry out in certain patients such as children, and is predominately used in research settings (11).

1.2.2.2.2 MRI (Magnetic Resonance Imaging) and CT (Computerised Tomography) Scans

MRI and CT scans can assess not only the overall fat mass but also its regional distribution, by using strong electromagnetic fields and a series of x-rays respectively. While they can both produce cross sectional high-resolution images, they are expensive to use, and CT scans involve radiation exposure (11).

1.2.2.2.3 Dual Energy X-ray Absorptiometry (DEXA)

DEXA is used to calculate both fat and fat-free mass, and total and regional body composition, using a series of transverse scans with low energy X-ray

beams moving across the body inch by inch, collected by an external detector. The beams are differentially absorbed by various tissues in the body. DEXA has a high degree of accuracy compared to hydrodensitometry, and is primarily used to measure bone mineral density, although it can be used to indirectly measure fat mass. DEXA is simple and non-invasive and can be used over a wide range of ages and body sizes, although its use in assessing adiposity in children is mostly limited to research settings, as there is a cost implication, radiation, and it requires technical expertise (11).

1.2.2.2.4 Bioelectrical Impedance (BIA)

The human body consists primarily of water with ions, through which an electric current can flow, and water in the body is either extracellular (approximately 45%) or intracellular (approximately 55%). The body also contains non-conducting materials, such as body fat, which provides resistance to the flow of current, with adipose tissue being significantly less conductive than bone or muscle. Bioelectrical impedance is based on the principle that electric current flows at different rates through the body depending on its composition, and there is a direct relationship between the concentrations of ions and the electrical conductivity, and an indirect relationship between the ion concentration and the resistance of the solution (22).

Impedance is a drop in voltage when a small constant current, with a fixed frequency, passes between electrodes spanning the body (22). Lean body mass and fat mass can be calculated from the difference in conductivity. Advances in technology in BIA over the last decade have included increasing the number of contact electrodes from four to eight, using multi-frequency electrical levels to estimate the intracellular and extracellular fluids, and incorporating a digital scale to measure body mass (23).

BIA is simple to perform, quick to carry out and non-invasive. The results are available immediately and are reproducible with <1% error when repeated. It also gives reliable measurements of body composition with minimal inter and intra-observer variability (22). However, there are factors which impact on the BIA results. BIA measurements assume that the body is a cylindrical shaped ionic conductor with homogenous composition, a fixed cross-sectional area, and a uniform distribution of current density. In reality, the human body is not uniform in length, cross-sectional area or ionic composition, and ethnic variation in terms of different body density and proportional limb length also influences the accuracy of BIA measurements, and needs to be taken into account. Empirical equations have been developed for estimating Fat Free

Mass (FFM) and Body cell mass by using sex, age, weight, height and race, but are not accurate when a generalised equation is applied for different ethnic groups (24).

BIA measurements are affected by the consumption of food and drink, which is why an overnight fast prior to taking readings is recommended. Results can also be altered by moderate to intense physical activity 2-3 hours before the measurement, as well as the ambient temperature of the room (cold increases impedance). In addition, certain individual characteristics, such as where in the menstrual cycle a women is, may affect results, but this can be more challenging to standardise (22).

1.2.2.2.5 Tanita BC-418 versus DEXA

The Tanita BC-418 MA segmental body composition analyser (Tanita, Japan) is a single frequency hand to foot BIA device that uses 8 electrodes (figure 1.3). It uses a single-point load cell weighing system in the scale platform, and estimates body composition in the standing position, and measures whole body and segmental estimates of fat (right arm, left arm, trunk, right leg, left leg). It uses an algorithm incorporating impedance, age, gender and height to estimate percentage Fat Mass (%FM) and categorizes individuals into two activity levels: standard and athlete. The athlete mode is characterised as a person 17 years or above, who should be involved in intense aerobic exercises for at least 10 hours a week, with a resting pulse rate of <60 bpm (23). The precision (coefficient of variation) of repeated measures for the Tanita BC-418 is on average 0.3% for %FM.

Figure 1.3. The Tanita BC-418 bioelectrical impedance device

There are conflicting results regarding single frequency BIA, as some studies have shown that it is a valid estimator of body composition in healthy individuals, while others state that it overestimates the FFM, thereby underestimating %FM of the obese and overestimating %FM of athletes. The criterion for selecting the athletic mode in the Tanita BC-418 is also questionable as it may not capture individuals who are more physically active than mainstream population, but do not meet the criteria as suggested by the manufacturer.

Compared to DEXA, BIA provides systematically lower values for truncal and total %FM. The discrepancies increased with the degree of adiposity, suggesting that the accuracy of BIA is negatively influenced by obesity, although this was more evident in BIA devices using 4 electrodes rather than 8 electrodes. Results from a study comparing DEXA (Prodigy GE Lunar model) specifically to the Tanita BC-418 found that the Tanita provided on average 2-6% lower values for %FM in men with a normal BMI and in women in all BMI categories (23). However, the study used the Prodigy GE Lunar DEXA as the reference standard, but the relative validity of this compared to a four component DEXA model (4C: water, bone mineral mass, fat and residual), which provides a more accurate measurement of FM and FFM, reported a non-differential overestimate of %FM compared to the four compartment model i.e. the Prodigy GE Lunar provided a higher estimate of %FM (23).

1.2.3 Lifestyle Induced Obesity

Children need a careful balance between energy consumption and energy expenditure in order to maintain their recommended body weight, and to ensure adequate growth (figure 1.4). However, a small but persistent average daily energy imbalance can cause an individual to become overweight, because over a number of years it can lead to considerable gains in body weight. In childhood, the energy requirements vary during the different development stages, and there are several nutritional factors such as dietary behaviour, food selection and nutrient supply, which contribute to the development of adiposity.

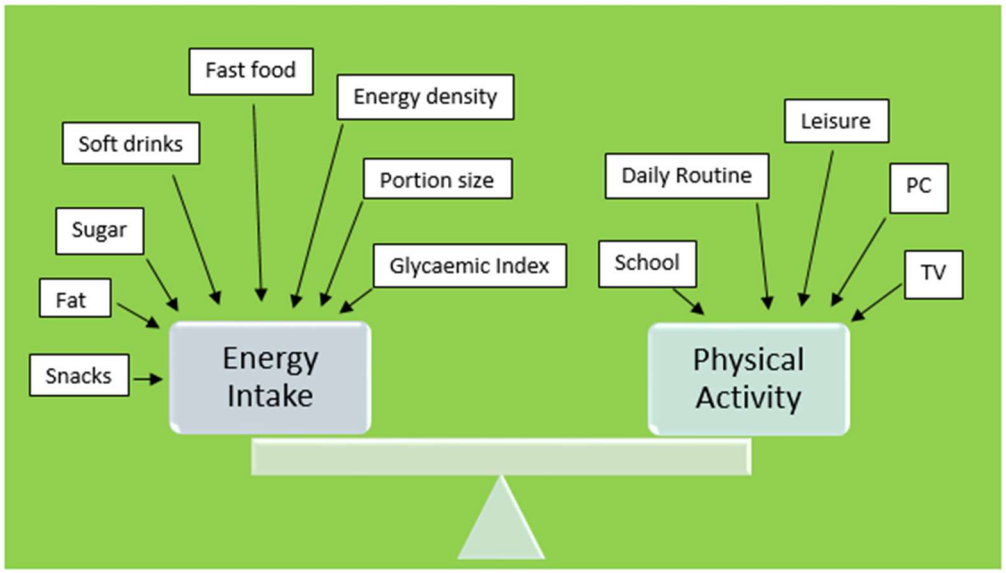


Figure 1.4. Components of dietary energy intake and energy expenditure that may impact the development of overweight and obesity in children and adolescents.

1.2.3.1 Nutrition

The offspring of overweight and obese women are at increased risk of being born large for gestational age, and of becoming overweight or obese as children and adults. The risk relates to maternal pre-conceptual weight, weight gain during pregnancy and maternal glucose metabolism during pregnancy and breastfeeding (25).

During infancy, dietary intake has a greater impact on nutrient balance than energy expenditure. Early childhood feeding appears to be a major contributor

to childhood obesity. The type of infant feeding (breast milk vs formula milk) and also the rate of weight gain in infancy in the first year of life, are important factors linked to weight status in later childhood. A meta-analysis comparing breastfed with non-breastfed infants found a slight decrease in the odds of becoming overweight due to breast feeding (26). In addition, high protein intake from formula in infants is associated with a higher BMI, which is evident until 6 years of age (27). There is also some evidence that the early introduction of solid food when weaning infants may increase the odds of overweight in children (26).

In the Kiel Obesity Prevention Study (KOPS), energy gain was calculated from the collected longitudinal data in normal weight children who maintained their weight and in those who became overweight. The mean energy gains were approximately 25 kcal/day for the children aged 6-10 years and approximately 40 kcal/day for those aged 10 to 14 years (28). Therefore in children and adolescents, a small but regular decrease in daily energy intake, ideally combined with a moderate increase in daily physical energy expenditure, would in theory be enough to stop the progression of obesity. However, a Cochrane systematic review of 55 published interventions to prevent obesity in children found on average a small but significant relative reduction in BMI or a 0.3kg relative reduction during the intervention period (29), (as all children will gain weight as they grow, comparisons must be relative). Although an intervention achieving 0.3kg relative weight reduction would be of value in younger children, in older children a 0.3kg weight reduction would only represent 3-4% of the average change needed (to no longer be overweight or obese), and it would be an even lower percentage if the intervention were targeted at older children who were overweight or obese (30).

1.2.3.2 Food types

An important factor contributing to the nutritional cause of childhood overweight and obesity is the energy density of food, which is defined as the energy content (in kcal of KJ) per unit weight (g or 100g). Foods with a greater energy density are typically high in sugar and/or fat, contain little water or dietary fibre and are often low in micronutrients. Children and adolescents obtain approximately 20% of their energy intake from the added sugars in foods and beverages, with the long-term high consumption of sugar-sweetened drinks in particular being associated with high body weight in adolescents, especially in females (31).

Fruits and vegetables have a high water and dietary fibre content, which makes them low energy density foods. Their consumption helps to reduce total energy intake by displacing high energy density foods from the diet, and the reduction in the glycaemic load of food may also alter the postprandial hormones involved in satiety initiation (32). Increasing dietary water intake is another way to reduce energy by displacing the consumption of sugar-sweetened beverages. However, the association between increasing water intake and reducing daily energy by decreasing food intake is difficult to prove, and studies have yielded inconsistent results, primarily because of a lack of good quality studies (33) .

It has also been suggested that increasing the amount of dairy in the diet leads to reduced adiposity in children (34).

1.2.3.3 Environmental factors

Parental dietary intake and familial nutritional behaviour are important components in the development of childhood nutritional habits. The parental intake of fruit and vegetables for example is positively correlated with the consumption of these foods in their offspring (35). Family meals are also associated with a number of health benefits for children, and significant associations have been found between positive family and parent level food related dynamics (parental food positive reinforcement, group enjoyment, food communication) and reduced risk of childhood obesity (36). Parents are encouraged to emphasize healthy food choices rather than restrictive eating patterns and to advocate moderation rather than overconsumption of food (37).

The frequency of eating is also important, and children and adolescents who eat breakfast have more favourable adiposity indices and better nutrient intake than those who skip breakfast (38). In fact, observational studies have shown that adolescents, particularly males, who eat more frequently are more likely to have a lower body weight during childhood (39). However, it is essential that food portion sizes are also taken into consideration, as multiple, well controlled studies have shown that providing subjects with larger portions of food in a research setting leads to significantly higher energy intake (40, 41), although participants reported similar ratings of hunger and fullness despite eating larger portions of food (40). In children, portion size alone can account for 17 – 19% of the variance in energy intake (42), and children with a higher

BMI have been shown to consume portions of food that were as much as 100% larger than those consumed by children with a lower BMI (43).

There are several factors influencing why particular foods are selected, and food choices are usually made on the basis of taste, cost, convenience (which refers to the time spent on buying, preparing and cooking food) and to a lesser extent health and variety (44). Regarding the increasing risk of obesity, portion size in particular appears to be the primary determinant of energy intake, more so than energy density (42). Increasing portion sizes are particularly evident in pre-prepared convenience foods, and these foods are often high in fat, high in energy density, deficient in nutrients and have a greater number of food additives and flavourings. In addition, the practise of supersizing portions is relatively common in fast food outlets, with French fries and sugary drinks being typical items chosen. Another reason for the increased consumption of convenience foods is that in many developed countries they are relatively cheap, which is an important consideration when there are economic constraints, whereas the more nutrient dense lean meats, fish, fresh vegetables and fruit, often cost more (45). In a recent study looking at the household availability of ultra-processed foods and obesity in nineteen European countries between 1991 – 2008, the average household availability of ultra-processed foods ranged from 10.2% in Portugal to 50.4% in the UK. The study found a significant positive association between national household availability of ultra-processed foods and national prevalence of obesity among adults, and highlighted the need for public policies to promote the consumption of unprocessed foods, while also making ultra-processed foods less available and affordable (46).

The food industry also targets children and adolescents through their marketing of convenience foods, and with early and repetitive exposure to advertisements, with the goal being to develop brand loyalty at a young age (47).

1.2.3.4 Management and prevention

To date, no country has managed to reverse its obesity epidemic, and while there have been areas of improvement, this is predominately from the plateauing of childhood obesity in countries where the prevalence was high (47). Based on research and practice, there is a consensus regarding the core policy actions that are needed to promote healthy diets, and these have been brought together in the NOURISHING framework, created by the World

Cancer Research Fund International (48). The framework identifies 3 domains (food environment, the food system, and behaviour-change communication), which cover 10 areas where policy actions can be taken, and the NOURISHING framework can be adapted to different countries around the world (figure 1.5).

In order to prevent the development of obesity, it is important to take into consideration dietary patterns. For children and adolescents (2-18 year olds), it has been recommended that slow absorbing carbohydrates and plant-based foods should be the main dietary constituents, and plain water should be the main source of fluids. In addition, children should eat at least 4 meals a day, which should include breakfast, and the consumption of convenience food is discouraged (49).

Domain		Policy area	Examples of potential policy actions
Food environment	N	Nutrition label standards and regulations on the use of claims and implied claims on foods	eg, nutrient lists on food packages; clearly visible "interpretive" and calorie labels; menu, shelf labels; rules on nutrient and health claims
	O	Offer healthy foods and set standards in public institutions and other specific settings	eg, fruit and vegetable programmes; standards in education, work, health facilities; award schemes; choice architecture
	U	Use economic tools to address food affordability and purchase incentives	eg, targeted subsidies; price promotions at point of sale; unit pricing; health-related food taxes
	R	Restrict food advertising and other forms of commercial promotion	eg, restrict advertising to children that promotes unhealthy diets in all forms of media; sales promotions; packaging; sponsorship
	I	Improve the nutritional quality of the whole food supply	eg, reformulation to reduce salt and fats; elimination of trans fats; reduce energy density of processed foods; portion size limits
	S	Set incentives and rules to create a healthy retail and food service environment	eg, incentives for shops to locate in underserved areas; planning restrictions on food outlets; in-store promotions
Food system	H	Harness the food supply chain and actions across sectors to ensure coherence with health	eg, supply-chain incentives for production; public procurement through "short" chains; health-in-all policies; governance structures for multi-sectoral engagement
Behaviour-change communication	I	Inform people about food and nutrition through public awareness	eg, education about food-based dietary guidelines, mass media, social marketing; community and public information campaigns
	N	Nutrition advice and counselling in health-care settings	eg, nutrition advice for at-risk individuals; telephone advice and support; clinical guidelines for health professionals on effective interventions for nutrition
	G	Give nutrition education and skills	eg, nutrition, cooking/food production skills on education curricula; workplace health schemes; health literacy programmes

Figure 1.5. World Cancer Research Fund International NOURISHING framework (food policy framework for healthy diets and the prevention of obesity in children and adolescents).

To have balanced diet, several different food types need to be combined from each of the main food groups, in the correct quantities. This information can be presented visually in different ways, one of which is the Food Pyramid, which was originally pioneered by the Harvard School of Public Health in America (50), and this has been adapted by the Irish Department of Health to provide guidance for adults, teenagers and children aged 5 years and over (figure 1.6). It also includes a serving size guide based on the hand rule, where the palm of the hand represents the recommended serving size of meat or

fish, whereas a clenched fist represents the amount of carbohydrate. In the UK, similar information is provided in the Eatwell guide, which uses a pie chart to represent the types of foods that should be eaten and in what proportion (figure 1.7) (51).

Environmental factors associated with childhood obesity risk also need to be addressed, such as the marketing of foods aimed at children, regulating the nutritional quality of foods and their availability in schools, taxing sugar-sweetened beverages and labelling the front of packages with nutritional values, to name a few.

Children from overweight and/or low socioeconomic status parents also have an increased risk of childhood obesity, and future intervention need to give specific attention to these children (52).

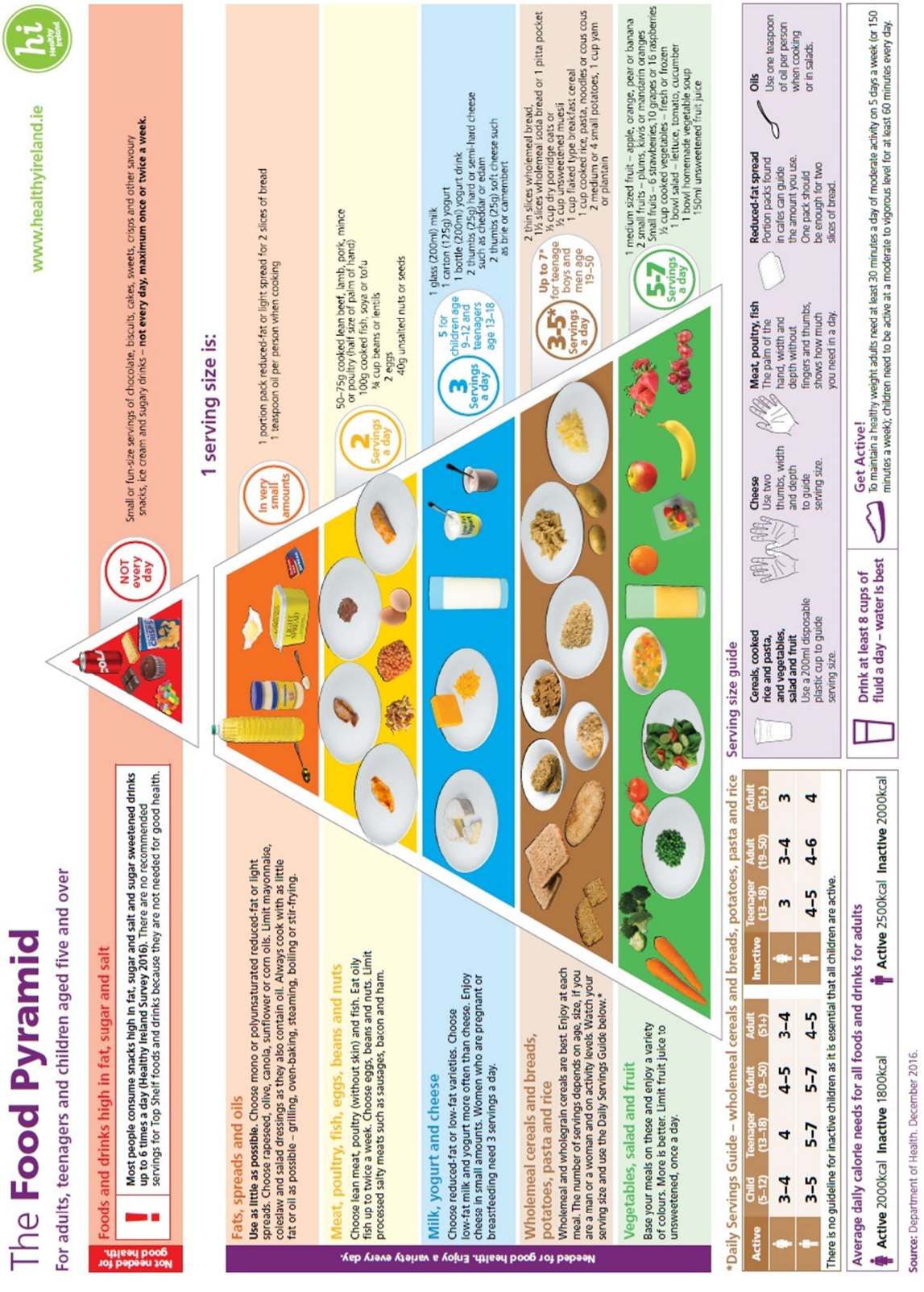


Figure 1.6. The Food Pyramid for adults, teenagers and children over 5 years

Figure 1.7. The Eatwell guide (used in the UK to give a visual representation of the types and proportions of foods needed for a healthy and well balanced diet).

1.2.4 Hypothalamic Obesity

Hypothalamic Obesity (HyOb) has been defined as an intractable form of obesity that was initially described in patients with hypothalamic tumours and surgical damage. However, obesity that develops after a variety of insults to the hypothalamus, such as infections, trauma, infiltration, vascular problems and hydrocephalus, is now included in the definition, as well as acquired or congenital functional defects in central energy homeostasis (53).

1.2.4.1 Aetiology

The mechanisms underlying HyOb are complex and multifactorial, but it is damage to the Ventromedial hypothalamus that causes weight gain as a result of hyperphagia. Hypothalamic damage in HyOb also compromises other functions of the hypothalamus and can lead to a lower resting metabolic rate, hypomobility, autonomic imbalance, insomnia and secondary hypopituitarism (growth hormone deficiency, hypogonadotrophic hypogonadism, secondary adrenal insufficiency, central hypothyroidism and diabetes insipidus) (53).

Leptin, which is a hormone synthesized in fat cells, is involved in the regulation of food intake and energy expenditure, by acting as a signal from the peripheral circulatory system to the hypothalamus. In HyOb, the disruption of leptin signalling, caused by the death of neurones in the ventromedial hypothalamus, prevents the integration of afferent leptin signalling, so that patients do not experience satiety or a sense of energy sufficiency (53). Consequently, children with HyOb exhibit intense hyperphagia with food seeking behaviours, which results in excessive weight gain, and occurs despite forced calorie restriction. The disruption in leptin signally is also associated with increased vagal tone, which results in insulin hypersecretion, which is in contrast to simple common obesity, where peripheral insulin resistance is the primary defect, leading to a compensatory β-cell response. Children with HyOb therefore have a higher insulin response to glycaemic load, although they frequently have normal fasting insulin levels (54).

Craniopharyngiomas and Hypothalamic Obesity

The survival rate for patients with Craniopharyngiomas, which are sellar embryonic malformations of low grade histological malignancy and low incidence (0.5-2/million/year), is high (92%). However, the quality of survival is frequently affected by HyOb, with 40-50% of patients with Craniopharyngiomas suffering from obesity or eating disorders following surgery. It is therefore vital that hypothalamic integrity is preserved in the surgical strategies used to treat craniopharyngiomas, and neurosurgeons now prefer stereotactic biopsy with radiation as the initial treatment of these tumours (55).

Prader-Willi Syndrome

Obesity is the leading cause of morbidity and mortality in Prader-Willi Syndrome (PWS), which is a genetic neurological disorder due to loss of paternally expressed, maternally imprinted genes within the long arm of chromosome 15 (q11 – q13), occurring in 1 in 16,000 births (56). These genes are widely expressed throughout the brain and hypothalamus. PWS has distinct nutritional phases, and hyperphagia and obesity develop after an initial phase of poor feeding and hypotonia. Although PWS is the most common cause of syndromal obesity, the exact mechanism for the development of obesity in PWS is still largely unknown, but it is thought that disruption in hypothalamic pathways of satiety control, in conjunction with abnormal satiety hormone responses to food intake, lead to hyperphagia and obesity (57). Patients with PWS have markedly increased levels of the satiety hormone Ghrelin (which is an appetite stimulant), in both the fasting and post-prandial

state when compared to controls (3-4 fold increase). However, the evidence linking hyperghrelinaemia with the development of hyperphagia in children with PWS is contradictory, and possible alternate causes of elevated ghrelin levels may be abnormal parasympathetic vagal innervation of the stomach and sympathetic tone (58). These patients also have reduced energy expenditure because of hypotonia, and altered behaviour, such as temper tantrums if food is restricted. Obesity in PWS can only be prevented by strict supervision of food intake, and so far pharmalogical treatments of hyperphagia and surgical procedures have proved ineffective or with significant side effects e.g. Octreotide and increased risk of gallstone formation (59) .

1.2.4.2 Treatment of Hypothalamic Obesity

HyOb is usually progressive and unresponsive to attempts to modify lifestyle through diet and exercise. Pharmacological treatment is also difficult and while several agents have been used to treat HyOb for limited periods (Octreotide, Triiodothyronine, Growth Hormone), no pharmaceutical therapy has had a consistent positive effect, although GLP-1 analogues may offer a new treatment of moderate to severe HyOb (60).

It has been suggested that HyOb could be regarded as an extreme form of common obesity, as the efferent signals downstream of the leptin receptor neurone are similarly attenuated, therefore the study of HyOb has the potential to provide further clarification on the pathogenesis of common obesity in the general population (53).

1.2.5 Genetics of Obesity

Obesity is caused by both genetic and non-genetic factors. While the prevalence of rare monogenic forms of obesity has not increased significantly, there has been an increase in the prevalence of common obesity, which is a complex polygenic disease with both genetic and environmental components (61).

1.2.5.1 Monogenic and Polygenic Obesity

The evidence for the genetic component to obesity comes from family, twin and adoption studies. In one study, which was designed to assess genetic and

environmental influences involving 114 monozygotic twins, 81 dizygotic twins and 98 virtual twins (same age but unrelated siblings), the genetic variance contributed approximately 65% to heritability of BMI, while environmental factors contributed to the remaining balance (62). In childhood obesity, one of the crucial predictors for an increased risk is parental obesity, with one study involving 8,234 children demonstrating a four-fold increased risk of childhood obesity if one parent was obese and a 10 fold increased risk if both parents were obese (63). In addition, heritability estimates increase from early childhood through adolescence, owing to the genetic susceptibility genes interacting more strongly with environmental factors, such as the easy access to high calorie foods and the general reduction in energy expenditure (64).

There is also a growing body of evidence demonstrating ethnic differences in the genetic predisposition to obesity, although many of the genetic variants responsible remain unidentified. Notable differences in the prevalence of obesity have been observed across diverse ethnic groups, with the Oslo immigration health study finding the highest prevalence of obesity among Turks (51%) and the lowest prevalence among the Vietnamese (2.7%), despite adjusting for lifestyle and socio-demographic factors (65). The Pima Indians of Arizona, have the highest prevalence of obesity (64% in men and 75% in women), and are an example of the detrimental effects of transitioning from a traditional farming lifestyle to a more modern sedentary lifestyle (66). It is hypothesised that they possess thrifty genes, which were thought to exist in certain groups of people with hunter-gatherer evolutionary lifestyles, who had experienced periods of plenty and of famine, which had resulted in the natural selection of thrifty genes. These genes encode proteins that are involved in maintaining energy balance i.e. the conversion of food calories into fat when supplies are plentiful, but these genes no longer provide a survival advantage against starvation and instead they make the population more susceptible to obesity (67) .

Explaining the heritability of common polygenic obesity has been a challenge because of the polygenic nature of the condition, combined with the strong influence of environmental factors.

The two main approaches to investigate the genetics of obesity have been:

1) **Candidate Gene Approach**: the analysis of candidate genes based on their known biological function related to regulation of metabolism.
2) **Genome Wide Strategies**: This includes *linkage analysis*, where family based genome wide linkage scans assess the co-segregation of highly polymorphic genetic markers with a phenotypic trait/disease.

While this has been very efficient for monogenic forms of obesity, it has been less successful in polygenic obesity. This has led to the development of more advanced molecular biological techniques, and researchers are now able to employ *Genome-Wide Association Studies (GWAS),* which are a powerful tool in identifying genetic variants with a moderate effect size, and so far more than 30 polymorphisms that are significantly associated with obesity have been revealed (68) .

1.2.5.2 Childhood Obesity Susceptibility Genes

Based on distinct genetic and phenotypic characteristics, there are 3 different types of childhood obesity, which are syndromic, non-syndromic and common obesity (69).

Syndromic Obesity

Well-known examples of syndromic obesity include Prader-Willi, Alstrom, Carpenter, Rubinstein-Taybi and Bardet-Biedel. There are approximately 30 unidentified susceptibility genes responsible for rare monogenic forms of syndromic obesity, and generally, these children have extreme adiposity, intellectual disability and are physically dysmorphic (68). They can also have undefined neuroendocrine abnormalities, which may adversely affect the function of the hypothalamus, which has an important role in regulating energy balance. Children with syndromic obesity often have severe hyperphagia and reduce satiety, which then promotes weight gain. These syndromes often have complex genetics with several overlapping and undefined loci, which are thought to contribute to the altered regulation of energy balance (70).

Regarding possible gene-diet interaction, a study involving patients with Prader-Willi Syndrome demonstrated that weight gain could be reduced and prevented by giving a low fat and modified carbohydrate diet (25% protein, 20% fat and 55% modified carbohydrate). Children with Prader-Willi Syndrome have delayed gastric emptying because of ineffective stomach contractions. The diet was effective because of the increased absorption of carbohydrates, which then prevented hypoglycaemia and food craving behaviour (71).

Non-Syndromic Obesity

Non-syndromic obesity is defined as weight gain in the absence of other clinical symptoms. There are approximately 8 susceptibility genes that are responsible for rare monogenic forms of non-syndromic obesity, and they

include Leptin (LEP), Leptin Receptor (LEPR), Melanocortin-4 Receptor (MC4R), Proopiomelanocortin (POMC), Brain-Derived Neurotrophic factor (BDNF), Neurotrophic Tyrosine Kinase Receptor Type 2 (NTKR2), Prohormone Convertase 1 (PCSK1) and Single-minded Homolog 1 (SIM1) (69). These genes code for proteins that are involved in integrating peripheral and central neural signals in the hypothalamus via the leptin/melanocortin pathway, so are responsible for maintaining energy balance by regulating food intake and energy expenditure (72). Mutations in these genes cause severe hyperphagia and reduced satiety, which then leads to severe childhood obesity. While the restriction of a high fat diet may initially be partially successful in non-syndromic obesity, in the long-term weight reduction is difficult to maintain and is usually unsuccessful (69).

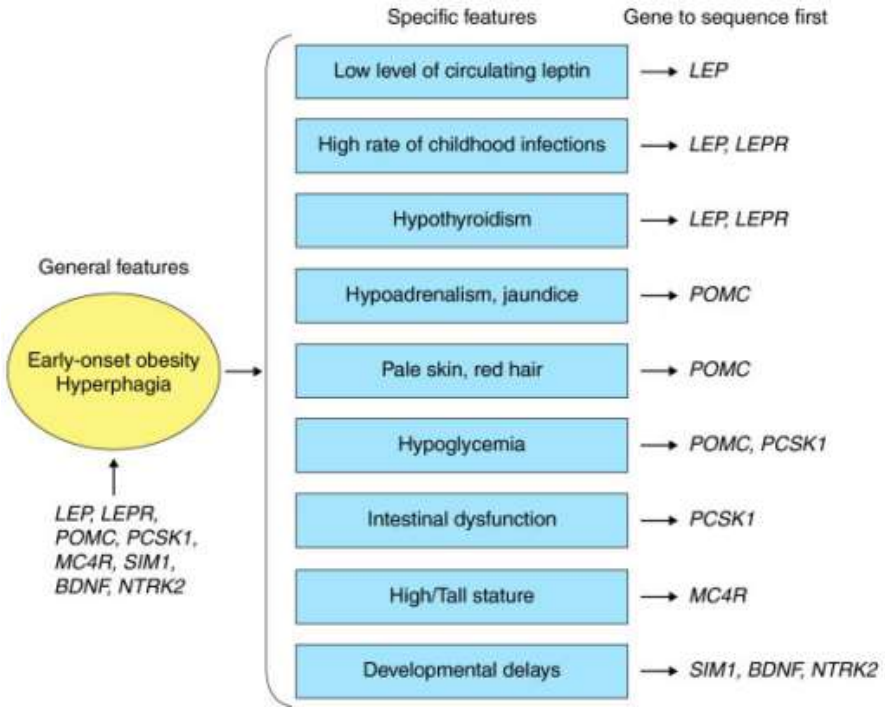


Figure 1.8. Prioritisation for screening for monogenic obesity in children with early-onset severe obesity and hyperphagia. (73)

Common Polygenic Obesity

The GWAS approach enables a more comprehensive and unbiased strategy to identify causal genes related to obesity. It is also well established that in non-coding regions of the genome there are regulatory elements called enhancers and silencers, as well as genetics variants, which disrupt these elements and could confer susceptibility to certain complex diseases such as obesity (74).

Since 2007, many genetic loci have been implicated for BMI from the outcome of GWAS. Insulin-induced gene 2 (INSIG2) was the first locus to have a role in obesity to be reported using this method, although replication attempts yielded inconsistent outcomes (74). The fat mass and obesity association gene (FTO) was the second locus to be identified, and its primary influence is on BMI determination, which then in turn impairs glycaemic control, although the mechanism by which variants in FTO influences the risk of obesity remains largely unknown (75). Evidence that FTO is directly involved in the regulation of energy intake and metabolism comes from studies involving both FTO knockout and FTO overexpression mouse models, where lack of FTO expression leads to leanness, whereas enhanced expression of FTO leads to obesity (76, 77) .

In childhood obesity, children's phenotypes are less affected by co-morbidities, treatment and environmental factors, which is in contrast to adults. Children also provide the opportunity to uncover genetic factors determining the risk of obesity, as the chronology of events and changes in metabolic alterations which ultimately result in obesity, can be recorded. Regarding genetic research studies in children, there have been fewer studies carried out compared to adults, and the studies have mainly focused on the replication of associations observed in adults i.e. variants of FTO and MC4R. However, two new loci (SGCCAG8 and TNKS/MSRA) have been identified for body weight regulation in a joint analysis of GWAS data for early onset extreme obesity (BMI ≥ 99th), in French and German study groups (78). As these two loci have never shown up in meta-analysis of GWAS on obesity in adults, they may represent susceptibility loci exclusively influencing childhood obesity.

1.2.5.3 GOOS (Genetics Of Obesity Study)

Following the discovery of Congenital Leptin Deficiency caused by mutations in the Leptin gene, the Genetics of Obesity Study (GOOS) was established (79). Led by Professor Farooqi, it aims to inform therapeutic strategies by understanding the fundamental mechanisms controlling body weight. To date, approximately 7000 patients have been recruited and 12 different gene disorders have been identified that can cause severe obesity in childhood. Recruitment criteria to the study includes severe obesity (BMI SDS >3), and early onset (before 10 years of age), with a particular interest in consanguineous families, families with a history of early obesity and children with developmental delay. In addition, the group are interested in exploring

how and why some individuals remain thin despite living in an obesogenic environment. So far, 2000 people (BMI < 18kg/m^2) have been recruited to a UK cohort called STILTS (Study Into Lean and Thin Subjects) to explore the genes that protect them from gaining weight, and may allow them to burn calories more easily.

1.3 Biology of Weight Regulation

Historically, obesity has been viewed as a behavioural disorder, caused by a disrupted pattern of food intake. As such, the behavioural therapies employed to treat obesity have focused on dieting and modifying existing eating behaviours, and these have constituted the most common forms of treatment. However, the resistance of obesity to such lifestyle modifications has raised questions about the origin of obesity, and that obesity may actually be the natural physiological state for some individuals.

Although the body weight of members of the general population can vary substantially, an individual's body weight is relatively stable and varies typically only 0.5% over a 6-10 week period, with even weight changes over longer periods remaining relatively modest (80). This stability in body weight is suggestive of a homeostatic control, similar to body water and body temperature control. However, the only way to maintain a stable level of body weight is to balance the daily intake of energy with its expenditure. Therefore, if body weight is regulated, we would expect to see body energy perturbations to be met by compensatory adjustments in the intake and/or energy expenditure, leading to the active defence of an individual's weight in order to maintain it at its normal level (81).

An individual's set-point for regulated body weight appears to be adjustable and will vary considerably over the life course in conjunction to naturally occurring physiological changes. The reason why adolescents and young adults appear to defend against weight loss more than children and older adults may simply represent an evolutionary adaptation associated with reproductive status, as future fertility requires the achievement and maintenance of a critical body weight/ or fat mass (82). In addition, it has been shown that the body is more efficient in protecting against weight loss during caloric deprivation compared to conditions of weight gain with overfeeding, which also suggests an adaptive role of protection in times of low food intake (83). Experimentally, by manipulating specific hypothalamic sites, the set-point for body weight can be adjusted, which highlights the primary role that

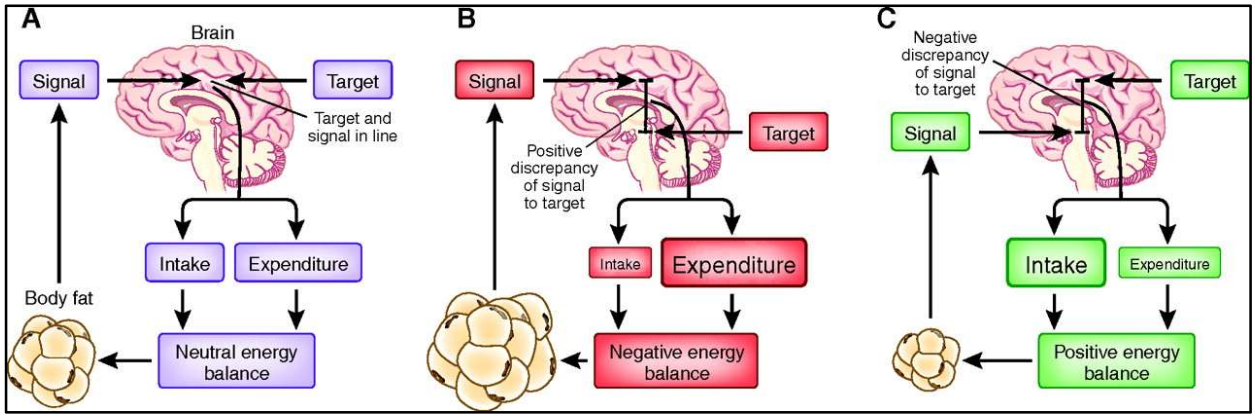
hypothalamic mechanisms play in setting the level at which individuals regulate body weight, with genetic, environmental and dietary influences on body weight also being expressed through these mechanisms (80).

1.3.1 Set-point theory of body weight regulation

It has been suggested that adults have a weight that is inherently determined and this is referred to as a 'set-point' for weight, and occurs through a combination of genetics, lifestyle and environmental factors (83). Several studies have shown that this set-point for body weight is strongly defended, despite variability in energy intake and expenditure (83).

The set-point regulation model based on the concept of a negative feedback system around a target set-point, was first proposed in 1953 by Kennedy (figure 1.9) (84). However, it was the discovery of leptin in 1994, a hormone which is predominately produced by adipocytes and which interacts with receptors in areas of the brain that are linked to the regulation of energy balance, that provided compelling molecular evidence to support the feedback mechanism, and helped to re-establish Kennedy's model for the regulation of body fatness, with leptin in its central role (84). In addition, the discovery of individuals with loss of function mutations in the gene encoding leptin, or in other genes in the neural pathways downstream from leptin, also provided support for the set-point model, as these individuals were extremely hyperphagic and obese (72).

Figure 1.9. Set-point theory



The Lipostatic model of body fat regulation based on a negative feedback system around a target set-point (84).

1.3.1.1 Evidence to support set-point theory

There are many experimental studies in both animal and human models to support the existence of the set-point theory and the active defence of body weight. In rat experiments involving the restriction of the daily caloric intake for a group of male rats over several weeks to restrict their growth, once allowed to feed freely the rats quickly restored their weight to a level appropriate to their age and gender (85). Also, in a different study where rats had their body weights experimentally elevated by electrical stimulation of the lateral hypothalamus, a similar rapid and precise restoration to a normal body weight was observed once they were allowed to feed freely (86). Similar studies in humans have replicated these results, such as the semi-starvation study of *Keys et al*, where 32 male participants aged between 22 and 33 years, of normal weight, demonstrated a hyperphagic response to significant weight loss (25% of their body weight over a 24 week period) once released from their dietary restrictions, and the weight that they lost was regained in a relatively short time (87). Comparable studies involving people gaining weight have found that when overfeeding ceases, they lose the accumulated fat and return to a level approximating their original fatness, and also modulate their energy expenditure to resist the change in their food intake. These findings support the set-point model because the amount of weight loss or gain is less, and the speed that the weight returns to its baseline value is faster, than would be predicted in a passive system which is only regulated by unchanging average intake and expenditure levels, and it strongly suggests there is some active control over intake that is related to changes in body composition i.e. a discrepancy between adiposity and a set-point target (84). Indeed this phenomenon of weight regain following acute weight loss, and the failure of dieting as a long term strategy to tackle sustained weight loss, is often explained by using the set-point model and its defence of a specific body weight.

1.3.1.2 Evidence against set-point theory

Opponents of the set-point theory highlight its inability to account for the social and environmental factors associated with obesity, specifically the increasing prevalence of obesity that has occurred over the last 40 years, or why obesity tends to occur more frequently in certain groups, such as the least affluent members of Western populations (88). One explanation that has been suggested for the gradual increase in prevalence may be that people with high

metabolic susceptibility are the first to experience weight gain in response to an increasingly obesogenic environment.

In addition, certain somatic and neuropsychiatric diseases and disorders such as tumour cachexia or anorexia nervosa, can rapidly affect weight gain or loss, implying that the tight regulatory system within the set-point model, can be substantially perturbed, and these perturbations can have long-term implications for body weight (84).

Although individuals with mutations in the gene encoding leptin are obese, the majority of obese humans do not have any mutations in the leptin gene (89). In fact obese individuals with large levels of stored lipids actually produce abundant amounts of leptin, and while daily injections of leptin reduce body mass in a dose-dependent manner, the effect is much smaller than would be expected if a set-point system with leptin at the centre was in place (84).

Finally, fat mass is integral to the set-point theory of body weight regulation. However, fat mass only accounts for a fraction of total body mass (ranging from as low as 5% to > 45%). The relatively constant body weight experienced by healthy individuals cannot be solely explained by the feedback loop between adipose tissue and the Central Nervous System (CNS), and if body weight is closely regulated then fat free mass must also be tightly regulated (84).

