Cardiovascular risk and stress in adolescents with obesity

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Submitted for examination for PhD

Declaration

I, Lee Duncan Hudson confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

14th July 2018.

Abstract

Background

Cardiovascular risk prediction is problematic in adolescents with obesity. Pulse wave velocity (PWV) can contemporaneously capture arterial stiffening in obesity. Stress has been implicated in the aetiology of obesity and cardiovascular risk. This thesis examines these relationships within a community sample of obese adolescents recruited to an obesity intervention (the HELP trial).

Methods

Two systematic reviews were performed for: i) associations between PWV and obesity; ii) associations between stress, obesity and metabolic risk. In the HELP trial PWV, adiposity measures, blood pressure, cardiovascular blood testing, stress measures (salivary cortisol, A-FILE questionnaire) were measured longitudinally. Baseline and longitudinal analyses investigated associations between adiposity, blood pressure, and blood markers with stress and PWV.

Results

Systematic reviews found: i)moderate evidence for increased arterial stiffening in obese children, especially in central arteries; ii)mixed findings for associations between stress, obesity and cardiovascular risk. 174 adolescents were recruited to the HELP study. Baseline findings: i)PWV was associated with adiposity; ii)PWV was not associated with BP or blood tests; iii) severe obesity groups had greater average PWV however overlap between groups was large; iv)stress measures were not associated with adiposity, blood pressure or blood tests; v)stress exposure was associated with risk of binge eating. Longitudinal findings: i)PWV in the group did not change; ii)multi-level models showed no association between stress measures, adiposity or blood pressure over time; iii)blood pressure and adiposity were associated over time.

Conclusions

Greater adiposity was associated with greater arterial stiffness. Partitioning by obesity severity was unreliable. Lack of associations between BP, blood testing and arterial stiffness questions their reliability for predicting cardiovascular risk in obese adolescents. Increases in adiposity and blood pressure were linked. The thesis did not demonstrate associations between stress and adiposity, blood pressure or PWV. Reducing BMI in adolescents with obesity may be an effective way to reduce cardiovascular risk.

Impact Statement

Potential benefits of the research presented in this thesis are as follows:

1. Direct clinical care. This thesis showed that above all, BMIz, i.e. degree of obesity in adolescents appears to be most associated with arterial stiffening. In clinical contexts, in particular obesity services, the findings of this thesis can benefit approaches to assessment and advice by directing emphasis on overall BMI/BMIz rather than blood risk parameters and acanthosis nigricans. In particular the emotional impact of discussing acanthosis nigricans with young people should take precedence over perceived risk. Potential use of PWV in clinical settings to identify those young people more at risk may be more of use than blood testing.

2. Public health. The research presented in this thesis adds to the evidence that there is potential cardiovascular risk for young people with obesity, with future health, well-being and financial implications for this current generation of adolescents. This research should be used to maintain and further the argument for public health approaches and investment into prevention and reduction of obesity in children and adolescents.

3. Further research. Using PWV as a modality should take places as this thesis has shown it is a practical research tool. Further discussion of future possible research is presented in detail in the discussion of the thesis.

4. A number of papers have been published from this thesis which are a key part of its impact.

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smiles, cries and laughter are a daily reminder as to why I chose to work in, and research, child health in the first place.

Publications arising from/linked with thesis and comment on permissions for figures and tables used in papers.

Publications from the thesis

The following papers were published from this thesis, and are signposted at the relevant points in the thesis. These papers are also presented in appendix 9.3. Permission to use figures and tables published in papers in this thesis were kindly given by each of the journals cited below. I created all figures (including illustrations) and tables found in this thesis.

Hudson L, Rapala A, Khan T, Williams B,Viner RM. Evidence for contemporary arterial stiffening in obese children and adolescents using pulse wave velocity: a systematic review and meta-analysis. *Atherosclerosis* 2015 [published Online First: doi: 10.1016/ j.atherosclerosis.2015.05.014.] *This is comprised of work presented in Chapter 1.*

Hudson LD, Kinra S, Wong ICK, et al. Arterial stiffening, insulin resistance and acanthosis nigricans in a community sample of adolescents with obesity. *International journal of obesity (2005)* 2017;41(9):1454-56. doi: 10.1038/ijo.2017.105 [published Online First: 2017/05/04] *This is comprised of work presented in Chapter 1,3 and 4.*

Hudson L, Kinra S, Wong I, et al. Is arterial stiffening associated with adiposity, severity of obesity and other contemporary cardiometabolic markers in a community sample of adolescents with obesity in the UK? BMJ Paediatrics Open

2017;**1**:e000061. doi:10.1136/ bmjpo-2017-000061 This is comprised of work presented in Chapter 1,3 and 4.

Other publications associated with the thesis

Hudson L, Christie D, Kessel A. Impact of health system reforms on primary care research. *The British journal of general practice : the journal of the Royal College of General Practitioners* 2012;62(600):349. doi: 10.3399/bjgp12X652247 [published Online First:2012/07/12]

Christie D, Hudson LD, Kinra S, et al. A community-based motivational personalized lifestyle intervention to reduce BMI in obese adolescents: results from the Healthy Eating and Lifestyle Programme (HELP) randomized controlled trial. *Arch Dis Child* 2017;102(8):695-701. doi: 10.1136/archdischild-2016-311586 [published Online First: 2017/07/09]

Research questions

In a group of adolescents with obesity:

1. How is degree of adiposity associated with pulse wave velocity as a proxy of arterial stiffening at a cross-sectional and longitudinal level?
2. How is blood pressure associated with pulse wave velocity as a proxy of arterial stiffening at a cross-sectional and longitudinal level?
3. How are other conventional measures of cardio-metabolic risk such as conventional blood markers (e.g. cholesterol) and acanthosis nigricans associated with pulse wave velocity as a proxy of arterial stiffening at a cross-sectional and longitudinal level?
4. How are measures of stress associated with degree of adiposity at a cross-sectional and longitudinal level?
5. How are measures of stress associated with blood pressure at a cross-sectional and longitudinal level?
6. How are measures of stress associated with pulse wave velocity at a cross-sectional and longitudinal level?

Research questions 1-3 are collectively addressed in chapters 1, 3,4 and 6 (with chapter 4 and 6 presenting cross-sectional and longitudinal data respectively).

Research questions 4-6 are collectively addressed in chapters 2,3,5 and 6 (with chapter 4 and 6 presenting cross-sectional and longitudinal data respectively).

All research questions are addressed in the discussion chapter 7.

Table of commonly used abbreviations within the thesis.