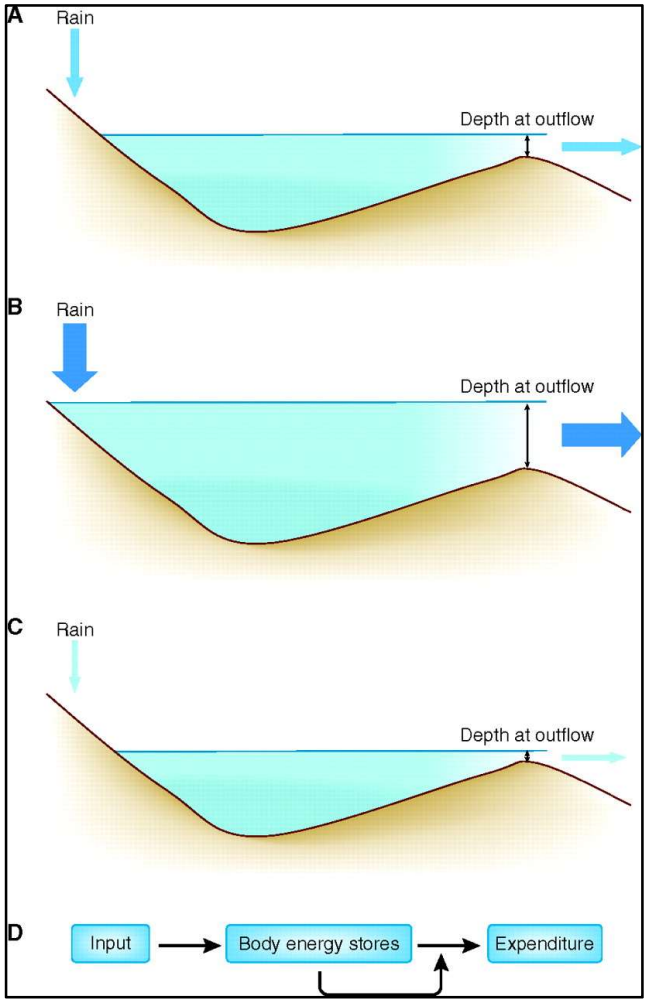
1.3.2 Alternative theories of body weight regulation

An alternative theory is the settling point model, which proposes that there is little active regulation towards a predefined body weight, but that body weight settles based on a number of factors, represented by the individual's genetic predisposition, their interaction with their environment, and socioeconomic factors such as diet and lifestyle. Body weight then drifts around the level at which the group of factors that determine energy expenditure and food consumption achieve an equilibrium (83, 90). An individual's body weight would therefore remain stable as long as there were no long lasting changes in any of the factors that influence it. Therefore, using the settling point model, an obese person should experience a downward drift of their settling point without active resistance from the body, if they make long-term changes in their eating or exercising habits (83). An analogy for body weight regulation in the settling point model is the level of water in a lake, where a natural equilibrium is present due to extra inflow of water, and the water in the lake will rise until outflow equals inflow (figure 1.10). The body energy stores

represent the lake, while rain is translated into energy input, and depth at outflow represents energy expenditure (90). Studies in humans involving subjects switching from a normal fat diet to a low fat diet eaten *ad libitum*, found that subjects lost body weight, but then regained their body weight when they returned to a normal fat diet, but did not exhibit any persistent effects of overfeeding (91), which would be expected in the set-point theory. However, in the settling point model it is difficult to establish exactly which factors are responsible for keeping weight balance, and it is unlikely that there is one single contributor. Therefore, while this model adequately accommodates social and environmental factors and gives an explanation for the world-wide increase in overweight and obesity, it struggles to take into account biological and genetic influences (84).

Consequently, newer models for body weight regulation have been put forward, such as the “general model of intake regulation” and the “dual intervention point model”. These models aim to combine the uncompensated factors of the settling point model with the negative feedback concept, which is inherent in the set-point model (84).

Figure 1.10. Settling point system using levels of water in a lake.(84)



A - In this schematic, the input to the lake is rain falling in the hills. The output of water from the lake is directly related to the depth of water at the outflow. The depth of the water in the lake reaches a settling point at which the outflow is equal to the inflow (indicated by the sizes of the arrows).

B - If the amount of rainfall increases (denoted by the larger arrow), the level of water in the lake increases until a new settling point is reached, at which the outflow is equal to the inflow.

C - Conversely, if the amount of rainfall decreases, the water level in the lake falls until a new settling point is reached, again where the outflow matches inflow.

D - The key characteristics of the settling point system are that a parameter of interest (e.g. body energy stores) has both inputs (energy intake) and outputs (energy expenditure). Importantly, for a settling point system to operate, one of these parameters must be independent of the size of the parameter of interest, and the other must vary in direct relation to the size of the given parameter (in this case the expenditure). The resulting settling point of the system varies in direct proportion to the unregulated flow.

1.3.3 Evidence supporting early plasticity in the regulation of set-points for body weight

Obese adults who lose weight exhibit strong physiological mechanisms which promote weight regain (81), and even extended treatment protocols are only associated with marginally improved outcomes (92).

However in children, long-term maintenance of weight loss is highly achievable (93) and failure appears to be more associated with adverse social factors rather than strong physiological mechanisms acting to defend body weight (94).

Additional evidence to support the physiological flexibility in body composition during the early years comes from studies that have examined the relationship between the timing of adiposity rebound and later obesity (95). This period, which is the inflection point at which BMI increases rather than decreases during normal growth, usually occurs at about 4-6 years of age, and may represent a critical window of opportunity for weight management in young children (96).

It has been suggested that obesity in adulthood is merely a condition of altered energy regulation at an elevated set-point, and there is a wealth of evidence from genetically transmitted and diet induced forms of obesity in animal studies to support this (80). There is also limited data to indicate that these set-points for the regulation of body weight can be adjusted by early life factors, such as in rat studies, where maternal obesity during gestation increases the risk of later obesity in the rat offspring by altering neural pathways involved in energy homeostasis regulation (97, 98). Also, early post-natal exposure of obesity-resistant offspring to the milk of genetically obese dams (female parent rats), alters the hypothalamic pathways involved in energy homeostasis causing them to become obese when fed a high fat diet as adult rats (97).

1.3.4 Influence of obesity on pubertal development

1.3.4.1 Puberty

Puberty is the process of physical changes through which a child's body matures into an adult body capable of sexual reproduction. It is initiated by hormonal signals from the brain to the gonad; the testes in a boy and the ovaries in a girl. On average, girls begin puberty at ages 9-11 years, and usually complete puberty by 15-17 years, whereas boys begin puberty at ages

10-12 years, and usually complete puberty by 16-17 years. Puberty which starts earlier than 8 years in a girl and 9 years in a boy is known as precocious puberty, and if there is an absence of secondary sexual characteristics by 13 years in a girl or by 14 years in a boy, this is referred to as delayed puberty.

Puberty follows a typical pattern of development. In girls, puberty usually starts with breast development, followed by pubic hair growth and finishes with menarche. In boys, true puberty starts with testicular enlargement, followed by pubic hair growth and enlargement of the penis. Pubertal development begins with activation of the hypothalamic-pituitary-gonadal axis (HPG), and the increasing production of Gonadotrophin-releasing hormone (GnRH) from the hypothalamus and the gonadotrophins Luteinising Hormone (LH) and Follicle Stimulating Hormone (FSH) from the pituitary gland. Clinically, pubertal status is classified using Tanner Stages; B1-5 for breast development, P1-6 for pubic hair development, and G1-5 for male genital development (99). However, as Tanner staging is dependent on the subjective evaluation of the examiner, there may be an element of bias, and in certain cases such as female obesity, there may be pseudo- or lipogynaecomastia instead of actual thelarche, which should be taken into consideration. The duration of pubertal development is usually over 4-5 years, although the actual timing of puberty is influenced by several factors such as genetics and ethnicity. On average black children enter puberty at an earlier age than Hispanic children, who have an earlier puberty than Caucasian children (100), although there is a large variation in the timing of puberty of up to 4 years within any given population.

1.3.4.2 Effects of obesity on pubertal development

The timing of puberty is integrally linked to weight gain and may play an important role in determining the degree of future weight gain (101). A rapid weight gain during infancy is associated with early pubertal development in both sexes, which supports the hypothesis that fast body growth and rapid weight gain during infancy influences pubertal development and brings about earlier maturation (102). Normal pubertal development, and future fertility requires the achievement and maintenance of a critical body weight and/or fat mass (82). This forms part of the critical mass theory, where a critical fat mass of approximately 17% is required for menarche and a higher fat mass of 22% body weight is needed to maintain reproductive capacity. Therefore Anorexia nervosa and other medical conditions that negatively impact on weight, such as inflammatory bowel disease, delay puberty and are associated with

reductions in fertility in both men and women (103) whereas obesity is associated with earlier puberty, particularly in girls (101). However, there is a subset of obese children who can present with late puberty as detailed in a German study involving 1,383 overweight and obese children aged 10-16 years, which found that obese children had later pubarche (where pubic hair develops independently from the activation of the HPG axis), menarche and voice break than their lean control group (104).

The results of studies researching how childhood obesity influences the timing of puberty can be controversial, although there is agreement that the connection between obesity and pubertal development has pronounced sex-dependent differences.

1.3.4.3 Molecular mechanisms of puberty

At a molecular level, evidence for a direct link between the regulation of weight and reproduction has been the finding that a hormone called the Gonadotropin Inhibitory Hormone (GIH) which acts as a molecular switch in the hypothalamus between reproduction and feeding (105). If weight is suboptimal, then increases in hypothalamic GIH occur which drive feeding and inhibit reproduction. Conversely, GIH levels decline when weight goes up, leading to an increase in gonadotropins and a decrease in feeding behaviours.

In puberty, and also in pregnancy, leptin resistance is thought to have an important physiological role by allowing the build-up of fat mass that is essential for subsequent reproductive function (106). Serum leptin levels correlate with the amount of adipose tissue mass, and it is an important signal in human energy regulation, although there can be considerable variation in leptin levels between individuals with similar fat masses, implying that leptin's role as a feed-back signal in the regulation of body weight is complex. The production of leptin is stimulated by insulin, and fasting insulin, when adjusted by age, sex and BMI, correlates with leptin, but only at selected pubertal stages and predominantly in females (107).

Leptin levels are much higher in obese children compared to children with a normal BMI, and pubertal female children have higher levels than their male counterparts, even when obesity is corrected for (107). Several studies have shown a rise in serum leptin levels in girls starting from 7 years of age, through puberty and up until 15 years of age, whereas in boys, leptin levels temporarily rise and then reduce from approximately Tanner stage 2 back to pre-pubertal levels. The rise in leptin correlates with the increase in body fat in females,

whereas the amount of fat decreases in male puberty, as muscle mass increases driven by testosterone secretion. It is thought that a threshold blood level of leptin in girls may be required in order to establish menses, with one study (108), finding that higher leptin levels up to 12ng/mL were associated with a decline in the age of menarche by 1 month for every 1ng/mL increase in leptin (109).

The biological mechanisms that drive weight regain are highly complicated, and so far have only been studied in adult humans or animal models (81). However, it is plausible that the drive to weight regain, after acute weight loss, may be more pronounced during the reproductive years.

1.3.4.4 Pubertal development in females

In females, the study data evaluating the connection between childhood obesity and pubertal development is consistent and clear, with girls that have a high BMI SDS (>1.88) having a greater probability of menstruating sooner and presenting with an earlier pubertal development than lean girls (110, 111), although, many of the studies focus on outcomes rather than determining the causality of the earlier pubertal development.

One study, which explored the effect of maternal obesity on the pubertal development of female off-spring, indicated that the daughters of mothers who were obese during pregnancy menstruated earlier than daughters of lean mothers (112). In addition, a longitudinal study from Switzerland examined 650 girls aged 6-18 years. Although they did not find any significant differences in pubarche between lean and obese girls, there were significant differences in the timing of breast development, with obese girls reaching Tanner stage B3 at 11.6 years, whereas normal weight girls were 12.2 years (113).

Height and weight however also directly affect the age of menarche, as demonstrated in the National Longitudinal Survey of Youth (114), where significant differences in BMI and height at the age of 6 years in different ethnicities were found.

1.3.4.5 Pubertal development in males

The link between male puberty and fat mass is more tenuous than in females, maybe because the evolutionary role of males in a successful pregnancy is the provision of sperm, so that their subsequent fat mass is irrelevant to the

progression of the pregnancy. However, there is insufficient data currently to rule out the role of leptin in the initiation of male puberty (109).

Unfortunately, data connecting paediatric obesity and pubertal development in boys is limited and controversial. Two studies from Denmark have investigated obesity and pubertal timing in males. The first study investigated the secular trend of pubertal timing in boys over a 15-year period comparing 1991-1993 with 2006 – 2008 (115). Puberty, which was defined as a testicular volume >3mls, was found to have started earlier in 2006 – 2008, and the BMI SDS was also significantly higher in that group. The study concluded that earlier pubertal development was associated with increasing BMI (115). The second study evaluated the voice break, which is another sign of pubertal maturation, in 436 choir-boys. The researchers found that the age at voice break decreased from 14 to 13.7 years within a 10-year period, and that an increasing BMI led to an earlier voice break (116).

While many studies have connected childhood obesity with the earlier timing of pubertal development in boys, further studies are required in order to definitively prove this link.

1.4 Satiety Signalling

1.4.1 Regulation of satiety signalling

An intimate knowledge of the changes in hormones and peptides that regulate body weight and control appetite is crucial to understanding and managing paediatric obesity. Appetite, which is the desire to eat, is controlled by interactions which form part of a psychobiological system which can be conceptualised on 3 levels:

1. Psychological (hunger perception, cravings and hedonic sensations) and behavioural levels (meals, snacks, energy intake),
2. Peripheral physiology and metabolic levels,
3. Neurotransmitter level and metabolic interactions in the brain (117).

Appetite is the synchronous operation of events and processes within these 3 levels, and when appetite is disrupted, which can occur in certain eating disorders, these 3 levels become desynchronised.

In order to maintain a stable body weight over a long period of time, food intake must be continually balanced with energy expenditure, and the hypothalamus plays a crucial role in this homeostatic process (118). Within the hypothalamus, the Arcuate Nucleus (ARC) is involved in the integration of

signals that regulate appetite and contains neuronal populations with opposing effects on food (figure 1.11) (118). Feeding is suppressed by the neurons co-expressing Proopiomelanocortin (POMC) and Cocaine and Amphetamine-regulated Transcript (CART), whereas food intake is stimulated by neurons which co-express Neuropeptide Y (NPY) and Agouti-related Peptide (AgRP). Both neuronal populations project to the Paraventricular Nucleus (PVN), although the ARC also communicates with other hypothalamic nuclei such as the Dorsomedial Nucleus (DMN), Lateral Hypothalamic area (LHA) and the Ventromedial Nucleus (VMN) (119). Of note, within the POMC neurons, α-melanocyte-stimulating hormone is produced which binds to melanocortin-4 receptors (MC4R) in the PVN to suppress food intake, and individuals who have MC4R mutations present with severe early-onset obesity (120), although abnormalities in the processing of, as well as mutations within the POMC gene can result in early-onset obesity, adenal insufficiency and red hair in humans (118).

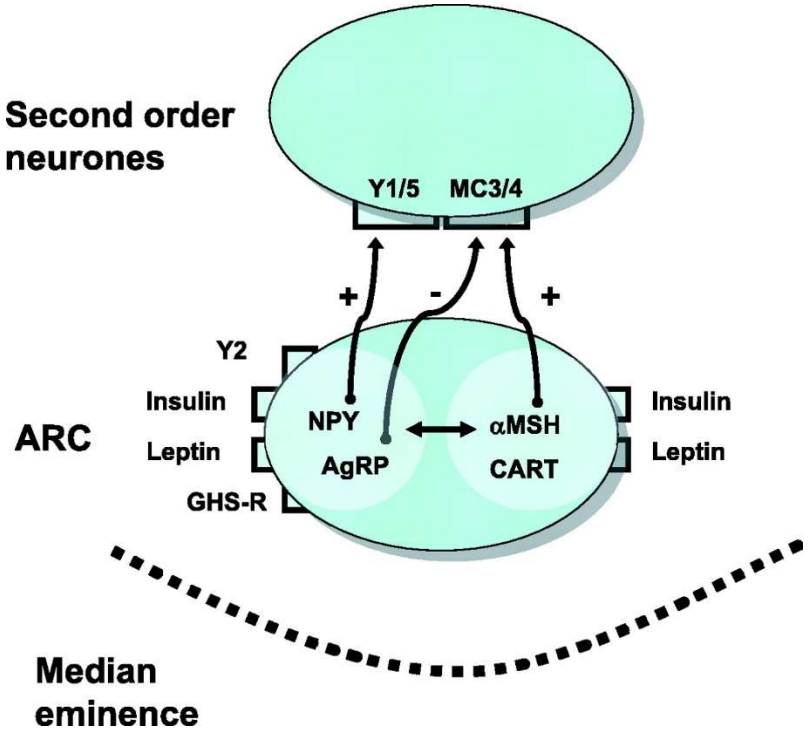


Figure 1.11. The ARC and the control of appetite. (118)

(α-MSH, α-Melanocyte-Stimulating Hormone; GHS-R, Growth Hormone Secretagogue Receptor)

The ARC is also accessible to peripheral circulating signals of energy balance via the underlying Median Eminence, and this part of the brain is not protected by the blood brain barrier. The blood brain barrier therefore has a regulatory

role in the passage of some circulating energy signals, as some of the Gastrointestinal hormones such as GLP-1 (Glucagon Like Peptide-1) and Peptide YY can cross the blood brain barrier, but others such as insulin and leptin cannot.

The hypothalamus and the brainstem have extensive reciprocal connections, and within the brainstem, the Dorsal Vagal Complex, which consists of the Dorsal motor nucleus of vagus, Area Postrema and the Nucleus of the Tractus Solitarus (NTS), is critical in the interpretation and relaying of peripheral signals via the vagal afferent nerve fibres from the gut to the hypothalamus. Like the ARC and the Median Eminence, the NTS is in close anatomical proximity to the Area Postrema which also has an incomplete blood brain barrier, and can respond to peripheral circulating signals from the gut (119).

The vagal afferent neurones within the brainstem express a variety of receptors including Cholecystokin 1 receptor (CCK1R), at which Cholecystokinin acts, Y2R, GLP-1 and Growth Hormone Secretagogue Receptor (GHS-R1) at which ghrelin acts (120). The NTS has a high density of Neuropetide Y binding sites including Y_1 and Y_5 receptors, but POMC neurons also exist within the NTS. The POMC neurons demonstrate Signal Tranducer and Activation of Transcription-3 (STAT-3) activation in response to the administration of leptin, which supresses food intake (119).

Leptin is also able to influence the reward pathways of the brain and there is considerable evidence that the mesoaccumbal dopamine system is a key target for leptin. The rewarding nature of food can act as a stimulus to increase food intake and can overide the homeostatic requirements of the body. The mesoaccumbal dopamine system, which encompasses the Ventral Tegmental Area (VTA) of the midbrain to the Nucleus Accumbens, is central to reward-associated feeding behaviour, and it receives and integrates information about the rewarding value of foods with information about metabolic status (figure 1.12) (121). Endocannabinoid and opioid receptors also play an important role in increasing feeding related to reward. Stimulation of μ-opioid receptors in the nucleus accumbens has been shown to increase sweet, high fat food intake, and suppression of the endocannabinoid receptors has resulted in the successful anti-obesity treatment, Rimonabant (119). Unfortunately the drug was withdrawn in 2008 due to serious psychiatric side effects. Endocannabinoid receptors within the hypothalamus have also been shown to be reduced by leptin (118).

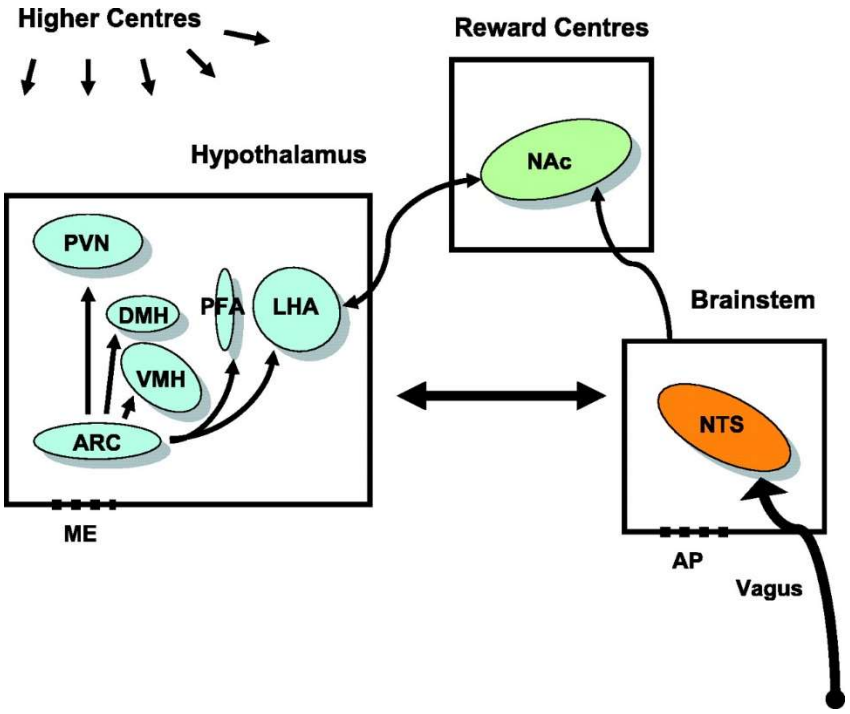
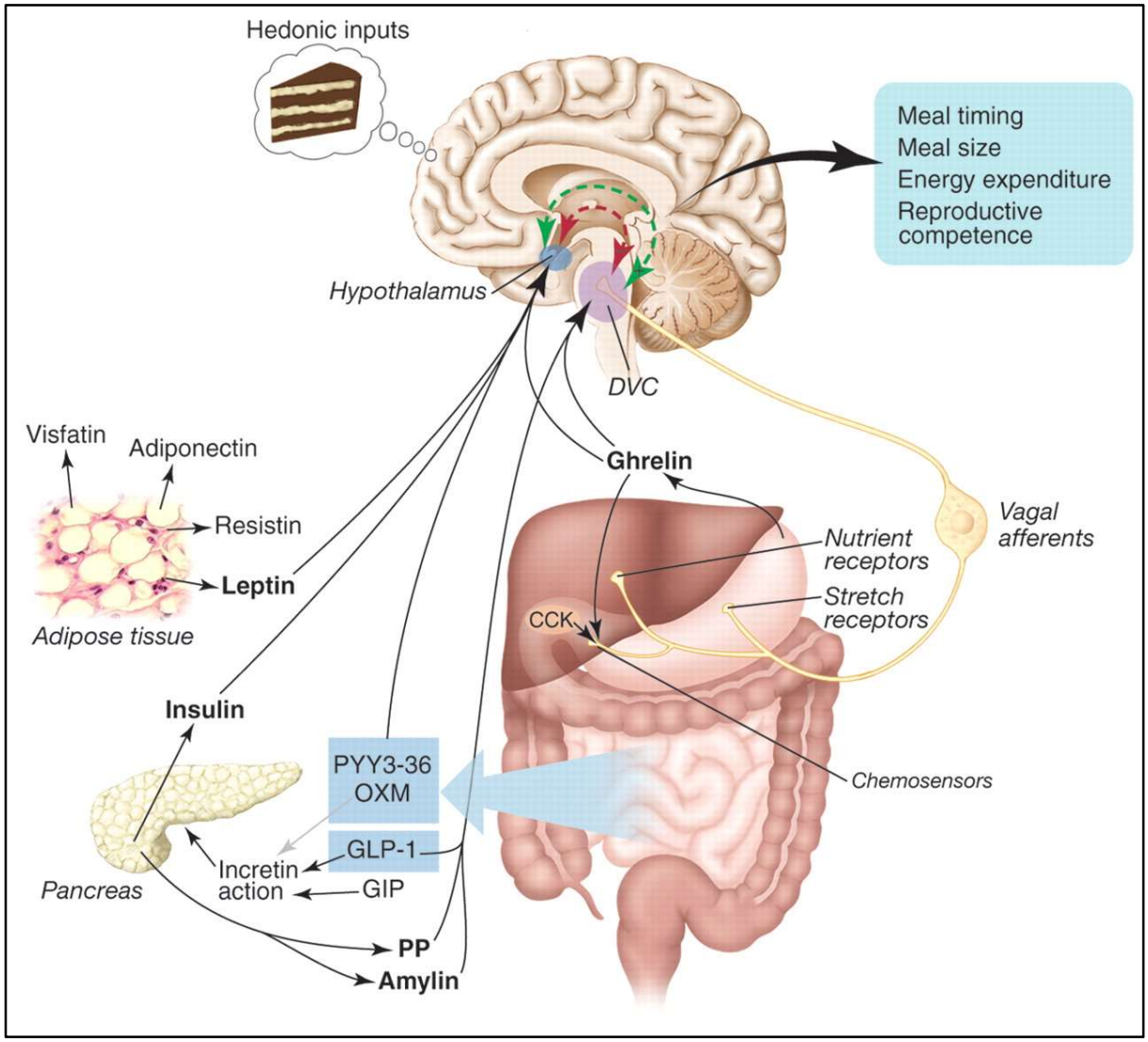


Figure 1.12. The central control of appetite. (118)

(AP, Area postrema; ME, Median Eminance; NAc, Nucleus Accumbens; PFA, Perifornical Area; ARC, Arcuate Nucleus; PVN, Paraventricular Nucleus; LHA, Lateral Hypothalamic Area; NTS, Nucleus of the Tractus Solitarus)

Problematic over-eating may therefore reflect a changing balance in reward circuits versus the control exerted by the hypothalamus, in addition to an altered hedonic “set-point”, such that susceptible individuals have a heightened responsiveness of the reward circuits to rewarding foods (121). Alternatively, it may be that increased signalling by the orexigenic gut hormone ghrelin may affect metabolic control, as ghrelin has also been shown to activate the mesoaccumbal dopamine system (121). While the exact mechanisms by which appetite is controlled remain to be fully elucidated, it is clear that the decision to eat is very complex and involves genetic, psychosocial, environmental and physiological processes.

Figure 1.13. Peripheral satiety signals relating to long-term energy stores



Peripheral signals relating to long-term energy stores are produced by adipose tissue (leptin) and the pancreas (insulin). Feedback relating to recent nutritional state takes the form of absorbed nutrients, neuronal signals, and gut peptides. Neuronal pathways, primarily by way of the vagus nerve, relate information about stomach distention and chemical and hormonal milieu in the upper small bowel to the Nucleus of the Tractus Solitarius (NTS) within the dorsal vagal complex (DVC). Hormones released by the gut have incretin-, hunger-, and satiety-stimulating actions. The incretin hormones GLP-1, GIP (Gatric Inhibitory Polypeptide), and potentially Oxyntomodulin (OXM) improve the response of the endocrine pancreas to absorbed nutrients. GLP-1 and OXM also reduce food intake. Ghrelin is released by the stomach and stimulates appetite. Gut hormones stimulating satiety include CCK (Cholecystokinin) released from the gut to feedback by way of Vagus nerves. OXM and PYY (Peptide YY) are released from the lower gastrointestinal tract and PP (Pancreatic Polypeptide) is released from the islets of Langerhans (122).

1.4.2 Satiety hormones and peptide neurotransmitters

The gastrointestinal tract (GI) is the largest endocrine organ, producing more than a 100 bioactive peptides from specialised endocrine cells that are interspersed throughout the luminal digestive tract and the pancreas (123). Hormones are secreted from the endocrine cells into the blood where they are carried to distant targets, whereas neurones secrete peptides into synapses or onto other cell types. Many of the same chemical transmitters in the GI tract are produced by endocrine and neural cells, such as Cholecystokinin (CCK) which plays important physiological roles both as a neuropeptide in the central nervous system and as a peptide hormone in the gut, where it is involved in a diverse number of processes such as satiety, digestion, memory and anxiety (124).

Endocrine transmitters of the GI tract consist predominately of peptides (short chains of amino acids) that have extremely short half-lives, which allows for rapid initiation and termination of signalling. GI peptides that function mainly as hormones include: GIP (Gastroinhibitory Polypeptide), GLP-1, PP (Pancreatic Polypeptide), Peptide YY, and Insulin (124).

The majority of the GI hormones are anorexigenic and are associated with satiety, except for ghrelin, which is an orexigenic hormone that promotes hunger (125).

Table 1.1. Effects of different gut and adipose tissue hormones on food intake and their changes in childhood obesity after weight loss. (125)

Source	Hormone	Effect on food intake	Post meal changes vs fasting	Changes in childhood obesity *	Changes after weight loss
GI Tract	Ghrelin	Orexigen	Decreased	Decreased	Increase or stable
	PYY	Anorexigen	Increased	Decreased	Increase
	GLP-1	Anorexigen	Increased	Unchanged	Controversial
	CCK	Anorexigen	Increased	----------	----------
	GIP	Anorexigen	Increased	----------	----------
Pancreas	Insulin	Anorexigen	Increased	Increased	Decrease
	PP	Anorexigen	Increased	Decreased	Increase
	Amylin	Anorexigen	Increased	Increased	Decrease
Adipose tissue	Leptin	Anorexigen	Increased (after hours)	Increased	Decrease

*Serum levels compared with lean

Anorexigen (appetite suppressant) Orexigen (appetite stimulant)

1.4.2.1 Ghrelin

Ghrelin is a 28 amino acid peptide that was first identified in 1999 as a ligand for the secretion of growth hormone secretagogue receptor (GHS-R1a) (126). The highest concentrations of ghrelin are found in the stomach, followed by the duodenum, and it is the only known circulating orexigen, stimulating rather than inhibiting feeding behaviour.

Acylated ghrelin is the active form (126), and it exerts its orexigenic effects via the arcuate nucleus in the hypothalamus. While ghrelin also stimulates growth hormone release via its action on the type 1a receptor in the hypothalamus, its orexigenic action is independent of its effect on growth hormone (118).

It has been suggested than ghrelin has a role in both meal initiation and weight gain, as endogenous levels of ghrelin increase before meals and decrease after food intake (125). Ghrelin is thought to act as a hunger signal, and ghrelin

levels correlate negatively with BMI, so increase following weight loss. Prader-Willi-Syndrome, a rare genetic condition characterised by severe obesity, is an exception to this, as ghrelin levels are approximately 4.5 times higher than in obese controls, although it is unknown whether the high ghrelin levels are a consequence or a cause of their obesity (126).

In addition to lower circulating ghrelin levels, obese subjects also have an attenuated suppression of ghrelin i.e. a greater number of calories are needed before a significant suppression of fasting ghrelin levels are seen in the obese compared to the lean. It has been suggested that the attenuated ghrelin suppression in obese subjects may contribute to a lack of satiety after smaller meals (126).

1.4.2.2 Peptide YY (PYY)

Peptide YY belongs to a family of peptides that includes Pancreatic Polypeptide (PP) and Neuropeptide Y (NPY) (118). Circulating Peptide YY has two major forms: PYY_{1-36} which is cleaved to PYY_{3-36} by dipeptidyl peptidase, with the latter being the more physiologically active. Peptide YY is secreted predominately by L cells of the distal GI tract, and the amount secreted is directly proportional to the number of calories consumed, although the levels are also influenced by meal composition, with greatest levels seen after high fat meals (125). Peptide YY post-prandially inhibits gastric acid secretion and motility via neural pathways, and it exerts its anorexic effects through its agonistic properties on the Y2 receptors in the ARC, which inhibits NPY neurons, and leads to reduced food intake (127). Peptide YY is also thought to effect energy expenditure and levels can remain raised for 6 hours post-prandially, with a peak at 1-2 hours. Peripheral Peptide YY is able to cross the blood-brain barrier freely via non-saturable mechanisms, and it has been shown to decrease ghrelin levels, which also contributes to its effect on appetite (118). In addition, obese subjects have a blunted rise in Peptide YY after a meal which also impairs satiety and results in greater food intake, in contrast to subjects with anorexia nervosa, who have high Peptide YY levels (119).

1.4.2.3 Pancreatic Polypeptide (PP)

Unlike Peptide YY, circulating PP is unable to cross the blood-brain barrier, but it may exert its anorexigenic effects on the ARC via the area postrema and

also via the vagal pathway to the brainstem (118). It is secreted from cells in the pancreatic islets of Langerhans and in a similar manner to Peptide YY, circulating concentrations rise in proportion to the calorific load (119) and remain elevated for up to 6 hours post-prandially (118). PP inhibits the gastric emptying rate, exocrine pancreatic secretion, and gallbladder motility, and like Peptide YY it also reduces ghrelin levels.

The release of PP is diurnal, with circulating concentrations being low in the early hours of the morning and highest in the evening. The levels of PP are thought to reflect long-term energy stores, with obese subjects having lower levels, although some studies have found no difference in PP levels between lean and obese subjects (118).

1.4.2.4 Amylin

Amylin is a pancreatic hormone, which is stored and released together with insulin by beta cells (119). Amylin reduces food intake, slows gastric emptying and reduces postprandial glucagon secretion in humans. When Pramlintide, which is a human amylin analogue, is administered it has been shown to reduce food intake and body weight by reducing meal size and inhibiting gastric emptying, and in patients with type 2 diabetes it has been shown to improve glycaemic control and cause weight loss (119). Amylin, which is an anorexigenic hormone, acts by inhibiting NYP release, although its anorectic action is also associated with the serotonin, histamine and dopaminergic system in the brain. Obese subjects have higher circulating levels of amylin than lean subjects, and increasing amylin levels in childhood is related to hypersecretion of insulin (125).

1.4.2.5 Cholecystokinin (CCK)

CCK was the first gut hormone found to be involved with appetite control. It is a meal termination signal that reduces both meal size and meal duration. It is found mostly in the upper small intestine and has multiple bioactive forms, and after eating CCK levels can remain elevated for up to 5 hours (125).

CCK co-ordinates digestion by stimulating gallbladder contraction, enzyme release from the pancreas, increased intestinal mobility and by inhibiting gastric emptying (125). CCK acts via CCK_A and CCK_B receptors, with the former being found throughout the brain including the dorsomedial nucleus of the hypothalamus and in the pancreas, vagal afferent and enteric neurons,

whereas the latter receptors are also distributed in the brain, afferent vagus nerve and stomach. The CCK_A receptor subtype mediates the anorexigenic effect on appetite by reducing levels of neuropeptide Y, a potent appetite stimulating peptide. Signals of adiposity such as leptin also enhance the satiating effect of CCK (118).

1.4.2.6 Gastric Inhibitory Polypeptide (GIP)

Gastric Inhibitory Peptide, which is also known as Glucose-dependent Insulinotropic Peptide belongs, along with GLP-1, to a class of peptides called incretins, which stimulate a decrease in blood glucose levels, in response to nutrient intake that increases or enhances glucose stimulated insulin secretion (128). Both hormones are proglucagon derived peptides (118).

GIP is secreted by K cells in the proximal small intestine, and the GIP receptor is widely expressed throughout the intestinal tract, in adipose tissue and several areas of the brain (119). GIP induces insulin secretion, which is stimulated in response to glucose, and the amount of insulin secreted is greater when glucose is administered orally rather than intravenously. GIP also has significant effects on fatty acid metabolism via stimulation of lipoprotein lipase activity in adipocytes (128).

1.4.2.7 Glucagon-like Peptide 1 (GLP-1)

Proglucagon is expressed in the pancreas, NTS of the brainstem and the L-cells of the small intestine, and depending on the tissue, the enzymes prohormone convertase 1 and 2 cleave proglucagon into different products; glucagon in the pancreas, GLP-1, GLP-2 and Oxyntomodulin in the brain and intestines (119).

GLP-1 and Peptide YY are co-secreted from the L-cells of the intestine, and GLP-1 has a potent incretin effect i.e a greater stimulation of insulin secretion elicited by oral glucose administration compared to an intravenous glucose infusion, despite inducing similar levels of glycaemia. This incretin effect is defective in patients with type 2 diabetes (129).

GLP-1 has two biologically active forms with equipotent biological activity, $GLP\text{-}1_{7\text{-}36}$ and $GLP\text{-}1_{7\text{-}37}$, with the former being the major circulating type in humans. GLP-1 binds to receptors in the hypothalamus (ARC and dorsomedial nucleus) and the brainstem (NTS), which are key appetite related sites (118). GLP-1 levels increase after a meal and fall in the fasted state, but

they also rise in anticipation of a meal (119). GLP-1 inhibits gastric emptying and acid secretion, it supresses glucagon secretion and may also reduce energy intake and enhance satiety (125). GLP-1 is rapidly degraded by the enzyme Dipeptidyl Peptidase-IV (DPP-IV), resulting in a short circulating half-life, and Exendin-V, which is a DPP-IV resistant GLP-1 receptor agonist that occurs naturally in the saliva of the lizard *heloderma*, has been developed for the treatment of type 2 diabetes (118).

While leptin stimulates the release of GLP-1, obese individuals are often leptin resistant which may explain why obese individuals have lower GLP-1 levels (128), and intravenous administration of GLP-1 in both lean and obese individuals decreases food intake in a dose-dependent manner (118). However, the role of GLP-1 in childhood obesity remains poorly understood, with conflicting post weight loss level changes reported in the literature (125).

1.4.2.8 Insulin

Insulin plays an important role in the long-term regulation of energy balance (119). Like leptin, levels of plasma insulin are proportional to adipose tissue, so that plasma insulin levels increase at times of positive energy balance and decrease at times of negative energy balance (118). However, unlike leptin, insulin secretion rapidly increases after a meal, with insulin crossing the blood brain barrier via a saturable receptor-mediated process, at levels that are proportional to the circulating insulin levels. It then acts at the ARC, where insulin receptors are highly expressed, to decrease food intake and body weight (119).

Insulin levels are determined by peripheral insulin sensitivity, of which visceral fat is a key determinant. In paediatric obesity, increased blood insulin levels indicate peripheral and central insulin resistance, with weight loss resulting in improved insulin sensitivity, and a reduction in hyperinsulinaemia (125).

1.4.2.9 Leptin

The obesity gene (*ob*) encodes a peptide hormone called leptin, which is produced by adipose tissue, and was discovered in 1994 (107). Leptin production is stimulated by glucocorticoids and insulin (125), and the amount of leptin produced correlates positively with adipose tissue mass. Food intake also affects leptin levels with restriction over a number of days resulting in a suppression of leptin levels, whereas refeeding reverses the effects, so that

circulating leptin levels reflect both energy stores and food intake. Leptin is also secreted in a diurnal and pulsatile pattern, with a peak at night, but leptin levels are dependent more on daytime feeding rather than the endogenous circadian clock (119).

Leptin is transported across the blood brain barrier by a saturable process, and it acts by inhibiting the NPY/AgRP neurons and stimulating the POMC/CART neurons in the hypothalamus to exert its anorexigenic effect. The leptin-receptor (Ob-Rb) has multiple isoforms resulting from alternative mRNA splicing and post-translational processing, and these can be divided into 3 classes; long, short and secreted (118). The long form, has a long intracellular domain which is needed for the action of leptin on appetite, and this intracellular domain binds to Janus Kinases (JAK) and to STAT3 transcription factors, which are needed for signal transduction i.e activates the JAK/STAT pathway to inhibit signalling (118).

Starvation reduces the transportation of leptin across the blood-brain barrier, and it is the short form of the receptor that is proposed to have a role in this mechanism, whereas the secreted form of the receptor modulates the biological activity by binding to leptin in the circulation (118).

Leptin deficiency in humans caused by a homozygous ob gene mutation is rare, and individuals have low leptin levels, hyperphagia and obesity, which can be reversed by subcutaneous administration of recombinant leptin, which reduces fat mass, hyperinsulinaemia and hyperlipidaemia (119). Obesity in humans however is associated with high leptin levels, which because of the increased leptin resistance and decreased leptin signalling in the brain, does not lead to appetite suppression and lower food intake that might be expected with higher leptin levels (130). The leptin resistance may then lead to ineffective appetite suppression and changes in the set-point of energy homeostasis which would then result in the defence of a higher level of body fat i.e. lipostatic model of regulation of body fat regulation (125). While leptin deficiency has profound effects on body weight, the high leptin levels seen in obesity are less potent at restoring weight, implying that leptin is primarily important in periods of starvation, and has a lesser role in times of plenty (118).

1.4.3 Effect of different macronutrients on satiety hormones

Studies have been conducted in both adults and younger children and adolescents to investigate the effect that different meal compositions have on

the postprandial response of various satiety hormones. Of the paediatric studies carried out, a Randomised Control Trial (RCT) (131) examined the postprandial responses of active ghrelin and PYY in obese and normal-weight adolescent girls following specific macronutrient dense standardised meals. 26 girls (13 obese and 13 non-obese) aged between 12 and 18 were recruited. The test meal was based on metabolic needs and calculated as 130% of calories based on the Resting Energy Expenditure (REE), which was determined during the baseline visit using indirect calorimetry. The caloric content of the breakfast test meal was 25-30% of the metabolic needs and 60-65% was either fat, protein or carbohydrate with the remaining 35-40% split between the other two macronutrients. Subjects were then randomised to the order that they would receive the high fat, high protein or high carbohydrate breakfast. The results showed that obese girls had a greater percent increase in active ghrelin after the high carbohydrate breakfast than normal weight girls and a lower percent increase in PYY after high fat. Obese girls then ate more at lunch 4 hours afterwards following a high fat and high carbohydrate meal, but not after high protein. A similar study in 32 pre-pubertal children (7-11 years) (132), also found that the PYY response following a high protein meal (44%) was significantly greater than after the high carbohydrate (88%) or high fat (81%) meal.

Another study (133), examined 10 pre-pubertal obese boys and compared high fat (52%) and moderate fat (27%) meals with changes in gastrointestinal hormones and appetite. They found that the moderate fat meal was able to more sufficiently suppress appetite, and that the glucose and insulin increases were higher. In contrast with adult studies the post prandial PYY levels were similar after high fat and moderate fat meals, as were the CCK and ghrelin levels, although GLP-1 was significantly higher following the high fat meal. The authors concluded that a moderate fat meal is preferable to a high fat meal as it induced a better postprandial metabolic nutrient balance and appetite suppression.

With respect to mixed meal post prandial responses in children, a study (134) in adolescent females demonstrated a blunted post prandial response in the obese subjects compared to the control group after consuming a meal containing 55% carbohydrate, 25% protein and 20% fat.

The implications of the findings in the paediatric studies therefore point, at least in part, towards high protein meals (>40%) as a possible strategy in managing paediatric obesity because of the advantageous increase in post prandial PYY levels that may be elicited (135).

1.4.4 Effect of weight changes in the circulating levels of satiety hormones involved in the homeostatic regulation of body weight

While several studies have been carried out to explore the different physiological adaptations to weight loss between children/young adolescents and adults in terms of serum levels of the major known gut and adipose tissue derived hormones, the results obtained are often inconsistent and controversial. In addition, some satiety hormones have been studied in greater depth than others. For example, Peptide YY levels may increase following weight loss in obese children, which is in contrast to obese adults, and as Peptide YY has been shown to reduce food intake, this potentially has important health implications for the treatment and prevention of obesity.

One study (127), looked at total PYY and insulin levels in obese and normal weight children aged 9 - 13 years, and the effect that weight loss had in the obese cohort following completion of a 1-year outpatient weight reduction programme. They found that obese children had significantly lower PYY levels than lean children, and that PYY levels increased significantly in children with the most effective weight loss, which would potentially help them to maintain their lower weight, but that PYY levels decreased in children who gained weight. There were no differences in PYY levels between girls and boys or between pre-pubertal and pubertal children (127). Similarly, another study, whose patient cohort had a mean age of 10.9 years, found that PYY levels also increased in obese children who lost weight after a 1 year lifestyle intervention (136). These findings however, were not replicated in research looking at PYY, ghrelin and GIP responses following a mixed meal in 30 female adolescents with anorexia nervosa, obesity or normal weight (134), or in an American study with 32 children, of whom 12 were obese and 20 were normal weight (137). The age range of the children in the study cohorts were 12 - 18 years and 7 – 11 years respectively. The older mean age of 14 years may have had an impact on the results obtained in the former study, who reported that the postprandial peak of PYY was attenuated in obese school age female children compared to normal weight controls, which is similar to the findings in adult studies (134). In the latter study, (137) which was looking at younger children, a positive correlation was found between percentage fat, BMI and PYY levels in children, although there was a significant difference in ethnic background and race between the normal weight children and the overweight children. This may account for the disparate findings, as ethnicity can significantly affect PYY levels, with African-American children having

lower fasting and post prandial PYY levels (135). The data obtained from these studies on PYY highlight how contradictory the findings can be, and that different variables such as age, sex and ethnicity should be taken into account when interpreting results.

Ghrelin, which is the only known orexigenic satiety hormone, is involved in meal initiation, as demonstrated by increasing levels before meals and decreasing levels after food intake. During weight loss in obese adults, a compensatory increase in ghrelin occurs which contributes to the challenges experienced in maintaining weight loss (125). While a Spanish study found increasing ghrelin levels in obese children who had successfully lost weight over a 3-month period, akin to that found in adult studies (138), this was in contrast to a German study involving 37 obese children with a median age of 10 years. Instead, they found no significant change in ghrelin levels in their obese children who experienced substantial weight loss following their 1-year lifestyle intervention weight loss programme (139) the findings of which would support the different physiological response to weight loss experienced in children compared to adults.

Several of the other satiety hormone responses in children and adolescents were also analysed during the same German study, before and after participation in their 1-year lifestyle intervention program called “Obeldicks”. They found that PP levels, while lower at baseline in obese children compared to lean controls, increased significantly and tended to normalise in those children who had experienced substantial weight loss when compared with children who had not managed to lose weight, although PP levels did not appear to correlate with insulin or leptin concentrations (140). As PP is an anorexigenic hormone, this finding would be advantageous in maintaining weight loss. The levels of Amylin, another anorexigenic satiety hormone, which causes a reduction in meal size and inhibits gastric emptying, were found to be significantly higher in obese children compared to normal weight controls and following significant reduction in weight, a pronounced decrease in amylin was also seen i.e. this would act to promote weight regain (141).

While studies have been carried out to investigate the effect of weight loss on different satiety hormones, there remains a paucity of literature involving children and Cholecystokinin, which controls appetite by reducing food intake, and also the role of GLP-1 in childhood obesity. Therefore, additional studies are still required to provide further evidence to support the theory that the energy homeostasis mechanisms regulated via satiety hormones following weight loss in children, are different to those seen in adults (142).

1.5 Resting Energy Expenditure

1.5.1 Definition of Resting Energy Expenditure

Resting Energy Expenditure (REE) is the energy needed by the body to maintain basic biological functions such as controlling normal body temperature, breathing air and pumping blood, and in the majority of individuals it constitutes up to 70% of the Total Energy Expenditure (TEE). The TEE comprises the REE, the Activity Energy Expenditure (AEE), which constitutes 15-30% of the TEE, and the Thermic Effect of Feeding (TEF), which is the increase in energy expenditure associated with digestion and storage of food, and constitutes 5-10% of the TEE (figure 1.14). The TEE can be influenced by physical activity, with energy expenditure increasing during exercise, which then raises the TEE and may also increase the REE (83). In children, growth on a daily basis is too small to measure, except in rapidly growing infants (143).

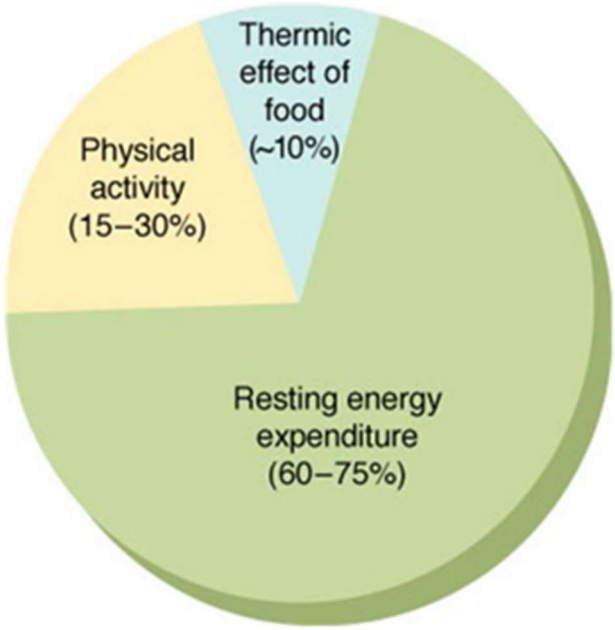


Figure 1.14. Components of Total Energy Expenditure

The Basal Metabolic Rate (BMR), is the rate at which the human body consumes energy when it is physically and mentally resting, in order to maintain important bodily functions such as cell division, breathing and nerve cell function. While the terms REE and BMR are often used interchangeably, they are different, with the REE being approximately 5% higher than the BMR, due to the increased energy demands of the brain and muscle components when awake compared to the metabolic rate expended during sleep. There is

also a difference in the way the BMR and REE are measured. Basal metabolic measurements take place under rigorous conditions, and require the patient to sleep in the laboratory, to undergo a fast of 12-14 hours, with the test conducted under controlled conditions of humidity and temperature. REE (which is also referred to as the Resting Metabolic Rate), requires less restrictions in its measurement compared to the BMR, as subjects do not need to sleep for 12-14 hours in an energy metabolism laboratory with controlled humidity and temperature, or to fast for 12 hours prior to measurement. REE is not as accurate as BMR, and gives slightly higher calorie estimations compared to BMR. However, REE provides an easy and realistic measurement and is significantly cheaper to carry out (143-145).

1.5.2 Measurement of Resting Energy Expenditure

The individual variation in REE is due to several different factors, such as age, gender, ethnicity, physical fitness level, body size and body composition. REE typically reduces with age due to loss of lean body mass and reduction in metabolic activity. Adult females have more fat in proportion to muscle compared to males and they have a metabolic rate that is 5-10% lower than males of the same height and weight. Larger individuals have more tissue, therefore their metabolic activity is greater than that of smaller individuals. However, most of the inter-individual variation in REE can be accounted for by differences in Fat Free Mass (FFM), as body composition plays a significant role in REE, of which FFM is the primary determinant (146).

The ability to accurately assess energy expenditure is important in the management of nutritional obesity, in terms of creating a negative energy balance by providing well-balanced, calorie-reduced diets in combination with increasing physical activity. However, it can also be useful in guiding nutritional support in children with chronic illnesses and malnutrition (146, 147).

There are 3 different approaches to measuring energy expenditure and the accuracy, reproducibility and reliability of the measurements obtained vary considerably, as does the complexity and cost of the different techniques.

1.5.2.1 Direct Calorimetry

In direct calorimetry, the rate of heat loss from the subject to the calorimeter is measured. It enables the heat produced from both aerobic and anaerobic

metabolism to be quantified by measuring heat exchange between the body and the environment. Radioactive and convective heat losses account for approximately 80% of the total heat loss, while evaporative heat loss accounts for 20%. The 3 main types of direct calorimeter are isothermal, heat sink and convection systems. The measurement error of each of these techniques is 1%, 3% and 1-2% respectively. While direct calorimetry remains the gold standard means of measuring human metabolic rate, direct calorimetry techniques are in general very expensive to run and require significant expertise, and offer relatively little extra compared to the cheaper and less complex indirect calorimetry methods (148, 149).

1.5.2.2 Indirect Calorimetry

In indirect calorimetry, oxygen consumption and/or carbon dioxide production is measured and converted to energy expenditure using formulae. There are 4 main ways to measure energy expenditure by indirect calorimetry.

Total Collecting Systems

This is where the expired air is collected in either an airtight rigid structure, such as the Tissot Gasometer, or in a portable flexible bag, such as the Douglas bag. In the Douglas bag, the volume of the expired respiratory gases in the bag is measured (using a mass flow meter) and the sample is analysed to determine the concentration of oxygen and/or carbon dioxide (149). The energy expenditure measurements taken with the Douglas bag incur a very small error (<3%) and it is often referred to as the gold standard for indirect calorimetry, because each variable is measured independently via calibrated and traceable instrumentation (150).

Open Circuit Indirect Calorimeter Systems

In open-circuit systems, energy expenditure can be recorded over several hours or days, and the person inspires air, and then the expired gases are analysed. There are two types of open-circuit system. In the *Ventilated open-circuit system*, the subject breathes into a container through which air is drawn, and the expired air is drawn out of the collection device using a pump, and the flow rate is accurately measured, and then the air is analysed for oxygen and/or carbon dioxide content. The methods used to collect the expired air vary considerably, with the least complex ways being to use a mouthpiece or mask, canopy or transparent hood. A more complex approach is to have the subject placed in a chamber/room of known volume, which also contains sensing equipment that can quantify physical activity. In an

Expiratory collection system, the subject inspires from the atmosphere and expires via a non-return valve into a measureable unit (149).

Confinement Systems (Respiratory Chambers)

Confinement systems are rarely used now as they have been superseded by other techniques. The subject is placed in a gas tight sealed container of known volume, and oxygen consumption and carbon dioxide production are estimated by changes in the concentration of these gases in the chamber air over a period of time (149).

Closed Circuit Systems

Closed circuit systems are also now seldom used. They consist of a sealed respiratory gas circuit in which concentrations of gas are measured over time (149).

1.5.2.3 Non-Calorimetric measures

Energy expenditure is predicted by extrapolation from physiological measurements and observations.

Isotope Dilution, Doubly Labelled Water

In this method, both the hydrogen and oxygen of water are labelled with stable, non-radioactive isotopes called Deuterium and oxygen-18 (D_2O^{18}). The stable isotope dilution and elimination of D_2O^{18} forms the basis for measuring body composition and energy expenditure (151). Subjects are given doubly labelled water orally, once baseline samples of blood, urine and saliva have been collected. The isotopes then mix within the body water space, and repeat samples of blood, urine and saliva are collected 7-21 days later, and D_2 and O^{18} are measured using mass spectrometry. The changes in D_2 and O^{18} in body water can then be calculated over time, so that CO_2 and energy expenditure can be worked out. Using this technique, energy expenditure can be measured over 7-21 days with an error of 6-8% (149).

Physiological Measurements

Heart rate and energy expenditure are related, but not linearly related, because cardiac stroke volume changes with changing heart rate. The precision of heart rate prediction of energy expenditure is dependent on several co-variables that can affect heart rate such as exercise, posture and emotion. However, despite the errors incurred, heart rate monitors are

portable, unobtrusive and measurements can be taken over several days (149).

Predictive Equations for REE

REE can be calculated by different predictive equations, which are based on anthropometric and body composition parameters. However, some of the equations were developed in groups of normal weight subjects while others were developed in overweight subjects. Equations used include McDuffie, Derumeaux, Schofield, FAO/WHO/UNU, Harris-Benedict and Lazzer-Satorio. The accuracy of the equations at predicting the REE varies considerably, particularly when used in severely obese children and adolescents, with Harris-Benedict and Derumeaux significantly underestimating the REE, while Schofield and McDuffie overestimate the REE. The Lazzer-Sartorio equations have been found to give a more accurate estimation of REE in severely obese children and adolescents, compared to the other equations (152).

1.5.2.4 MedGem® Handheld Indirect Calorimetry Device

The MedGem® (MG) is a portable handheld indirect calorimetry device, and was developed as an alternative to the traditional indirect calorimetry systems. It displays the REE (also known as the Resting Metabolic Rate) in calories/day and the VO_2 in ml/day. It measures the VO_2 and then calculates the VCO_2 based on an assumed respiratory quotient (RQ) of 0.85 (where the RQ is derived as a ratio of VCO_2 / VO_2), which is a value considered to be representative of a typical Western diet or mixed diet (153).

Before each measurement, the MG is autocalibrated, and it is programmed to collect data when the first breath is detected, and it continues until a either a steady state or 10 minutes is reached, however, data collected in the first 2 minutes are not used in the subsequent calculations. Sensors measure the relative humidity, temperature and barometric pressure, and all of this information is used in the internal calculations from which the REE is derived using a modified Weir equation, and assumes a constant Respiratory Quotient (RQ) of 0.85 (154).

The principle mechanism underlying the MG is the deactivation of ruthenium in the presence of oxygen, where the amount of ruthenium deactivated is proportional to the concentration of oxygen. The volume of inspired and expired air is measured using ultrasonic sensing technology (154).

When using the MG, each subject either sits upright or is supine, while wearing a nose clip, and places a disposable mouthpiece in position while ensuring they maintain a firm seal (figure 1.15). Subjects should rest for 10-15 minutes before the measurement is taken, and measurements should be carried out at least 4 hours after exercise and after eating food or consuming caffeine. In patients who smoke, the measurement should be performed at least 1 hour after nicotine use (150, 154).

The position the subject is in when the measurement is recorded needs to be taken into consideration as it can affect the REE value, as previous research has shown that there is a 70 kcal/day increase in REE when the subject is seated rather than supine (150).

Figure 1.15. How to use a MedGem® handheld indirect calorimetry device to measure REE.

There have been several validation and comparison studies conducted to determine if the MG is accurate and reliable at measuring REE. A systematic review (150) looking at this included 12 studies (10 adult and 2 paediatric), 4 of which (3 adult and 1 paediatric) compared the MG to the Douglas Bag system (which is often referred to as the gold standard for indirect calorimetry). The MG measurement was not significantly different from the Douglas bag system (mean difference in adults ± 1% i.e. 1559 vs 1568 kcal/d and in paediatrics ± 1%). The intraclass reliability of the MG ranged from 0.97 to 0.98, and the interclass reliability of the MG ranged from 0.91 to 0.97 i.e. the MG

was found to have excellent repeatability and agreement with the Douglas bag (150). Based on this data, the MG was found to be a valid and reliable indirect calorimeter for energy assessment in most children and adults.

More recent studies have looked at the validity of the MG in assessing REE in overweight and obese children and adolescents. In one study (155) involving 39 overweight and obese children and adolescents (age 15.2 ± 1.9 years) and 15 normal weight adolescents, subjects had their REE measured in the supine position using the MG and also by standard indirect calorimeter (SensorMedics VMax). The MG was found to have a lower REE than the VMax (1600 ± 372 kcal/d compared to 1727 ± 327 kcal/d respectively) in the overweight and obese adolescents. This particular study concluded that the MG significantly underestimates the REE in overweight adolescents compared to standard indirect calorimetry (155). However, other studies have shown conflicting results regarding REE comparisons between portable and standard indirect calorimeters. In a study involving 100 non-obese children aged 10-13 years, the MG was found to overestimate the REE by 8% when compared to a traditional indirect calorimeter, although the researchers concluded that the MG was a valid tool in the assessment of REE in children (153). Another study with 19 overweight and obese subjects aged 17-19 years, compared portable to traditional indirect calorimeters found no significant difference in REE measurement between the two devices ($P = 0.22$), with an intraclass correlation coefficient of 0.91, indicating the portable indirect calorimeter to be reliable and valid for assessing the REE in overweight and obese adolescents (156).

Despite the inconsistencies, most studies agree that there are benefits in using the MG in overweight and obese children and adolescents, to produce an individually measured REE, which can serve as a starting caloric goal in diet prescription. This can provide additional motivation to make small changes to the nutritional intake, and if combined with an increase in physical activity, it may help to prevent weight gain (155). The MG remains a practical alternative to traditional indirect calorimetry, but further studies are required to assess its effectiveness as a weight loss tool.

1.5.3 Fat Free Mass and Resting Energy Expenditure

The body is composed of water, protein, minerals and fat. A two-component model of body composition divides the body into a fat component and a fat-free component. The total amount of fat consists of essential fat and storage

fat. Essential fat serves as metabolic fuel for energy production and normal bodily functions, and includes fat found in bone marrow, the heart, muscles and in the lipid-rich tissues of the CNS, whereas storage fat is found in adipose tissue and is also located around internal organs, where it serves to provide protection and insulation.

Lean Body Mass (LBM) is different to Fat Free Mass, although the terms are sometimes used interchangeably, because LBM represents the weight of bones, muscles, ligaments, tendons and internal organs, so also includes a small percentage of essential fat, as there is essential fat in bone marrow and in internal organs. FFM does not contain any fat, so in the two-model component of body composition, the sources of essential fat are estimated and are subtracted from the total body weight to obtain the FFM.

There is a strong relationship between FFM and REE, as FFM consists of metabolically active tissue, and is the largest contributor to REE. REE to FFM is therefore a function of the volume of energetic cells relative to the FFM compartment, and is influenced by age, body weight and gender (157). During adolescence, FFM is composed of 42% skeletal muscle, 8% organ tissue, with the remainder being made up of bone and extracellular fluid. Nearly all of the REE is accounted for by the liver, heart, kidneys and brain, which generate approximately 450 kcal/day, and skeletal muscle (approximately 15 kcal/day), while bone and extracellular fluid contribute <1 kcal/day (158). The REE of organs is therefore considerably greater than that of skeletal muscle, and as adolescents transition to adulthood, they increase their skeletal muscle to organ ratio, which results in a decreased REE to FFM (157). However, during puberty, children have higher levels of REE, and this is due to the effects of growth, higher Na+-K+ ATPase activity (159), changes in hormonal status and higher proportions of skeletal glycolytic fibres (160). Young obese adolescents have 1.8% greater hydration of FFM compared to their normal weight counterparts, which suggests that obese youth have a relatively smaller contribution of the more metabolically active tissues to FFM, so that the ratio of REE to FFM is decreased (158).

While the preferred measurement of body composition in young people would include a combination of measures of fat, water, protein and mineral content, which is referred to as a 4-compartment model, this is expensive and technically challenging to carry out. The less accurate but more practical 2-compartment model, which divides the body into FM and FFM is used instead, and FFM is often calculated by DEXA or BIA. Reference data for children and

adolescents has since been established for body composition, which is necessary to standardise measurements for age, gender and size (161, 162).

While BMI is widely used as a surrogate measure of adiposity, it is a measure of excess weight relative to height, rather than excess body fat. In children and adolescents, the interpretation of BMI is also complicated by the changes that occur in weight, height and body composition during growth. BMI levels are highly correlated with % body fat in adults, but there is a weaker association in children and adolescents, most likely because of the effect of growth (163).

As FFM and FM vary with height, weight and age, it can be difficult to determine whether an individual has a high or low FFM or FM. In order to assess the relationship between BMI, FM and FFM among children, the FFM and FM can be normalised for height by dividing the FFM and FM by the height squared to give the Fat Mass Index (FMI measured in kg of fat mass/height^2) and the Fat Free Mass Index (FFMI measured in Kg of fat-free mass/height^2). This allows the evaluation of FFM and FM relative to body size. As the BMI, FM and FFM are all standardised for height, the contribution of FM and FFM to BMI can then be easily assessed, and one set of recommended ranges, independent of height and age, can be used (161).

1.5.4 Impact of obesity on Resting Energy Expenditure

Obesity has an effect on energy expenditure and several studies have shown that the TEE is elevated with increasing BMI, and that some people who are obese may have a TEE that is approximately 40% higher than that found in non-obese individuals (164). Energy expenditure is higher in obese individuals because of the increase in FM and FFM that accompanies obesity. However, some obese patients have an inability to respond to overfeeding with the normal increase of energy expenditure, with evidence suggesting that obese people may have individual variations in energy expenditure (83).

Recent studies have indicated that in obese people FFM is strongly positively associated with daily energy intake and meal size, so the greater the amount of FFM in a person, the greater the daily energy consumed and the larger the individual meal size. In contrast, fat mass (whose primary function is storage), has a mildly negative association with food intake in obese patients i.e. fat mass has a strong inhibitory negative effect on food intake in lean subjects, and while this effect remains in obese individuals, it significantly weakens as the amount of adipose tissue increases. Therefore, appetite control would

appear to be influenced by metabolism associated with FFM and the energy requirements associated with REE, in addition to signals from adipose tissue and gastrointestinal peptides (165).

Obese individuals often continue to feel hungry and are driven to eat, despite the large amounts of stored energy in the body. It has been suggested that as obese individuals have large amounts of adipose tissue and also additional FFM, it would be expected that they would have a persistent drive to eat that would be stronger than a non-obese individual of the same age, due to the larger FFM and higher REE. Consequently, as people become fatter it becomes easier for them to overeat, and the fatter they become the weaker the inhibitory action of their stored fat is to help them resist the drive to eat (165).

1.5.4.1 Sedentary Behaviour

Childhood obesity has been attributed to a decline in TEE, which has been shown to be secondary to an increasingly sedentary lifestyle amongst children, which is often established at an early age (166).

Physical activity and sedentary behaviour in adolescents has been studied extensively. In two international studies; the Global school based student health study (167), and the Health behaviour in school-aged children study (168), the main findings were that 80.3% of 13-15 year olds were physically active for less than 60 minutes a day, and that 66% of the boys and 68% of girls aged 13-15 years spent at least 2 hours a day watching Television (TV) i.e. excessively sedentary behaviour.

There appears to be a dose-response relationship between increasing sedentary behaviour and unfavourable health in young people, with TV viewing being the sedentary behaviour with the most negative impact on health. The reason screen time increases adiposity is because it reduces the time spent on physical activity, and increases energy intake (169). TV watching in particular has been associated with eating foods that have a high calorie density, and this has been linked to TV advertising (170). One study found that when children watched televised food advertisements, they increased their food intake by 45% when they were allowed to eat freely (171).

There are several other factors that also determine sedentary behaviour, such as age, gender, socioeconomic status, parental habits and environmental factors.

Sedentary behaviour tends to increase with age. Longitudinal studies have shown that screen time as well as the total sedentary time increase during early adolescence, and a UK study found that 5 years after entering adolescence the weekly time spent in front of screens increased by 2.5 hours, which was matched by a significant decrease in physical activity, that was more marked in girls than in boys (172). These results were confirmed in the HELENA study (173), which showed that girls watch more TV than boys and that boys engage in more sustained physical activity than girls.

Low socioeconomic status is associated with high screen time, particularly TV viewing, and before the age of 7 years, this association is a consistent finding (174). Children from a high socioeconomic status however are not exempt from sedentary behaviour, and one study demonstrated that they were in fact more sedentary than children from a lower status (175). They engaged in activities other than TV viewing though, highlighting that the type of sedentary behaviour is more important than the total sedentary time, as time spent watching TV is particularly harmful. Results from studies concerning the association between TV viewing time and whether mothers work have been inconsistent (176), although the crucial role that parents play regarding the development of sedentary behaviours is indisputable.

Parental lifestyle encourages certain behaviours in children. Children whose parents spent more than 2 hours watching TV, were 5 times more likely to also spend more than 2 hours watching TV. Half of the families in the study (177) were found to own more than 11 multimedia devices, and it has been shown that as the number of devices rises so does the amount of time that parents spend watching TV. Parents' perception of the safety of the proximal environment also impacts on children's sedentary behaviours, as children in rural areas were more likely to play outdoors compared to their inner city counterparts, and screen time has been found to rise as traffic density increases (178).

It has been shown that parental ability to set limits on children's screen time directly correlates with a lower level of sedentary behaviour (179). It is therefore essential that management and prevention strategies involve parents, to enhance their awareness of the impact their behaviour has on their children and to encourage them to promote outdoor activities.

1.5.5 Effect of weight loss on Resting Energy Expenditure

When weight loss occurs, resting metabolism decreases by an amount that is significantly greater than would be expected from the loss in metabolically active tissue. It is this compensatory adaptive down regulation in REE, coupled with a decrease in the serum concentrations of thyroid hormones (180), that is frequently cited as the cause for weight regain after weight loss through calorie restriction in obese adults. It is also accompanied by feelings of hunger, driven by changes in satiety hormone responses, which leads to an increase in food intake (181).

These factors act to minimise weight loss while facilitating a rapid restoration of the previously lost weight, which would support the theory that body energy is also regulated around a set-point. However, it is possible that if weight loss is maintained for a prolonged period of time, then a new set-point may be established with long term adjustments to energy expenditure, so that the lost weight is not regained (180).

In adolescent obesity, the major objectives of weight reduction programmes are to:

1. Decrease fat mass in order to reduce the metabolic disorders which predispose obese adolescents to severe metabolic complications and comorbidities, such as hypertension, type 2 diabetes and cardiovascular disease.
2. Promote physical activity and support/provide motivation to enable individuals to continue to practise physical activities.
3. Preserve and possibly increase FFM to enhance daily energy expenditure and improve long-term weight regulation.
4. Influence food choices and behaviour so that energy balance can be improved.

In obese adolescents, severe energy restrictions result in significant decreases in FM and FFM (182), which causes reductions in energy expenditure and leads to weight regain. However, physical training without energy restrictions has been shown to preserve and even increase FFM (and energy expenditure), but the reduction in FM is not as significant (183).

In a study (184) involving 26 obese adolescents aged 12-16 years (12 male, 14 female), weight was lost over a 9 month period (mean BMI decrease of 8.1 kg/m^2 and 6.3 kg/m^2 in males and females respectively). Energy expenditure was affected by weight loss, with REE, BMR and sedentary energy expenditure significantly lower at the end of the 9-month weight reduction

programme, even after adjusting for FFM. In the 4-month follow-up period after the weight reduction programme, 12 of the 26 adolescents maintained their weight loss, while 10 gained 6.6kg of body weight (5.8kg of FFM) (185).

The results of the study confirmed that a Multidisciplinary Team (MDT) approach to weight loss can be beneficial, but acknowledged that reductions in energy expenditure contributed to the weight regain in the adolescents following the weight loss programme.

Physical activity has been shown to be important in maintaining weight loss, although some obese adolescents are limited in the physical activity they can perform. Ideally physical activity which promotes considerable energy expenditure is preferred i.e. promotes substantial fat oxidation with minimal subjective perception of effort and exercise (186). Walking and jogging would therefore be preferred over cycling and swimming, but in some obese children and adolescents with joint pain, cycling and swimming would be more suitable in the first instance.

There is no definite consensus on the amount of physical activity needed to prevent weight gain or weight regain, and 60 minutes a day of either moderate or vigorous intensity aerobic activity is recommended, although the intensity, duration and type of exercise needs to be taken into consideration (187). It is also important that the physical activity is age appropriate and enjoyable, if longer lasting effects are to be seen.

Chapter 2 Childhood and adolescent obesity

2.1 Background.

2.1.1 Prevalence of obesity

Obesity, and particularly paediatric obesity is a major health concern, and is estimated to cost the NHS in England £6.1 billion per year (188). In Australia the situation is similar, with obesity costing the Australian economy $21 billion/year, and this figure is likely to increase substantially in the next few decades, as evidence shows that school children are still getting larger, with currently 2.3 million children and adolescents being classified as either overweight or obese (189).

In England, the National Child Measurement Program (NCMP), provides children's weight status at ages 4-5 years and 10-11 years. It began in 2006, and currently involves 17000 schools and over a million children. Data collected from the program has shown that more than a third of children are now leaving primary school overweight or obese. The prevalence of childhood obesity is also closely correlated to socioeconomic status, with a child living in the most deprived 10% areas in England being more than twice as likely to be obese as a child living in the least deprived 10% areas in England (188). A recent study has investigated how height, weight and BMI have changed between 1953 and 2015 using data from 4 British cohort studies of children born in 1946, 1958, 1970 and 2001 (190). They assessed changes from childhood to adolescence at age 7, 11 and 15 years, and also changes in socioeconomic inequalities over time. The study found that in the late twentieth and early twenty-first centuries that socioeconomic equalities in weight reversed i.e. lower socioeconomic position was associated with lower weights in 1946, 1958 and 1970 cohorts but it was associated with a higher weight in the 2001 cohort. The authors concluded that changes in dietary intake and eating habits since the 1970's, compounded by an increase in sedentary behaviours, had contributed to the inequalities in weight and BMI (190). However, the study was not able to accommodate for the change in ethnic diversity which has occurred, as children from most ethnic minority groups (Black African, Caribbean, and Pakistani) in England are more likely to be obese than white caucasian children. In addition, approximately 7% of children and young aged 5-15 years in the UK have obesity at a level likely to be associated with comorbidities and over 1% of adolescents have extreme obesity with a BMI SDS of ≥ 3 (191).

2.1.2 Significance of paediatric obesity

Extreme paediatric obesity (>99th percentile of BMI for age) now affects more children than those affected by cancer, cystic fibrosis, HIV, and Type 1 diabetes combined (192). Detrimental effects as a consequence of obesity include a clustering of cardiovascular risk factors termed the “metabolic syndrome” (193), which affects around 1 in 4 obese children and increases their medium term chance of heart disease by a factor of 10 (194), while approximately 1 in 10 children will have pre-diabetes (impaired glucose tolerance) (193), a condition which progresses to type 2 diabetes as weight continues to increase (195).

Adolescent type 2 diabetes is also an emerging problem in both Australia (196) and the UK, and although it remains less common than Type 1 diabetes at the present time (197), it is associated with the development of early complications (198). In the only long-term follow-up study of individuals who developed obesity-related Type 2 diabetes in adolescence, it was found that within 15 years of diagnosis, 1 in 20 had developed a need for permanent kidney dialysis, and 10% had died (199).

2.2 Metabolic Syndrome

2.2.1 Definition of Metabolic Syndrome

The metabolic syndrome has been described using several other names throughout the years, such as Syndrome X (1988) and Insulin resistance syndrome (1992), and it has long been recognised that certain risk factors for cardiovascular disease occur in clusters. In 2001, the National Cholesterol Education programme (NCEP) first coined the term “metabolic syndrome” as the presence of 3 out of 5 risk factors: central obesity, hyperglycaemia, hypertriglyceridaemia, high-density lipoprotein (HDL) and hypertension (200). However, due to varying definitions, a consensus statement made by the Joint Task Force in 2009, provided clarification on the diagnostic criteria for metabolic syndrome in adults, which included 3 of the following 5 criteria (201):

1. Elevated waist circumference
2. Systolic blood pressure (BP) of ≥130mmHg / diastolic BP of ≥ 85mmHg or on anti-hypertensive treatment.

3. Fasting blood glucose of ≥100 mg/dL (5.6 mmol/L) or on treatment for hyperglycaemia.
4. Triglycerides of ≥150 mg/dL (≥1.7 mmol/L) or on treatment for elevated triglycerides.
5. HDL (High Density Lipoprotein) cholesterol ≤ 40 mg/dL (≤1.03mmol/L) in males and ≤ 50 mg/dL (≤1.29mmol/L) in females or on treatment for reduction of HDL cholesterol.

Currently, there are no consensus guidelines or diagnostic criteria for metabolic syndrome in the paediatric population. This is because definitions are more complicated due to the different ranges that need to be covered, and also because of the limitations in the normal cut-off and reference ranges used for various parameters. In 2007, the International Diabetes Federation (IDF) proposed a definition of metabolic syndrome in children and adolescents aged 10 – 16 years, which is clinically applicable, and also allows for the development of symptoms with age (table 2.1) (202). However, it is considered to be quite conservative, but until better definitions of the metabolic syndrome in children and adolescents are available, it is proposed that the IDF definition is used in practise. Owing to the many definitions of paediatric metabolic syndrome, it is difficult to estimate the true prevalence of metabolic syndrome in children and adolescents. In a systematic review of 85 studies in children (203), the median prevalence of metabolic syndrome in whole populations was 3.3% (range 0 – 19.2%), in overweight children it was 11.9% (range 2.8 – 29.3%) and in obese children it was 29.2% (range 10 – 66%), with non-obese, non-overweight populations having a range of 0.1 – 1%. The authors also found evidence that ethnicity and geography are important to metabolic syndrome prevalence in children, and a clustering of risk factors associated with metabolic syndrome has been demonstrated in certain adult populations, such as East Asians, Asian Indians, Native Americans, Japanese Americans and Hispanics (204).

Characteristics	Age		
	6 to <10 years	10 to <16 years	≥ 16 years
Definition of adiposity	WC >90th percentile	WC >90th percentile	WC >90th percentile
Blood glucose	No set value	FBG >5.6mmol/L (100mg/dL)	FBG >5.6mmol/L (100mg/dL)
Dyslipidaemia	No set value	TG >1.7mmol/L (150mg/dL) HDL <1.03mmol/L (40mg/dL)	TG >1.7mmol/L (150mg/dL) HDL <1.03mmol/L (40mg/dL)
Blood pressure	No set value	Systolic >130mmHg Diastolic >85mmHg	Systolic >130mmHg Diastolic >85mmHg

Table 2.1. International Diabetes Federation (IDF) criteria for the definition of metabolic syndrome in children and adolescents. (202)

(WC, Waist circumference; FGB, Fasting Blood Glucose; TG, Triglyceride; HDL, High Density Lipoprotein)

2.2.2 Pathogenesis of Metabolic Syndrome

Although obesity is commonly thought to be the antecedent of metabolic syndrome, lean individuals can also have metabolic syndrome, suggesting that obesity is a marker of metabolic syndrome rather than a cause. This is clearly illustrated in the syndrome of lipodystrophy (an absence of subcutaneous fat) where all fat is stored in liver and muscle, causing severe insulin resistance and diabetes (205). Metabolic syndrome can therefore arise from too much or too little fat, and 40% of the normal weight population have the same metabolic dysfunction as the obese, although 20% of the obese population are actually metabolically normal (206).

While the pathogenesis of metabolic syndrome is still not completely understood, the interaction between insulin resistance, inflammation and also fat, are thought to play a key role in its development.

2.2.2.1 Insulin Resistance

The central pathogenetic feature of the metabolic syndrome is insulin resistance, which refers to reduced glucose uptake in the whole body, in response to physiological insulin levels.

Insulin mediates its effect on target tissues via specific membrane receptors, and it stimulates glycogen synthesis and inhibits hepatic gluconeogenesis and glycogenolysis (207).

In metabolic syndrome, the number of insulin receptors is reduced, and this down-regulation is triggered by existing hyperinsulinism. Insulin is also an anabolic hormone, so is a negative regulator of β-adrenergic stimulated lipolysis, and with insulin resistance, leads to reduced insulin-mediated inhibition of lipolysis. In the presence of increased body fat mass, this causes an increase in the level of circulating free fatty acids (FFA). FFAs precipitate and perpetuate insulin resistance and hyperinsulinism in 3 ways:

1. They reduce insulin binding to its receptor, so decrease insulin clearance in the liver.
2. High levels of FFAs significantly impair glucose utilization in muscle.
3. They impair glucose uptake by inhibiting the tyrosine phosphorylation of insulin receptor substrate-1.

The resulting hyperglycaemia causes permanent stimulation of insulin secretion, which results in a reactive hyperinsulinaemia, and high levels of FFAs also directly stimulate insulin secretion by the β-cells (207).

There is however a clear association between the degree of obesity i.e. visceral fat mass, and adverse metabolic changes. Visceral fat mass in particular contributes to the development of insulin resistance and impaired β-cell function, as well as to the development of dyslipidaemia.

Adipose tissue also secretes adipokines, many of which interfere with the regulation of glucose homeostasis, and visceral adipose tissue in particular produces large amounts of insulin resistance-promoting adipokines. One of the features of obesity-associated insulin resistance is chronic inflammation of the adipose tissue, and these inflammatory changes can be observed very early on in the development of obesity-related comorbidity i.e. before a significant increase in the circulating insulin concentration (200).

Exercise and physical activity however have a positive effective on insulin sensitivity and on pro-inflammatory activity i.e. reduction of serum interleukin-

6 concentrations. Life style intervention programmes have been found to be more effective than pharmacotherapy for the prevention of progression from impaired glucose tolerance to type 2 diabetes in obese adults, and it is thought that the same is true for obese children and adolescents. It has also been shown that functionally healthy adipose tissue, which is characterized by high insulin sensitivity, and a high fat storage capacity, is essential for healthy metabolism and the prevention of the development of the metabolic syndrome (208).

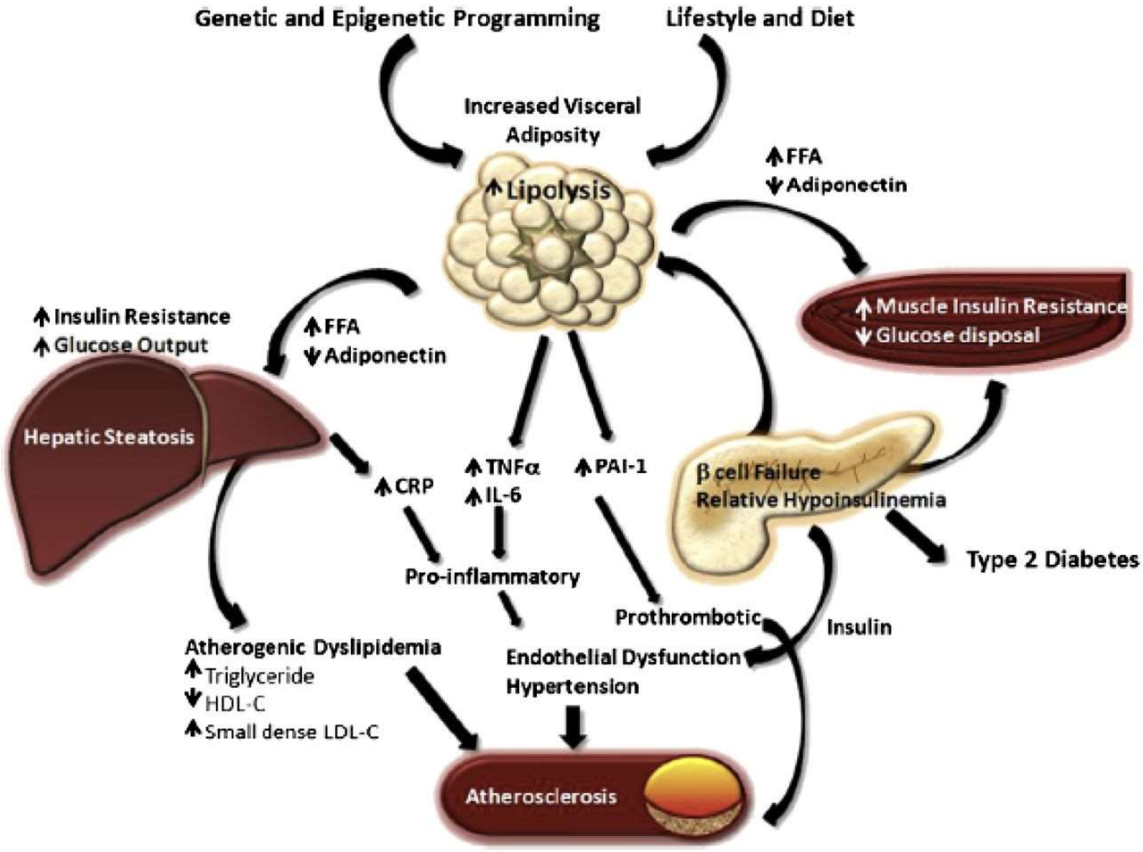


Figure 2.1. Proposed mechanism for the clustering of metabolic syndrome traits and the increased risk of type 2 diabetes mellitus and cardiovascular disease. (209)

(CRP, C-Reactive Protein; FFA , Free Fatty Acids; IL-6 , Interleukin-6; LDL-C , Low Density lipoprotein Cholesterol; PAI-1, Plasma Activator Inhibitor 1; TNF-α , Tumour Necrosis Factor α)

2.2.3 Clinical Features of Metabolic Syndrome

Clinical features seen in metabolic syndrome include:

2.2.3.1 Visceral obesity

Obesity is an important component of the metabolic syndrome and the development of type 2 diabetes and cardiovascular disease. Visceral fat accumulation, independent of the degree of obesity, is strongly associated with both childhood metabolic syndrome and cardiovascular disease later on in life. Visceral adiposity can be estimated using waist circumference, waist to hip and waist to height ratios and MRI, and it has been suggested that waist measurements should be incorporated routinely in paediatric screening, as it could improve cardiometabolic risk stratification among children (210).

2.2.3.2 Dyslipidaemia

In adolescents, an increased triglyceride to HDL ratio can be used as a marker for elevated LDL, with a ratio of ≥ 3 indicative of more LDL particles and a higher risk for cardiovascular disease, owing to the atherogenic effect of LDL (211). The risk of pancreatitis is increased with concentrations of triglycerides >5 mmol/L.

2.2.3.3 Hypertension

Blood Pressure should be measured with the patient in an upright sitting position using the right arm, and using the correct cuff width (this needs to cover at least 80% of the upper arm length). In children and adolescents, the measured values should be interpreted using reference values which take into account age, sex and body height (209).