ACTH	adrenocorticotropic hormone
AI	Augmentation index
ALT	Alanine aminotransferase
AN	Acanthosis nigricans
AUC	Area under the curve
BMI	Body mass index (Kg/m2)
BMI z	BMI z-score for age and sex
BP	Blood pressure
C-dayAUC	Total day cortisol from awakening to evening, area under the curve (See figure 3.1)
C-evening	Cortisol evening sample (See figure 3.1)
C-ratio	Ratio of evening cortisol / wakening cortisol (See figure 3.1)
C-wake	Cortisol on wakening sample (See figure 3.1)
CAR	Cortisol awakening response

CAR-AUC	Cortisol awakening response, area under curve (See figure 3.1)
CAR-rate	Cortisol awakening response, rate of change (See figure 3.1)
CRF-GOSH	Clinical research facility at Great Ormond Street Hospital
DAWBA	Development and wellbeing assessment
DBP	Diastolic blood pressure
Diastolic z	Systolic blood pressure z-score for age and sex.
DM	Diabetes mellitus
FMI	Fat mass index (Kg/m2)
HbA1c	Percentage glycosylated Haemoglobin (units %)
HDL	High density lipoprotein
HDL/C ratio	HDL/total cholesterol ratio
HELP	Health eating and lifestyle program (original trial from which thesis comes from)
HOMA-IR	Homeostatic model assessment-Insulin resistance.
HPA Axis	Hypothalamic-pituitary-adrenal axis
IMD	Index of multiple deprivation
IOTF	International obesity Task Force
LDL	Low density lipoprotein
MetS	Metabolic syndrome

PWV	Pulse wave velocity (units m/s)
RSE	Rosenberg's self esteem score
SAD	Sagittal-abdominal diameter (cm)
SBP	Systolic blood pressure
Systolic z	Systolic blood pressure z-score for age and sex.
SD	standard deviation
t	Time (various time points, e.g. t0 = time 0)
TBFM	Truncal body fat mass
UFC	Urinary free cortisol
Waist z	Waist circumference z-score for age and sex.
WBTM	Whole body fat mass
WHO	World Health Organisation
WMD	Weighted mean difference (in meta-analysis)
z	Z-score

Chapter 1: Evidence for a long-term cardiovascular risk associated with obesity in adolescence.

1.1 Introduction

As a clinician who has worked with adolescents with obesity in a weight management service, the validity of perceived risk associated with degree of overweight and associated cardio-metabolic co-morbidities has long interested me. Within such clinical settings, it is standard practice to partition by degree of obesity, and use adult-based cardio-metabolic markers to conceptualize risk for later cardiovascular disease, and moreover to communicate these findings to counsel young people and their families.[1] Yet when working in these settings, I often wondered about the validity of this well-established practice in terms of scientific evidence, particularly where they were used to support interventions. In 2010 I was employed as the medical research fellow for a randomized controlled trial of a weight management intervention for adolescents with obesity at the UCL Institute of Child Health (The HELP trial which is now published).[2] The study itself is described in more detail in chapter 3, however the study also provided me with the opportunity to explore evidence for potential long-term cardiovascular risk in an obese group of adolescents with cross-sectional and longitudinal methods, which is a key focus of this thesis.

In this first chapter, I will address the background to research questions 1-3 (namely 1) how degree of adiposity is associated with pulse wave velocity as a proxy of arterial stiffening at a cross-sectional and longitudinal level; 2) how blood pressure is associated with pulse wave velocity as a proxy of arterial stiffening at a cross-

sectional and longitudinal level; and 3) how other conventional measures of cardio-metabolic risk such as blood testing and acanthosis nigricans are associated with pulse wave velocity as a proxy of arterial stiffening at a cross-sectional and longitudinal level). I will firstly examine what is currently known about the relationship between obesity in young people and long-term cardiovascular risk, and outline important key concepts and definitions necessary to explore this relationship, and then present a systematic review of the literature on pulse wave analysis. Finally I will outline the relevance of information presented in this chapter as the basis for the research questions 1-3 studied in this thesis.

1.2 Obesity definitions

The term overweight strictly refers to an excess of body fat rather than body weight per se. By convention, international definitions of overweight and obesity in both children and adults use the body mass index (BMI), a function of weight (in kg) divided the square of height (in m). Obesity, and severe obesity, are increasing grades of overweight. In adults an individual is described as having overweight when their BMI is equal to or exceeds 25Kg/m^2 and obesity when their BMI is equal to or exceeds 30 kg/m^2; with severe (or "morbid") obesity greater than 40. These cut-offs have been validated to long-term outcome data in adults, correlated with mortality risk. Whitlock et al published data on relative risks of death in 35-79 year olds related to their BMI.[3] This demonstrated that there was an increase in risk of death in adults with a BMI over 25, with each increase of 5 kg/m^2 over 25 shown to increase mortality by 30%. Adults with a BMI in the range of 30–35 were shown to have their lives shortened on average by 2–4 years, while those at 40–45 are reduced by 8–10 years.

Definitions of overweight and obesity are complicated in children because of a paucity of similar longitudinal data to validate them. Moreover, the normal distribution of BMI varies by age and sex throughout childhood and using the same cut-offs for all ages and sexes would therefore be inappropriate. Definitions of obesity in childhood have utilized the convention in child health of applying extremes of normal distributions of the BMI distribution.[4] However there is variation

clinically and in research over which cut-offs are used internationally – with cut-offs including above the 95th centile,[5] and the International Obesity Task Force (IOTF) cut-offs based on combining international data sets of BMI distributions.[6] Obesity degree itself has been categorised into severity levels using >2.5 standard deviations of BMI[7] and also >3.5 SD.[1] It is important to note that because definitions of obesity in childhood utilize extremes of the normal population, by definition such cut-offs will include a large number of children who are potentially healthy. Thus, in effect the higher the cut-off centile for BMI is placed, the more specific it will become at picking up children with higher body fat.

The number of children and young people reaching cut-offs used to define obesity has increased dramatically in the last 30 years internationally in both high and low income countries.[8-12] In England, the national child measurement program measures all school age children at school reception (aged 4/5) and school year 6 (aged 10/11 years) and categorizes children by BMI centile.[13] The 2011/12 results (which incorporated over 1 million children; with 93% of eligible children included) showed that 13.1% of reception children had overweight (>85th centile) and 9.5% had obesity (>95th centile), with 22.6% of this age group of children with either overweight or obesity. Proportions of obesity across the United Kingdom vary by region, with London carrying the greatest burden,[14] the region that the data used in this thesis were drawn from. In London, around 11% of children entering reception year at school have obesity, and by the age of 10-11 (the age just before the lowest age of subjects in this thesis) 25% of black children will have obesity, 22% of south Asian children and just under 20% of white children.[14] Obesity is also more common in boys than girls in London at age 10-11 at 24% versus 19%.[14] Obesity is greater in regions of London with the highest degrees of deprivation.[14]

1.3 Measures and proxies for body fat in children

BMI is a pragmatic, inexpensive proxy of stratifying children and adolescents by degree of body fat, and as described above carries the drawback of not being able to directly measure body fat. Wells has demonstrated the normal variation of fat mass in healthy children by using Dual-energy X-ray absorptiometry (DEXA), underwater

weighing, deuterium dilution, bioelectrical impedance analysis, and anthropometry.[15] Theoretically, centile charts could be developed for fat content using these methods but so far these do not exist, and moreover given the complexities and expense of measuring using these techniques, its use would be limited. Other, easier methods for trying to measure body fat more accurately in clinical and research contexts, which can be used directly on young people (and are used in this thesis – see chapter 3), are measuring waist circumference, which offers the advantage of attempting to measure and stratify central adiposity which is associated with cardio-metabolic risk (see below) and also measuring bio-impedance. I will outline these here, and the background to them as they are used in this thesis as measures of fat.

Waist circumference

Waist circumference is most commonly measured using a non-flexible measuring tape midway between the 10th rib and iliac crest, and centiles have been developed to standardize measurements for each age and sex.[16] Another method of measuring intra-abdominal fat is to measure Anterior-posterior sagittal abdominal dimension (SAD) using a Holtain-Kahn abdominal caliper with young people in a supine position.[17] This method has been shown to correlate better than waist circumference in adults for cardio-vascular risk factors such as lipids, although it does not have standardized measures/centiles unlike waist circumference.[18].