2.2.3.4 Glucose intolerance and Type 2 diabetes

Acanthosis nigricans is a brown hyperpigmentation of the skin, which typically occurs in the neck and armpits, and is a clinical sign associated with hyperinsulinaemia (figure 2.2). Glucose intolerance (impaired fasting glucose) and T2DM develop as a result of deterioration of β-cell function and subsequent reduction in insulin secretion capacity.

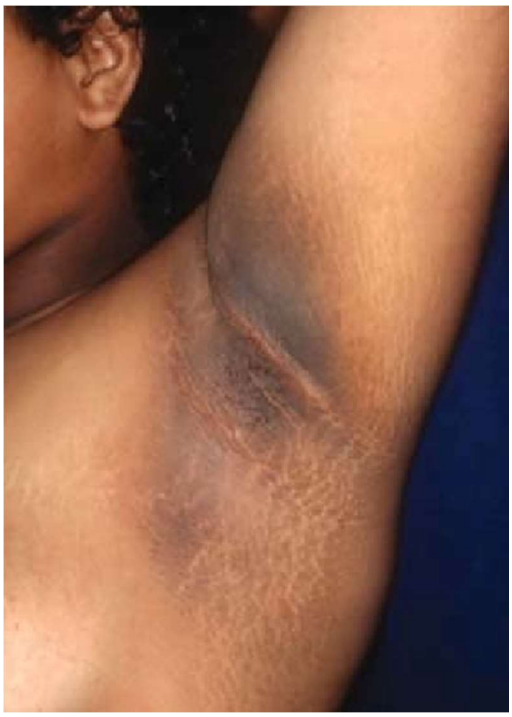


Figure 2.2. Acanthosis nigricans

While the gold standard for measuring insulin resistance is the euglycaemic hyperinsulinaemia clamp, this is an invasive and expensive method. The oral glucose tolerance test (OGTT) is a more clinically applicable test, and is performed following the guidelines of the American Diabetes Association (212). Fasting levels of glucose and insulin are measured, and then an oral dose of liquid glucose (1 gram of glucose/kg body weight to a maximum of 75 grams) is given to the patient, and the blood glucose is measured at 120 minutes.

Impaired fasting glucose is defined as a fasting glucose ≥ 100 and < 126 mg/dL (5.6 – 6.9 mmol/L).

Impaired glucose tolerance is diagnosed if the blood glucose is ≥ 140 and < 200 mg/dL (7.8 – 11 mmol/L) at 120 minutes (table 2.2) (212).

In the absence of unequivocal hyperglycaemia, results can be confirmed by repeating the test. However, OGTT results have shown high intra-individual variability. In addition, the test does not consider insulin response and so cannot detect hyperinsulinaemia, which is the first sign of impaired glucose-insulin metabolism (213). As puberty is a major factor influencing glucose tolerance in children and adolescents, with the increased secretion of growth hormone in puberty contributing to the differences in insulin sensitivity, screening for type 2 diabetes usually begins at age 10 years or at the onset

of puberty (whichever comes first) to 19 years of age, as per the recommendations of the American Diabetes Association (212). However, progression from insulin resistance to glucose intolerance and/or T2DM is variable among individuals, which is why routine monitoring for the development of T2DM symptoms is essential.

Table 2.2. Cut-off values for glucose and HbA1c

Parameter	HbA1c (%)	FPG	2h PG OGTT
Diabetes	>6.5	>7.0 mmol/L >126 mg/dL	>11.0 mmol/L >200 mg/dL
Impaired Glucose Tolerance	5.7 – 6.4	5.6-6.9 mmol/L 100-125 mg/dL	7.8-11.0 mmol/L 140-199 mg/dL
Normal	≤5.7	≤5.6 mmol/L ≤99 mg/dL	≤7.8 mmol/L ≤139 mg/dL

FPG = fasting plasma glucose; 2h PG OGTT = plasma glucose at 120 mins after ingestion of 1g/kg (max 75g) glucose

2.2.3.5 Non-alcoholic Fatty Liver Disease (NAFLD)

NAFLD represents a spectrum of damage to the liver that varies in severity from asymptomatic steatosis, to non-alcoholic steatohepatitis (NASH) with inflammation, to advanced fibrosis with cirrhosis, which can lead to hepatocellular carcinoma. NAFLD is defined by having liver fat >5% liver weight (not caused by alcohol consumption) and is strongly associated with insulin resistance (214). Owing to the increase in obesity prevalence in children and adolescents, there has been a corresponding increase in the incidence of NAFLD, and it is now the most common cause of liver disease in children. Diagnosis of NAFLD is challenging as a liver biopsy is required, although suggested recommendations are biannual screening for NAFLD by measuring aspartate aminotransferase (AST) and alanine amino transferase (ALT), and ultrasound imaging of the liver may also be helpful (200).

2.2.3.6 Polycystic Ovary Syndrome (PCOS)

PCOS is characterised by hyperandrogenism, menstrual irregularities and/or ovulatory dysfunction, and polycystic ovaries. It is commonly associated with

obesity and insulin resistance in adolescent females, and monitoring and frequent screening for the development of metabolic syndrome in affected females is recommended (215).

2.2.3.7 Inflammatory Markers

Obesity is associated with a pro-inflammatory state secondary to the increased production of inflammatory cytokines by hypertrophic adipose cells. These cells are more resistant to insulin's action to suppress lipolysis and they secrete increased amounts of pro-inflammatory chemokine monocyte chemoattractant protein-1. Other inflammatory markers secreted by adipocytes include Interleukin-6, tumour necrosis factor-α, and C-Reactive Protein (CRP) (216). While CRP can be used for cardiovascular risk stratification in adults, the exact relationship between CRP and metabolic syndrome in children remains unclear (217).

2.2.4 Screening for Metabolic Syndrome in children and adolescents

History and examination constitute the first step in screening for metabolic syndrome, and the presence of parental obesity, which is a major risk factor, should also be included in the evaluation (218). The signs and symptoms for associated comorbidities such as obstructive sleep apnoea, which can be identified with polysomnography, should also be included (218). It is recommended that screening for fatty liver disease using ALT and AST levels, should be performed bi-annually, starting at the age of 10 years for children with obesity, or for those who are overweight with other risk factors (219).

In a study published in 2015 that examined trends in dyslipidaemia in youth, 20.2% were found to have abnormal levels of total and HDL cholesterol. (220) The US National Lipid Association provides annual updates of lipid lowering therapies, as well as hyperlinks to resources and separate recommendations for children, adolescents and adults <21 years of age (221).

Routine screening for dyslipidaemia with non-fasting HDL lipid profile is recommended for all obese children between 9 and 11 years and should be repeated between 12 and 16 years. For those children aged 2 – 8 years, a fasting lipid profile should be carried out (222). In those whom lifestyle modification is ineffective, and aggravating factors have been excluded, omega-3 fatty acids, fibrates or niacin should be trialled. (221)

Hypertension prevalence ranges from 3.8% to 24.8% in overweight and obese youth, with the rates of hypertension increasing in a graded fashion as adiposity increases (223). BP should be obtained annually using an appropriately sized BP cuff, starting at 3 years of age, and results should be compared to age, height and gender adjusted reference ranges (222). Any elevated BP should be confirmed with repeated measurements, and any child with a sustained elevation in their BP at or above the 95th percentile when measured by manual auscultation, should undergo evaluation for a secondary cause (224). Ambulatory Blood Pressure Monitoring (ABPM) is also a valuable tool in the diagnosis of hypertension in children with obesity, because of the higher prevalence of masked hypertension. While lifestyle interventions are recommended to lower BP, pharmacotherapy should not be withheld in obese youth if weight loss does not occur, and the updated 2017 American Pediatric guidelines for screening and management of hypertension in children and adolescents provides a comprehensive guide and discussion about drug choices (223).

Screening for T2DM is recommended in in overweight and obese children with any 2 of the following risk factors:

1. Family history of T2DM in first or second degree relative.
2. Specific race/ethnicity (Native American, African, Asian, pacific Islander).
3. Signs of insulin resistance (e.g. acanthosis nigricans).
4. History of being small for gestational age.
5. Maternal history of diabetes or gestational diabetes during the child's gestation.

Screening should begin at 10 years of age or at the onset of puberty, whichever occurs first, and should be repeated every 3 years. OGTT remains the gold standard for the diagnosis of diabetes (225).

2.2.5 Tracking of Paediatric Obesity

The term "tracking", which is often used in relation to metabolic syndrome, means the persistence of an individual's risk factors from childhood to adulthood, in addition to the increasing expression of those risk factors with age i.e. if evidence of metabolic syndrome is found in a child, symptoms are likely to persist to later in life and the clinical symptoms will continue to develop with age (226).

Obesity is associated with widespread, damaging effects on health (227, 228), mainly because of the tracking of weight across the life-course (226), meaning that if we can improve outcomes from paediatric obesity programmes, then large scale reduction in obesity-related adult disease will follow (229).

2.3 Major complications associated with childhood obesity

The most common obesity associated complications in children and adolescents are primarily related to metabolic abnormalities and mental health concerns (figure 2.3) (230). In addition to the clinical features of metabolic syndrome, other obesity associated comorbidities include Obstructive Sleep Apnoea (OSA), Orthopaediatic complications such as Slipped Capital Femoral Epiphyisis (SCFE) and urogenital complications (urolithiasis and stress urinary incontinence) (230).

The prevalence of OSA amongst obese children and adolescents can be as high as 60% (231), and currently polysomnography (sleep study) is the gold standard for diagnosing OSA. It is characterised by snoring, and recurrent partial (hypopneas) or complete (apnoeas) obstruction of the upper airway. The number of apnoeic and hypopneic events per hour of sleep is expressed as apnoeic/hypopnea index (AHI) on polysomnography. This is then used to characterised the severity of OSA; AHI up to 1.5 events/hour is mild, 1.5 – 5 events/hour is moderate and >5 events/hour is severe (232). In OSA, the episodes of airway obstruction can be due to increased airway collapsibility due to mechanical or neuronal factors, with hypertrophy of the adenoids +/- tonsils being the most common mechanical factor. OSA is also associated with systemic hypertension, changes in ventricular structure and function and arterial stiffness i.e. increased cardiovascular burden, and OSA is also frequently associated with sleep disruption and fragmentation. Adeno-tonsillectomy is recommended as the first step in the management of OSA and it can improve obstructive symptoms in up to 80% of obese children with OSA (233), however, morbidly obese children are more likely to fail treatment and up to 50% continue to have OSA. Consequently, Positive Airway Pressure has become the standard of care, in addition to weight loss strategies (234).

Cross-sectional studies have also shown an association between short sleep duration and an increased risk of overweight or obesity in children and adolescents, with a systematic review of 12 studies giving a pooled odds ratio of 1.89 (95% CI 1.43 – 1.68) for short sleep duration and obesity (235). Interventions targeting sleep may result in improved weight and body

composition in adolescents with obesity, and strategies might include an earlier bedtime and removal of electronic screen devices from the bedroom. Improving sleep quality may also have additional benefits, such as improved mood, school performance and general health (230) .

After smoking, obesity is the second biggest preventable cause of cancer in the UK. Obese children are more likely to become obese adults and obese adults are more at risk of cancer, with the risk being greater the longer a person is overweight, and with the more weight they gain. The evidence linking obesity to cancer risk comes predominantly from large cohort studies, which means it can be difficult to establish cause and effect. However, despite limitations in study designs, there is consistent evidence that higher amounts of body fat are associated with increased risks of a number of cancers (236) including endometrial cancer (237), oesophageal adenocarcinoma (238), gastric cardia cancer (239), liver, kidney, pancreatic and colorectal cancer (240), multiple myeloma (241), meningioma (242) and breast cancer in both men and women (243, 244). Possible mechanisms by which obesity could affect cancer risk include:

- Obese individuals have chronic low-level inflammation, which over time can cause DNA damage which can lead to cancer (245).
- Testosterone is converted to oestrogen in adipose tissue and adipose tissue also produces excess amounts of oestrogen and higher levels have been linked to increased risks of breast, endometrial and ovarian cancer.
- Obese individuals have increased levels of insulin and IGF-1 (hyperinsulinaemia) and many cancer cells express elevated levels of IR-A, a form of insulin receptor with a high affinity for insulin and related growth factors. High levels of insulin and IGF-1 have been linked to the development of colon, kidney and endometrial cancers (246).
- Adipose cells produce adipokines which can stimulate or inhibit cell growth
- Adipose cells also have direct and indirect effects on other cell growth regulators such as mTOR (Mammalian Target of Rapamycin) and AMP-activated protein kinase (247).

Currently, several areas of research are exploring mechanisms that link obesity with cancer, and how avoiding weight gain or losing weight can affect the risk of cancer.

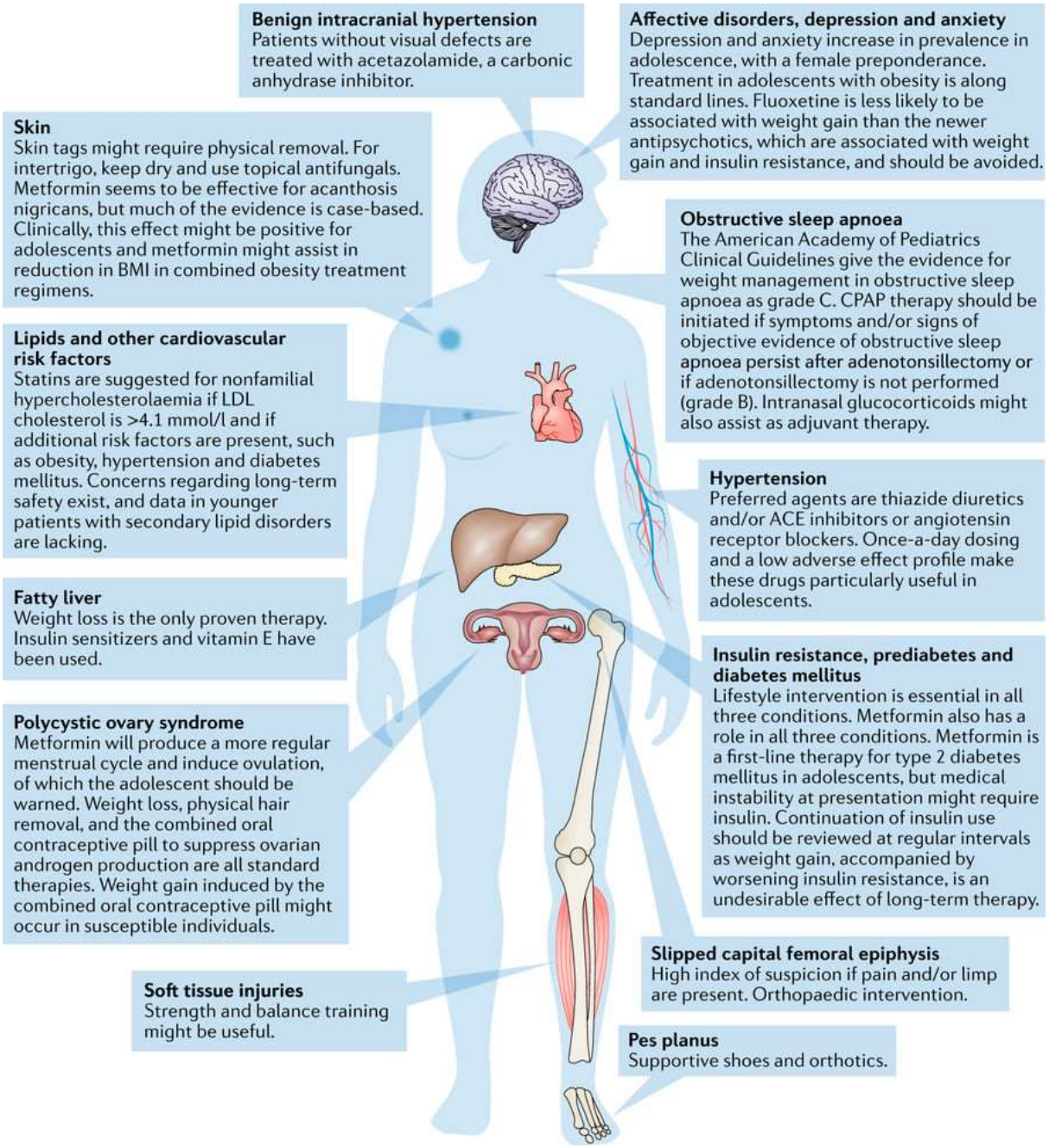


Figure 2.3. Management of obesity associated comorbidities. (230)

(ACE – Angiotensin Converting Enzyme, CPAP – Continuous Positive Airway Pressure)

2.4 Prevention and treatment of childhood and adolescent obesity

Obesity is a complex and multifactorial disease, so requires a multifaceted treatment approach to managing it, which takes into account both the external environment and the internal physiological mechanisms underpinning it, as well as the chronic nature of the condition.

2.4.1 Obesity treatment programmes

Obesity treatment programmes usually comprise of exercise and dietary modifications with behavioural therapy techniques, and they are the first line of treatment in paediatric obesity (248). A plethora of different weight management programmes are available, such as WATCH IT, HENRY and MEND (Mind, Exercise, Nutrition, Do it!), although there is a wide variation in local provision (249). Most weight reduction programmes are provided via outpatient clinics, and interventions lasting at least 6-12 months are needed to produce longer-term success (250, 251). However, obesity interventions programmes in schools, via the internet and using telephone coaching have not been associated with changes of BMI in obese children (252). Their limited effect can be explained partly by the absence of parental involvement. Parents control the health behaviour of their children and are important role models with respect to eating and exercise behaviour, therefore their involvement in any lifestyle intervention is important if it is to be effective (252). In addition, many existing paediatric obesity programmes which involve parents often fail to engage fathers, and innovative strategies are needed to make participation more accessible to fathers (253).

In children, a strict hypocaloric diet is no longer recommended as a weight reduction intervention, as it can adversely affect growth and development (254). In addition, energy requirements of children, even of the same age and sex, can vary significantly, owing to differences in genetic background and levels of physical activity (255). Despite insufficient supporting evidence, most dietary lifestyle modifications in children aim to reduce the calorie intake by approximately 30% (248), although whether a low-fat or low-carbohydrate diet is more advantageous is debatable. Of note, a reduction in the intake of sweetened drinks is the only dietary intervention in children proven in RCTs to be effective (256).

Sporting activities also constitute an important part of obesity treatment programmes, with the aim of the sessions being to reduce body weight but maintain lean body mass by improving muscle strength, and also increasing aerobic and anaerobic fitness. However, physical activity interventions have been found in RCTs and meta-analyses to have no effect on the BMI of obese children (250, 251), although it is associated with more favourable cardiovascular risk factors (252). Reducing sedentary behaviour by limiting screen time has become the latest focus, and has demonstrated favourable results on weight status in younger children (257). In obese adolescents, NICE guidance recommends aiming for weight loss of 0.5 – 1.0 kg per month, based on British Dietetic Association recommendations (258), however in younger children, weight maintenance is preferred over weight loss so as not to affect normal development and growth.

Behavioural strategies are routinely used to support changes in diet and physical activity, and help to facilitate long-term maintenance of these changes. Behavioural therapy approaches have been proven to be effective in several RCTs and meta-analyses (259), and commonly used techniques include impulse control techniques, self-reflection curves, problem-solving strategies and model learning via parents. More recently, behavioural therapies have moved towards solution-focused theories and family based interventions, which avoid assigning blame and instead highlight strengths rather than weaknesses. Motivational interviewing can also be used to increase motivation and help with the setting of goals (260), although goals must be concrete, developmentally relevant and achievable, and need to be reviewed regularly in clinical sessions in order to be effective (230).

2.4.1.1 Services for the management and treatment of overweight and obesity in children

A classical commissioning pyramid as shown in figure 2.4, is used to illustrate how services for the management and treatment of overweight and obesity in children is organised within NHS England (261). There are 4 levels or tiers.

Tier 1: Primary care and community advice. Public health measures, simple interventions and opportunistic advice to families based within primary care.

Tier 2: Primary care with community interventions. There is a wide variation in the weight management programmes available, some of which are commissioned such as MEND, Alive & Kicking and More Life. Typically, the

weight management programmes last for 10-12 weeks, and are based in the community, with children and young people attending once or twice a week.

Tier 3: A specialist MDT to provide an intensive level of input. NICE recommends referral to tier 3 specialist obesity services for children with early onset severe obesity, those with very severe obesity, those who have failed to lose weight with Tier 2 programmes, those who have relapsed or children with significant comorbidities (258). Unfortunately, tier 3 services are seldom commissioned and the lifestyle interventions are often indistinguishable from those at tier 2. Service provision in children is often fragmented and specialist MDT obesity clinics are rarely commissioned.

Tier 4: Specialised complex obesity services (medical management, obesity surgery and other elements of specialised MDT care). The commissioning of tier 4 bariatric services for obese adolescents is currently being reviewed by NHS England, and bariatric surgery in exceptional circumstances is included in the NICE recommendations for childhood obesity (262).

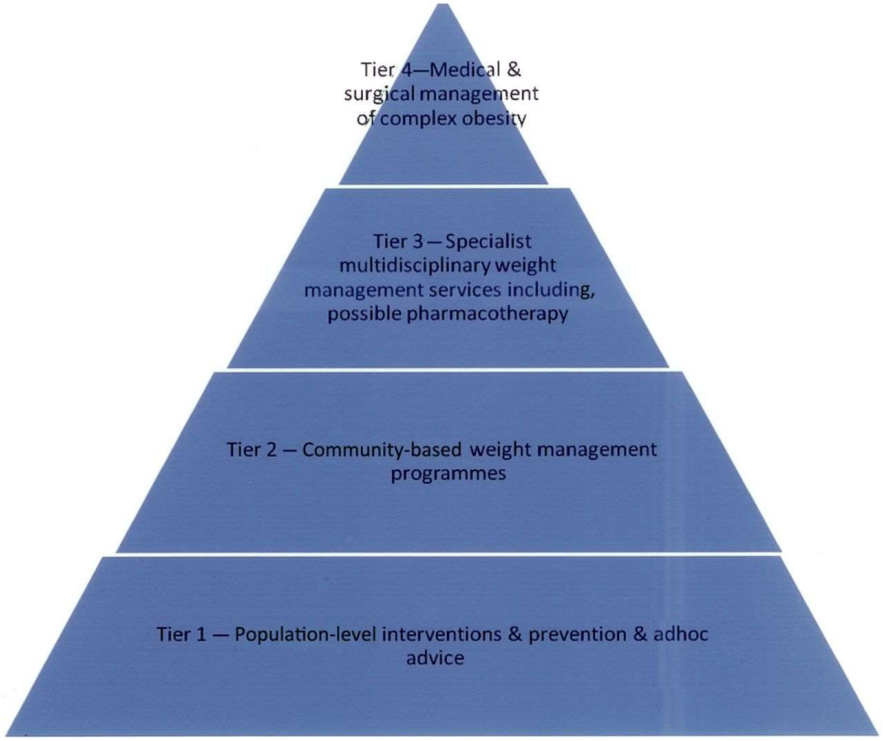


Figure 2.4. Commissioning pyramid for the organisation of weight management services for children. (262)

It is also important that the identification and assessment of obesity related co-morbidities are considered by referring clinicians (table 2.3), as often community weight management programmes do not routinely screen for weight-related co-morbidities (262).

Enquiry/investigation	Rationale
Family history and age-onset obesity	Children with a very strong parental history of obesity and early-onset obesity (<5 years) are more likely to have a monogenic cause for their obesity.
Assess growth and puberty	Children with endocrinopathy tend to be short and overweight, while those with nutritional obesity tend to be tall and overweight (compared with mid-parental height). Individuals with endocrinopathies such as Cushing's show arrested growth.
Blood pressure	Up to 20% children with severe obesity may have hypertension.
Liver function tests	Non-alcoholic fatty liver disease (NAFLD) as a consequence of obesity is common. An ALT >twice the upper limit of normal range suggests fatty liver.
Lipids	Risk of raised cholesterol and triglycerides is increased sevenfold in severe obesity.
Fasting insulin and fasting glucose	If the fasting insulin and glucose are raised with a high HOMA score (>4.5), consider an oral glucose tolerance test. HOMA is a measure of insulin resistance $\text{HOMA} = \frac{\text{Fasting insulin (mU/L)} \times \text{Fasting glucose mmol/L}}{22.5}$
Thyroid function	Exclude hypothyroidism though modest rises in TSH are common in obese children. It is not recommended that mild subclinical hypothyroidism (TSH <10.0) is treated.
Consider obstructive sleep apnoea	Enquiring about snoring and consideration of a screening questionnaire may assist in identifying those who may need further investigation for obstructive sleep apnoea.
Enquire about menstrual irregularities and hirsutism	In overweight/obese girls, ovarian hyperandrogenism is common and menstrual irregularities and hirsutism frequently occur. Investigation of follicle stimulating hormone (FSH), lutenising hormone (LH), sex hormone binding globulin (SHBG) and testosterone may be indicated.
Consider screening for vitamin D deficiency	Vitamin D deficiency is common in overweight/obese children as a consequence of vitamin D deposition in fatty tissues and diet.

Table 2.3. Investigations to consider for screening of weight related comorbidities. (262) (HOMA, Homeostatic Model Assessment)

2.4.1.2 Effectiveness of obesity treatment programmes

The effectiveness of obesity treatment programmes can be defined in various ways, such as a reduction in BMI or weight outcomes, improvements in obesity-associated complications, or a change in weight gain trajectory.

Obesity treatment programmes have been found to be effective in reducing obesity when compared to standard care (i.e. self-help), following analysis of >60 RCTs involving >5,500 children (250, 251). Success rates are higher in children compared to adults, with the best results achieved by younger children, (aged 8-12 years), who are less overweight (263). The mean reduction of BMI-SDS of obesity treatment programmes for obese children 12 months after onset ranges from -0.2 to -0.6 BMI-SDS (250-252, 264). In addition, a decrease in BMI SDS of ≥ 0.25 (equivalent to a reduction of 1 BMI or a stable weight of over 1 year in a growing child) has been associated with improvements in cardiovascular risk factors, intima-media thickness, androgen excess in PCOS and NAFLD (248, 264, 265).

Interestingly, in the few studies carried out in children to analyse the long-term effect of obesity treatment programmes i.e. changes in weight status ≥ 5 years after the end of treatment, the achieved weight loss secondary to the intervention was sustained for 5 – 10 years, which is in contrast to adult studies, where the majority of participants regained weight (263). However, obesity treatment programmes have not been found to be successful in extremely obese adolescents (252).

It is important to recognise that there are several limitations of obesity treatment trials, and also many reasons why obesity treatment programmes fail to be effective. The trials tend to have relatively short follow-up periods, have small sample sizes and have insufficient numbers of children from low socioeconomic status or from different ethnic groups, which may explain why RCTs tend to overestimate the effectiveness of obesity treatment programmes (250). Some of the reasons for intervention failure include a reluctance by families to participate in obesity treatment programmes, high rates of attrition from clinical trials, and the premature cessation of exercise programmes, with several meta-analyses in children and adults supporting the continuation of exercise in order to sustain long-term (3 – 5 years) weight loss and weight maintenance (266). Genetic background also influences the response to treatment and children who already have features of the metabolic syndrome are less likely to respond (252). Finally, it can be difficult to assess the effectiveness of obesity prevention programmes because while there is an evidence base for the effectiveness of different single parameters (e.g.

negative correlation between weight status and high media time), the effectiveness of combined parameters still needs to be evaluated. However, the time needed to really show preventative effects and whether they are cost-effective may take many decades, and research funding is usually restricted in both time and amount (267).

2.4.2 Prevention of paediatric obesity and barriers to implementing effective prevention strategies

2.4.2.1 Prevention of paediatric obesity

Prevention is better than cure forms the crux of many public health initiatives, and is the strategy employed for managing several chronic diseases. As so many people are affected by obesity and its associated health, social and economic consequences, all levels of prevention are required to effectively tackle the problem.

Public health science defines 3 different levels of prevention, which include:

1. Universal prevention and health promotion – This is where information is directed towards the whole population, irrespective of their weight status, with the most common method for disseminating information being provided by government funded institutions e.g. TV advertising, posters, leaflets and websites.
2. Selective prevention – these measures only address those groups who are at risk of obesity e.g. low socioeconomic status, genetic predisposition, families with obese parents.
3. Targeted or Indicated prevention – efforts are directed towards children and young people who are already obese with the aim being to prevent further increases in body weight.

Behavioural and environmental prevention are 2 modes of prevention which are applicable to all 3 levels of prevention. The former addresses the individual person or family, and there is an expectation that health behaviour will change (micro level of prevention). The latter is concerned principally with creating health promoting living spaces and surroundings, which are accessible to the whole population (macro level of prevention) (267).

The UK spends only around £638 million on obesity prevention programmes each year, whereas the cost of treating obesity and its consequences alone costs the NHS at least £5.1 billion, although more recent figures put this at £6.1 billion. By 2050, the UK wide costs attributable to overweight and obesity

are projected to reach £9.7 billion, with costs to the wider economy estimated to reach £49.9 billion per year (268).

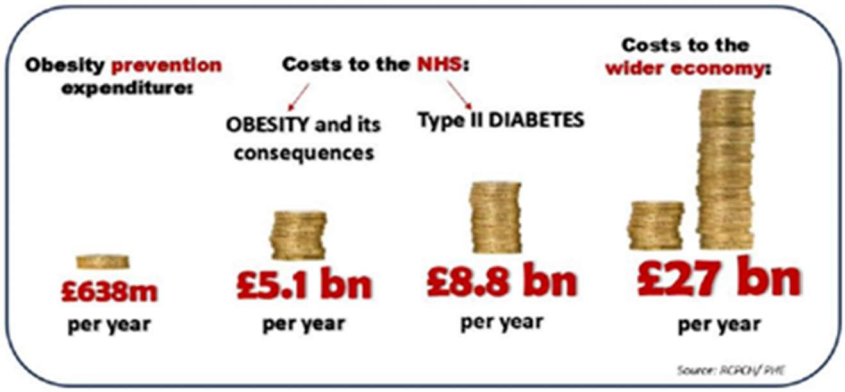


Figure 2.5. Costs of obesity. (268)

The sugar intake for many children is still too high, with 4-10 year olds consuming twice as much sugar as they should, and teenagers having three times as much (figure 2.6). Current national policies to tackle childhood obesity include the introduction of a soft drinks sugar levy across the UK, which came into force in April 2018. According to the National Diet and Nutrition study, sugary soft drinks provide 26% of the total sugar intakes for 11 – 18 years in England, and a single 330ml can of a soft drink with added sugar can contain 35 grams of sugar. The levy relates to the total sugar content on drinks with more than 5g per 100 ml, while a heavier levy is imposed on drinks with 8g per 100ml. Other countries have introduced similar measures, such as Mexico, which has one of the world's worst weight problems, and introduced a 10% tax on sugar-sweetened drinks in 2014, and saw a 12% reduction in the sales of fizzy drinks in the first year (269). Several other countries have already imposed levies on unhealthy foods, such as chocolate and sweets in Norway. Some argue that the policy did not go far enough and should have included fruit smoothies and milk-based drinks, while opponents argue that the levy will lead to job losses and a significant fall in the economic contribution from the soft drink industry.

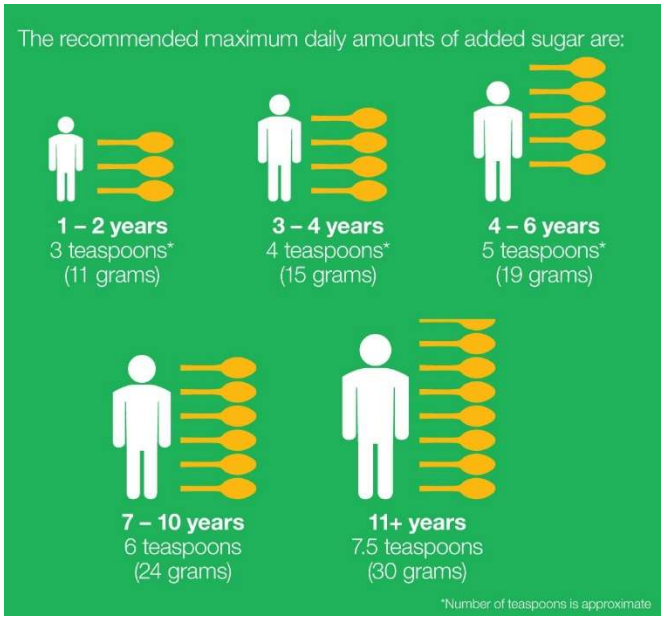


Figure 2.6. Recommended maximum daily amounts of added sugar for children. (270)

The revenue the levy generates will be invested in programmes to reduce obesity and increase physical activity and encourage healthy eating in school age children. Public Health England (PHE) also have a broader structured sugar reduction programme, and have set a target of reducing the sugar intake from a number of foods that children commonly eat by a fifth by 2020. PHE have identified 10 types of products: yogurts, sweet spreads, breakfast cereals, ice creams and lollies, sweet and chocolate confectionary, biscuits and puddings, which covers all of the 10 groups of foods except for breakfast pastries and cakes (270).

In 2013, the government brought in new consistent, front of pack, food labelling, combining red, amber, green colour-coding and nutritional information to show how much fat, saturated fat, salt and sugar, and calories were in food products (figure 2.7). The traffic light system for labelling foods was part of the governments work to reduce obesity levels by making it easier for people to make healthier food choices. It was also a way to get businesses to take action to reduce the amount of calories, salt and trans-unsaturated fat in foods, by signing up to the Responsibility deal pledge and using the traffic light labelling system on the front of their packaging (271). However, food and drink companies are under no obligation to use it, and unfortunately, some companies use the minimal amount of nutritional labelling required on their packaging, which some consumers find confusing and misleading i.e. the Nutrition Labelling and Education act of 1990 requires the disclosure of *total*

sugars on the nutrition facts label for processed food, however, there are 56 different names for sugar, most of which are unknown to the public (272).

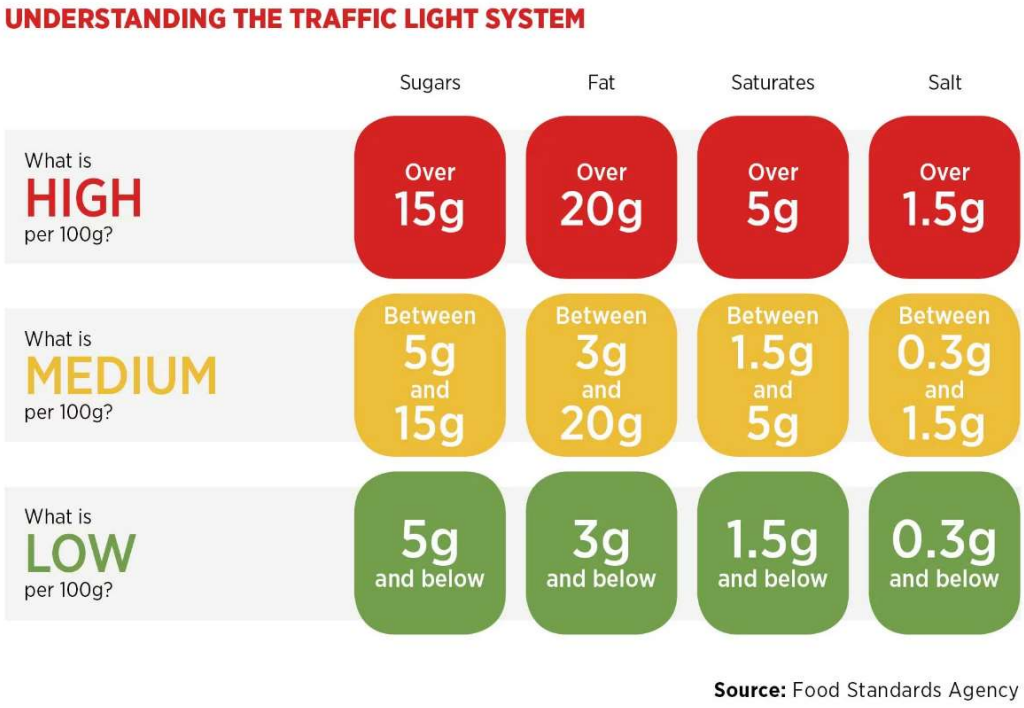


Figure 2.7. Traffic light system for labelling foods. (271)

Finally, campaigns led by celebrity chefs have helped to raise the profile of paediatric obesity and have led to successful interventions such as the introduction of healthier school dinners (Jamie Oliver) and documentaries (Britain's Fat Fight by Hugh Fearnley-Whittingstall). Both chefs also recently gave evidence to MPs at the Health and Social Care Committee, along with academics and health and fiscal experts, regarding their assessment of the governments' childhood obesity strategy, and advising on what they expect from the next part of the plan.

2.4.2.2 Barriers within paediatric obesity

Parents play a crucial role in the prevention of childhood obesity, however up to 75% of parents do not recognise that their child is overweight, and even when they do, 40% do not appreciate the health risks associated with obesity (273). In families with paediatric obesity, the parents appear to lack confidence in their own abilities to manage their child's weight, and this is evident in their higher expectations towards schools, where they expect their child to be educated in how to eat healthily. They also want the clinician and other members of the MDT involved in their child's care to adopt a mediating role.

Most affected families also have a lower socioeconomic status with the accompanying financial constraints this brings (274).

The social economic status of an area is commonly measured using one or more of the following indicators; unemployment rate, proportion of educational levels, material possessions, median income and single parents. Evidence from studies (275, 276) suggests that low socioeconomic status is related to an increased prevalence of overweight or obesity in children and adolescents. Reasons for this may be:

- A lack of resources, such as parks or recreational facilities for physical activities.
- A decrease in formal and informal institutions that monitor and supervise children's behaviour e.g. parents modelling healthy food and exercise behaviour and schools' influence on healthy behaviour
- Unhealthy behaviour in peers e.g. fast food consumption, physical inactivity and shared perceptions regarding body shape (277).

In the last decade, consumption of food away from the home has increased by 29% in the UK, while the number of fast food outlets has increased dramatically (278). In a study looking at area deprivation and the food environment over 18 years in Norfolk, the number of takeaway food outlets rose by 45%, with the highest absolute increase in density of outlets in areas of highest deprivation (43% increase) as oppose to areas of least deprivation, which saw a 30% increase over the same time period (279). Research has linked exposure to takeaway food outlets to increased consumption of takeaway food, and overconsumption of takeaway food has been strongly linked to low diet quality and to weight gain (280). An association has also been found between consumption of fast food at lunchtime and exposure to fast food outlets around schools for children (281). Diets and body weight could therefore be improved if policies restricting takeaway food access were instigated, with particular focus around the workplace and schools.

Industrialised consumer societies show high prevalence rates of overweight and obesity, which is shaped by the priorities and influence of different stakeholders (government, health, industries, media and general public). The government of a country has a fiduciary duty to the health and welfare of its population, and through the NHS, the government in the UK provides healthcare to all legal residents, so has an important role in setting the agenda for obesity prevention and management. Industries, such as food industries, pharmaceutical industries and electronic industries, are driven by wanting to sell their products to all potential consumer groups, including children, and are

supported by commercial advertising. The media also aim to share their information, but are often funded by the commercial advertising of the industries (272).

High calorie, low nutrient “junk” food brands last year spent 27.5 times more on advertising their products than the amount available for the UK government’s flagship healthy eating campaign (Change 4 life), with the top 18 companies (crisps, confectionary and sugary drink brands) spending over £143 million/year on advertising (282). Marketing strongly influences children’s food preferences, requests and consumption. While marketing to children is not new, methods currently employed are more intense and pervasive. Television still predominates, but product placement in toys, games, educational materials, songs and films, cartoon and celebrity endorsements, and stealth campaigns (word of mouth, text messages and the internet) all aim to teach children to recognise brands and pester their parents to buy them (283). Currently, the efforts of the food industry and government agencies to promote healthier foods falls woefully short of their potential, and if the food industry does not change its practice voluntarily then government must enact legislation mandating the shift. Initiatives such as banning food advertising meant for children before 9pm or prohibiting the use of cartoon characters to promote unhealthy foods to children younger than 12 years, have been employed by other countries, and while these actions have not eliminating childhood obesity in these countries, they have helped to slow current trends (283). While the general public are free to make their own decisions regarding their health choices, the present infrastructure within industrialised countries promotes obesogenic behaviour, making the long-term implementation of a healthy lifestyle difficult. Therefore, in order to make long-term changes towards health promotion there needs to be a public will and public pressure, as some of the involved stakeholders within industrialised societies have a conflict of interest, so cannot be relied upon to fully support universal obesity prevention and health promotion (284).

2.4.3 Pharmacological management of paediatric obesity

Pharmacotherapy is one example of a biologically based treatment that can be used as an adjunct to lifestyle modification to improve long-term weight loss outcomes. Despite the important role that pharmacotherapy could potentially have in the treatment of obesity, and the increasing number of obesity medications approved in adults, few agents have been evaluated in

children and adolescents for this indication, and the therapeutic options remain limited. Orlistat remains the only medication available with weight loss as a specific licensed indication, and although the Federal Drug Administration (FDA) has approved Orlistat for use in individuals aged over 12 years, its use in the UK and the rest of Europe, remains off licence. Two medications, Rimonabant and Sibutramine, had been used previously for weight loss in children with good effect, but were withdrawn several years ago due to adverse effects on mental health and increased cardiovascular risks respectively (249). A 2016 Cochrane review provided data for trials of current anti-obesity pharmacotheraphy in children and adolescents. In addition to Orlistat and Metformin, which both result in a reduction in weight in favour of active intervention (of around 2 kg), ongoing trials for Topiramate and GLP-1 receptor agonists (GLP1R) were identified (285).

In contrast to adult obesity, where anti-obesity drugs are recommended when lifestyle changes have failed to help the person lose weight or there are associated comorbidities, NICE does not recommend drug therapy in children unless (258):

a) there are severe life-threatening comorbidities like sleep apnoea or raised intracranial pressure in a child less than 12 years, or
b) when physical or significant psychological comorbidities are present in children 12 years or older.

In addition, it is recommended that they are referred to and managed within a specialist clinic (multidisciplinary team) with experience of prescribing in this age group. Despite the limited data available regarding the use of anti-obesity drugs in children and adolescents, there is reported to have been a 15-fold increase in the prescribing of these medications in children < 18 years in the UK between 1999 and 2006 (286).