Bio-impedance (bio-electrical impedance)

This method models the body of an individual as a cylindrical conductor, and uses bio-electrical impedance measures to derive total free fat mass (FFM). This method is readily available using commercially available equipment, and in this thesis the Tanita BC 418MA (Tanita, UK) is used. The reason this particular equipment was used in the thesis is because it has been validated against the more complex four component model described above, in a published study which also provided a more

precise formula for free fat mass for adolescents using height and impedance measured using this model of Tanita (the use of this in this thesis is described in more detail in chapter 3).[19]

1.4 relationship between obesity and long term cardiovascular risk

As described above, Whitlock et al's study provided evidence of risk of death predicted by increasing BMI in adults.[3] However, error bars were wide, and there is therefore variation in risk within individuals at the same BMI. Variation may be due to presence or not of obesity-associated cardio-metabolic risk factors: namely hypertension, central adiposity, dyslipidaemia and insulin resistance. From the MUNSTER study, Schulte et al demonstrated that whilst mortality increased with increasing BMI in adults in this study, the increases in mortality could, in analyses, be explained by the presence of additional cardio-metabolic risk factors such as hypertension and dyslipidaemia.[20] The importance of the clustering of risk factors within individuals with obesity has led to the concept of both the "metabolic syndrome" (MetS)[21 22] and "metabolically healthy obese adults".[23] However, whilst risk in "metabolically healthy obese adults" may be lower than in obese individuals with co-existing cardio-metabolic risk factors, in a systematic review including 10 studies, Kramer et al showed that in obese adults without co-existing cardio-metabolic factors (so called "metabolically healthy obese adults), the relative risk for cardiovascular events was still greater compared to metabolically healthy normal weight adults (RR 1.24 (1.02 -1.55)) – thus being obese alone still carries cardiovascular risk.[24]

There is now a well-established evidence for the interaction between individual cardio-metabolic risk factors and long-term cardiovascular risk in obese adults, in particular location of adiposity (central versus more peripherally placed adiposity), hypertension, dyslipidaemia and insulin resistance:

- *Location of adiposity* : Padwal et al showed that those with central adiposity using weight circumference and DEXA had greater risk of death from cardiovascular events compared to those with alternate distributions. [25]

- *Hypertension*: A recent Cochrane review showed that drops in weight from weight reducing diets led to reductions in systolic and diastolic blood pressure.[26] Data from the Framington study showed that a 6.8 kg loss of weight in obese participants led to a 22-26% relative risk reduction of developing hypertension over time.[27]

- *Dyslipidaemia :* Higher levels of total cholesterol, triglycerides and lower levels of HDL (higher levels being cardio-protective) have been demonstrated in adults with obesity; and all of these risk factors are associated with coronary artery disease.[28]

- *Insulin resistance and diabetes* has been shown to be more common in adults with obesity,[29] especially those with central obesity,[30] and carries increased risk for cardiovascular disease.[31]

1.5 Metabolic syndrome in children and conventional markers of cardiovascular risk in children and adolescents with obesity.

With the emerging increase internationally of obesity in children and young people, definitions of MetS in children and adolescents have been developed (table 1.1), with an increased incidence of children and adolescents meeting these criteria with increasing levels of obesity.[1 32] Such definitions however have been the subject of debate, principally because of an observed temporal instability of definitions through growth and development, particularly during puberty, limiting their contemporaneous use to predict future cardiovascular risk (see below).[2] In their large, school-based study of over 1000 adolescents over 3 years, Goodman and colleagues found that around half of adolescents meeting the AHA and IDF

definitions of MetS at entry to the study no longer fitted such definitions at exit at 3 years.[2] As well as the challenge of predicting which adolescents will continue to have the MetS into their adulthood, this instability also calls into question the basis of pharmacological treatments in this age group, treatments which themselves carry cost and potential side effects.

In the United Kingdom, consensus guidelines for assessing cardiovascular risk have been published in the format of the Obesity Services for Children and Adolescents (OSCA) guidelines.[1] These guidelines provide specific guidance on absolute values to define abnormalities of important cardio-metabolic co-morbidities in children and young people with obesity. These are summarized in table 1.2. These guidelines are important for the thesis, as they will be used as the definitions for cardio-metabolic risk in the thesis and various cut-offs (as outlined further in chapter 3).

Table 1.1: Summary of current guidelines for the MetS in children and adolescents.

Parameters	**Modified ATP III**	**IDF (10-16 years)**	**NHANES III**
Waist circumference	Not required	≥ 90th percentile (but ethnic specific)	≥ 90th percentile
Number of abnormalities required	≥ 3	≥2	ALL
Triglycerides	> 95th percentile	≥1.7 mmol/L	≥ 1.24 mmol/L
HDL	< 5th percentile	< 1.03 mmol/L	< 1.03 mmol/L
BP	Either systolic or	Either systolic or	Both ≥90th

Parameters	Modified ATP III	IDF (10-16 years)	NHANES III
	diastolic raised	diastolic raised	percentile
Systolic	> 95th percentile	> 95th percentile	
Diastolic	> 95th percentile	≥85 mm Hg	
Glucose	Impaired measured glucose tolerance	≥ 5.6 mmol fasting	≥ 6.1 mmol/L fasting

Key: ATP = Adult treatment panel; IDF = International Diabetes Federation; NHANES = National Health and Nutrition Examination Survey

Table 1.2 : Summary of OSCA guideline[1] definitions of cardio-metabolic risk factor co-morbidities associated with obesity.

Risk factor	Definition
Extreme obesity	>3.5 BMI z score for age and sex
Hypertension	Systolic or diastolic blood pressure >98th centile for age and sex (equivalent to > 2.06 z-score_
Impaired fasting glucose	6.1-6.9 mmol/L
Abnormal HOMA-IR (calculated by insulin * glucose/22.5)	> 4.4,
Insulin resistance / raised insulin.	Dependent upon pubertal staging : >10 mU/L pre/early puberty, >30 mU/L mid puberty, mU/L

Risk factor	Definition
	>20 late and complete puberty
Low LDL	<0.9 mmol
Raised triglycerides	>1.47 mmol/L
Raised cholesterol	>5.2 mmol/L
"Abnormal HDL/C ratio"	> 4.3.

A final subject in this section that I am also interested in, as a marker for cardio-metabolic risk in children and young people with obesity, is the relevance of Acanthosis nigricans (AN). AN is a dark, velvety skin pigmentation associated with obesity[33] and frequently encountered in clinical practice in young people with obesity. Correctly interpreting its relevance and communicating this to patients and families is important. Studies have identified AN in adolescence as a marker for type 2 Diabetes Mellitus (DM)[34 35] and insulin resistance (IR)[36-39] yet it is unclear whether AN in adolescent obesity is associated with increased cardiovascular risk separately to risk for diabetes. The OSCA guidelines recommend using it as a marker of co-morbid risk in obesity. There is evidence that there may be emotional impact from acanthosis nigricans. Huang el al recently reported in one study that depression was greater in adolescents with obesity and AN compared to those with obesity alone.[40] and Pirgon et al have also shown that adolescent girls with obesity and acanthosis have lower self esteem scores compared to those with obesity without AN, and healthy weight controls.[41] It is my own clinical experience that AN causes stress for young people. Both their own, and their family's knowledge of it is also anecdotally low in my experience, and young people and their families have frequently thought it to be removable with cleaning. As I will discuss below, I was interested in investigating the longer-term cardiovascular relevance of acanthosis nigricans and

whether it added anything more helpful than degree of obesity alone, within an obese group.

1.6 Current longitudinal evidence for long-term cardiovascular risk, obesity and cardio-metabolic risk factors in children and adolescents with obesity.

The evidence presented above for the implications of cardio-metabolic risk factors either individually, or in clusters in adults with obesity does not necessarily translate in helping understand long term cardiovascular risk in children and adolescents with obesity – firstly because they have not been validated in the long term in children and young people; but also because, as discussed above, they are temporally unstable. A key reason for the paucity of long-term evidence for outcomes of both obesity alone, and obesity with cardio-metabolic risk factors such as hypertension and dyslipidaemia in children and adolescents is because the obesity epidemic in children and adolescents is recent.

There are however some longitudinal studies to provide information which have tracked children and adolescents into adulthood to see what differences are evident in later adulthood, though there are currently limits as to how late these studies can follow-up for. Juonala et al followed 6328 subjects from childhood to adulthood (mean follow up of 23 years later).[42] They reported that children with obesity at entry into the study who continued to be obese versus healthy weight children who continued to be healthy weight adults, had relative risks for type 2 diabetes of 5.4, hypertension of 2.1 and raised triglycerides of 3, and increased intima media thickness in the carotid arteries of 1.7. The risk of these factors in children with obesity who became healthy weight children in adulthood was the same as those who developed obesity. Thus one answer to understanding risk is to track the associations of cardiovascular outcomes with persistence of obesity in childhood into adulthood, where we have more data. Twig et al followed older adolescents, which allowed for follow up at older ages than younger children.[43] They followed 2.3 million adolescents from a mean age of 17.5 into adult life and looked at risk of death from cardiovascular causes. The hazard ratio for death from coronary artery

disease in later life in obese (greater than the 95th centile) versus healthy weight adolescents was 2.6 (1.7-4.1) and 2.6 (1.7-4.1) for stroke. Baker et al followed children and adolescents (range 7-13 years) into adult life (greater than 25 years) and found a linear association between BMI and coronary artery disease related events and death.[44] They also found that the association was stronger, the older the participant was at entry into their study.

1.7 Evidence for arterial stiffening in childhood and adolescence as a contemporaneous indication of future cardio-vascular risk.

Given the current limitations of long-term follow-up studies, an alternative approach to predicting long-term cardiovascular risk is to look for contemporaneous evidence of pathological changes known to be involved with cardiovascular disease. It has been known for some time that pathological arterial processes, in particular atherosclerosis, begin in childhood and adolescence. For example, Berenson et al reported in the 1990s on 204 autopsies in a group being followed up as part of a longitudinal cardiovascular risk study, in which they found raised fibrous plaque lesions present in the aorta in 20% of the 2-15 age group, and that the amount of atherosclerotic changes was associated with pre-morbid body mass index, blood pressure and lipid concentrations.[45] Similar findings were also found by McGill et al on a large number of autopsies in young people.[46]

Although a degree of stiffness in an artery is necessary and helpful so as to transmit blood during diastole; elasticity is also important so that arteries are compliant and can receive blood from the upstream artery, or in the aorta directly from the left ventricle.[47] Increased stiffening, especially over age, represents pathological changes related to cardiovascular disease.[48] Arterial stiffening is thought to be primarily related to changes in the elastin function and organization in the extra-cellular, media component of an artery and atherosclerosis, a process where the internal component of arteries are altered deleteriously involves the intima of artery (see figure 1.1).[48] However atherosclerosis and stiffening tend to co-exist with the same risk factors over time, the areas affected are in very close proximity, and the process of stiffening is thought to have a damaging effect on the atherosclerotic process.

Thus arterial stiffening and atherosclerosis are better viewed as processes which are inter-related, although the complex relationship between each other is still not fully understood.[48] Indeed, adult studies of pulse wave velocity, a measure of arterial stiffening (see below), has been shown to be associated with degree of arterial plaques in coronary arteries in adults, the hallmark of athersclerosis.[49] Measurement of arterial stiffness is now recognized as a way of studying cardiovascular risk in people with obesity with established scientific guidance.[50 51]

A number of reasons have been cited for potential explanations for increased arterial stiffness in obesity such as associated insulin resistance,[52 53] dyslipidaemia[54] and hypertension.[53 54] The importance of hypertension in this process, especially in the context of obesity will be discussed at various points throughout the thesis.

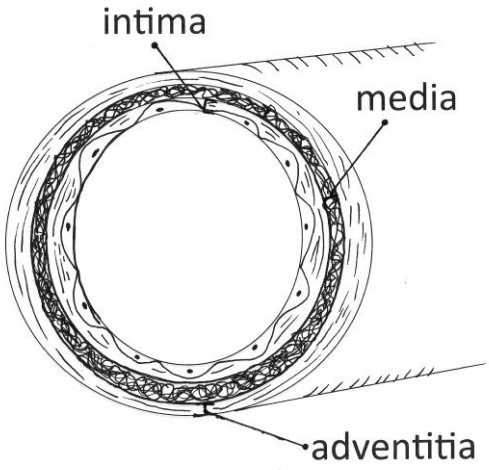


Figure 1.1 : The anatomy of an artery*:*

An artery comprises 3 main regions: the intima which is most internal and is in proximity to blood contents, the media which contains mostly elastin, collagen and smooth muscle; and the adventitia.[55] It is the media which is thought to be the most involved in arterial stiffening due to changes in elastin and collagen structure and function and the intima which is associated with atherosclerotic changes. However in reality, given their proximity, the two

processes are thought to be inter-related.[48]

Whilst working with a group of adolescents with obesity in the HELP program I was interested in understanding arterial stiffening within this group and how it interacted with co-existing cardio-metabolic risk factors. There are a number of modalities available to study early evidence of atherosclerosis in children and young people which have been applied in obesity; in particular carotid intimal-media thickness, measurement of carotid artery calcification and proxies of arterial stiffening using pulse waves. It is beyond the scope of this thesis to explore all of these modalities, however the reader is directed to two important consensus guidelines on the topic led by Urbina et al[50]and Laurent et al.[51] Whilst working as the fellow for the HELP trial, I had the opportunity to access equipment and training to measure and examine pulse waves (Sphygmocor, AtCor Medical, Sydney Australia) – see below); and this offered a non-invasive and relatively swift method for me to study arterial stiffness in the group of adolescents with obesity studied in the HELP trial. I will therefore now specifically discuss arterial stiffness and how this can be measured by analysis of pulse waves in more detail.

1.8 Pulse wave analysis: pulse wave velocity and augmentation index.

Pulse wave velocity

Pulse wave velocity (PWV) is a technique of measuring arterial stiffening between two regions. The concept of PWV was first defined in the 19th century, independently by Moens and Korteweg (later defined as the Moens-Korteweg equation), which defines PWV based on Newtonian mechanics using the Young's elastic modulus applied to an artery (E), arterial thickness (h), radius (r) and blood density (ρ)[56]:

$$PWV = \sqrt{\frac{E \cdot h}{}}$$

$$2\,r\,\rho$$

It can be seen from this equation that as a measure, PWV is indirectly proportional to the distensibility of the artery (given it is a function of the square of the inverse of the radius), which explains why a more positive value of PWV implies a stiffer artery.[57 58] In practical terms, the measurement of pulse wave velocity has been developed most effectively and conveniently by measuring the delay in transit of the pulse wave at two sites, and the distance between the two sites, with PWV being calculated as a function of the distance divided by the time delay in meters/second; with validated algorithms used to detect the foot of waves at different sites (see 1. 2). PWV can be measured between short segments, as has been done using MRI and USS, but more commonly has been used for longer segments between two peripheral arterial sites using tonometry (e.g. carotid:radial) due to the more straightforward nature of peripheral measures in ambulatory research settings.[57]