2.4.3.1 Orlistat

Orlistat is a reversible gastric and pancreatic lipase inhibitor that limits the gastrointestinal absorption of dietary cholesterols by approximately 30%, in patients eating a 30% fat diet (i.e. a reduction of approximately 200 calories per day) (287). As Orlistat causes a reduction in plasma fat-soluble vitamin levels, which may affect adolescent growth and development, the concomitant administration of a daily multivitamin is recommended (288). The usual dose of Orlistat is 120mg three times daily with meals or within an hour of the meal, and the main side effects are gastrointestinal (such as increased stool

frequency, fatty/oily stools), but these are generally mild to moderate, and transient in nature (289).

A number of trials have evaluated the use of Orlistat in children and adolescents, but many of the studies involved small numbers of participants and were of short duration. A systematic review looking at the safety and efficacy of anti-obesity drugs in children and adolescents (290), included one of the largest published trials of Orlistat in adolescents (aged 12-18 years) with patients randomised to receive either Orlistat (n=357) or placebo (n=182) three times daily for a year, in conjunction with diet, behavioural and lifestyle modifications (289). All participants were maintained on a nutritionally balanced hypocaloric diet, which produced an initial weight loss of up to 1kg per week. By the end of the study , the mean BMI was 0.55 kg/m^2 lower in the Orlistat group, but higher in the placebo group by 0.31 kg/m^2 (p=0.001) and no significant changes were seen in serum cholesterol levels or in insulin and glucose (markers of insulin sensitivity). While Orlistat has been studied in a relatively small number of paediatric patients, trials have reported a significant decrease in BMI from baseline varying from 0.5 to 4.09 kg/m^2, with study completion rates of 65 – 100%, and reported medication adherence rates between 73 and 98%, with the variation in results being due to differences in study design and patient populations (291). However, one UK study suggested that 45% of individuals prescribed Orlistat take it for less than a month, suggesting that gastrointestinal side effects may be more significant than reported in other studies (286).

Currently, data suggesting the extended use of Orlistat is lacking, with evaluations varying between 3 to 15 months (291), and a trial period of Orlistat of 6-12 months is currently recommended by NICE. In addition, data from paediatric trials suggest that Orlistat may be safe and effective as an adjunct to diet and behavioural modification in obese children aged >8 years. One paediatric study looking at Orlistat use in pre-pubertal children (n=11, aged 8 – 12 years), found a total weight loss of approximately 4kg (292), however longer term studies to confirm the safety of Orlistat in this younger population are still required.

Cetilistat is another gastrointestinal lipase inhibitor that is currently being investigated. In a multi-centre study involving 612 adults, similar weight reduction was seen for Cetilistat and Orlistat over 12 weeks in obese adults with type 2 diabetes treated with Metformin (293). While fewer GI side effects were experienced with Cetilistat compared to Orlistat, the weight reduction was similarly modest.

2.4.3.2 Metformin

Metformin is a biguanide derivative, which is approved for the treatment of type 2 diabetes in adults and children over 10 years, but not for obesity. It activates adenosine monophosphate-activated protein kinase, to reduce hepatic glucose production, decrease intestinal glucose absorption, and increase insulin sensitivity, by improving peripheral glucose uptake and use. Metformin also inhibits fat cell lipogenesis, and by increasing glucagon-like peptides, it may also reduce food intake. Modest weight loss and a reduction in insulin resistance is associated with its administration, and it has been shown to prevent or delay the onset of type 2 diabetes. Its main side effects are gastrointestinal disturbances, with lactic acidosis being a very rare complication (0.05 per 1000 patient years) (249, 294).

There are very few studies on the effect of Metformin as a weight loss treatment, and most are of short duration, ≤ 6 months. The longest study (295) was for 48 weeks with daily Metformin (extended-release) or placebo in conjunction with a lifestyle intervention programme. It was a multicentre, randomised, double-blind placebo-controlled trial, involving 77 obese adolescents, which were the subjects who had shown 80% medication compliance following a 4-week single blind placebo run-in phase. The BMI change in those patients who completed the trial was significantly different, with -0.9 kg/m^2 in the Metformin group versus +2.2 kg/m^2 in the placebo arm, but there was no significant change in total fat mass, abdominal fat or insulin with Metformin treatment. There have been two recent paediatric Metformin trials, the first of which included 66 children and adolescents (aged 7-8 years) with obesity, randomised to either lifestyle modification alone or lifestyle modification plus Metformin, for 6 months (296). Compared to the control group, Metformin reduced BMI (-1.3 kg/m^2 control-subtracted difference) and waist circumference, but did not reduce markers of inflammation. The second trial included 151 children and adolescents (aged 8 – 18 years) with obesity who were randomised to either Metformin (1500mg per day) or placebo for 6 months (297). Metformin significantly reduced BMI (-1.07 kg/m^2 placebo-subtracted difference) and BMI SDS (-0.1 SDS units placebo-subtracted difference) compared with placebo, but there were no statistically significant differences between groups in cardiometabolic risk factors at 6 months.

In conclusion, Metformin has a modest impact on weight, but does not appear to be particularly efficacious for weight reduction, and larger, long-term randomised placebo controlled trials are needed to evaluate its effect on weight loss alone.

2.4.3.3 Exenatide

GLP-1 is a naturally occurring incretin produced in the GI tract. GLP1R agonists were originally used for the treatment of type 2 diabetes, because they enhance glucose-stimulated insulin secretion, but obesity management has become an additional indication for this drug class, owing to the effect they have on appetite suppression and weight loss, via delayed gastric emptying and reduced food intake (249). Exanitide is a GLP-1 agonist that has been trialled in youth with severe obesity. During the 3 month, randomised, placebo-controlled phase of the trial involving 26 obese adolescents, Exanitide elicited a greater reduction in percentage change in BMI (-2.7% placebo-subtracted difference) and absolute BMI (-1.13 kg/m^2 placebo-subtracted difference) compared with placebo. BMI was further reduced (cumulative reduction of -4%) during the following 3-month open label extension (298). Liraglutide, which is another GLP-1 agonist, has been associated with a dose-dependent mean weight loss of 4.8 – 7.2kg, as compared with 2.8 kg with placebo after 20 weeks in obese individuals without type 2 diabetes (299). However, studies documenting the long-term safety, efficiency and side effects of GLP-1 agonists in children and adolescents are still required.

2.4.3.4 Potential future paediatric therapies

A number of potential therapies are currently under investigation.

Octreotide, is a somatostatin analogue that inhibits glucose-dependent insulin secretion from the pancreatic β-cells. There are 3 studies (294, 300, 301) that have evaluated Octreotide for weight loss in paediatric patients with hypothalamic obesity, who are thought to have increased insulin production in response to the stimulation of hepatic glucose production, as a consequence of their hypothalamic damage. The studies demonstrated either small weight losses or reduced weight gain in Octreotide-treated patients. The major adverse effect from Octreotide is the development of cholelithiasis (occurs in up to 44% of subjects), and its subcutaneous administration is a major obstacle to its wide spread use, and currently Octreotide is not recommended for treatment of obesity outside of clinical trials (301).

Pramlintide, is a synthetic analogue of Amylin, and is approved for the treatment of type 1 and type 2 diabetes, and produces small amounts of weight loss in obese and diabetic adults. In one study in adults with and without type 2 diabetes, a placebo-subtracted weight loss of up to 2.7kg was found after 16 weeks of Pramlintide 240μg three times a day (302). Although

small trials of Pramlintide have been reported in adolescents with type 1 diabetes, there have not been any paediatric weight loss studies involving the drug. Pramlintide has also been used in combination with Metreleptin, which is a leptin analogue that works by increasing insulin sensitivity by reducing the accumulation of fat in organs, and the combination of these two drugs has resulted in significant weight loss (303).

As body weight is defended by multiple internal physiological mechanisms, it would seem reasonable to tailor obesity treatment to the targeting of multiple weight-regulating pathways at the same time. This has led to the development of combination therapies. Since 2012, four new obesity medications have been approved for use in adults by the FDA in the US. Lorcaserin (selective serotonin receptor agonist), the combination of Phentermine (norepinephrine reuptake inhibitor) and Topiramate (GABA-ergic anticonvulsant drug), the combination of Naltrexone (opioid receptor blocker) with Bupropion (dopamine reuptake inhibitor) and Liraglutide. At 1 year, the placebo-subtracted weight loss with these agents varied from approximately 3 -10% (304). The manufacturers of these medications have stated that they plan to start performing paediatric trials in the next few years, although the pace of paediatric evaluation is very slow, and very few paediatric obesity trials have been reported.

With respect to future pharmacological treatments, research needs to focus on conducting trials with sufficient power and long-term follow-up to ensure that the long-term effects of any pharmaceutical intervention are comprehensively assessed, which in turn should generate and drive an increase in high quality trials of new medications in paediatric obesity (285).

2.4.4 Psychological management of paediatric obesity

The impact of childhood obesity on psychological health and general well-being is often underestimated. In children with obesity, internalizing and externalizing disorders and behaviours such as anxiety and hyperactivity are common, and on a day to day basis they are more relevant to families than the metabolic risk factors that are associated with obesity (305). These psychological issues can also complicate the implementation of and adherence to management strategies, and the assessment and treatment of mental health by professionals (e.g. psychologist) is a vital part of the weight management team.

There is a clear correlation between obesity and earlier pubertal development, with the latter causing psychological burden for girls and boys. Obese boys may also have lipogynaecomastia, which can have significant negative effects on quality of life and self-esteem (306). Childhood obesity has been found to be negatively associated with psychological comorbidities such as depression, emotional and behavioural disorders, compromised perceived quality of life and self-esteem, and these can be enduring in nature and may continue into adult life (307). Adolescence is a particularly complex developmental stage, during which self-identity is developing, and a sense of belonging is central. Complicated positive or negative psychological sequelae are linked to rapid changes in BMI, and are related to increases in social acceptance or continued marginalisation.

Unfortunately weight stigmatisation is widespread, and leads to psychological, social and physical health consequences. However, health-care professionals have an important role to play in reducing the negative effects of obesity stigmatisation, and clinical care can be improved through role modelling, using appropriate language and terminology, and by ensuring a safe and welcoming environment (308).

2.4.5 Adolescent bariatric surgery

The number of adolescents having bariatric surgery has increased over the past decade, most likely due to increasing rates of adult bariatric surgery, coupled with the fact that in children with severe obesity, non-surgical treatments have a limited effect (309). The most common types of bariatric procedures performed in adolescents include Roux-en-Y gastric bypass (RYGB), Laparoscopic Adjustable Gastric Banding (LAGB), and Sleeve Gastrectomy (SG), and in the majority of cases all 3 procedures are performed laparoscopically. RYGB involves creating a small gastric pouch below the gastroesophageal junction and connecting a Roux-en-Y limb of jejunum to the pouch. It is both a restrictive and malabsorptive procedure. LAGB and SG however are both restrictive procedures. In LAGB, a band is placed around the proximal aspect of the stomach, below the gastroesophageal junction, and a catheter is attached to the band which is connected to an infusion port on the abdominal wall, and by injecting saline the band can be tightened or loosened. In SG, most of the stomach is removed, leaving a tabularized stomach which is 85-90% smaller than its original size (310) (figure 2.8). More extreme malabsorption accompanies the biliopancreatic diversion operation,

in which a short distal common channel length of small intestine severely limits caloric absorption, with a sleeve gastrectomy also being carried out (311). Owing to concerns about long-term nutrient deficiencies associated with malabsorption procedures, as well as lower long-term weight loss associated with LAGB procedures, SG, which is a relatively new procedure, is often recommended for adolescent patients (312). In some patients, an intragastric balloon can be inserted endoscopically to provide short-term weight loss. The silicon balloon is filled with liquid and partially fills the stomach, creating a feeling of fullness. The maximum time it can be left in place is 6 months, and indications for use may be in morbidly obese adults prior to obesity surgery, younger children, or in patients with learning difficulties (261).

Best-practice guidelines on adolescent bariatric surgery were published in 2012 by the American Society for Metabolic and Bariatric surgery pediatric committee (313), in order to provide more uniformity and guidance in this complex area of obesity management in children. Adolescent patient criteria includes:

- BMI ≥35kg/m^2 and with major co-morbidities (T2DM, moderate to severe OSA, pseudotumor cerebri or severe NASH).
- BMI ≥40kg/m^2 and with other co-morbidities (hypertension, glucose intolerance, dyslipidaemia).
- Post-pubertal or have completed 95% of estimated growth.
- Demonstrate an understanding of the lifestyle changes needed after surgery.
- Psychosocial assessment to demonstrate they are capable of making an informed decision and understand the risks and benefits of surgery.
- Social support so that together with their family they are able to adhere to the pre- and post-operative recommendations.

In addition, all operations should be performed in a specialised bariatric surgery centre (313).

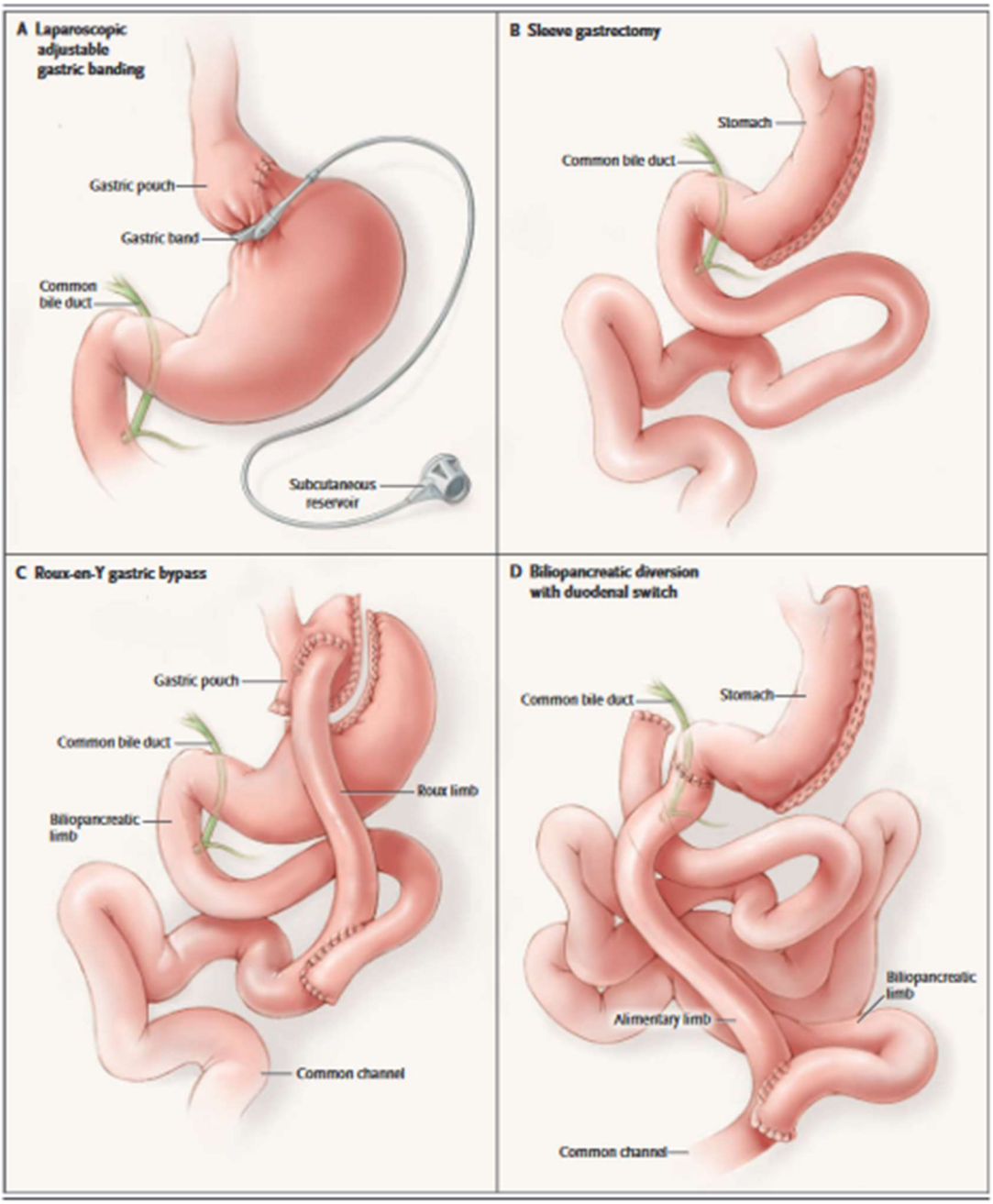


Figure 2.8. Common surgical procedures for weight loss. (311)

The results of outcomes following adolescent bariatric surgery are limited by the small sample size and short follow-up times of several of the studies, coupled with the heterogeneity among studies and their retrospective nature. The longest follow-up to date is from a retrospective analysis of 33 adolescents who had been followed-up for 21 years after a variety of gastric bypass surgery modifications (314). The mean age of the cohort was 16 years with a mean BMI of 52 kg/m^2, and after 14 years the average BMI was maintained at 38 kg/m^2. However, 5 patients (15%) regained all or most of

their lost weight (314). While it is difficult to ascertain the degree of weight regain in adolescent patients after RYGB, it is thought to be as high as 20% (315). In a meta-analysis of bariatric surgery in 637 paediatric patients (316), the average weighted mean BMI difference from baseline to 1 year was shown to be -13.5 kg/m^2. When analysed by surgery type, RYGB, LAGB and SG were associated with an average BMI loss at 12 months of -17.2 kg/m^2, -10.5 kg/m^2 and -14.5 kg/m^2 respectively, based on 3 studies (316). Improvements in obesity-related comorbidities are quickly seen in adolescents following bariatric surgery, with one German study reporting rates of diabetes, hypertension, and sleep apnoea reduced by approximately half in 167 adolescents and young people following gastric bypass, LAGB, or SG at 18 months of follow-up (317). In fact glycaemic control in patients with diabetes often improves almost immediately after surgery, preceding any significant weight loss, which suggests that alterations in gastric hormones that augment insulin secretion (the incretins) may be altered in gastric bypass, and this phenomenon is also seen in adults (128). The largest report on outcomes after SG involved 108 children and adolescents aged 5 – 21 years, and found that resolution rates for dyslipidaemia were 70%, hypertension was 75%, sleep apnoea was 91% and diabetes and prediabetes was 94% and 100% respectively (318).

Unfortunately there is a paucity of long-term safety data in adolescents undergoing bariatric surgery, although shorter-term information is available. The Teen-Longitudinal Assessment of Bariatric Surgery study (319) collected standardized data from multiple centres performing bariatric surgery, and reported on the perioperative outcomes of 242 adolescents aged 19 years or younger who had bariatric surgery in the U.S from 2007 – 2011. The average age was 17.1 years, with a median BMI of 50.5 kg/m^2, and 51% of patients had ≥ 4 major co-morbidities. There were no deaths reported in the 30 days following the procedure, although the majority of complications occurred before discharge from hospital, with 5% (13 patients) having a major complication (bowel obstruction, gastrointestinal leak/sepsis, transfusion for post-operative bleeding, splenectomy for intraoperative splenic injury, and anticoagulation for Deep Vein Thrombosis), and 8% having a minor complication, with urinary tract infection being the most common. Overall, within 30 days of surgery, 19 patients (8%) experienced a major complication and 36 (15%) had a minor complication (319). However, the main long-term risks of bariatric surgery in adolescents are nutritional, with LAGB and SG this is related to reduced intake of food, and in RYGB it is malabsorption as well as reduced food intake. Adolescents have a variable but low rate of adherence

to vitamin supplementation, so are more vulnerable to developing nutritional deficiencies (320). Recommended supplementation includes a chewable multivitamin, calcium citrate with vitamin D, vitamin B12 and iron, with annual monitoring for iron deficiency anaemia, calcium, vitamin B12, vitamin D and Parathyroid Hormone recommended (128).

NHS England guidance recommends that follow-up post gastric band insertion should be for a minimum of 5 years in a specialised unit, and in patients who have received a gastric bypass or sleeve gastrectomy, then life-long follow-up is required, although this can be in a shared care agreement with local paediatric or adult tier 3 units (261).

Post-operatively there can be an improvement in psychosocial functioning, with a short-term study from Sweden (321) involving 37 adolescents, demonstrating an overall significant improvement in symptoms of depression, anxiety and self-concept from baseline to 4 months after RYGB, with no change in anger or disruptive behaviour. However, 16% of adolescents went on to exhibit deterioration in 2 or more of these aspects, highlighting the importance of close psychological monitoring following bariatric surgery. There can also be negative sequelae following bariatric surgery, such as the development of disordered eating (binge eating, night eating and purging), the presence of excess skin following significant weight loss, and increased fertility which can leave vulnerable females at increased risk of unwanted pregnancies (322). It is also important to ensure equitable access to this treatment, as disparities have been identified in adult studies, with fewer African Americans, Hispanics, low-income individuals and males undergoing bariatric surgery than would be expected (323).

While adolescent bariatric surgery appears to be an effective treatment option for certain patients for whom other interventions have failed, further long-term studies are required in order to monitor potential complications, to identify if weight loss and improvements in metabolic risks are maintained into adulthood, (324) and also to determine which patients benefit most from surgery.

2.4.6 Evidence for the importance of satiety hormones in body-weight regulation from bariatric surgical studies

Changes in the enteric hormones involved in the gut-brain axis have also been implicated in the anorexigenic and weight reducing effects of bariatric surgery (128). It is thought that in RYGB the re-arrangement of the GI tract alters the release of different GI satiety hormones, thereby eliciting an endocrine response that results in profound changes in metabolism and body weight as demonstrated by significant reductions in appetite and earlier sensation of satiety following meals. It is therefore hypothesised that non mechanical factors may play a part in the long-term weight loss experienced following RYGB, possibly through the establishment of a new set-point for body weight (128).

RYGB produces a greater reduction in obesity related co-morbidities compared to gastric banding or sleeve gastrectomy, and patients are more likely to maintain their weight loss following the procedure. Several studies have shown that RYGB alters the release of several of the satiety hormones produced in the gastrointestinal system, and of particular significance is the dramatic increase in the post-prandial rise in the anorexigenic hormone PYY, possibly due to a more rapid delivery of nutrients to the distal ileum, which is only seen after RYGB and not LAGB (128). The effects of bariatric surgery on ghrelin however, remain inconsistent, with some adult studies demonstrating a decrease in ghrelin levels within 24 hours of RYGB surgery (325) and also several months following surgery (326). In contrast, other studies have found an increase in ghrelin levels following RYGB (327) while others have reported no differences in pre or post- surgical ghrelin levels (328). There is also insufficient data in adolescents, as there is only a single study in an adolescent with hypothalamic obesity secondary to radiation therapy for craniopharyngioma, which evaluated the change in ghrelin after RYGB, and although a reduction in peak and basal active ghrelin and insulin levels were observed, overall ghrelin profiles were not affected (329).

Following RYGB surgery, an increase in REE after the procedure has been observed in animal models, which is different to the response after weight loss through food restriction, where a compensatory reduction in REE occurs. This difference, in addition to the favourable changes in satiety hormone responses, may explain why patients are more successful in maintaining long-term weight loss following RYGB, in contrast to purely dietary restriction methods, although as yet no definitive association in human models have been made between energy expenditure changes and RYGB. However, a

study from Chile (330) demonstrated that after a RYGB, patients who had an initial lower REE before surgery had an increase in their REE, while those patients who had a normal or increased REE before surgery did not experience a significant change in their REE afterwards, even when FFM was adjusted for.

Chapter 3 Identifying differences in the defence of body weight across the life course

3.1 Concept behind the research.

While most obese adults can lose weight in the short term, the majority regain their weight in the longer term (81). It has been suggested that adults have a weight that is inherently determined, and this is referred to as a set-point for weight, and occurs through a combination of genetics, lifestyle and environmental factors (83). Several studies have shown that this set-point for weight is vigorously defended despite variability in energy intake and expenditure (83). While the set-point for body weight in adults is maintained at a relatively stable level for long periods, this trend is not seen in children. Clinical evidence supports the view that young obese children who lose weight following simple lifestyle intervention, appear to slim down (331) and keep the weight off (93, 250). This raises the question of when does an individual's set-point for weight become determined. In fact differences in outcomes from obesity interventions exist across the whole of the life-course, with life-style based obesity programmes achieving good long-term outcomes in childhood (93, 250), but are associated with weight regain in post-pubertal adolescents (332) and young adults (333), but not in older adults (334), prompting questions about what determines these age-related differences. It was therefore postulated that set-points for weight are flexible in early life, but become fixed at around the time of puberty, and remain so until post-reproductive years. This novel idea had never before been tested and formed the concept behind the research.

This led to the hypothesis that plasticity exists in the regulation of body weight in young children, which allows for alterations in long-term weight status following a period of clinical obesity, but that this 'flexibility' disappears by post-pubertal adolescence, and then re-emerges again later in adult life.

Two key studies provided direct evidence to support this hypothesis. In 2009, an adult obesity study by *Proietto et al* recruited 50 obese adults from a specialist obesity clinic to study the physiological adaptations which occur with weight change (335). A ten week intervention delivered an extremely low-calorie diet, providing just 500 to 550 calories a day, to those with a mean baseline weight of 95.4kg and BMI of 34.7 kg/m2. By the end of the intervention, the mean (Standard Error) weight loss was 13.5 (0.5) kg. At that point the 34 participants who remained in the study stopped dieting and began

working to maintain the lower weight. Counselling was provided, delivering nutritional and physical activity advice, but despite the patient's best efforts, they slowly began to regain weight, and after a year the mean (SE) weight regained was 5.5 (1.0) kg. The participants also reported feeling more hungry and pre-occupied with food than before they had lost weight. However, the comparison of circulating changes in satiety hormones in response to a standard breakfast meal revealed the most interesting findings. In association with the weight loss there was a significant increase in ghrelin (associated with feelings of hunger) and decreases in PYY, amylin and CCK (all associated with reduced feelings of fullness) following the meal (figure 3.1). Furthermore these changes were still evident 1 year after the intervention, highlighting just how vehemently the adult body protects against weight loss.

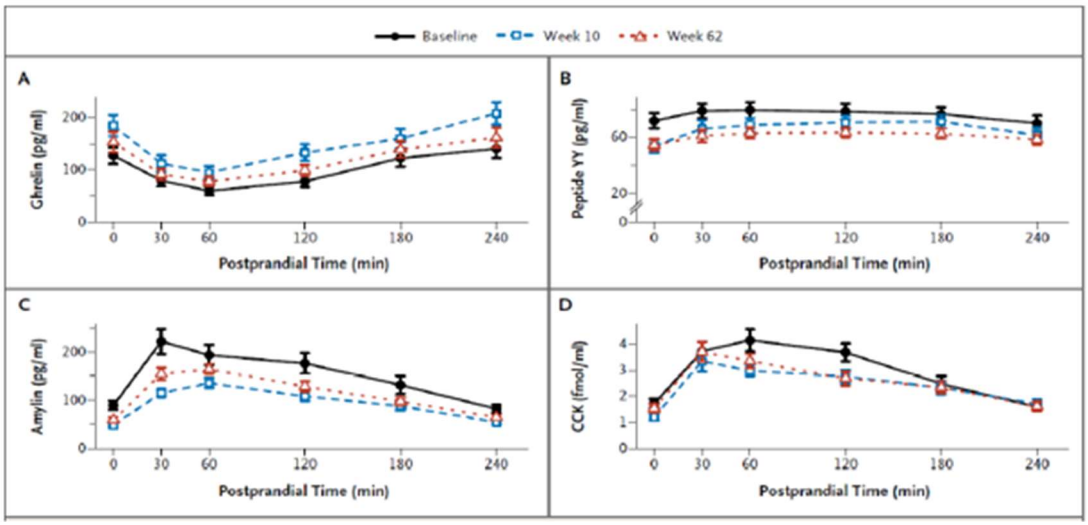


Figure 3.1. Mean (+/- SE) postprandial levels of ghrelin, PYY, Amylin and CCK at baseline (black circles), 10 weeks following weight loss (blue squares) and 62 weeks (red triangles). (335)

At approximately the same time, the clinical utility of a novel device called a Mandometer®, aimed at retraining eating behaviours, was being studied in obese adolescents. It was initially developed to encourage individuals with anorexia and bulimia nervosa to eat more (336). The device consists of a portable weighing scale (upon which a plate of food is placed) which is connected to a small computer that shows the patient how quickly they are eating. The study investigators were interested to see whether using this device, alongside provision of general lifestyle advice, could improve weight loss in obese adolescents. The study was an RCT involving 106 peri-pubertal obese adolescents (337), and the baseline BMI was almost identical to the adult study described above (33.8kg/m^2). The use of the Mandometer® was associated with significant reductions in weight when compared to standard

life-style based treatment alone (338), with reductions in BMI almost identical to the adult study described above (-2.5kg/m^2 vs -2.8g/m^2). However, following the intervention in the adolescent obesity study, the adolescents continued to keep the weight off, and satiety hormone profiles following an oral glucose load moved in the opposite direction to that seen in the adult study, where weight regain rather than sustained weight loss, had been the more common outcome. Instead of increases in ghrelin, levels were significantly reduced, and instead of reduction in PYY there were significant increases, both of which would be compatible with a state of enhanced satiety which would favour long term maintenance of weight loss (337). These data are shown in figure 3.2, and with the adult data in figure 3.1, emphasize the different physiological adaptations to weight loss between peri-pubertal adolescents and adults.

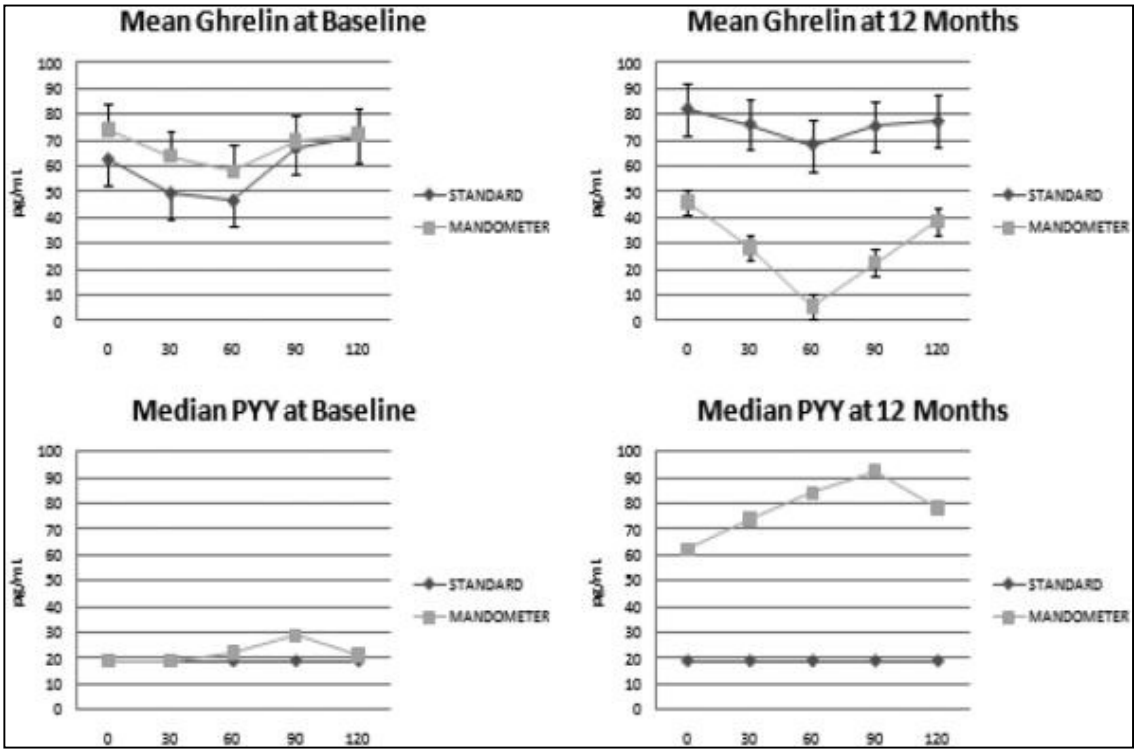


Figure 3.2. Ghrelin and PYY levels during the oral glucose tolerance test at baseline and 12 months after the intervention. (337)

It is not possible however to make direct comparisons between the two studies, as the interventions were very different, with markedly different study designs, and they were undertaken over different time periods. Therefore it was felt that a single study assessing weight loss, weight regain and associated physiological responses in young children, adolescents, younger and older adults was warranted.

The original aim of the study was to compare future weight trajectories, and associated metabolic adaptations, following a period of weight loss in obese children, adolescents, young and older adults.

The experimental paradigm would be to delineate future weight trajectories, and associated physiological drivers to weight regain in 4 age groups of obese individuals (pre-pubertal children, post-pubertal adolescents, young and older adults) following a 6-12 month intervention which achieved >10% relative or absolute weight loss (see figure 3.3), to compare:

a) Age-related patterns of weight regain, and
b) The homeostatic mechanisms associated with these weight changes.

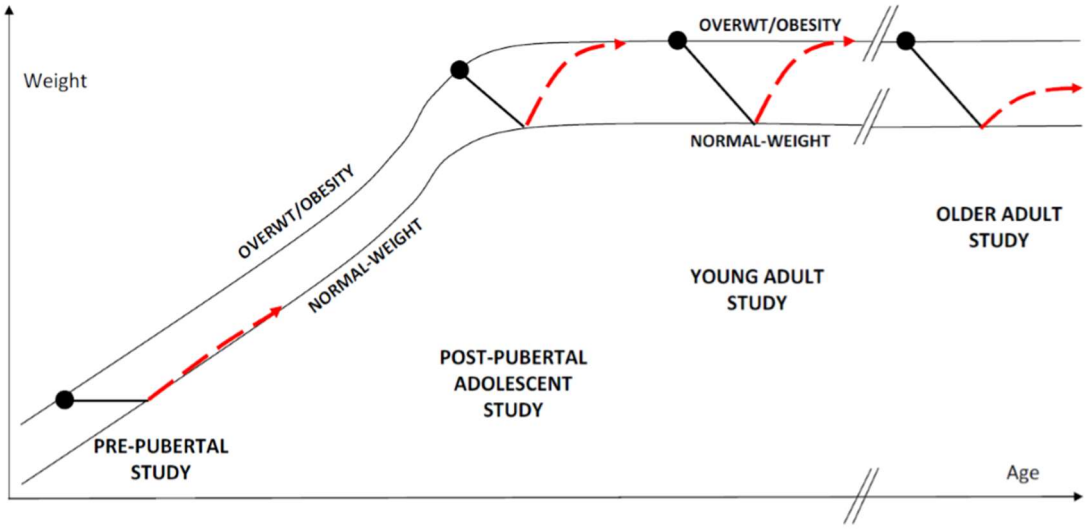


Figure 3.3. Experimental paradigm for study design. Black dots represent obese patients undergoing a 1 year programme (solid line), and the hashed red arrowed lines represent hypothesised outcomes.

This research plan was submitted as a multicentre National Health and Medical Research Council (NHMRC Australia) grant application in the 2012 funding round, but was not successful primarily because of an absence of supporting preliminary data in children and adolescents.

Consequently, this led to the current study looking at satiety signalling in obese children and adolescents before and after puberty, to provide important data for a future multi-centre study in Melbourne, Australia, involving both children and adults.

3.1.1 Life-style based obesity interventions in adults

Although initial weight loss is achievable with lifestyle change in the majority of obese adults, more than 90% will ultimately regain weight after dieting, highlighting the tight regulatory set-points for body weight acting to prevent against weight loss (97). This has led to change in direction in adult obesity research away from simply quantifying degrees of weight loss using different approaches, and more to understanding their role in prevention of weight regain and achieving long-term maintenance of weight loss (333).

There is evidence that older adults have better outcomes with lifestyle orientated weight loss programmes. In a RCT which evaluated the effects of diet and/or exercise in 107 obese adults aged ≥ 65 years, those randomised to dietary restriction obtained a mean persistent reduction in weight of 10% at 1 year, compared with <1% in the control group (334), which was a favourable amount compared with outcomes in younger adults over the same duration (339). However, no follow-up data are available to show whether these individuals are at the same risk of even longer-term weight regain when compared with younger adults. The physiological differences in metabolism between younger and older adults would suggest that older adults are less likely to regain weight than younger adults (340, 341), but meta-analysis of weight maintenance in obese adults who have lost weight, have not directly examined the effects of baseline age or of the duration of pre-existing obesity (263). To date, no studies have directly compared outcomes relating to weight gain in younger versus older adults.

3.1.2 Significance of Research

The successful prevention and treatment of paediatric obesity is critical to the prevention of adult obesity. The longer an individual is obese, the greater is their risk of weight related disease or death (342). However, an epidemiological study has shown that overweight and obese children and adolescents who grow up to be non-obese adults, have the same weight-related disease in adulthood as if they had never carried any excess weight (226). The problem is that most overweight and obese children grow up to be obese adults (343), as weight tracks across the life course (344, 345).

In addition, it has been shown that a diagnosis of metabolic syndrome in childhood which resolves by adult life, is associated with complete resolution of risk for later carotid-intima media thickness, which is a marker for cardiovascular damage, and type 2 diabetes (346), emphasizing the plasticity

that the childhood years offer in recovering from a weight related metabolic insult.

Together, these findings provide the greatest incentive to developing effective programmes to successfully prevent and treat overweight and obese youth, as they promise the greatest long-term health benefits (229).

3.1.3 Weight Management Service (WMS) at The Royal Children's Hospital, Melbourne

The Weight Management Service (WMS) at The Royal Children's Hospital in Melbourne, Australia, is the largest paediatric obesity service in the Southern hemisphere. The Multidisciplinary Team includes a consultant paediatric endocrinologist and paediatric gastroenterologist, a weight management nurse, a dietician, a psychologist, a social worker and an exercise physiologist. There are currently 450 patients aged ≤ 16 years in the WMS, and approximately 6 new and 20 follow-up patients are seen each week. Referral criteria to the service includes:

- Age ≤ 10 years with obesity (BMI > 95th centile).
- Age > 10 years with obesity (BMI > 95th centile) and established comorbidity (obstructive sleep apnoea, hyperlipidaemia, orthopaedic problems such as slipped upper femoral epiphysis, polycystic ovary syndrome, hypertension, non-alcoholic fatty liver disease, type 2 diabetes/insulin resistance).
- Aged <5 years with rapid weight gain.

3.1.4 Childhood Overweight BioRepository of Australia (COBRA)

All of the patients within this study had originally been recruited to the Childhood Overweight BioRepository of Australia (termed "COBRA"). COBRA collects baseline environmental, clinical and anthropometric data, whilst also simultaneously storing blood samples from patients for genetic, metabolic and hormonal profiles. It was established in 2009 to investigate the development of weight related co-morbidity in target populations with established obesity, with the aim of early identification of both risk factors and potential solutions, which would allow further refinement of obesity prevention and treatment programmes (347). The Royal Children's Hospital (RCH) at Melbourne is the parent site, and all overweight and obese patients referred to the Weight Management Service are approached for enrolment to COBRA. This project

has full ethical approval and is now well established. The response rate among eligible participants has been high, as has the level of compliance, ensuring a comprehensive level of data collection (347). As of 2016, approximately 500 patients have been enrolled to COBRA.

3.1.5 Scoping Project: Auxological data looking at all male patients attending the WMS from 2008 – 2014 who had attended clinic 5 or more times

Owing to inherent differences in body composition between maturing males and females (348), and also the associations that exist between obesity and puberty (101), it was originally proposed that the study would only be undertaken in males.

Using the COBRA database, a scoping project looking at auxological data collected from all male patients attending the WMS at The Royal Children's Hospital, Melbourne, between 2008 and 2014, who had attended the WMS five times or more were reviewed, with the aim of observing trends in weight and BMI trajectories over time in males at different stages of childhood and adolescence.

The male patients were divided into 3 groups; post-pubertal males aged ≥ 14 years, peri-pubertal males aged 10 – 14 years, and pre-pubertal males aged < 10 years. In each group, the weight and also the BMI SDS was plotted against the decimal age for each patient, in order to observe how the trajectories changed over time for each group.

The post-pubertal group had 6 patients and the general trend for the majority of patients was that both the weight and BMI SDS increased over time (figures 3.4 and 3.5). In the peri-pubertal group there were 18 patients, and both the weight and BMI SDS remained static in the majority of patients (figures 3.6 and 3.7). Finally, in the pre-pubertal group there were 16 patients and the weight increased over time but the BMI SDS decreased (figures 3.8 and 3.9).

These observations appeared to support our hypothesis that pre-pubertal children had greater flexibility in changing their body weight trajectories compared to post-pubertal adolescents. It also became apparent from the scoping project that there would be insufficient post-pubertal males to recruit to the study for it to be undertaken only in males. It was therefore decided that females would also be recruited.