The recent recognition of the value of PWV has been its evident usefulness as a predictor of cardiovascular outcomes in adults. Beyond research showing PWV measures correlate with more direct measures of arterial plaques mentioned above,[49] two important, recent systematic reviews have been published on adult outcomes which have demonstrated the importance of PWV in the prediction of cardiovascular outcomes. Ben-Sholomo et al meta-analysed 16 studies, pooling a total of 17,635 participants and looked at the predictive value of aortic PWV (aPWV) for cardiovascular disease. After adjusting for age and sex, they found that a 1 standard deviation increase in log aPWV was associated with a 1.5 hazard ratio of coronary artery disease and 1.45 for cardiovascular disease. Even after controlling for contemporary conventional risk factors such as lipids and blood pressure, aPWV remained an independent risk predictor for coronary artery disease, stroke and cardiovascular disease events (eg MI). Vlachopoulos et al published a meta-analysis of 17 longitudinal studies measuring aPWV, pooling 15,877 participants. They

reported that the relative risk of cardiovascular events increases linearly by tertile of PWV. Relative risk for cardiovascular event mortality was 2.2 for higher versus lower aortic PWV groups. They reported that an increase in aortic PWV by 1 standard deviation resulted in a 47% increase in total cardiovascular events and 47% increase in cardiovascular mortality (after adjustment for age, sex and conventional risk factors). PWV in adults therefore appears to be a reliable predictor of cardiovascular events and mortality, separate to the measures of cardio-metabolic risk such as lipids and blood pressure discussed above.

Arterial stiffening is a process which increases with age, and in children, cross sectional data have been published for PWV in healthy children which have show a steady rise with age.[59] The normative changes in PWV in children and adolescents is discussed in more detail in section 1.9.4.

Augmentation index

Another way of using pulse waves to study arterial stiffness is to use the peripheral pulse to generate a picture of the central arterial waveform, and then use its structure to assess systemic arterial stiffness. This is done most commonly by using tonometry at the radial pulse to measure pressure waveforms at the radial pulse which are then subjected to a validated transfer function which generates a representation of the arterial waveform. The arterial wave form can then be used to derive the augmentation index (AI).[60]

The aortic waveform is made up of two component waves – the forward and reflective waves.[47] The first, the forward wave, is generated by the pressure caused by ventricular ejection; the second due to the elastic properties of the whole distal arterial tree (from aorta to arterioles), called the reflective wave.[60] These feature as two noticeable points during the waveform – but differ based on age. In young adults, the first wave component and the reflective wave look distinct (see figure 1.3), with the peak of the reflective wave occurring after the peak of systole;

however as individuals age and arterial stiffness increases, the waveform changes shape and the wave starts to look more homogenous with the inflection of the reflective wave occurring before peak systolic pressure. As a result, the difference between the inflection point of the reflective wave and peak systolic pressure of the wave increases as a proportion of the total wave pressure (systole minus diastole).[60] The augmentation index is calculated by the ratio of the peak systolic pressure – the pressure at inflection, divided by the total aortic amplitude (which is the difference between peak systolic pressure and peak diastolic pressure) as a percentage. As arterial stiffness in the arteries increases, the inflection and systolic difference will become larger compared to the systolic-diastolic pressure, thus the augmentation index increases proportionally to arterial stiffness. [61] This is illustrated in figure 1.3.

The augmentaion index has been demonstrated to be reproducible,[62] and has been shown in adults to be associated with future cardiac disease and events.[63] However, the value as a predictor for cardiovascular disease in adults has not been consistent. [64 65] Because the aortic pulse pressure wave is generated from the radial pulse, it is not as direct a measure of arterial stiffness as pulse wave velocity is.

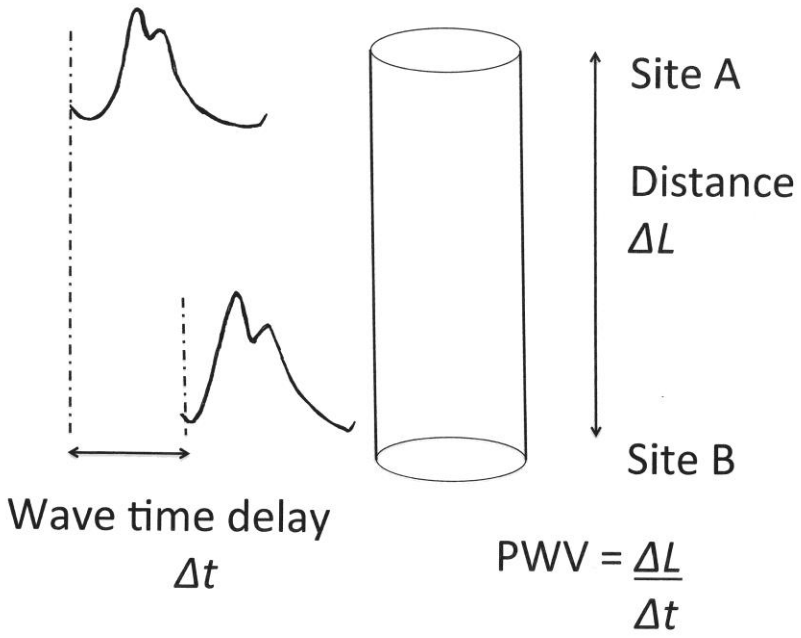


Figure 1.2 Measurement of PWV

Pulse wave velocity (PWV) is measured by measuring pulse wave forms at two arterial sites, and then calculated by dividing the distance between the two measuring sites (L) by the difference in wave commencement delay (in seconds) between the two waveforms at the two different sites, resulting in a velocity in metres/second. The technologies which have been developed to measure PWV have developed specific algorithms to detect the start of wave forms.[57]

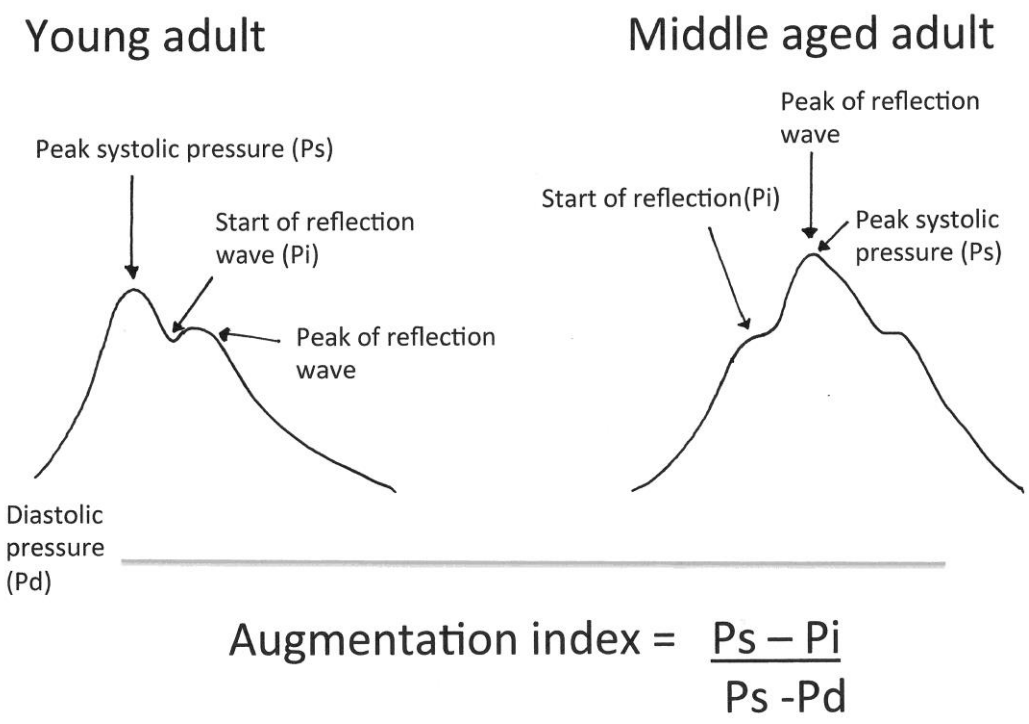


Figure 1.3 : Measurement of Augmentation Index

The Augmentation index is calculated after the aortic wave is generated from algorithms using the radial pulse wave. The aortic wave comprises two distinct components: the initial wave from ventricular ejection during systole, and the reflective wave which is due to systemic pressure. In younger people the reflective wave occurs after peak systolic pressure, whereas in older adults it occurs before. The augmentation index is the ratio of the difference in the pressure between the start of the reflective wave and systolic pressure to the total pulse amplitude (systolic – diastolic pressure) expressed as a percentage. Augmentation index increases with age, and with arterial stiffening.

1.9 Pulse wave analysis and Augmentation index systematic review

Upon initial exploration of the literature on the effects of obesity on PWV and augmentation index in children and adolescents, findings appeared to vary between individual studies. A systematic synthesis of the evidence in this area had never been performed before, and systematic review techniques and meta-analysis offered the potential to summarize existing knowledge of this area. As a part of this thesis I led a systematic review and meta-analysis to evaluate associations between obesity and PWV and AI in children and adolescents. I collaborated with Ms. Alicja Rapala (AR) and my supervisor Professor Viner for this. This systematic review was subsequently published (see publications arising from this thesis section above and the publication is included in appendix 9.2) : *Hudson L, Rapala A, Khan T, Williams B,Viner RM. Evidence for contemporary arterial stiffening in obese children and adolescents using pulse wave velocity: a systematic review and meta-analysis. Atherosclerosis 2015 [published Online First: doi: 10.1016/ j.atherosclerosis.2015.05.014.]*

1.9.1 Systematic review Methods

Search strategy

We performed a systematic search using PRISMA guidelines to identify studies that compared PWV and/or AI in both obese and non-obese children (< age 18). Two reviewers (LH and AR) searched PubMed, Embase and Web of Science electronic databases in September 2014 (see table 1.3 for search terms) independently. Inclusion criteria were: 1) studies measuring PWV and/or AI at any site; 2) participants <age 18 years; 3) use of accepted criteria to define childhood obesity. Exclusion criteria were: 1) genetic, syndromic or endocrine causes of obesity; 2) Studies of participants with chronic illness which might independently affect arterial stiffness 3) Studies using self-reported BMI.

Table 1.3 : Summary of search terms for systematic review for each database.

Database	Search terms

Database	Search terms
Pubmed	("Pulse Wave Analysis"[Majr] OR "Vascular Stiffness"[Majr] OR augmentation index OR arterial stiffness) AND ("Child"[Mesh] OR "Adolescent"[Mesh]) AND ("Obesity"[Mesh] OR "Overweight"[Majr]) Note pulse wave velocity not included in search as was a component of PWA MESH
Embase	(child or adolescent) and (obesity or overweight) and (pulse wave analysis or pulse wave velocity or arterial stiffness or vascular stiffness or augmentation index)
Web of knowledge (combines web of science, MEDLINE and BIOSIS)	Topic=((obesity) OR (overweight)) AND Topic=((pulse wave analysis) OR (pulse wave velocity) OR (arterial stiffness) OR (vascular stiffness) OR augmentation index) AND Topic=((child OR adolescent))

Two reviewers (LH and AR) screened abstracts for inclusion separately, which were then adjudicated by RV. Potentially eligible studies were independently reviewed by LH and AR and data extracted and adjudicated by RV. Reviews and retrieved studies were hand-searched for additional eligible studies. Where inadequate information was provided, primary authors were contacted. Translations of non-English language studies were sought from authors or interpreters used.

Bias assessment and grading

We decided to grade studies for bias risk using a method used in a previous review[32] as low, medium risk or high risk of bias, combining domains from the Cochrane Collaboration guidelines[66] (inadequate blinding of outcome assessment, incomplete outcome data, and selective reporting) with two additional domains - "inappropriate measurement methods" (split into a. suitability and b. reliability) and use of an unrepresentative sample. These are summarized in table 1.4. A priori standards were agreed. Where it was not possible to grade as low or high bias, a medium risk was assigned. Although each study was assessed in each category, an overall bias assignment was assigned based on the highest risk score across all categories. Studies were independently assessed for bias by LH and AR and adjudicated by RV.

Table 1.4 : Bias classification criteria for studies in systematic review

Quality Assessment criteria	Low risk of bias	High risk of bias
1. Blinding of outcome assessment	• Researchers measuring observations blinded to BMI status or absence of blinding considered unlikely to have affected results	• No blinding of researchers measuring observations to BMI status and absence of blinding considered likely to have affected results
2.Incomplete outcome data	• No missing data for participants or missing outcome data unlikely to be related to outcome • OR missing data	• Some missing data for participants or missing outcome data likely to be related to outcome • OR missing data not balanced

Quality Assessment criteria	Low risk of bias	High risk of bias
	balanced across both groups • OR missing data methods used • OR effects of missing data unlikely to impact on an effect size that has a clinically significant implication.	across both groups • OR effects of missing data likely to impact on an effect size that has a clinically significant implication.
3. Selective Reporting	• Study appears to report all of the pre-specified outcomes that were measured.	• Study does not appear to report all of the pre-specified outcomes that were measured.
4a. Measurement methods that were suitable	• Appropriate protocols for pulse wave measurement used (e.g. resting beforehand) • Appropriate BP cuff sizes • Measurement of weight and height done using standardized equipment	• No protocol for pulse wave measurement used (eg resting beforehand) • Inappropriate BP cuff sizes • Measurement of weight and height done without using standardized equipment

Quality Assessment criteria	Low risk of bias	High risk of bias
4b.Measurement methods that were reliable	• Trained operators used • Reproducibility quality assurance in place or sensitivity analysis done for multiple operators • Quality indices for PWV and AI used and reported	• Number of operators not reported, or sensitivity analysis or reproducibility quality assurance not provided. • No evidence that quality indices were used.
5. Use of a representative sample	• Community drawn sample or from existing community cohort? • Statistically equal mixture of males and females between obese and non-obese subjects (within 10%) • Statistically equal age range between obese and non-obese subjects; and ideally groups would have a narrow age range within 2 years SD.	• Non-community sample, eg clinic sample (especially obesity clinic) • Unequal mixture of males and females between obese and non-obese (greater than 10%) • Unequal age range between obese and non-obese subjects; with a large age range greater than 2 years SD. • Mixed ethnicities not taken

Quality Assessment criteria	Low risk of bias	High risk of bias
	• Single ethnicity or data presented by ethnicity	into consideration in analyses.