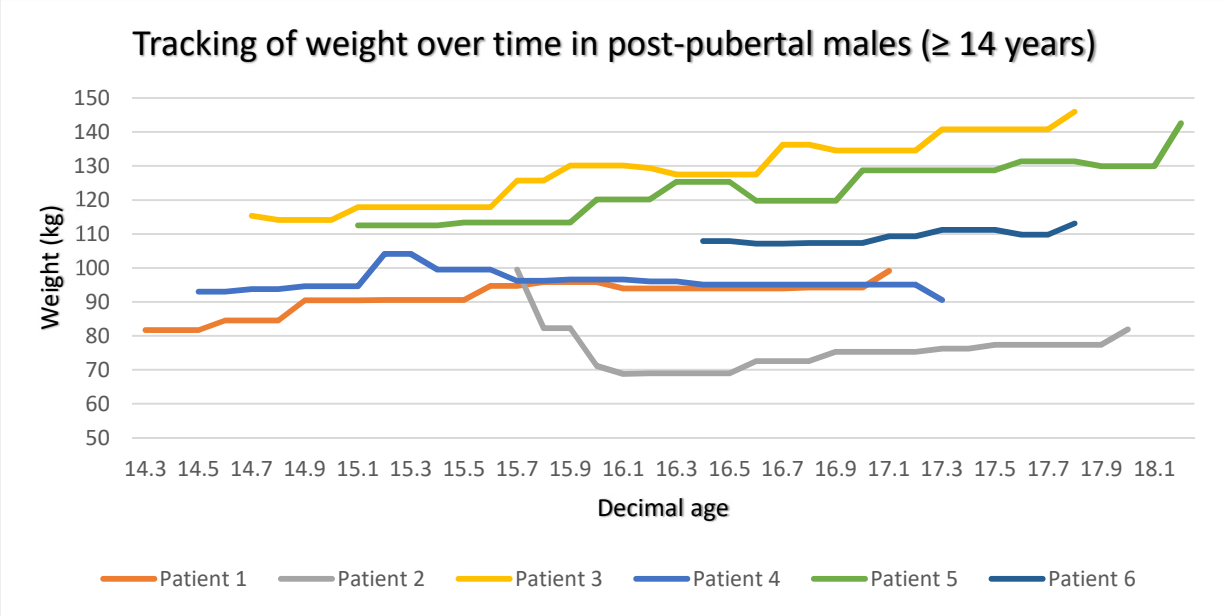


Figure 3.4. Tracking of weight over time in post-pubertal males aged ≥ 14 years recruited to COBRA between 2008 and 2014

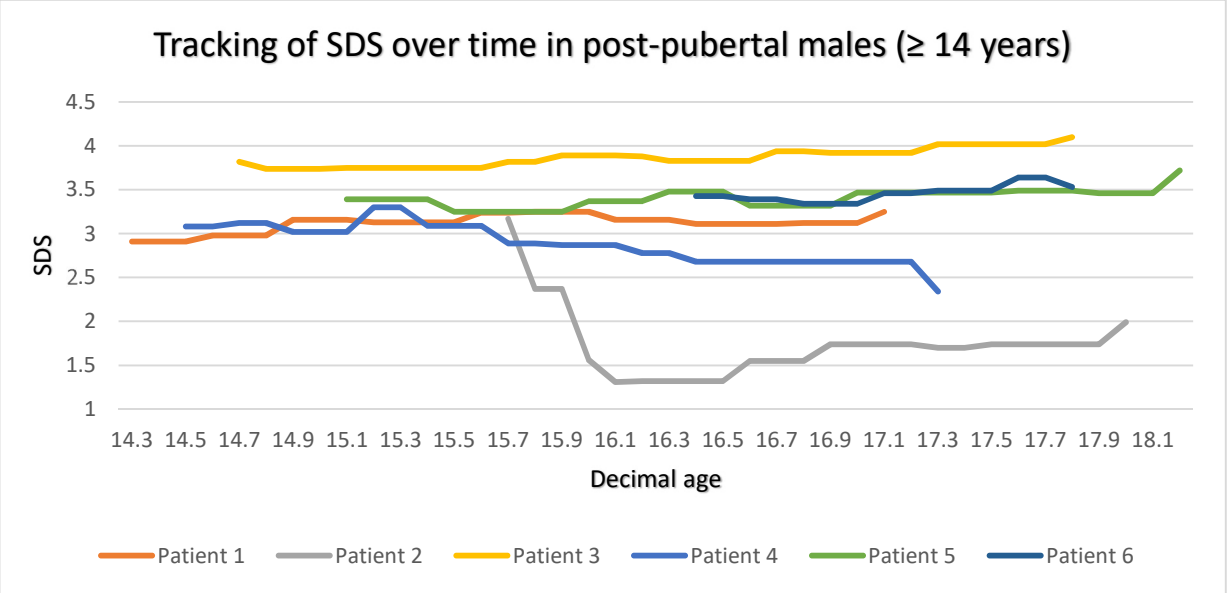


Figure 3.5. Tracking of SDS over time in post-pubertal males aged ≥ 14 years recruited to COBRA between 2008 and 2014

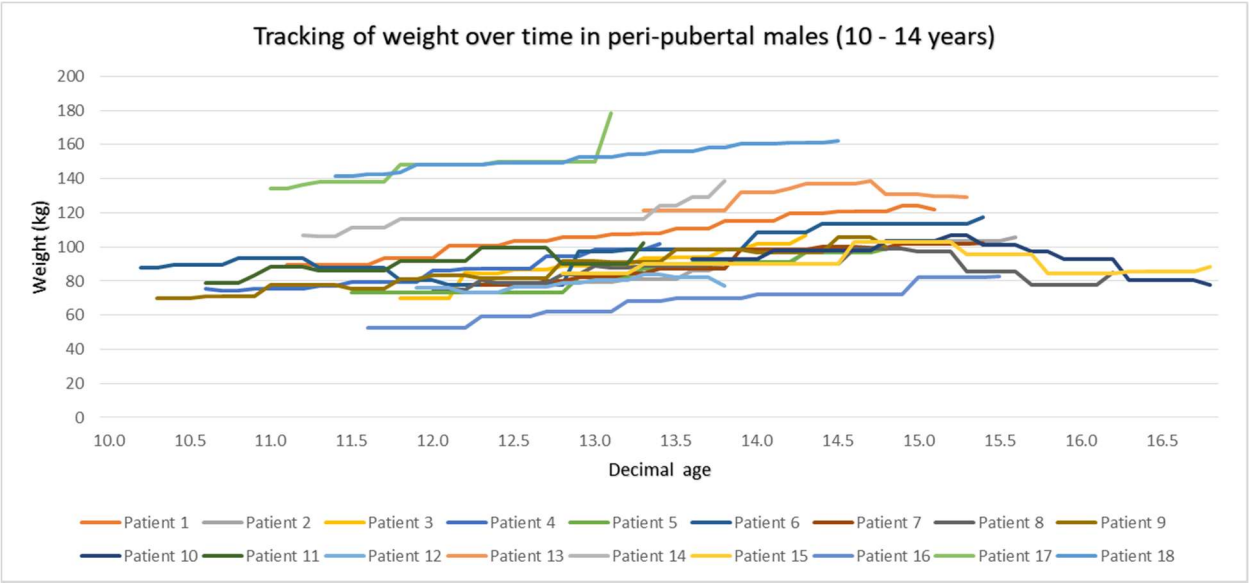


Figure 3.6. Tracking of weight over time in peri-pubertal males aged 10 - 14 years recruited to COBRA between 2008 and 2014

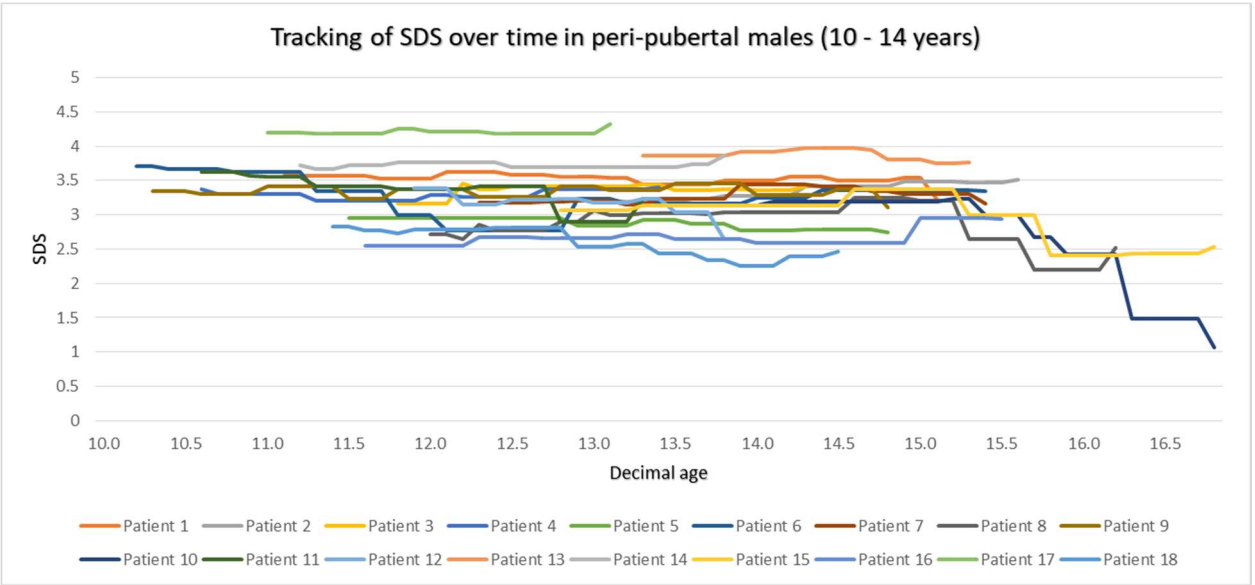


Figure 3.7. Tracking of SDS over time in peri-pubertal males aged 10 - 14 years recruited to COBRA between 2008 and 2014

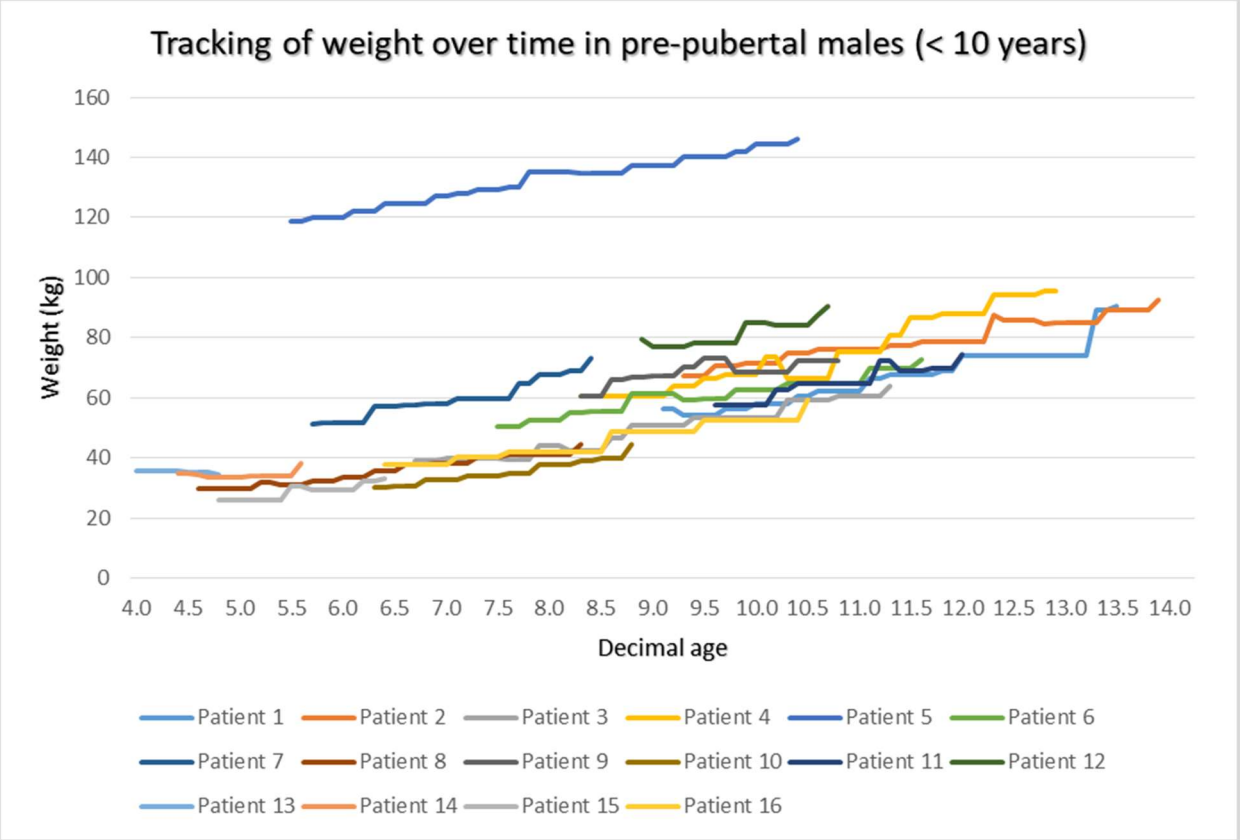


Figure 3.8. Tracking of weight over time in pre-pubertal males aged < 10 years recruited to COBRA between 2008 and 2014

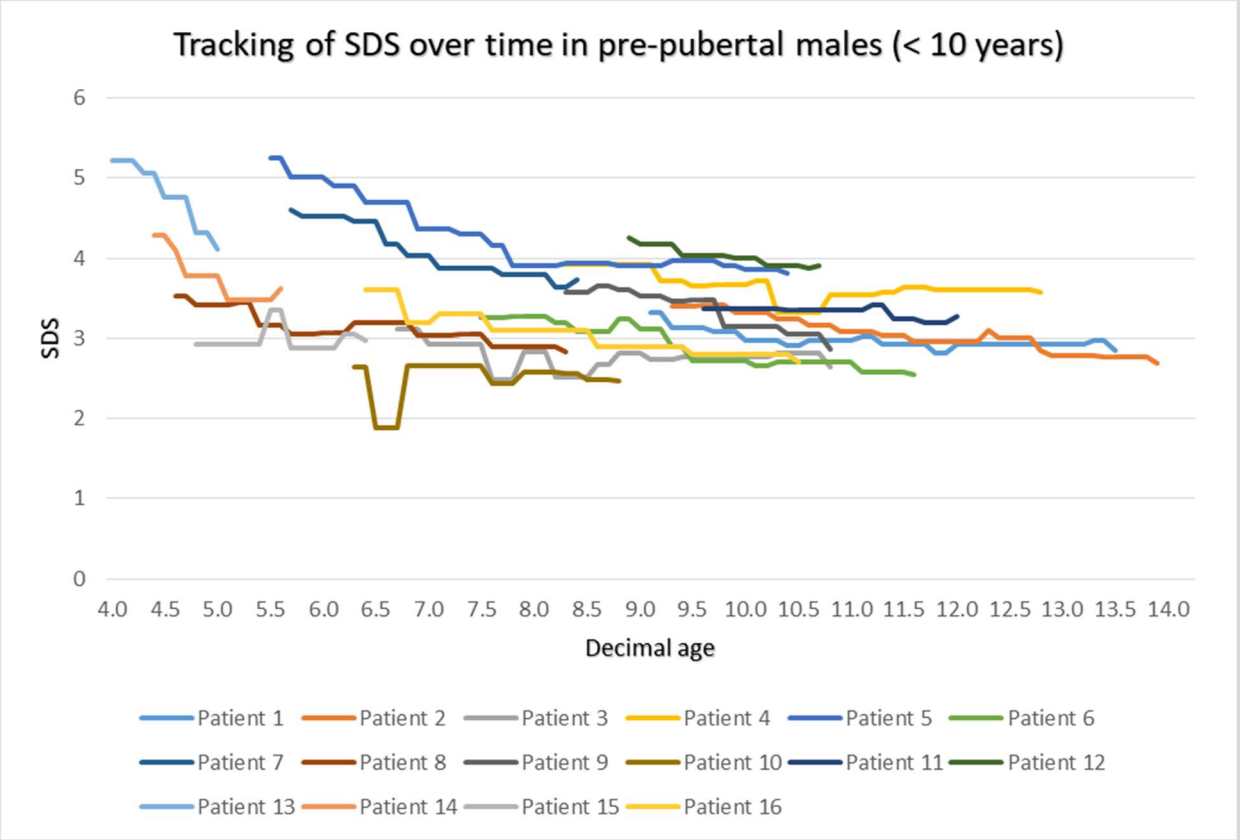


Figure 3.9. Tracking of SDS over time in pre-pubertal males aged < 10 years recruited to COBRA between 2008 and 2014

3.1.6 Preliminary data leading to this study

It has been shown that:

- Childhood obesity is associated with significant morbidity (193), particularly through the tracking of obesity through the life-course (349), but that long-term disease risk can be completely ameliorated by the prevention of tracking of obesity from childhood to adulthood (226).
- A diagnosis of metabolic syndrome in childhood is significantly more labile than in adulthood (350), possibly due to the changes in BMI trajectories which occur throughout the childhood years, requiring a different approach to its treatment in young people compared with adults (351).
- Although the detrimental effects of obesity on the juvenile cardiovascular system are evident at a young age, only risk factors present in later childhood and adolescence are associated with long-term carotid-intima media thickness (352).
- Younger age at presentation to a paediatric obesity service is associated with the greatest reductions in BMI SDS (331).
- The timing of puberty is integrally linked to weight gain and may play an important role in determining the degree of future weight gain (101).

3.2 Aims of Thesis

Aim:

The aim of this research was to carry out a pilot study to observe how satiety signalling varies in obese children and adolescents for a given level of preceding BMI SDS change, and to compare future weight trajectories and associated metabolic adaptations.

Primary Outcome:

The primary outcome was the subsequent change in BMI SDS following the period of attempted weight change, in pre-pubertal children versus post-pubertal adolescents.

Secondary Outcomes:

The secondary outcomes included a) differences in circulating satiety hormone concentrations for a given level of BMI change in obese pre-pubertal children and post-pubertal adolescents, by comparing the circulating changes in satiety hormones in response to a standard test meal in both groups after a period of weight loss, b) changes in body composition, resting energy expenditure and fasting Triiodothyronine (T3) levels.

Chapter 4 Satiety signalling in obese children and adolescents

4.1 Methodology

4.1.1 Study Design

This was a prospective cohort study, with a retrospective element.

4.1.2 Study Participants

Patients from COBRA (347) (Childhood Overweight BioRepository of Australia), who were already regularly attending the Weight Management Service, were considered for recruitment to this study.

41 subjects; 21 obese pre-pubertal children (aged 3-7 years; 11 male) and 20 obese adolescents (aged 14-18 years; 10 male) were recruited from the paediatric obesity service at The Royal Children's Hospital in Melbourne. The age ranges for the two groups were chosen to avoid the main period of time that pubertal maturation most commonly occurs (10 -13.99 years in males and 9 -12.99 years in females). Pubertal development was clinically assessed using Tanner stages and those in true puberty i.e. testicular volume ≥ 4mls to <15mls in males and Tanner breast stage >1 to pre-menarche in females, were excluded.

Power for the study was calculated from a similar study in adults (335), and a formal statistical power calculation was not carried out.

4.1.2.1 Inclusion criteria

Inclusion criteria to this study required a Body Mass Index Standard Deviation Score (BMI SDS calculated using the UK 1990 growth reference data) of ≥ 2.4 taken 9 – 15 months prior to when the satiety hormone profile would be carried out.

The SDS value of 2.4 as the cut off for obesity was based on the paper by *Cole et al* (6). Dataset 4.1 is taken from the paper and shows the centiles and z scores for obesity corresponding to a BMI of 30 kg/m^2 at age 18 years in 6 datasets, derived from fitted LMS (Lambda Mu Sigma) curves. As shown in the dataset, the z score of 2.4 is above the threshold for obesity in the UK

population, and Australia has a similar dataset. If an SDS of 2 was used as the threshold the concern would be that the cohort were not strictly obese, which is why a value of 2.4 was used instead.

Dataset 4.1. Centile and z scores for obesity corresponding to a BMI of 30 kg/m^2 at age 18 years in 6 datasets, derived from fitted LMS curves. (6)

Country	Males			Females		
	Centile	Z score	% above cut off	Centile	Z score	% above cut off
Brazil	99.9	3.1	0.1	98.0	2.1	2.0
UK	99.1	2.37	0.9	98.8	2.25	1.2
Hong Kong	96.9	1.86	3.1	98.2	2.1	1.8
Netherlands	99.7	2.71	0.3	99.7	2.73	0.3
Singapore	98.3	2.12	1.7	99.0	2.33	1.0
USA	96.7	1.84	3.3	96.0	1.76	4.0

The study included both males and females, as the initial scoping project had highlighted that there were insufficient post-pubertal males to carry out the study with only male participants.

As oestradiol exerts a cyclical effect on some of the satiety hormone responses it was thought that it may affect the results obtained in the post-pubertal females (353) depending on where in their menstrual cycle they were when they had their bloods taken. It was therefore decided that where possible, the post-pubertal females would have their blood tests taken for their satiety hormone profiles during the second half of their menstrual cycle.

There is no evidence for a gender effect on satiety hormone responses in pre-pubertal children.

4.1.2.2 Exclusion criteria

Patients with identified hormonal, medical or genetic reasons were excluded, and also those with significant illness, including diabetes, or on medications known to affect body weight or appetite. Smoking, present or past history of eating disorders, and previous bariatric surgery also resulted in exclusion. In addition, those in true puberty, and at a stage where increases in circulating

testosterone or oestrogen and growth velocity are typically seen were excluded.

4.1.2.3 Consent

As part of their enrolment to COBRA, all of the participants had already signed a consent form, which allowed the use of their data and biologically stored samples in any future ethically approved studies without requiring the COBRA research group to re-contact the participant for consent. In this study we were using the participant's original auxological and epidemiological data collected at their enrolment to COBRA, and we had also considered carrying out satiety hormone profiles on the original stored serum samples, but due to financial constraints we were not able to do this.

Participants to this study were still required to consent to having additional blood tests taken for the satiety hormone profiles before and after eating the standardised test meal, and to having an assessment of their Resting Energy Expenditure, as these represented additional measures to those normally carried out as part of the standard clinical practise at the WMS at The RCH. This study was therefore submitted as a new project to ethics, with separate information and consent forms, although the study is essentially a "run-on" project from COBRA.

Ethics approval for this study was authorised by The Royal Children's Hospital Human Research Ethics Committee (HREC No. 33115C), and written informed consent was obtained for all participants.

Children with the maturity to determine their own willingness to consent (approximately age 16 years and older) were permitted to sign their own informed consent, with a separate copy on record signed by the parent. In cases where informed consent had been provided by a parent/guardian, the child was given the opportunity to provide their own consent to participate, at age 18 years. This was to ensure explicit permission from the participant was obtained once they had reached the legally defined age of adulthood.

4.1.3 Data Collection

All patients recruited to this study received a comprehensive medical, auxological and biochemical review as part of their enrolment to COBRA, although the majority of the measures carried out represent standard clinical practice for patient management in the WMS at The RCH.

4.1.3.1 Medical History

A full medical history was obtained from each child on enrolment to COBRA using a standardised data collection sheet. Specific information collected included:

- Maternal and paternal obesity status (BMI ≥ 30)
- Maternal smoking during pregnancy
- Duration of breast feeding (exclusively for > 3 months)
- Birth weight >4kgs vs ≤ 4kgs
- Basic demographics
- Socioeconomic status and family dynamics
- Ethnicity, which was defined by self-identification.

This information allowed for confounding variables to be accounted for in order to minimise their impact on our findings.

4.1.3.2 Auxology

Every child was weighed (SECA chair scales, model no. 9567021099) and their height recorded using a stadiometer (Holton Ltd stadiometer, CRYMMYCH Pembs UK). BMI was calculated and BMI Standard Deviation Scores (SDS) were generated using UK90 standardised growth reference data. Body composition was assessed by bioimpedance, which was measured using a Tanita Body Composition Analyser (Model BC-418) (22). Pubertal staging was performed by the method of Tanner and Whitehouse. Baseline information from the participants' initial enrolment to COBRA was used.

4.1.3.3 Medical examination

A complete medical examination was carried out as part of routine clinical care, and also for recruitment to COBRA. Specific details relating to obesity and its causes and consequences were already documented. A paediatric endocrinologist carried out the pubertal assessment on each participant.

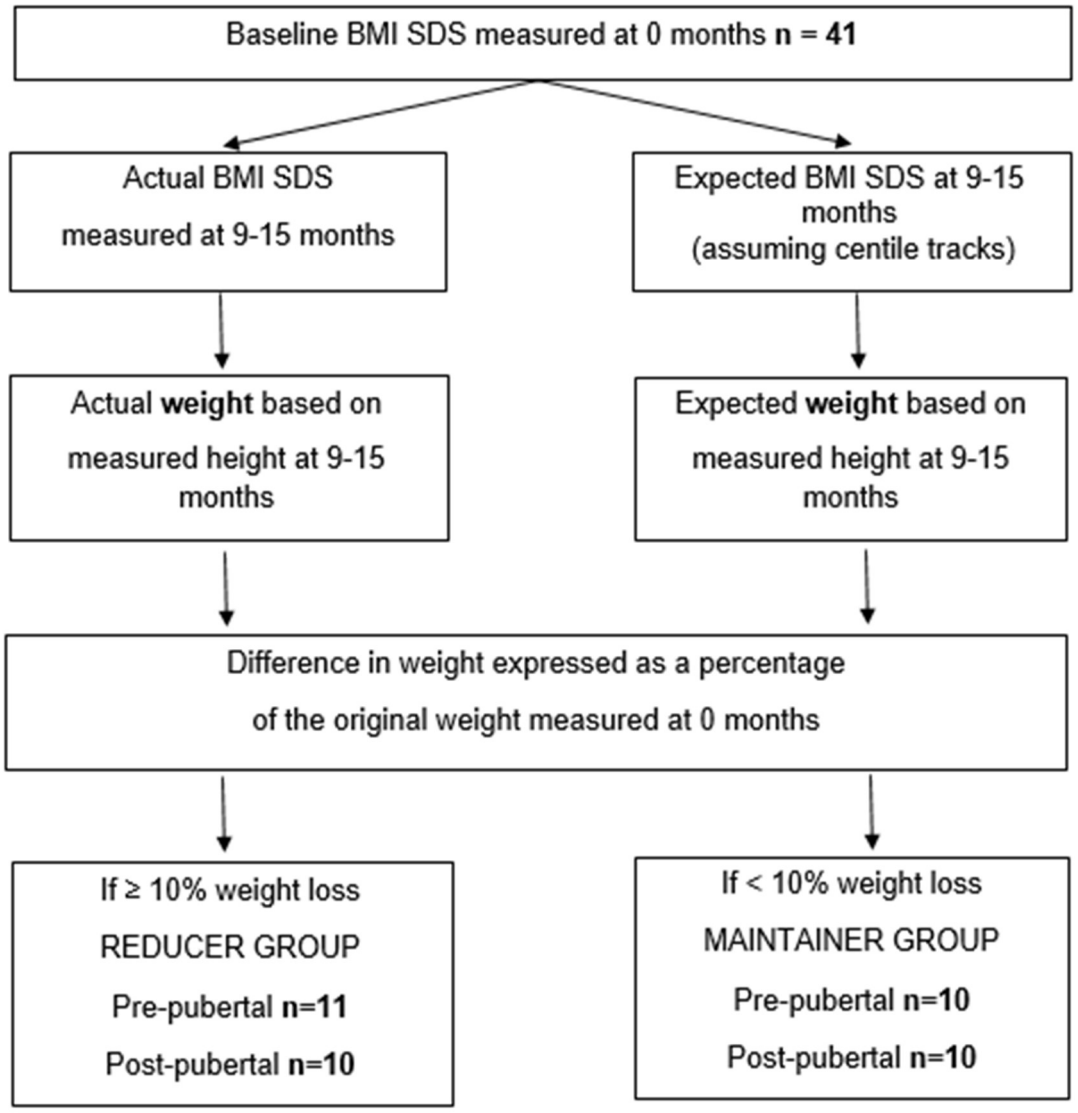
4.1.3.4 Grouping

Participants in pre (n=21) and post-pubertal (n=20) groups were divided into 4 groups of 10, with 2 "reducer" groups (one pre-pubertal and one post-

pubertal) and 2 “maintainer” groups (one pre-pubertal and one post-pubertal). The reducer groups comprised obese patients who had achieved a relative or absolute weight loss of ≥ 10 % in the preceding 9 – 15 months, whereas participants in the maintainer groups had not. This was calculated as follows. At baseline, the BMI SDS for each participant was calculated and the predicted weight 9-15 months later determined, assuming the same BMI SDS. We then calculated their weight change as the difference between their expected weight and their actual weight at the time of their baseline satiety hormone profiling. This was expressed as a percentage by dividing by the original observed weight, so that participants could be categorised as either weight reducers (≥10% weight loss) or weight maintainers (<10% weight loss) (figure 4.1).

The aim in the pre-pubertal children was that they would have achieved weight maintenance (leading to a 10% relative reduction in weight, alongside absolute reductions in BMI as they continue to grow) while in the post-pubertal adolescent group they would need a combination of absolute and relative weight reduction in order to achieve the 10% target.

Figure 4.1. Calculation of weight loss



4.1.3.5 Intervention

All of the participants in each of the 4 groups received the same standard clinical care through the specialist multidisciplinary WMS at RCH. This comprised assessment of their physical activity, sleep, psycho-social health and diet, while auxological measures (weight, height and waist circumference) were repeated at each 3 monthly clinic visit. However, while the intervention is the same for all 4 groups, those children in the maintainer groups (pre and post-pubertal) did not achieve a relative or absolute weight loss of ≥10% in the preceding 9 – 15 months, while those in the reducer groups (pre and post-pubertal) did.

4.1.4 Measures

4.1.4.1 Anthropometry

To minimise differences in levels of dehydration, participants initially had weight and height measured between 8-9am following an overnight fast. Body composition was then assessed by bioelectrical impedance (Tanita BC-418MA Segmental Body Composition Analyser - Tanita Corporation, Tokyo, Japan) (22). Age per se is not a limitation for using the Tanita BC-418 and the degree of resistance encountered (electrode placement) is the rate limiting factor. There are also derived predictive equations that can be applied to younger age ranges which will enable Bioimpedance based prediction of Free Fat Mass and Fat Mass values which are close to DXA results (354) .

4.1.4.2 Satiety hormone profiling

An indwelling peripheral cannula was sited following application of topical anaesthetic cream (+/- nitrous oxide sedation in younger children). Fasting blood samples were collected prior to a standard breakfast (details below), with samples then taken every 30 minutes afterwards (30, 60, 90, 120, 150 and 180 minutes). Fasting blood samples were analysed for concentrations of insulin, glucose, ghrelin, active Glucagon-like Peptide 1 (GLP-1), total Gastric Inhibitory Polypeptide (GIP), amylin, Pancreatic Polypeptide (PP) and total Peptide YY (PYY). Triiodothyronine (T3) was also measured as it appears to play an important role in the biological response to dieting (81) . Post prandial blood samples were analysed for circulating levels of ghrelin, active GLP-1, total GIP, amylin, PP and total PYY.

Blood was collected into prepared tubes, which were spun within 20 minutes of collection in a centrifuge at 23 degrees at 3000 rpm for 10 minutes. Plasma was divided into aliquots and frozen for later analysis. Collection tubes were lithium heparin (insulin, T3), glucose oxalate (glucose) and EDTA tubes containing dipeptidyl IV Inhibitor (Epitope), protease Inhibitor cocktail (Sigma) and serine protease inhibitor (Pefabloc SC; Roche Diagnostics) for GLP-1, amylin and ghrelin measurements respectively, as well as samples being taken for GIP, PYY and PP. All Aliquots were stored at -80^{o}C. Satiety hormones were analysed by Cardinal Bio-research. The Intra assay and Inter assay concentration variables respectively were similar.

The Intra assay and Inter assay concentration variables respectively were 4% and 3% for Ghrelin, 4% and 7% for GIP, 5% and 4% for PP, 4% and 3% for PYY, 4% for Amylin and 5% for active GLP-1.

4.1.4.3 Standardised breakfast meal

The standard breakfast consisted of cereal with milk and a milkshake, which was consumed within 15 minutes. One of the main challenges was palatability and food preferences in such a wide age range of children and adolescents (3-18 years). Other considerations included:

- Ability to eat the breakfast meal within a short 15 minute timeframe
- Food structure and the need to have a rapid gastric emptying time
- Requirement for minimal food handling and preparation with the utilisation of pre-prepared foods where possible, and
- Ability of the foods to be extremely consistent in terms of macronutrient composition.

The specific need to replicate macronutrient content from a published study in adults was the key driver of the macronutrient composition (335). As such, the proportions were 16% Protein, 51% Carbohydrate and 33% fat. A mid-point of Estimated Energy Requirements (EER) of 3-8 year olds and 9-17 year olds was calculated for both boys and girls. The breakfast meal was then deemed ‘ideal’ if it contained between 20-25% of EER across each age group. For 9-10 year olds only, the defined breakfast meal contains 29-30% of EER. For the reasons listed above, Coco Pops was chosen as the breakfast cereal, which may appear an unusual choice for a paediatric obesity study, but unfortunately healthier alternatives were not able to fulfil the essential criteria required of the standardised breakfast meal.

Breakfast for 3- 8 year olds (figure 2a)

25g Kellogg's Coco Pops with a 150 ml carton of ready to drink cows full cream milk, Milo milk shake containing 70ml full cream milk, 16 g full cream milk powder and 8g of Milo powder.

Breakfast for 9-17 year olds (figure 2b)

30g (1 individual packet) of Kellogg's Coco Pops with a 150 ml carton of ready to drink cows full cream milk, Milo milk shake containing 250ml full cream milk, 12g dry full cream milk powder and 25g Milo powder.

Four participants had special dietary needs and were provided with suitable alternative foods that kept the energy within 20-25% of requirements, but used the same percentage macronutrients as the standard breakfast meal.

Figure 4.2. Standardised breakfast meal for 3-8 year olds (a) and 9-17 year olds (b)

Figure 2a

Figure 2b

4.1.4.4 Hunger and satiety ratings

Each participant was asked to rate their subjective appetite using a standard visual analogue scale (VAS). This comprised of two questions, “how hungry do you feel?” and “how full do you feel?”, and subjects were asked to mark along the single 100mm line, which was anchored at the extremes with “not at all” and “extremely”. This was repeated every 30 minutes, just prior to blood sample collection, over a 3 hour period.

4.1.4.5 Resting Energy Expenditure

REE was measured in all fasted participants (prior to the insertion of the indwelling cannula) using a MedGem® hand-held indirect calorimetry device (figure 4.3), which has been shown to be valid and reliable in adolescents and young children (150, 153). Age per se is not a rate limiting factor for using the MedGem®, instead it is the operating range of the device (upper limit for airflow cannot exceed 1.5L/min for 5 consecutive breaths, minimum peak airflow of 100mL/sec and a minimum breathing frequency of 2 breaths per minute) (355).

The children and adolescents rested quietly for 15 minutes before the procedure and were all seated in the same upright position holding the MedGem® to their mouth. After ensuring that there was a tight seal around the disposable mouthpiece and that the nose clip was in place, each participant breathed normally into the device for 10 minutes while either listening to music or watching television. Participants had 3 consecutive attempts to successfully complete the measurement; interruption to airflow due to coughing or talking being the most common reason for repeating the procedure.

Figure 4.3. MedGem® hand-held indirect calorimetry device

4.1.5 Follow-up Period

Following the standardised breakfast meal and blood sample collection for satiety hormone profiling, participants continued to receive routine follow up through the hospital's obesity service every 3 months. Anthropometric measures (weight, height, BMI, waist circumference and body composition) were recorded. At 6 - 18 months, participants fasted for 4 hours prior to their clinic appointment and the REE was repeated using the MedGem® hand-held indirect calorimetry device.

4.2 Statistics

For the preliminary data, Excel was used to produce the graphs showing how weight and BMI SDS tracks with age in male youth separated into 3 different age groups (< 10 years, 10 – 14 years, and >14 - 18 years).

Excel was also used to calculate the mean average and standard deviation values for the demographic characteristics for all the participants at baseline and at the time of the satiety hormone profiles as shown in Table 1.

Regarding the satiety hormone profiles, 41 participants (21 pre-pubertal and 20 post-pubertal) had 7 blood samples taken at 7 different time points, 30 minute apart over 3 hours; time 0 (fasting pre-prandial sample taken directly prior to eating the standardised breakfast), 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes and 180 minutes. Each blood sample was analysed for 6 different satiety hormones (Ghrelin, GLP-1, GIP, PP and Total Peptide YY). Satiety scores were also collected from all the participants at each of the 7 time points.

The analyses of the 3 hour satiety hormone profiles and the satiety scores were performed by fitting linear mixed-effect models, which was chosen because it takes into account the hierarchical model (also known as multilevel data structure), as we have multiple measurements on one person taken over 7 different time points. We did not use standard regression models because they require that all the observations are independent, however in this study, there are several measurements taken from each individual over different time points which means that the values are not independent, and are more similar within the same person than would be expected if measured on a different person. This then violates the assumption of independence and leads to incorrect standard errors, confidence intervals and p values, which is why in this study we have used linear mixed effect models for the analyses.

Area under the curve (AUC) analysis is another standard way to assess and compare differences in hormone profiles, but was not used in this study, as it was a less efficient use of the data collected i.e. less powerful, compared to the linear mixed effects models that were used, especially in light of the low numbers of participants involved. We did carry out AUC analysis as a sensitivity analysis, but as the results did not add anything, it was not included. In addition, AUC makes an assumption that it is an average over time that is the main feature, but in this study it was the rate of change in the different satiety hormone profiles, and the depth of the dip and the height of the peak

of the hormone concentrations that were of interest, and these cannot be modelled in an AUC approach.

The statistical analyses were performed using the Stata statistical software package rather than SPSS statistical software package, because of its ease and flexibility in fitting a range of linear mixed effects models.

All the satiety hormone concentrations were first log-transformed, because it is an assumption of the linear mixed effects models that were used. The methods of estimating the impact of the different variables in the linear mixed effects model used restricted maximum likelihood, as this is the most valid method for linear mixed effects models, owing to the small numbers in the study.

Pre and post-prandial hormone and satiety score profiles were modelled with pubertal status and weight loss status as fixed effects, and both child and time point as a random effect (to allow for random error between different children, as well as across different time points), with changes in hormone and satiety score profiles over time modelled by including the appropriate interaction terms. This allows for different mean hormones / satiety score profiles / trajectories over time between males and females, weight loss reducers and weight maintainers and pre-pubertal children and post-pubertal adolescents i.e. it formally models the different effects of these groups.

While the analysis was carried out to identify if the difference in satiety hormone profiles of children losing weight and those maintaining a similar weight varied between pre and post-pubertal children, owing to the small numbers in each of the 4 groups (11 in the pre-pubertal weight reducer group, 10 in the pre-pubertal weight maintainer group, 10 in the post-pubertal weight reducer group and 10 in the post-pubertal weight maintainer group), there was insufficient power to generate any significant P values when comparing these 4 different groups. However, by combining all the pre-pubertal children (weight reducers and weight maintainers with n=21) and comparing them to all the post-pubertal adolescents (weight reducers and weight maintainers with n=20) there was sufficient statistical power to generate significant P values.

We conducted a number of sensitivity analyses to assess the robustness of our results to choice of model (linear mixed effects model) by:

- Using exact weight loss included as a continuous variable rather than categorised, and,
- Combining all pre-pubertal children and comparing to all post-pubertal adolescents, combining all weight reducers (≥ 10% weight loss in pre-

and post-pubertal groups) and comparing with all weight maintainers (<10% weight loss in pre and post-pubertal groups), and comparing results for all males (pre and post-pubertal) and all females (pre and post-pubertal).

One participant had incomplete information for their 180 minute hormone concentrations, but was complete for all other measures. As a sensitivity analysis, analyses were repeated without this assumption. The observation at 150 minutes was imputed for this individual.

Where a hormone concentration was less than the lower limit of detection, a value equal to half that limit was used, which is a standard technique that is commonly employed.

Finally, no formal statistical power calculation was carried out for this research study, and instead the study sample size was derived from the adult study carried out by *Sumithran et al*. Power calculations are based on how much an outcome changes over time in one group relative to the other (the group by time interaction in the repeated measures design). This benefits from the increased precision of the measures repeated at seven time points (0, 30, 90, 120, 150 and 180 minutes post-prandially). For the 41 participants recruited in total (approximately 20 to 21 in each group being compared), the study had at least 80% power to detect a relative change (at $p<0.05$) between two approximately equal-sized groups of 15% per hour. For example, if Ghrelin started at 100 pg/ml in both groups at time zero, but had grown to 115 pg/ml in one group by 60 minutes post-prandially, to 130 pg/ml by 120 minutes, and to 145 pg/ml by 180 minutes. Similar power would be achieved for the other satiety hormones, with adjustment for their different correlation structures.

The study was therefore adequately powered to detect medium to large sized effects overall. The above effect size that the study was adequately powered to detect is similar to the change in post prandial AUC (the weighted average) for Ghrelin seen in *Sumithran et al*. So overall this study was adequately powered for a reasonably realistic effect that others have found before, albeit in a different context.

4.3 Results

4.3.1 Study Participants

Of 74 eligible patients, 43 were successfully recruited. Twelve were un-contactable, 17 declined and cannulation proved too difficult in 2 (1 pre-pubertal female and 1 post-pubertal female). There were no significant differences in baseline characteristics between those enrolled and not enrolled. Patient characteristics in the 9-15 months prior to the study, and at the time of the baseline satiety hormone profile are shown in Table 4.1. The only significant difference was that there were more males in the pre-pubertal reducer group than females, as it was not possible to balance for sex during the recruitment period.

One pre-pubertal male in the reducer group had been started on Topiramate for epilepsy between baseline values and satiety hormone profile. In addition, another pre-pubertal male also in the reducer group was in early puberty (4ml testes) at the time of the satiety hormone profile. The analyses were therefore re-run excluding these two patients and the estimates were similar. Three of the post-pubertal adolescents (one female reducer and two male maintainers) were also taking fluoxetine intermittently during the study.

Table 4.1. Characteristics of the participants at baseline and at the time of their satiety hormone profile*

Baseline (Values taken 9-15 months before satiety hormone profile)	**Characteristic**	**Pre-Pubertal Reducer (N = 11)**	**Pre-Pubertal Maintainer (N = 10)**	**Post-Pubertal Reducer (N = 10)**	**Post-Pubertal Maintainer (N = 10)**
	Age (yr)	5.3 ± 1.6	7.1 ± 0.7	15.1 ± 1.6	14.7 ± 0.9
	Weight (Kg)	45.1±10.5	45.7 ± 6.1	103.3±25.4	111.3±24.7
	Height (cm)	122.9±13.7	132.8 ± 7.7	164.6±10.1	169.8 ± 5.6
	BMI	29.8 ± 4.6	26.3 ± 4.1	37.6 ± 6.4	38.2 ± 6.5
	BMI SDS	4.9 ± 1.1	3.4 ± 0.8	3.4 ± 0.5	3.4 ± 0.5
	Time Interval (months) [a]	12.7 ± 2	11.1 ± 2.7	11.8 ± 3.2	11.5 ± 2.7
	Male Sex (%)	9 (82)	2 (20)	6 (60)	4 (40)
Satiety Hormone Profile	**Age (yr)**	6.3 ± 1.6	8 ± 0.6	16.1 ± 1.6	15.6 ± 0.8
	Weight (Kg)	48.3 ± 13.3	52.7 ± 6.1	99.8 ± 19.5	119.2±25.2
	Height (cm)	130.4±13.6	138 ± 7.7	166.8±11.0	170.8 ± 5.5
	BMI	28.4 ± 5.3	27.9 ± 4.6	35.5 ± 5.3	40.6 ± 6.6
	BMI SDS	4.2 ± 1.3	3.3 ± 0.8	3.1 ± 0.5	3.6 ± 0.5
	Fat (%)	38.5 ± 8	40.5 ± 8.9	42.2 ± 9.7	47.8 ± 6.4
	REE (Kcal/day)	1197 ± 247	1247 ±160	1852 ± 279	2104 ± 393
	O_2 Produced VO2 (ml/day)	181.3±35.8	185.5±36.5	266.9±40.3	319.2±76.4

*****Plus-minus values are means ±SD. BMI denotes body-mass index, calculated as the weight in Kg divided by the square of the height in metres. BMI SDS denotes body-mass index Standard Deviation Score and allows for comparisons between children of different ages and sex (UK90 growth reference).