Once agreement was reached for which studies should be included, I pooled individual studies for meta-analysis using STATA version 13 (StataCorp, Texas, USA). As arterial stiffening is potentially influenced by a number of factors, I made an a priori decision to use a random effects model (DerSimonian & Laird method[67]). Weighted mean differences (WMD) (with 95% confidence intervals) were calculated for a) all site arterial stiffness and b) by site (carotid-femoral, carotid-radial), to examine for differences by region measured. In studies with multiple measures over time, we used baseline data. Some studies did not provide means and standard deviations, but reported medians and ranges instead. Hozo et al have published validated formulae to allow estimation of mean from median, and standard deviation from the range depending on sample size, and these techniques were used to derive mean and standard deviation in such studies.[68] We also performed sensitivity analyses by performing analyses with or without studies with a higher risk of bias. Evidence of publication bias was examined using a funnel plot and Begg's test[69] and Egger's test [70]were used to additionally assess publication bias. I performed all analyses for the systematic review.

1.9.2 Systematic review results

Figure 1.4 shows the results of the search. 383 discrete studies were identified, and 81 retrieved and reviewed. Fifteen studies were included in the study (summarized in table 1.6).[71-85] One presented PWV data by sex separately and information for

males and females were entered into the meta-analysis separately. We contacted three authors for further data, which two authors[71 83] provided and were used for analysis. For one study which was published in Russian, I contacted the authors directly who provided a translation into English.

Quality

Bias assessments for each study are shown in table 1.5, and overall bias assessment is included in table 1.6. Risk of bias was generally high. Because no study blinded researchers to weight status, all studies were graded as at least medium risk - effectively meaning that no study was considered low risk across all domains. The majority of studies either had one domain classed as high bias risk or at least medium risk in at least one domain. Studies were therefore grouped by studies as low/medium or high risk of bias.

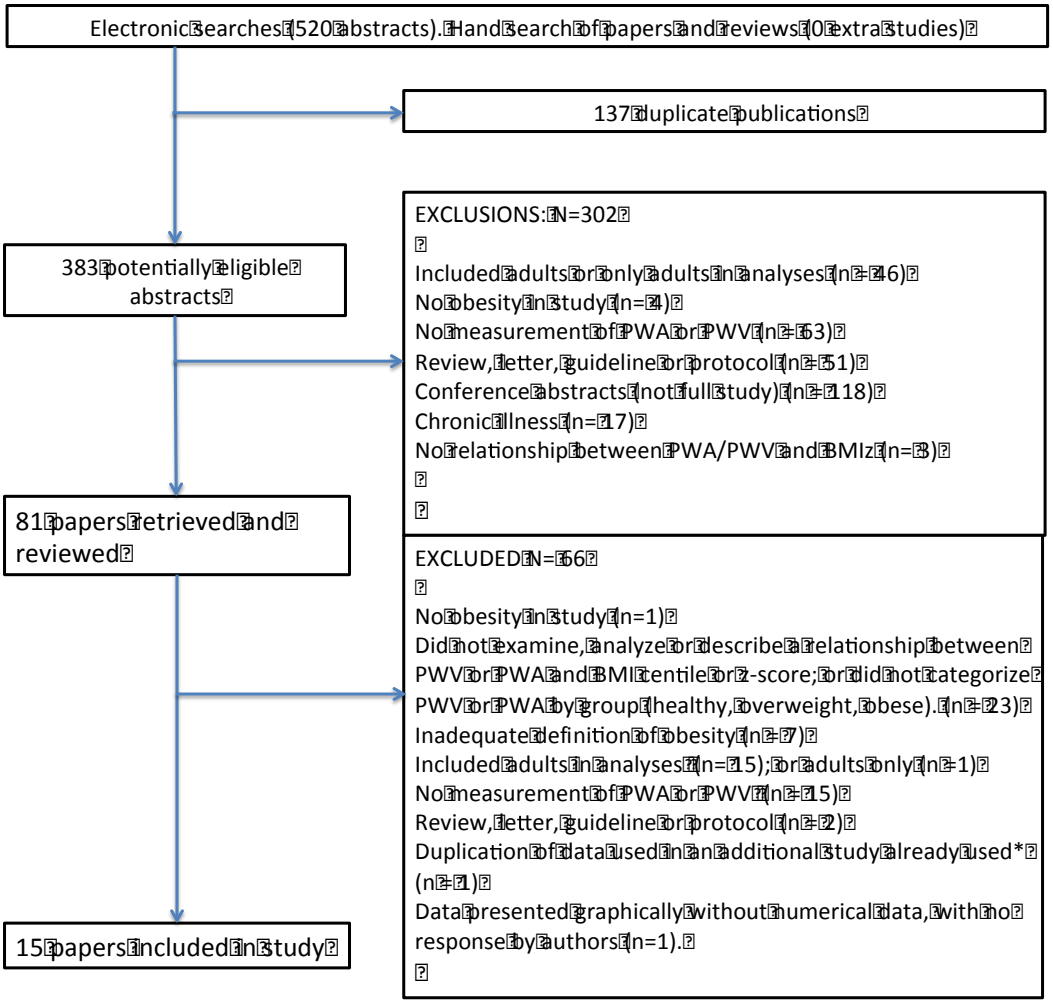


Figure 1.4 : Summary of findings from the Pulse wave velocity and augmentation index systematic review

Table 1.5 Summaries of bias assessment for each included study

	1. Blinding of outcome assessment	2. Incomplete outcome data	3. Selective Reporting	4a. Measurement methods that were suitable	5. Use of a representative sample
Bolotova	MEDIUM	LOW	LOW	HIGH RISK	HIGH RISK
Cabrera-Rego et al. (2014)	MEDIUM	LOW	LOW	MEDIUM	HIGH
Çelik et al (2011)	MEDIUM	LOW	LOW	LOW	HIGH
Charakida et al. (2012)	MEDIUM	LOW	LOW	LOW	MEDIUM
Dangardt et al. (2013)	MEDIUM	LOW	LOW	LOW	HIGH
Hacıhamdioğlu et al. (2014)	MEDIUM	LOW	LOW	LOW	HIGH
Harris et al. (2012)	MEDIUM	LOW	LOW	LOW	HIGH

	1. Blinding of outcome assessment	2. Incomplete outcome data	3. Selective Reporting	4a. Measurement methods that were suitable	5. Use of a representative sample
Jin et al. (2013)	MEDIUM	LOW	LOW	LOW	MEDIUM
Koopman et al. (2012)	MEDIUM	LOW	LOW	LOW	HIGH
Lurbe et al. (2012)	MEDIUM	LOW	LOW	MEDIUM	HIGH
Lydakis et al. (2012)	MEDIUM	LOW	LOW	MEDIUM	LOW
Martínez-Costa (2014)	MEDIUM	LOW	LOW	LOW	HIGH
Montero (20130	MEDIUM	LOW	LOW	LOW	HIGH
Nunez et al. (2010)	MEDIUM	LOW	LOW	MEDIUM	HIGH
Pandit et al. (2011)	MEDIUM	LOW	LOW	MEDIUM	MEDIUM

1.9.3 Meta-analysis results

14 studies including 6677 total participants (1120 obese and 5557 non-obese) were suitable for meta-analysis for PWV, and 5 studies including 728 participants (411 obese and 317 non-obese) for AI.

Pulse wave velocity

A forest plot for all studies investigating PWV is shown in Figure 1.4 with pooled estimates for low/medium risk of bias (3 studies studies; N= 5568, 418 obese and 5150 non-obese) and high risk of bias (11 studies; N=1109, 702 obese and 407 non-obese); and overall pooled estimate. A difference was found indicating higher PWV in obese versus non-obese subjects including all regions (WMD 0.45 (95% confidence interval 0.10 to 0.81 ms^{-1})); however this was not significant when analysis was restricted to low/medium risk of bias studies (0.34 (-0.49 to 1.16)).