[a] Time interval between characteristics taken at baseline and characteristics at the time of the satiety hormone profile.

The post-pubertal group only had 2 reducers who had achieved weight loss ≥10%. The remainder of the post-pubertal group was therefore made up of post pubertal adolescents who had lost weight (average weight loss in the post-pubertal reducer group was 7%), as opposed to the maintainer group which comprised post pubertal adolescents who had gained weight (average weight gain in the post-pubertal maintainer group was 5%). However, when we repeated our analysis incorporating actual percentage weight gain or loss, we found that interactions for GIP and Amylin between reducers and maintainers were the same, even when percentage weight loss was modelled as a continuous variable (Table 4.2).

Table 4.2. Overall differences in hormone concentrations and post-prandial hormone response trajectories between maintainers and reducers with weight as a continuous variable.

Group	Hormonal regulator of Appetite	Comparison of means	95% CI	Overall P-value	P-value for diverging trajectories
Reducers vs Maintainers	Ghrelin (pg/ml)[a]	1.008	0.99-1.03	P=0.39	P=0.35
	Peptide YY (pg/ml)[a]	1.003	0.99-1.01	P=0.51	P=0.41
	GIP (pg/ml)[a]	0.999	0.99-1.01	P=0.91	P=0.07
	PP (pg/ml)[a]	1.007	0.99-1.03	P=0.51	P=0.81
	Amylin (pg/ml)[a]	0.992	0.98-1.01	P=0.24	P=0.008
	Active GLP1 (pmol/l)[a]	1.00	0.97-1.03	P=0.99	P=0.16
	Insulin (mu/l)[a]	0.989	0.97-1.01	P=0.38	P=0.24
	Glucose (mmol/l)[b]	-0.002	-0.02 to 0.01	P=0.75	P=0.93
	T3 (pmol/l) [b]	0.04	-0.001 to 0.08	P=0.06	P=0.31
	How hungry	0.07	0.02-0.12	P=0.007	P=0.48
	How full	0.04	-0.10 to 0.01	P=0.11	P=0.81

[a] Values are log-transformed, so estimates represent the ratio of means. Estimates quoted are for mean hormone concentration in first named category divided by mean for second named category, i.e. post-pubertal / pre-pubertal.

[b] Values are not log-transformed, so estimates represent the difference in means. Estimates quoted are for mean hormone concentration in first named category minus mean for second named category, i.e. post-pubertal - pre-pubertal.

4.3.2 Satiety Hormone Profiles

4.3.2.1 Acylated Ghrelin

Post-pubertal adolescents had 31% lower ghrelin concentrations (95% CI: -4% to -51%, p=0.03) than pre-pubertal children, but displayed similar post-prandial profiles (figure 4.4, table 4.3). However, the difference in post-prandial response of ghrelin between maintainers and reducers in the pre-pubertal group was similar to the difference between maintainers and reducers in the post-pubertal group (p=0.79). Contrary to our hypothesis, the post-pubertal reducers did not exhibit a greater increase in ghrelin concentration compared to the other 3 groups (figure 4.6).

4.3.2.2 Peptide YY

When all pre-pubertal children were compared to all post-pubertal adolescents, there was no evidence of any difference in average Peptide YY concentrations (-6%, 95% CI: -23% to +14% higher, p=0.52), and both groups had similar post-prandial profiles (p=0.86). The association between Peptide YY concentration and weight change was similar for both pre and post-pubertal children (p=0.17). The pre-pubertal reducers did not demonstrate any greater increase in Peptide YY post-prandial concentrations compared to the other groups (figure 4.6).

Figure 4.4. Post prandial response trajectories for active Ghrelin, Peptide YY, GIP, PP, Total Amylin and Active GLP-1, comparing all pre-pubertal children to all post-pubertal adolescents over 3 hours post prandially

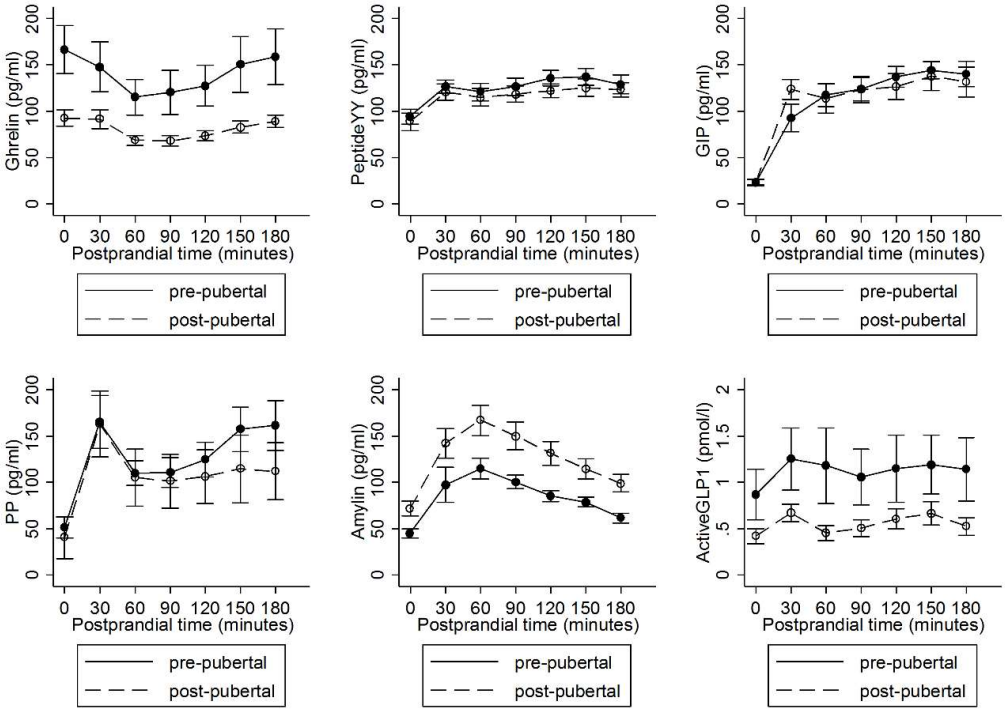


Table 4.3 . Overall differences in hormone concentrations and post-prandial hormone response trajectories between pre- and post-pubertal children, and all weight maintainers and reducers

Groups	Hormonal Regulators of Appetite	Comparisons of means[c]	95% CI	Overall P-value[c]	P-value for diverging trajectories [d]
Post-pubertal vs Pre-pubertal	Ghrelin (pg/ml)[a]	0.69	(0.49 - 0.96)	P=0.03	P=0.68
	Peptide YY (pg/ml)[a]	0.94	(0.77 - 1.14)	P=0.52	P=0.86
	GIP (pg/ml)[a]	1.02	(0.77 - 1.36)	P=0.87	P=0.09
	PP (pg/ml)[a]	0.67	(0.44 - 1.03)	P=0.07	P=0.93
	Amylin (pg/ml)[a]	1.50	(1.18 - 1.91)	P=0.001	P=0.55
	Active GLP1 (pmol/l)[a]	0.69	(0.41 - 1.17)	P=0.16	P=0.94
Reducers vs Maintainers	Ghrelin (pg/ml)[a]	1.05	(0.73 - 1.50)	P=0.80	P=0.28
	Peptide YY (pg/ml)[a]	0.93	(0.77 - 1.14)	P=0.50	P=0.50
	GIP (pg/ml)[a]	0.91	(0.69 - 1.20)	P=0.48	P=0.05
	PP (pg/ml)[a]	1.16	(0.75 - 1.79)	P=0.51	P=0.51
	Amylin (pg/ml)[a]	0.83	(0.63 - 1.08)	P=0.16	P=0.03
	Active GLP1 (pmol/l)[a]	0.69	(0.41 - 1.17)	P=0.17	P=0.31
Post-pubertal vs Pre-pubertal	Insulin (mu/l)[a]	2.35	(1.53 - 3.6)	P<0.0001	P=0.07
	Glucose (mmol/l)[b]	0.27	(0.01 - 0.54)	P=0.05	P=0.06
	T3 (pmol/l) [b]	0.75	(-1.6 - 0.11)	P=0.09	P=0.64
Reducers vs Maintainers	Insulin (mu/l)[a]	0.82	(0.50 - 1.35)	P=0.44	P=0.90
	Glucose (mmol/l) [b]	-0.02	(-0.3 - 0.26)	P=0.89	P=0.81
	T3 (pmol/l) [b]	0.57	(-0.31 - 1.4)	P=0.20	P=0.64

[a] Values are log-transformed, so estimates represent the ratio of means. Estimates quoted are for mean hormone concentration in first named category divided by mean for second named category, i.e. post-pubertal / pre-pubertal and reducer / maintainer.

[b] Values are not log-transformed, so estimates represent the difference in means. Estimates quoted are for mean hormone concentration in first named category minus mean for second named category, i.e. post-pubertal minus pre-pubertal and reducer minus maintainer.

[c] Comparisons are ratio of means for log-transformed outcomes (Ghrelin, Peptide YY, GIP, PP, Amylin, Active GLP-1, insulin), but difference in means for non-log-transformed outcomes (Glucose, T3).

[d] P-value for test of whether trajectories diverge post-prandially.

4.3.2.3 Gastric Inhibitory Polypeptide

When all the weight reducers (pre- and post-pubertal) were compared to all the weight maintainers, the reducer group was slower to reach maximum peak GIP concentration compared to maintainer groups (p=0.05) (figure 4.5). When all pre-pubertal children (reducers and maintainers) were compared to all post-pubertal adolescents (reducers and maintainers), there was no evidence of any overall difference in average GIP concentrations (+2%, 95% CI: -23% to +36%, p=0.87), and no evidence of a difference in post-prandial profiles (p=0.09). Furthermore, the association between GIP and weight change did not vary by age group (p=0.79) (figure 4.6).

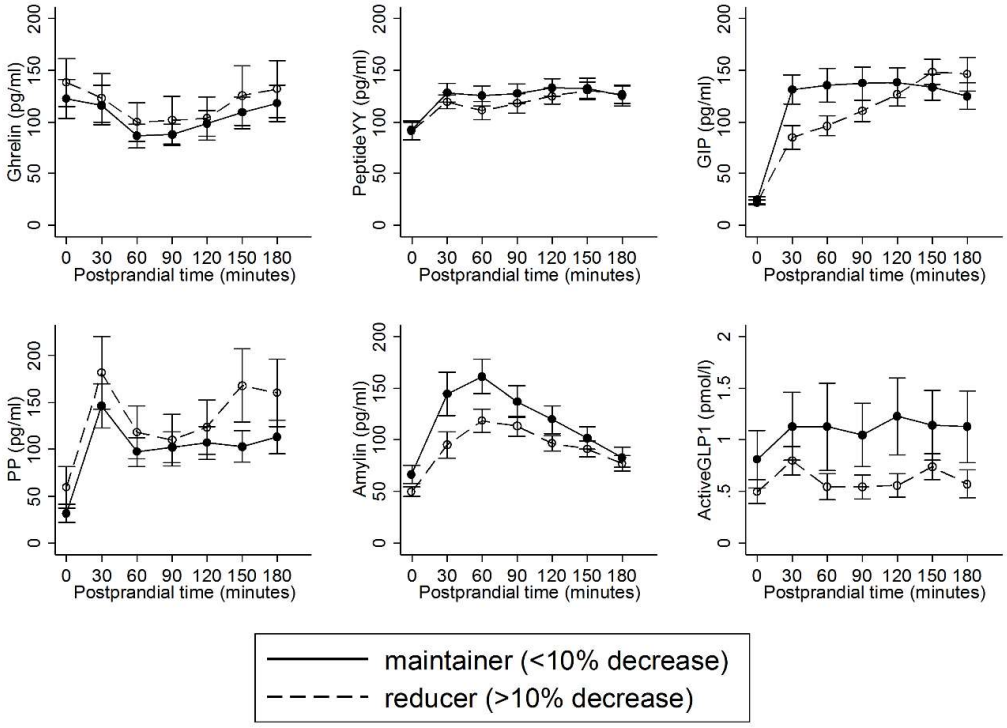
4.3.2.4 Pancreatic Polypeptide

There was no evidence of a mean difference in PP concentrations between pre-pubertal children and post-pubertal adolescents (-33%, 95% CI: -56% to +3%; p=0.07). There was also no evidence that post-prandial profiles diverged over time (p=0.93). The association between PP and weight change did not vary by age (p=0.81) (figure 4.6).

4.3.2.5 Amylin

Post-pubertal adolescents had substantially higher mean amylin concentrations (+50%) than pre-pubertal children (95% CI: +18% to +91%; p=0.001). However, there was no evidence that these post-prandial profiles diverged (p=0.55). The association between amylin concentration and weight change was similar for both pre- and post-pubertal children (p=0.39).

Figure 4.5 . Post prandial response trajectories for Active Ghrelin, Peptide YY, GIP, PP, Total Amylin and Active GLP-1, comparing all weight reducers (pre- and post-pubertal) to all weight maintainers (pre- and post- pubertal) over 3 hours post prandially.



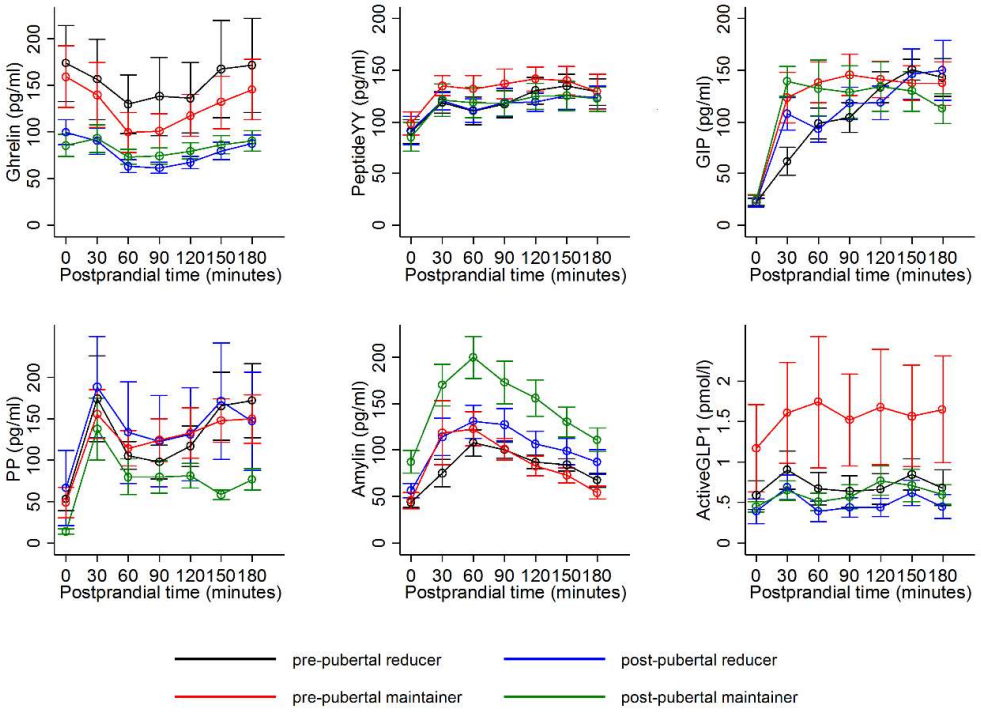
4.3.2.6 Active GLP-1

There was no evidence of any overall difference in active GLP-1 concentrations between pre- and post-pubertal subjects (31% lower, 95% CI: -59% to +17%; p=0.16), and no evidence that their combined profiles diverged (p=0.94). The difference in post-prandial response of active GLP-1 between maintainers and reducers in the pre-pubertal group was similar to the difference between maintainers and reducers in post-pubertal adolescents (p=0.53) (figure 4.6). The pre-pubertal maintainer group had higher concentrations of active GLP-1 than the other three groups, but post-prandial profiles did not differ.

While all participants had fasting glucose, insulin and T3 levels taken at the time of their satiety hormone profile, only some had baseline values taken 9-15 months before their satiety hormone profile was carried out, 41% (n=17) had baseline glucose, 49% (n=20) had baseline insulin and 22% (n=9) had baseline T3 levels.

As expected, insulin concentrations were twice as high in the post-pubertal group than the pre-pubertal group (ratio = 2.35, 95% CI 1.53 to 3.60, $p<0.001$) (table 4.3).

Figure 4.6. Post prandial response trajectories for active Ghrelin, Peptide YY, GIP, PP, Total Amylin and Active GLP-1, comparing pre-pubertal reducers and maintainers and post-pubertal reducers and maintainers over 3 hours post prandially



4.3.3 Appetite Ratings

Post-pubertal adolescents reported feeling less hungry and more full than pre-pubertal children. The post-pubertal group reported being less hungry by 2 points on the VAS (95% CI: 1 to 3 points lower, p<0.001) and more full by 1 point (95% CI: 0 to 2 points, p=0.03) (table 4). However, profiles did not diverge post-prandially (p=0.81 and 0.21 respectively).

The association between satiety and weight change did not vary by pubertal status (p=0.17 for hunger and p=0.23 for how full) (figure 4.7).

Figure 4.7. Ratings of appetite using validated visual analogue scales at baseline and over 3 hours post prandially, comparing pre-pubertal reducers and maintainers and post-pubertal reducers and maintainers

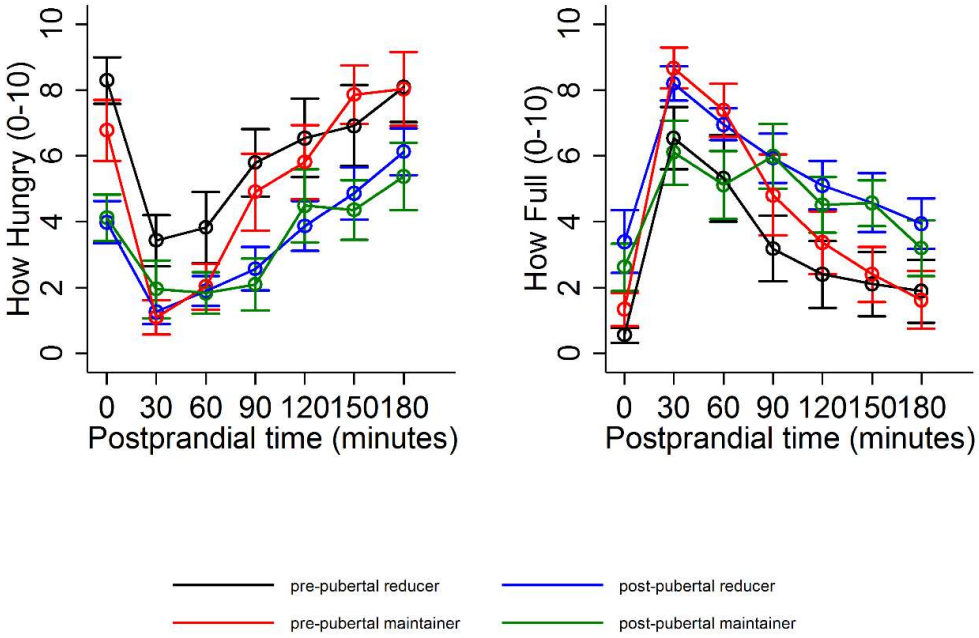


Table 4.4. Overall differences in post-prandial satiety scores and post-prandial satiety response trajectories between pre- and post-pubertal children and all weight maintainers and reducers

Group	Parameter	Overall difference in means	95% CI	P-value for difference	P-value for diverging trajectories
Post-pubertal Vs Pre-pubertal	How hungry	-2.15	-3.11 to -1.20	P<0.0001	P=0.81
	How full	1.20	0.15 to 2.26	P=0.03	P=0.21
Reducers Vs Maintainers	How hungry	0.69	-0.46 to 1.84	P=0.24	P=0.48
	How full	-0.20	-1.32 to 0.92	P=0.73	P=0.83

4.3.4 Correlations between appetite ratings and satiety hormones

Associations between all the hormone concentrations and satiety scores were calculated using Pearson's correlation (table 4.5). As expected, there were strong positive correlations between how hungry the participants were and ghrelin concentrations (r= 0.4), Amylin and Insulin concentrations (r= 0.7) and GIP and PYY concentrations (r= 0.5). Strongly negative correlations included ghrelin and insulin concentrations (r= -0.6) and PYY and Amylin concentrations (r= -0.3).

4.3.5 Resting Energy Expenditure

As expected, REE was higher in post-pubertal adolescents compared to pre-pubertal children (+757 kcal, 95% CI: +574 to +940, $p<0.001$). REE in pre-pubertal weight reducers and maintainers were similar (-50kcal, 95% CI: -242 to +143, p=0.6) but post-pubertal reducers had 250kcal lower REE compared to post-pubertal maintainers (95% CI: -572 to +68, p=0.1).

Table 4.5. Correlation between baseline hormone concentrations, satiety, insulin, T3 and glucose

	Ghrelin	PYY	GIP	PP	Amylin	GLP-1	How Hungry	How Full	Insulin	T3
PYY	r=0.00									
	p=0.98									
GIP	r=0.04	r=0.50								
	p=0.82	p=0.0008								
PP	r=-0.04	r=0.09	r=-0.02							
	p=0.80	p=0.57	p=0.91							
Amylin	r=-0.31	r=0.23	r=0.42	r=-0.32						
	p=0.05	p=0.15	p=0.007	p=0.04						
GLP-1	r=-0.00	r=0.16	r=0.00	r=-0.09	r=-0.06					
	p=0.98	p=0.31	p=0.98	p=0.59	p=0.72					
How Hungry	r=0.37	r=0.14	r=0.12	r=0.17	r=-0.27	r=0.06				
	p=0.02	p=0.38	p=0.44	p=0.29	p=0.09	p=0.70				
How Full	r=-0.17	r=0.08	r=0.11	r=-0.25	r=0.27	r=0.07	r=-0.53			
	p=0.29	p=0.63	p=0.51	p=0.12	p=0.09	p=0.66	p=0.0004			
Insulin	r=-0.58	r=-0.15	r=0.08	r=-0.19	r=0.74	r=-0.01	r=-0.44	r=0.1710		
	p=0.0001	p=0.34	p=0.60	p=0.24	p<0.0001	p=0.94	p=0.004	p=0.29		
T3	r=-0.06	r=0.17	r=0.37	r=0.27	r=0.03	r=-0.01	r=0.11	r=-0.02	r=0.05	
	p=0.72	p=0.30	p=0.02	p=0.09	p=0.88	p=0.96	p=0.48	p=0.89	p=0.76	
Glucose	r=-0.46	r=0.18	r=-0.01	r=0.07	r=0.29	r=0.00	r=-0.38	r=0.31	r=0.55	r=-0.07
	p=0.003	p=0.26	p=0.97	p=0.65	p=0.06	p=0.99	p=0.02	p=0.05	p=0.0002	p=0.67

4.4 Discussion

A large body of evidence supports adults having a 'set-point' for weight (83, 84, 335, 356) but no studies have yet evaluated the degree of drive to weight regain in young obese children following a period of weight loss, or undertaken comparative studies of weight regain and its physiological drivers before and after puberty. We studied the physiological changes in satiety hormone profiles between young and older obese children/adolescents who had lost weight. While we found distinct variations in several specific hormones, there were no demonstrable differences in satiety hormone profiles between the two groups.

Although body weight is tightly regulated, excess nutrient intake over long periods may alter energy balance set points, which includes the gut-brain axis and adipose tissue-brain axis. In obesity, this would make weight reduction even more challenging as evolutionary defense mechanisms actively try to maintain elevated levels of body fat (125). This has been clearly demonstrated in an adult obesity study (335), where weight loss resulted in changes in the levels of appetite regulating hormones (increased ghrelin and decreased PYY, leptin, amylin, GIP, PP and GLP-1 levels), and caused increased subjective sensations of appetite. These compensatory physiological adjustments act to promote weight gain and were still evident 12 months after the initial weight reduction. This contrasts to obese children, where long-term maintenance of weight loss appears more achievable (93).

The results of this study did not replicate those seen in adults. There was no post-prandial rise in ghrelin following weight loss in pre- or post-pubertal children, although other studies in obese children have shown ghrelin levels to be unaffected by preceding weight loss (139, 357).

Gastric Inhibitory Polypeptide is released from the gastrointestinal tract and promotes energy storage (335), and stimulates lipoprotein lipase activity in adipocytes (358). We found that when all reducers (pre and post-pubertal) were compared to all maintainers (pre and post-pubertal), the reducer group was slower to reach their maximum peak GIP concentration compared to the maintainer group. This is consistent with a previous study in obese children which demonstrated that after a dietary intervention lasting 3 – 7 months, fasting GIP levels also decreased (359). A slower rise in GIP concentrations may help to prevent an excessive insulin response in participants who lose weight (134). In contrast, obese adults experience a greater secretion of GIP

following diet induced weight loss (335), possibly to increase fat storage, which would act to promote weight regain.

Peptide YY is an anorexigenic peptide that reduces food intake (135), as is Pancreatic Polypeptide (PP), which slows gastric emptying and reduces ghrelin levels. Studies in obese adults have demonstrated that weight loss reduces fasting and post prandial Peptide YY levels (335), which would act as a compensatory response to promote weight gain. The results in children and adolescents however are conflicting. In our study, there was no evidence of any difference in average Peptide YY or PP concentrations when pre-pubertal children were compared to post-pubertal adolescents, and the pre-pubertal reducer group (BMI SDS decrease >0.5) did not demonstrate any greater increase in post-prandial Peptide YY concentrations compared to the other groups.

Findings in this study are similar to those found in two previous RCTs, where no association was seen between moderate weight loss (BMI SDS decrease <0.5) and fasting Peptide YY and GLP-1 levels in adolescents (360, 361), but is in contrast to another study which found that Peptide YY and PP levels increased significantly in obese children with the most effective weight loss (BMI SDS decrease >0.5) and that Peptide YY levels decreased in patients with weight gain (127). Possible explanations for these inconsistencies may be related to differences in study design, degree of weight loss, duration and types of interaction, whether total PYY or the active isoform PYY_{3-36} was measured and the pubertal status of the participants.

Amylin is synthesised and released with insulin from the beta cells in the pancreas, and inhibits nutritional intake by causing a reduction in meal size and inhibiting gastric emptying (125). High amylin levels in childhood are linked to hypersecretion of insulin, and amylin is thought to play a significant role in the development of type 2 diabetes (125). Previous studies have shown that in adolescents, amylin levels decrease with weight loss (141). In our study, the combined post-pubertal adolescent group had substantially higher levels of amylin compared to the pre-pubertal group, with the post-pubertal maintainer group having the highest concentrations of amylin.

Regarding subjective sensations of appetite, in this study, post pubertal adolescents felt less hungry and reported being more full than pre-pubertal children, which is concordant with the higher ghrelin levels seen in the pre-pubertal group. However, research using VAS in younger children have demonstrated that they are more likely to register extreme responses, being less able to distinguish between different levels of hunger (362), which may

have influenced the results. We also found that the association between satiety and weight change did not vary by pubertal status, which is consistent with other paediatric obesity studies (359, 361).

In obese adults, caloric restriction results in a profound reduction in energy expenditure (363) and this compensatory adaptive down-regulation is frequently cited as one of the causes of weight regain. In children, there are currently insufficient data to establish if this phenomenon also occurs. Our results show that while REE in pre-pubertal reducers and maintainers were similar, post-pubertal reducers had 250Kcal lower REE compared to post-pubertal maintainers. These findings indicate that obese adolescents who lose weight may experience a similar reduction in REE to that seen in obese adults who lose weight, which would act to promote weight regain, but that this reduction in REE is not evident in obese pre-pubertal children who lose weight.

This study has several strengths. As participants were already attending the Weight Management Service and were also enrolled in COBRA, we had complete data at baseline and at the time of the satiety hormone profiles, thus avoiding high attrition rates typically seen in studies involving long-term weight loss. Furthermore, we believe this is the first study to properly exclude puberty as a confounding variable, thus adding considerable new knowledge to the literature

There are however several important limitations. Although we achieved acceptable power for the main comparisons (all pre-pubertal children vs all post-pubertal adolescents, all weight reducers vs all weight maintainers, and all males vs all females), power to detect significant interactions between hormones and weight change was lower. The study was adequately powered to detect medium to large sized effects overall.

For subgroup analysis with approximately 20 subjects in total (approximately 10 in each of the 4 groups), the study would have had 80% power to detect a relative change (at $p<0.05$) only if the groups diverged by 22% per hour post-prandially. For example, if Ghrelin started at 100 pg/ml in each group at time zero, but had grown to 122 pg/ml after 60 minutes, to 144 pg/ml after 120 minutes and to 166 pg/ml after 180 minutes. Our study was therefore unlikely to have adequate power to detect more realistic sized effects within the subgroups. To achieve adequate power within the subgroups for more realistic effects would need approximately double the numbers to be recruited within each subgroup than those that were obtained i.e. 80 patients in total and 20

in each of the 4 subgroups (pre-pubertal reducer and maintainer and post-pubertal reducer and maintainer).

There were also more males in the pre-pubertal reducer group than females, as it was not possible to balance for sex during the recruitment period. This was reflected in our study results, with 82% of the participants in our pre-pubertal reducer group being male, but only 20% were male in our pre-pubertal maintainer group. There were also more males in our post-pubertal reducer group compared to the post-pubertal maintainer group (60% and 40% respectively).

Secondly, various drugs can affect body weight as a side effect, but often the weight change is statistically non-significant. However, Topiramate and Fluoxetine are associated with weight loss (364), and one of the patients within this study was started on Topiramate for his epilepsy while three others were intermittently taking Fluoxetine.

Thirdly, the post pubertal group only had 2 reducers who had achieved weight loss ≥10%. The post-pubertal adolescent group require a combination of absolute and relative weight reduction in order to achieve the 10% target, whereas the pre-pubertal group benefit more from the effects of growth. However, when we repeated our analysis incorporating actual percentage weight loss, we found that interactions for GIP and Amylin between all weight reducers compared to all weight maintainers still held even when percentage weight loss was modelled as a continuous variable.

Pre-pubertal children of both sexes from the age of 4 years, grow at approximately the same rate until the adolescent growth spurt (5 - 6 cm/year and 2.5 kg/year). Although pre-pubertal children have greater growth than post-pubertal adolescents, post-pubertal adolescents who have just attained their peak height velocity are still growing, and experience a period of decelerating height velocity until growth ceases because of epiphyseal fusion at approximately 15 years in females and 18 years in males, with males experiencing an average height velocity of 5.7cm/year two years after peak height velocity at 14 years (365, 366). The implication of this is that differences in growth between the pre-pubertal and post-pubertal groups would not have had a significant impact on the results obtained as both groups continued to experienced growth.

Finally, we did not have a group of adults in order to directly compare our findings within this study with those already published.

Both the MedGem® hand-held indirect calorimetry device and Tanita BC-418 have been validated for use in children aged 7 years and over. In this study children as young as 4 years of age were successfully able to follow instructions and cooperate during the measurements of both REE and Bioelectrical Impedance. However, age per se is not a rate limiting factor for using these devices but instead for the MedGem® it is the operating range of the device (355), and for the Tanita BC-418 it is the degree of resistance encountered (electrode placement) (354).

In conclusion, our study demonstrates that satiety hormone profiles are similar between pre and post-pubertal subjects, and appear to contrast with previously published adult data and our hypothesis, where weight reduction leads to sustained increases in Ghrelin and PP, and reductions in other satiety hormones. Consistent with adult obesity studies, and our hypothesis, the REE in post pubertal adolescents who had lost weight was lower than the post pubertal maintainer group who had not lost weight, while the REE in the pre-pubertal reducer and maintainer groups were similar. Larger studies exploring the role of REE in maintaining weight loss in younger children are therefore required. Taken together, these findings indicate that the physiological mechanisms which act to protect against weight change may develop later than in the adolescent years.

Chapter 5 Effect of weight loss on Resting Energy Expenditure

5.1 Methodology for the Follow-up period.

Following the standardised test meal and blood sample collection for satiety hormone profiling, participants continued to receive routine follow-up through The Royal Children's hospital's weight management service every 3 months. Anthropometric measures (weight, height, BMI, waist circumference and body composition) were routinely recorded at these clinic appointments.

5.1.1 Study Design

This was a prospective cohort study with a retrospective element.

5.1.2 Study Participants

The participants for the follow-up period of the study were taken from the original 21 obese pre-pubertal children aged 3-7 years and 20 obese post-pubertal children aged 14-18 years, who had been recruited from the weight management service at the RCH in Melbourne. All of the subjects that had been recruited had an initial BMI SDS >2.4, and the participants had been divided into pre- and post-pubertal groups, and then the groups had been further sub-divided into maintainer or reducer groups, giving 4 groups. The reducer groups comprised obese patients who had achieved a relative or absolute weight loss of ≥ 10% in the preceding 9-15 months, whereas participants in the maintainer group had not.

The participants had attended The RCH following an overnight fast, and had their body composition recorded using BIA, and their REE measured using the MedGem® hand-held calorimetry device. Blood samples had been collected to measure satiety hormone levels before and every 30 minutes after eating a standardised breakfast meal. Each participant had also been asked to rate their subjective appetite sensations using a standard visual analogue scale, which was completed every 30 minutes just before the blood sample collection over a 3 hour period.

Six – twelve months following their satiety hormone profile, participants were contacted by telephone by the weight management nurse, and asked if they

would fast for 4 hours prior to their routine morning clinic appointment, so that their REE could be repeated using the MedGem®, along with their BIA and auxological measurements. Consent for the re-assessment of the REE at the follow-up clinic appointment had already been obtained when participants had been recruited to the study, and the measurement of REE at the time of the satiety hormone profile and at follow-up had been included in the ethics application.

5.1.3 Data Collection

Every child was weighed (SECA chair scales, model no. 9567021099) and their height recorded using a stadiometer (Holton Ltd stadiometer, CRYMMYCH Pembs UK). BMI was calculated and BMI SDS were generated using UK90 standardised growth reference data. Body composition was assessed by bioimpedance, which was measured using a Tanita Body Composition Analyser (Model BC-418). Pubertal staging was not repeated at the follow-up appointment unless there was a clinical indication for it.

5.1.4 Measures

5.1.4.1 Anthropometry

To minimise differences in levels of dehydration, participants fasted for 4 hours before having their weight and BIA measured. All the clinic appointments were in the morning.

5.1.4.2 Resting Energy Expenditure

REE was measured in exactly the same way as it had been previously, when participants had attended for the satiety hormone profiles. The participants had all fasted for 4 hours and the post-pubertal group were asked about their smoking habits, as REE values are affected by smoking within 4 hours of the measurement being taken. Fasted participants had their REE measured using the MedGem® hand-held indirect calorimetry device. The children and adolescents rested quietly for 15 minutes before the procedure and were all seated in the same upright position holding the MedGem® to their mouth. After ensuring that there was a tight seal around the disposable mouthpiece and that the nose clip was in place, each participant breathed normally into the device for 10 minutes while either listening to music or watching television.

Participants had 3 consecutive attempts to successfully complete the measurement.

5.2 Results

5.2.1 Study participants

Of the original 41 participants, 23 subjects had their REE repeated at a routine clinic appointment 6-12 months after their satiety hormone profile, having fasted for 4 hours before their clinic appointment. In the pre-pubertal group there were 6 participants in the reducer and 7 in the maintainer sub-set. In the post-pubertal group there were 5 participants in both the reducer and maintainer groups (figure 5.1).

Reasons for subjects not having their REE values repeated at follow-up, included participants having left the service because the family had moved away from the area, or the child's weight had normalised and the family no longer required the weight management service. The most common reason was because participants had not fasted for 4 hours prior to attending their clinic appointment.

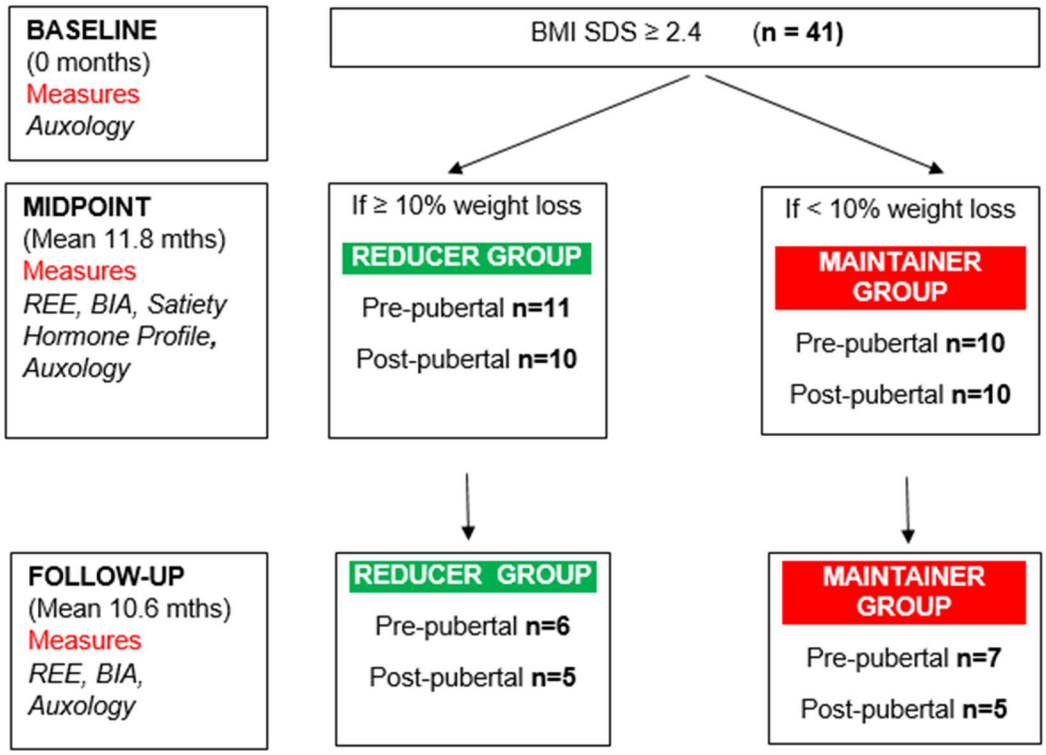


Figure 5.1. Time line and grouping for the study with participant numbers.

In table 5.1 are the characteristics of the 41 participants taken at the midpoint in the study, which was at the time of their satiety hormone profile, and also of the 23 participants at follow-up, which was on average 10.6 months after the midpoint. As expected, the REE was higher in post-pubertal adolescents compared to pre-pubertal children. The REE in pre-pubertal reducers and maintainers was similar at both time points, and post-pubertal reducers had 250kcal lower REE compared to post-pubertal maintainers at the midpoint, and the difference had reduced to 159 kcal by follow-up.

Table 5.1. Characteristics of the 41 participants taken at the midpoint in the study (at the time of the satiety hormone profile) and of the 23 participants at follow-up.

MID-POINT Satiety Hormone Profile	**Characteristic**	**Pre-Pubertal Reducer (N = 11)**	**Pre-Pubertal Maintainer (N = 10)**	**Post-Pubertal Reducer (N = 10)**	**Post-Pubertal Maintainer (N = 10)**
	Age (yr)	6.3 ± 1.6	8 ± 0.6	16.1 ± 1.6	15.6 ± 0.8
	Male (%)	9 (82)	2 (20)	6 (60)	4 (40)
	Weight (Kg)	48.3 ± 13.3	52.7 ± 6.1	99.8 ± 19.5	119.2 ± 25.2
	BMI	28.4 ± 5.3	27.9 ± 4.6	35.5 ± 5.3	40.6 ± 6.6
	BMI SDS	4.2 ± 1.3	3.3 ± 0.8	3.1 ± 0.5	3.6 ± 0.5
	Fat (%)	38.5 ± 8	40.5 ± 8.9	42.2 ± 9.7	47.8 ± 6.4
	REE (Kcal/d)	1197± 247	1247 ± 160	1852 ± 279	2104 ± 393
	O_2 Produced*	181.3± 35.8	185.5 ± 36.5	266.9± 40.3	319.2 ± 76.4
	REE/weight**	25.4 ± 4.2	23.7 ± 1.8	18.9 ± 3.2	17.8 ± 1.7
	REE/FFM**	41.4 ± 6	40.9 ± 8.4	32.9 ± 4.4	34.5 ± 4.5
FOLLOW UP (Average time to follow-up in months 10.6±5.3)		**N = 6**	**N = 7**	**N = 5**	**N = 5**
	Age (yrs)	6.4 ± 1.1	9.0 ± 0.9	17.3 ± 1.6	16.7 ± 0.7
	Male (%)	4 (60)	2 (29)	3 (50)	3 (60)
	Weight (Kg)	50.2 ± 7.8	59.9 ± 9.0	108.2± 18.8	129.0 ± 15.8
	BMI	29.7 ± 5.6	29.0 ± 5.8	38.4 ± 5.5	42.9 ± 3.5
	BMI SDS	4.4 ± 0.9	3.18 ± 0.7	3.4 ± 0.6	3.81 ± 0.2
	Fat (%)	32.9 ± 4.9	33.5 ± 11.0	39.1 ± 10.9	43.7 ± 6.8
	REE (Kcal/d)	1402 ± 236	1528 ± 491	1967 ± 453	2126 ± 312
	O_2 Produced*	212.3± 44.5	235.1 ± 45.3	277.6± 64.9	306.4 ± 45.0
	REE/weight**	28 ± 2.5	25.5 ± 6.6	18.4 ± 2.2	16.6 ± 2.7
	REE/FFM**	57.1 ± 17.8 **(N = 3)**	60.4 ± 19 **(N = 4)**	42.3 ± 12.8	31 ± 4.6

*VO2 in ml/day **kcal/kg/day

However, when the REE was adjusted to account for weight, the kcal/kg/day was greater in the pre-pubertal group compared to the post-pubertal group at the midpoint (at the time of the satiety hormone profile), and this trend was also seen at follow-up. As FFM is the metabolically active tissue and is the largest contributor to REE, changes in REE per kg of FFM were also looked at. At the midpoint, (which was when the participants entered the study), a complete data set for all 41 participants was available, and the REE/FFM values confirmed that the pre-pubertal groups had higher values compared to the post-pubertal groups.

Unfortunately, owing to the high attrition rates typically seen in studies involving long term weight loss, there were only 23 participants at follow-up, which made it harder to interpret the REE/FFM values, because results were limited by the small sample size. This also meant that there was insufficient power to generate significant P values at follow-up, when comparing the 4 different groups (pre- and post-pubertal reducer and maintainer) and also when the groups were combined e.g. all pre-pubertal (maintainer and reducer) compared to all post-pubertal (maintainer and reducer).

The characteristics of all the participants at all 3 time points in the study (baseline, midpoint and follow-up) are shown in table 5.2.

Table 5.2. Characteristics of all the participants taken at each time point in the study

Baseline (Values taken 9-15 months before satiety hormone profile)	**Characteristic**	**Pre-Pubertal Reducer (N = 11)**	**Pre-Pubertal Maintainer (N = 10)**	**Post-Pubertal Reducer (N = 10)**	**Post-Pubertal Maintainer (N = 10)**
	Age (yr)	5.3 ± 1.6	7.1 ± 0.7	15.1 ± 1.6	14.7 ± 0.9
	Weight (Kg)	45.1 ± 10.5	45.7 ± 6.1	103.3 ± 25.4	111.3 ± 24.7
	BMI	29.8 ± 4.6	26.3 ± 4.1	37.6 ± 6.4	38.2 ± 6.5
	BMI SDS	4.9 ± 1.1	3.4 ± 0.8	3.4 ± 0.5	3.4 ± 0.5
	Time Interval (months) †	12.7 ± 2	11.1 ± 2.7	11.8 ± 3.2	11.5 ± 2.7
Midpoint (Satiety Hormone Profile)	**Age (yr)**	6.3 ± 1.6	8 ± 0.6	16.1 ± 1.6	15.6 ± 0.8
	Weight (Kg)	48.3 ± 13.3	52.7 ± 6.1	99.8 ± 19.5	119.2 ± 25.2
	BMI	28.4 ± 5.3	27.9 ± 4.6	35.5 ± 5.3	40.6 ± 6.6
	BMI SDS	4.2 ± 1.3	3.3 ± 0.8	3.1 ± 0.5	3.6 ± 0.5
	Fat (%)	38.5 ± 8	40.5 ± 8.9	42.2 ± 9.7	47.8 ± 6.4
	REE (kcal/day)	1197 ± 247	1247 ± 160	1852 ± 279	2104 ± 393
	O_2 Produced VO2 (ml/day)	181.3± 35.8	185.5 ± 36.5	266.9 ± 40.3	319.2 ± 76.4
Follow-up		**N = 6**	**N = 7**	**N = 5**	**N = 5**
	Time Interval (months) #	9.6 ± 4.6	11.0 ± 5.9	10.3 ± 5.4	11.6 ± 5.3
	Age (yr)	6.4 ± 1.1	9.0 ± 0.9	17.3 ± 1.6	16.7 ± 0.7
	Weight (Kg)	50.2 ± 7.8	59.9 ± 9.0	108.2 ± 18.8	129.0 ± 15.8
	BMI	29.7 ± 5.6	29.0 ± 5.8	38.4 ± 5.5	42.9 ± 3.5
	BMI SDS	4.4 ± 0.9	3.18 ± 0.7	3.4 ± 0.6	3.81 ± 0.2
	Fat (%)	32.9 ± 4.9	33.5 ± 11.0	39.1 ± 10.9	43.7 ± 6.8
	REE (kcal/day)	1402 ± 236	1528 ± 491	1967 ± 453	2126 ± 312
	O_2 Produced VO2 (ml/day)	212.3± 44.5	235.1 ± 45.3	277.6 ± 64.9	306.4 ± 45.0
	Total Time interval (months)	22.3 ± 4.6	21.9 ± 6.1	20.8 ± 6.7	22.8 ± 4.2

*Plus-minus values are means ±SD. BMI denotes body-mass index, calculated as the weight in kg divided by the square of the height in metres. BMI SDS denotes body-mass index Standard Deviation Score and allows for comparisons between children of different ages and sex (UK90 growth reference).

† Time interval between characteristics taken at Baseline and characteristics at the time of the satiety hormone profile.

Time interval between characteristics taken at the time of the satiety hormone profile and follow-up

Figures 5.2, 5.3, and 5.4 depict different parameters (REE, BMI z-score, VO_2, Fat mass %, Truncal fat mass %), plotted against the date of measurement for the participants at the 3 different time points; baseline, satiety profile (mid-point) and follow-up.

Figure 5.2 shows the different parameters against the date of measurement for the participants at the 3 different time points, when all pre-pubertal children are compared to all post-pubertal adolescents. The pre-pubertal children had an increase in the REE and VO_2 from mid-point to follow-up.

Figure 5.3 shows the different parameters against the date of measurement for the participants at the 3 different time points when all maintainers (pre and post-pubertal) are compared to all reducers (pre and post-pubertal).

Figure 5.4 shows the different parameters against the date of measurement for the participants at the 3 different time points when all females (pre and post-pubertal) are compared to all males (pre and post-pubertal).

Figure 5.5 shows the different parameters against the date of measurement for the participants at the 3 different time points in each of the 4 groups (pre and post-pubertal reducers and pre and post-pubertal maintainers). While the post-pubertal adolescents have a higher REE compared to the post-pubertal children, the REE has not been adjusted for FFM or body weight.

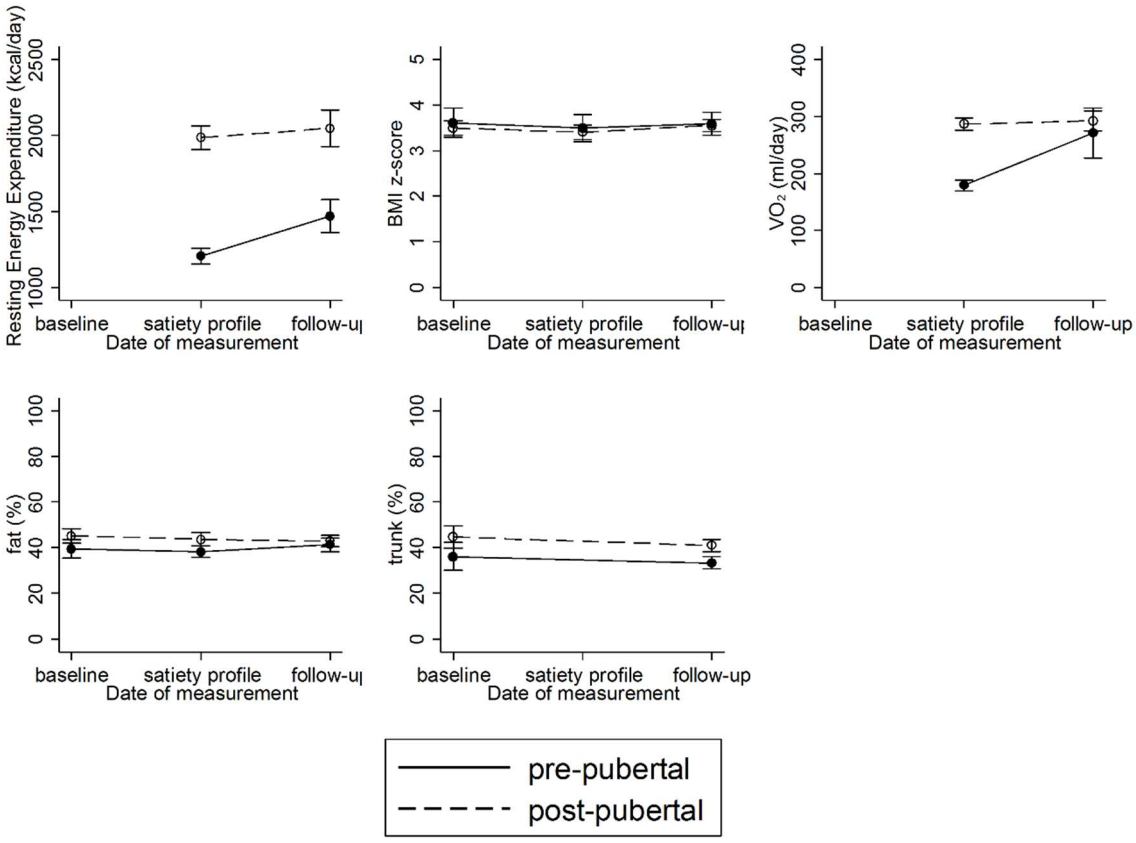


Figure 5.2. Different parameters, (REE, BMI z-score, VO_2 , Fat mass % and Truncal fat mass %), plotted against the date of measurement for the 3 different time points in the study (baseline, midpoint and follow-up) for all the pre-pubertal participants (reducers and maintainers) compared to all the post-pubertal participants (reducers and maintainers).

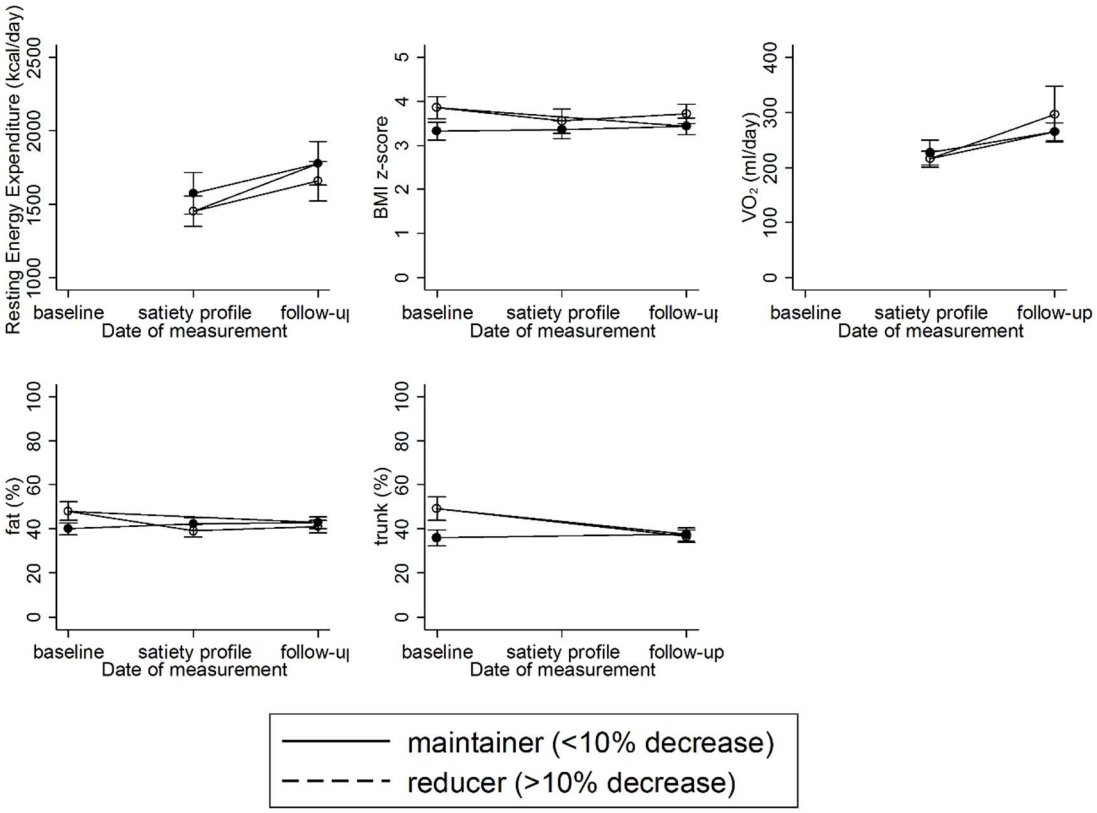


Figure 5.3. Different parameters (REE, BMI z-score, VO_2 , Fat mass % and Truncal fat mass %) plotted against the date of measurement for the 3 different time points in the study (baseline, midpoint and follow-up) for all the maintainers (pre and post-pubertal) compared to all the reducers (pre and post-pubertal).

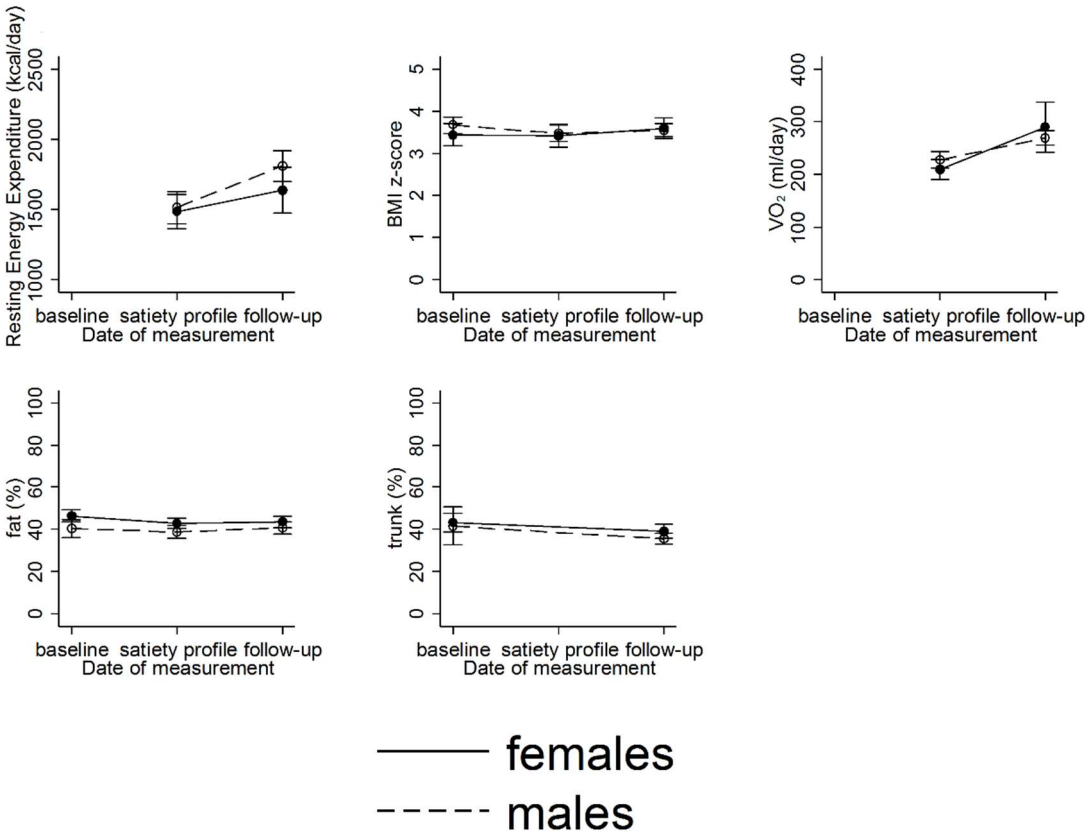


Figure 5.4. Different parameters (REE, BMI z-score, VO_2 , Fat mass % and Truncal fat mass %) plotted against the date of measurement for the 3 different time points in the study (baseline, midpoint and follow-up) for all females compared to all males.

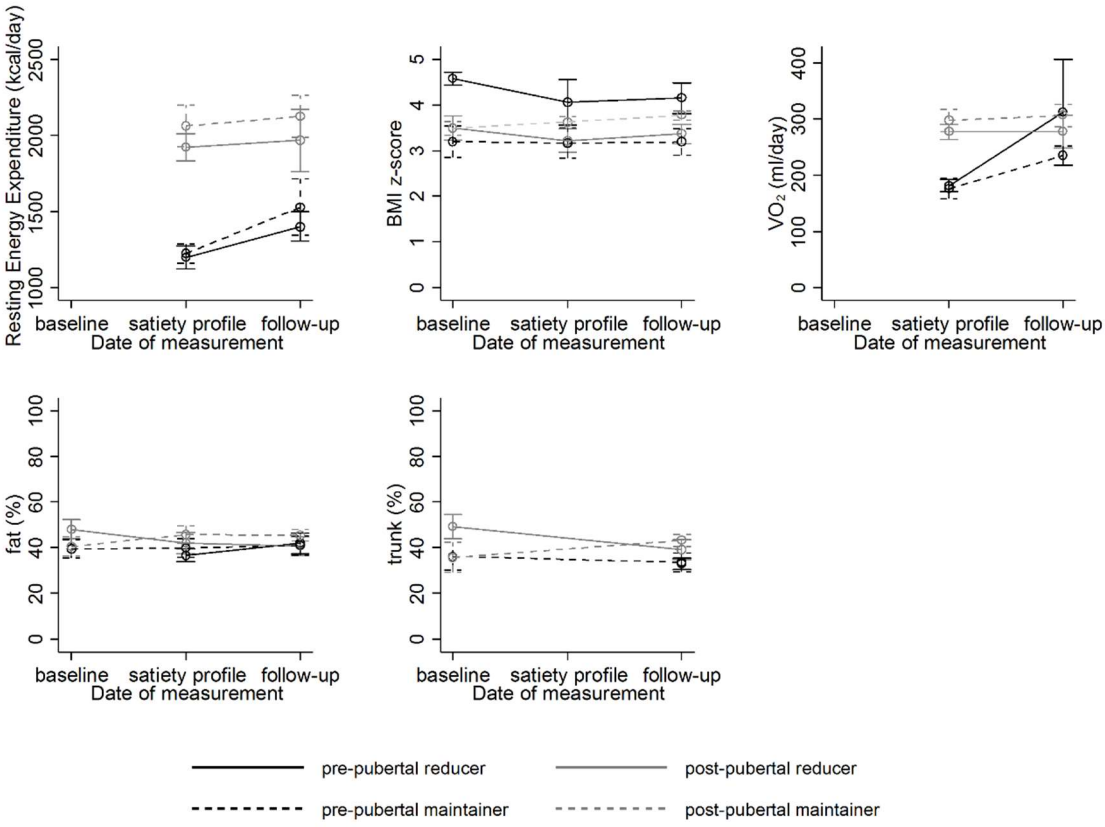


Figure 5.5. Different parameters (REE, BMI z-score, VO_2 , Fat mass % and Truncal fat mass %) plotted against the date of measurement for the 3 different time points in the study (baseline, midpoint and follow-up) for each of the 4 groups (pre-pubertal reducer and maintainer, and post-pubertal reducer and maintainer).

Figure 5.6, is a bar chart representing the FFM adjusted for REE taken from all 41 participants at the midpoint in the study, comparing the 4 groups (pre-pubertal reducers and maintainers and post-pubertal reducers and maintainers), and shows that the values are greater in the pre-pubertal groups compared to the post-pubertal groups.

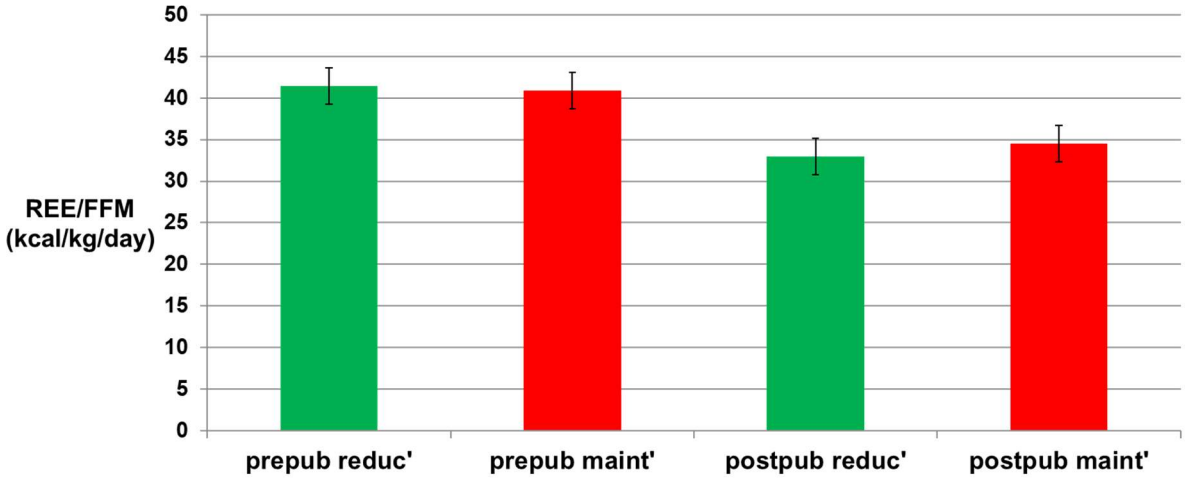


Figure 5.6. REE adjusted for Fat Free Mass in pre- and post-pubertal reducers and maintainers at the midpoint (at the time of the satiety hormone profile).

5.3 Discussion

In this study, we found that the REE in the post-pubertal adolescent reducers was lower than the post-pubertal maintainers, which is a finding that is consistent with adult obesity studies, where calorie restriction causes a profound reduction in energy expenditure, which leads to weight regain in adults (181). As expected, the post-pubertal adolescents had a higher REE than the pre-pubertal children, but when this was adjusted to account for body weight and also for FFM, the pre-pubertal groups (maintainer and reducer), had higher values in kcal/kg/day than the post-pubertal groups. In addition, the post-pubertal reducer group had a lower REE adjusted for FFM compared to the post-pubertal maintainer group at the time of the satiety hormone profile, as shown in figure 5.6, which is also consistent with results from adult studies (181).

Puberty was excluded in this study, one of the reasons for this was because pubertal children have a higher absolute REE and Total Daily Energy Expenditure than pre-pubertal children. The difference is predominately because of FFM, but rapid growth and puberty related hormones are also factors, although even in pubertal adolescents who are experiencing their growth spurt, growth only contributes 2-4% of their daily energy requirements (367).

Adolescents are at increased risk of developing obesity during puberty, as this is a period of growth, changing diet and also changing energy needs. Adolescent obesity often persists into adulthood, and females are particularly at risk owing to the pubertal increase in fat mass that they experience (368), and this was reflected in the higher proportion of females in the post-pubertal maintainer group. During adolescence the relative increase in fat mass is 13% in girls, while the level decreases by 4% in boys, so that adolescent boys have on average 20kg more FFM than girls (369).

At any given BMI, body fat and muscle mass can vary substantially. Therefore, an accurate assessment of body composition allows a more precise evaluation of nutritional status and exercise habits. Several studies (368, 370-372) have emphasised the importance of determining the fat mass and FFM by direct measures rather than relying on BMI when assessing obesity. The REE can then be adjusted for FFM, as FFM has a major impact on REE. When the REE is adjusted for FFM, the REE is not noticeably different between obese and lean subjects, suggesting that tissue and organ metabolic rate is not significantly different between subjects that are obese and lean.

As Fat mass and FFM vary with height, age and weight, it can be difficult to determine whether an individual subject has a low or high FFM or fat mass using only the absolute values of these parameters. Some researchers (162, 163) advocate the use Fat Free Mass Index (FFMI) and Fat Mass Index (FMI) to eliminate any differences in FFM or fat mass due to height, and to enable a more accurate nutritional assessment. Comprehensive reference data for paediatric body composition (SDS for 5 – 20 year olds) with which to interpret the individual measurements are now available (161). One study involving 1196 subjects aged 5-18 years, found the variability of FFMI to be approximately 50% of that in the FMI, and that the accuracy of BMI as a measure of adiposity varied greatly according to the degree of fatness, with a high BMI-for-age being a good indicator of excess fat, but less reliable in thinner children, where BMI differences were more likely due to differences in FFM (163). The FFMI also allows weight loss to be properly monitored. If for example a patient has a significantly lower FFMI than predicted, an increase in physical activity incorporating anaerobic resistance training may be useful. Or if a patient is losing weight but not changing their relative body fat, the FMI will show the amount of body fat store lost, which may act as a motivating factor to help patients to continue with their weight loss.

In this study, the REE was adjusted for weight and FFM, as each participant had their body composition measured using BIA. Several studies (163, 370, 371, 373, 374) have also adjusted the REE for body weight and FFM, as differences in absolute values of the REE often disappear once this is done. In research involving 221 subjects aged 6 – 17 years (113 males and 108 females) (375), REE was measured using a ventilated indirect calorimeter. The REE was first expressed as absolute values, and was found to increase with age in both genders, with a significant difference between genders in the 12-17 year age group. The REE was then adjusted for body weight and FFM, and was found to decrease with age in both genders, but a gender gap was still observed in the 12-17 year old group following the adjustment i.e. females had a lower REE adjusted for FFM compared to males (375). The researchers concluded that the metabolic activity of FFM decreases with age, due to an increase in skeletal mass which is less metabolically active, and that during puberty, the gender specific changes in body composition directly influences REE. Another study (371) which investigated the effect of body composition, age, sex and pubertal development on REE in 371 obese and non-obese children and adolescents aged 9.5 – 16.5 years, found that the absolute REE was significantly lower in non-obese compared to obese subjects. However, when the REE was adjusted for FFM, the difference became non-significant.

They also found a difference between genders which remained significant even after adjusting the REE for FFM, and there was a slight but significant decrease in REE adjusted for FFM in the older adolescents, demonstrating the effect that age has on REE (371).

While there is evidence to support younger children having a higher REE relative to body weight and FFM compared to older post-pubertal adolescents and adults, there is also evidence that there is a gender difference, and when the REE is adjusted for FFM (and expressed as per kg of FFM), it is significantly higher in boys than in girls, by 3% and 6% in pre-pubertal and pubertal subjects respectively (371).

REE is higher in obese individuals because of the increase in FM and FFM that accompanies obesity. Studies in adults and children linking the development of obesity to lower REE have been inconclusive, mainly because body composition is not routinely included in studies, but also because the follow-up period can be very variable. Certain groups of individuals such as the Pima Indians of Arizona, are uniquely prone to developing obesity, in part because they have a relatively low resting metabolic rate, and this has led to suggestions that the development of obesity is due to a lower than expected REE. However, this theory has been refuted by a UK study (376) involving 307 healthy school children (170 boys and 137 girls). REE and body composition were measured by indirect calorimetry and DEXA respectively on 7 occasions each year between 7 – 13 years, and the effect of REE on change in weight and body composition were analysed at 7 years. REE was also adjusted for FM and FFM. The results showed a small but statistically (negative) significant interaction between gain in fat and REE in boys but not girls i.e. in boys, fat was exchanged for FFM. However, they were unable to demonstrate any interaction between weight change and REE over 6 years, and their conclusion was that REE does not provide an explanation for childhood obesity (376).

It is known that obese children spend less time in physical activities and more time in sedentary activities than their age-matched counterparts (377). Sedentary and physical activities were assessed in a study of 50 non-obese and 27 obese adolescents, aged 14 years ± 0.3 years (377). As expected, the sleeping and sedentary energy expenditure were 19% higher in the obese subjects compared to the non-obese, but were similar following adjustment for body composition. However, energy expenditure associated with physical activity (walking on a treadmill in whole body calorimeters), was 51% lower in obese adolescents compared to non-obese subjects, after adjustment for

body composition (P<0.001) (377). Other studies have reported similar findings, with the energy expenditure of obese children and adolescents for moderate and sports activities being 20% and 25% of non-obese subjects respectively (369). It also appears that physical activity is essential for weight maintenance after diet induced weight loss in obese adolescents, particularly if the activity promotes substantial fat oxidation e.g. walking rather than cycling (152).

Limitations of the follow-up component of this study, included the small sample size at follow-up, which meant there was insufficient power to generate any significant P values when comparing the 4 separate groups (pre and post-pubertal reducers and pre and post-pubertal maintainers), or combinations of the groups (all pre-pubertal children vs all post-pubertal adolescents). We had also wanted to carry out REE measurements in the non-obese siblings of the paediatric diabetic patients at The RCH for comparison. However, owing to time restraints in terms of gaining ethics approval and also carrying out the investigations, this was not possible.

In conclusion, pre-pubertal children have a greater REE adjusted for weight and FFM compared to post-pubertal adolescents, which would help them to maintain a lower weight trajectory and prevent weight regain. In the last decade there has been a decline in research interest in energy expenditure, with the majority of studies occurring in the mid-1990's to mid-2000's, which most likely coincided with the emergence of relatively affordable indirect calorimeters. However, larger studies are still needed to explore the relationship between weight loss and REE in pre and post-pubertal children and adolescents, and in particular the effect that physical activity has on REE, as this will help to inform our strategies for preventing and treating childhood obesity.

Chapter 6 Final Discussion and Future Directions

6.1 Final discussion.

6.1.1 Study hypothesis

In this study we hypothesised that set-points for weight, and their physiological defence, are flexible in childhood but become fixed around puberty. We aimed to show that obese pre-pubertal children who lost weight had less 'reflex' changes in satiety hormone profiles (that would drive weight regain), compared with adolescents, who had experienced a similar degree of weight change. We therefore expected post-pubertal adolescents to have similar satiety hormone responses to obese adults who lose weight i.e. post-prandial increase in ghrelin (associated with feelings of hunger) and decreases in PYY and amylin (all associated with reduced feelings of fullness). However, while we found distinct variations in several specific hormones between young and older obese children/adolescents who had lost weight, there were no demonstrable differences in satiety hormone profiles between the two groups. Also in contrast with adult studies were the results from the subjective sensations of appetite. In this study post-pubertal adolescents felt less hungry and reported being more full than pre-pubertal children, whereas in adult obesity studies, participants reported feeling more hungry and pre-occupied with food than before they had lost weight, which is concordant with the higher ghrelin levels seen in obese adults following weight loss.

These findings therefore did not support our hypothesis, and it would appear that the physiological mechanisms involving satiety hormones responses, which act to protect against weight change, may develop later than in the adolescent years. These results however may be advantageous for post-pubertal adolescents trying to lose weight, as it means that their higher body weight is not as vigorously defended by internal physiological mechanisms, which in theory would make it easier for them to maintain a lower body weight compared to obese adults.

While the absence of a 19 to 25 year old group for comparison was a limitation of this study, the future multi-centre research study in Australia looking at weight trajectories, and associated physiological drivers to weight regain in 4 different age groups, will include a young adult cohort. This should provide further clarification on exactly when an individual's set-point for body weight becomes fixed.

Regarding REE, our results showed that while REE in pre-pubertal reducers and maintainers were similar, post-pubertal reducers had a lower REE compared to post-pubertal maintainers. These findings indicate that obese adolescents who lose weight may experience a similar reduction in REE to that seen in obese adults who lose weight, which would act to promote weight regain, but that this reduction in REE is not evident in obese pre-pubertal children who lose weight. In addition, pre-pubertal children have a greater REE adjusted for FFM compared to post-pubertal adolescents, which would also help to prevent weight regain.

REE is an area for potential future development, as the MedGem® hand-held indirect calorimetry device, which was used to measure REE in this study and has been shown to be valid and reliable in adolescents and young children, could be used in an outpatient clinic setting to monitor REE over time. Those children with the highest REE adjusted for FFM would in theory have the greatest chance of improving their future weight trajectory and maintaining their lower weight in the longer-term. Children identified as having a lower REE adjusted for FFM however may need earlier adjuvant obesity support with pharmacotherapy or more intense physical activity programmes in order to achieve and maintain a lower weight trajectory.

It is recognised that there remains a paucity of data looking at satiety hormone responses in children and adolescents following weight loss, and that study results are often confounded by the physiological changes that occur during puberty, such as increased insulin resistance and changes in FFM in relation to FM. We believe this was the first study to properly exclude puberty as a confounding variable, thus adding considerable new knowledge to the literature, although further research is needed to determine the pre and post-prandial satiety hormone responses to weight loss in children and adolescents.

6.2 Future direction

There have been several interesting discoveries and developments in the field of paediatric obesity recently, ranging from pharmacotherapy treatments to successful implementation of large-scale public health initiatives, to novel research findings, which may have significant implications for the future prevention and management of obesity.

6.2.1 New treatment developments

6.2.1.1 New developments in pharmacotherapy – Sustained release Oxyntomodulin analogue (OX-SR)

Oxyntomodulin is a gut hormone that causes weight loss by reducing appetite and increasing energy expenditure. Exactly how oxyntomodulin works remains unclear as it can activate both glucagon and GLP-1 receptors, although no specific receptor has been identified. The research team at University College London have developed a sustained release oxyntomodulin analogue (OX-SR), which produces a significant increase in energy expenditure in Wister rats. The energy expenditure increase occurs via activation of the glucagon receptor, and this receptor is essential for OX-SR's sustained effects on energy expenditure. OX-SR is slightly more potent at both the GLP-1 and Glucagon receptors than oxyntomodulin, and has a sustained release from a subcutaneous depot, taking 6 days for plasma levels to be undetectable, as opposed to 1 day for the same dose of oxyntomodulin. A single dose of OX-SR was found to increase energy expenditure in Wister rats, when measured by indirect calorimetry, with oxygen consumption increasing by 10% over 12 hours (378).

While many drugs can increase energy expenditure, their use as anti-obesity treatments have been withdrawn due to significant side effects (e.g. amphetamines and levothyroxine cause cardiovascular side effects). Dual and even triple agonist therapies combining GLP-1, GIP and glucagon receptor activities are actively being trialled for obesity and diabetes with encouraging results (379-381), but the development of future dual agonist analogues will need a careful balancing of GLP-1 and glucagon receptor activities, in order to preserve the optimal effects of both.

6.2.1.1 Successful implementation of Public Health Initiative – Amsterdam Healthy Weight programme

The childhood obesity and overweight rate in Amsterdam in 2012 was 21%, which was much higher than the national average in the Netherlands of 13%, and the highest percentage of overweight or obese children were of Turkish or Moroccan descent. To address this problem the city of Amsterdam (population 851,600), looked for a long-term sustainable change in behaviour, which culminated in the Amsterdam healthy weight programme (382). Preventative measures included the first 1000 day approach, making schools healthy, investing in neighbourhoods and communities, in order to construct the city in a way that would promote healthy lifestyles and create a healthy

food environment. They also focused on obesity management by targeting morbid obesity in children and developing better obesity treatment programmes. Finally, they invested in education, digital facilities and communication. They also worked with individuals to instigate actions at a community and population level. They did this by organising focus groups, panels and organised meetings, which resulted in the co-production of solutions to obesity with the communities. The combination of all of these efforts were required to bring about change.

Following the launch of the Amsterdam Healthy Weight Programme, the whole city managed to reduce the total number of overweight and obese children by 10% within the first 2 years, which equates to 2000 fewer overweight children. Importantly, the programme was especially successful for children from low socioeconomic backgrounds. The Amsterdam Healthy Weight Programme was started in 2013 and has a 20 year plan, and its final aim is for a healthy weight in all young people in Amsterdam by 2033 .

6.2.1.2 Novel research developments

Faecal Microbiota Transplantation (FMT): The human gut microbiome is the collection of microorganisms, their gene products and corresponding physiological functions found in the human GI tract (383). Following the discovery that the ratio of *Firmicutes* to *Bacteroidetes* (F:B ratio) and energy harvest capacity differs in obese versus lean animal and human subjects (384), the gut microbiome has been implicated as having an important role in obesity. Both obese animal and human subjects have been found to have altered gut microbiota (reduced bacterial diversity and altered colonic fermentation) compared to their lean counterparts. The gut microbiome can be classified into High Gene Count (HGC) or Low Gene Count (LGC). The former is most often seen in lean individuals and is associated with greater *F. prausnitzii* and butyate levels, whereas the later is seen more often in obese individuals and is associated with lower butyrate production but higher levels of *Bacteriodetes* (385). Diet induced weight loss interventions in obese individuals (LGC group) can partially restore these changes, which confirms that there is plasticity within the gut microbiome.

Faecal Microbiota Transplantation (FMT) aims to restore gut microbiota by transfering faeces from a healthy (lean) individual to a sick (obese) individual. It has been used extensively to treat *Clostridium difficile* infection, but there are only a small number of studies looking at its success in treating obesity. In an animal study involving the transfer of stool from monozygotic or dizygotic twins discordant for obesity into ‘humanized’ germ-free mice, the recipient

mice showed an increase in adiposity i.e stool was transferred from obese mice to normal weight mice, and the normal weight mice then became obese. This study confirmed that it is possible for obesity-associated metabolic phenotypes to be transferred (386) which means that in the future, obesity-related disorders could potentially be managed by the therapeutic manipulation of the gut microbiome. In a small human RCT investigating the effects of FMT on the metabolic syndrome, FMT was transferred from healthy lean subjects to patients with metabolic syndrome. 6 weeks after transfer, there was an increased insulin sensitivity and gut microbial diversity, particularly of *Roseburia intestinalis*, which produces butyrate (387), which is known to play an important role in promoting insulin sensitivity in mice (388). In the future targeted microbiome therapeutics could be used in clinical practice, but further studies are needed to assess the metabolic changes following FMT.

Body Weight homeostat that regulates fat mass independently of Leptin: Scientists in Sweden have discovered a new leptin-independent body weight homeostat (gravitostat) that regulates fat mass. There is epidemiological evidence for an association between the number of hours spent in a sitting position and several metabolic diseases such as obesity, diabetes and cardiovascular disease (389, 390). However, the mechanism for the antiobesity effect of standing is unclear. In this study (391), mice with diet induced obesity were loaded with capsules of different weights, which were implanted in their abdomen or subcutaneously on their backs. The increased loading was found to reversibly decrease the biological weight by reducing food intake, with the mice losing almost as much weight as the artificial load. The loading also resulted in an improvement in glucose tolerance and a reduction in fat mass.

It is well establised that osteocytes are able to sense changes in bone strain (392). In this study, it was hypothesised that the homeostatic regulation of body weight and fat mass by osteocytes in reponse to changes in body weight was mediated by a bone derived cirulating factor (sclerostin,osteocalcin, FGF23, and lipocalin 2). However, increased loading did not significantly alter the expression of any of the four main bone-derived circulating factors, although the body weight reducing effect of increased loading was was lost in mice who were depleted of osteocytes. The authors therefore concluded that increased body weight activates a sensor dependent on osteocytes of the weight-bearing bones, which induces an afferent signal to the brain, which decreases body-weight by reducing food intake, in order to keep body weight

constant. Conversely, excess sitting time would result in decreased loading of osteocytes in the weight-bearing long-bones, so that the homeostatic regulation of body weight does not activate the afferent signal to the brain, resulting in obesity i.e. obese patients need to spend more time stood up, weight-bearing, in order to activate afferent signals to the brain to reduce their food intake. Further studies exploring this novel body-weight regulation system are still warranted, and could potentially lead to a better understanding of the causes of obesity, and pave the way for new anti-obesity treatments.

Electronic implant to reduce obesity: Researchers from Imperial College in London have developed a microchip which can be attached to the Vagus nerve within the peritoneal cavity, which can process chemical impulses in order to suppress appetite (393). While gastric pacemakers and vagus nerve stimulators have been used previously with mixed results, this microchip can identify chemicals rather than just electrical impulses, which makes it a more selective and precise instrument. The intelligent implantable modulator is also different because it does not just send stimulating impulses, but instead reads and processes electrical and chemical signatures of appetite within the vagus nerve, and can send electrical impulses to the brain in order to reduce or stop the urge to eat i.e. mimics the signals the brain normally receives from the GI system following a meal to supress appetite. In the future, the electronic implant could provide a more effective alternative to bariatric surgery, and has the advantage of being reversible and potentially simpler and cheaper. The project, which is called, Intelligent implantable modulator of Vagus nerve function for treatment of obesity (i2MOVE), received over 7 million euros in funding from the European Research Council in 2013, and research is ongoing.

There are however other teams working on Vagus nerve implants to treat obesity. EnteroMedics, a US based company, have developed an implant (VBloc®) (394) which intermittently blocks the Vagus nerve using electrical impulses. A clinical trial of the VBloc® device involving 239 patients showed that more than half of those using it had lost at least 20% of their excess body weight, although results were not as good as had been expected. Another US company, IntraPace, has European approval for its Abiliti® device (395), which uses Vagus nerve stimulation to reduce food consumption.

6.2.2 Conclusion

The obesity epidemic is a looming crisis that requires immediate action. While some have questioned the forecasts of experts and have doubted the far-reaching impact of obesity, any residual scepticism is gradually being eroded away by accumulating evidence. Others would prefer to defer efforts directly addressing the problem and instead place hope in the development of new drugs or surgical procedures that might offer a quicker solution, or argue that the costs of action are too great, ignorant to the fact that future survival depends on solving the problem.

The good news is that solutions to the problem are readily available, but while broad consensus exists with respect to the dietary and lifestyle habits needed to prevent and treat childhood obesity, a co-ordinated and comprehensive strategy for encouraging children to eat healthily and exercise more is sadly lacking. A successful strategy would need to include the strict legislation and regulation of fast-food advertising, with incentives and farm-subsides for the promotion of nutrient-dense rather than calorie-dense produce, and the provision of healthy lunches and regular physical activities at school. Parental involvement is key, and parents must take responsibility for the welfare of their own children, by limiting their screen time, providing high quality food and modelling healthy eating and exercise behaviours. However, their efforts should not be undermined by the marketing campaigns of the manufacturers of unhealthy foods, and by governments who have a conflict of interest in regulating them. The early identification and implementation of effective, accessible, well-resourced obesity treatment programmes, which take into account the internal physiological mechanisms regulating body weight, are also an essential component to successfully preventing and treating overweight and obese youth, and are critical to the prevention of adult obesity.

In conclusion, childhood obesity can be cured, but only with the exercising of both social and personal responsibility, and only then will we be able to change the shape of things to come (396).


Obesity
Childhood
Childhood Obesity
obese
BMI
disorders
body
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Disease
overweight
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determining
problems
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affects
difficult
recognized
published
aged
cholesterol
however
emotional
public
fat
adults
esteem
normal
stigmatizing
mortality
greater
percentile
adolescence
prevalence
including
low
grow
diabetes
depression
levels
very
family
condition
high
pressure
directly
wellbeing
stereotypes
first
much
acceptable
less
well
many
life-threatening
abound
carbid
bulimia
Control
serious
two
harassed
include
Due
skin
infections

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