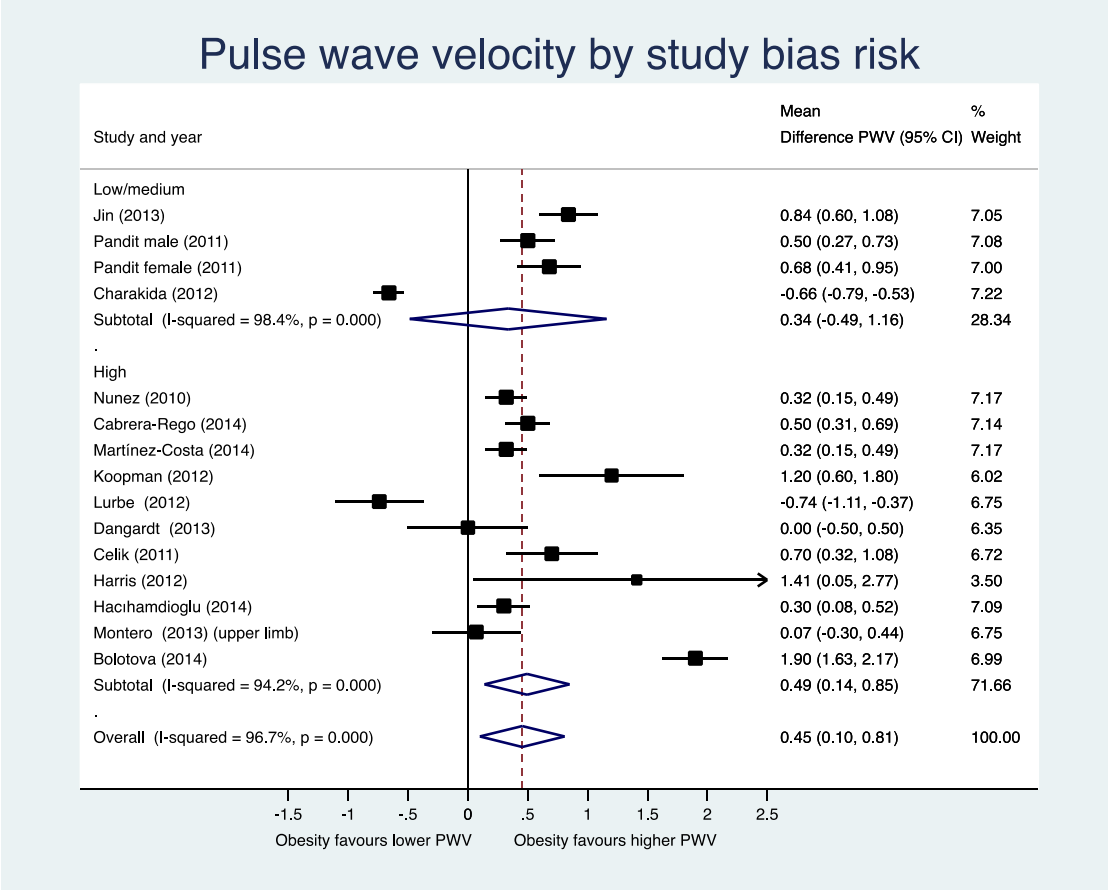


Figure 1.4 : Forest plot of PWV of all studies stratified by bias assessment

Forest plot of PWV stratified by bias risk assessment and overall pooled estimate. Note : Weighted Mean difference (ms^{-1}) between obese and non-obese subjects. Subgroup analysis was done for the studies that of low/medium and high potential for bias (Differences in mean change in PWV are calculated by inverse variance statistical method of random effects model)

Figure 1.5 shows PWV studies grouped by anatomical region (figure 3). In studies of PWV measured at the carotid (5 studies; N=849, obese 582, non-obese 267), obese subjects had higher PWV (WMD 0.51 (0.35 to 0.67 ms^{-1}) (figure 3). Restriction of this analysis studies at low/medium risk of bias (N = 282, obese 166, non-obese 116) increased the weighted mean difference (WMD 0.67 (0.47 to 0.87 ms^{-1}). No differences were found for PWV in studies measured at the aorta using all methods (combining direct aortic measurements and carotid-femoral measurements) but studies measuring the aortic directly (3 studies, N = 221, 116 obese and 105 non-

obese) showed greater PWV in obese subjects (WMD 1.33 (0.36 to 2.31),); all of these studies were however assessed as high risk for bias.

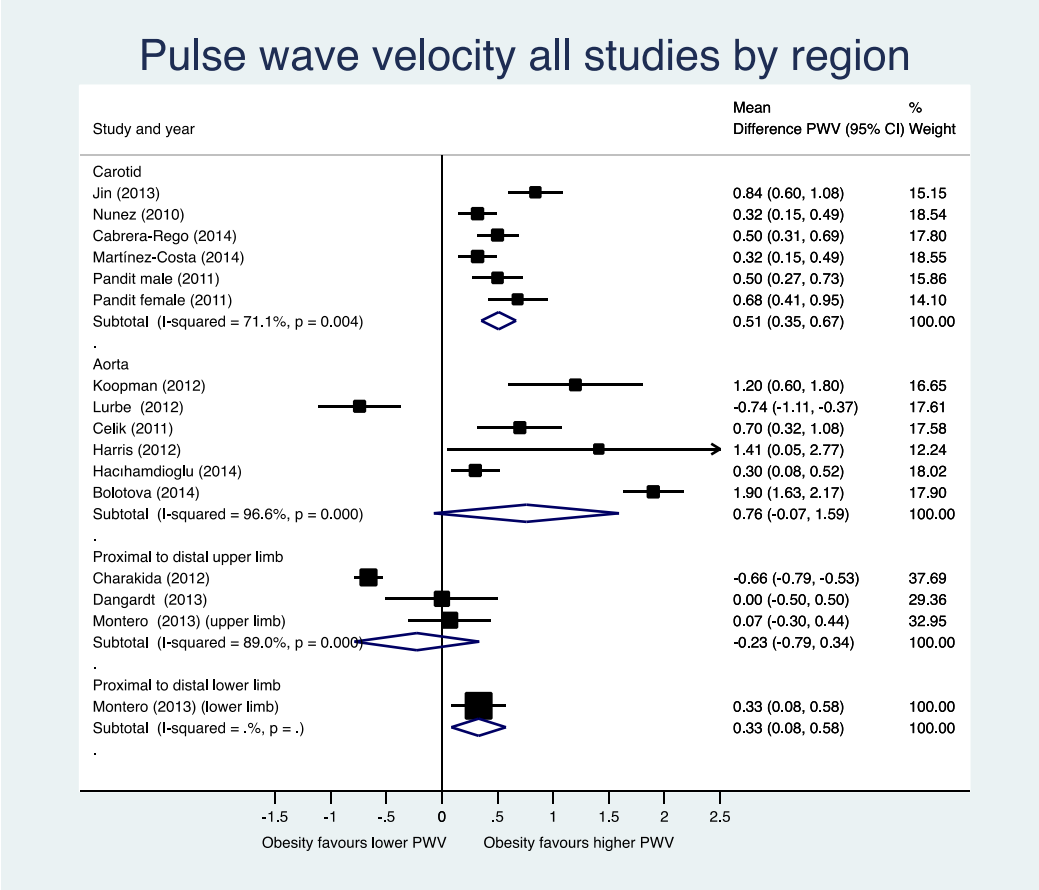


Figure 1.5 Forest plot of PWV by anatomical region

Forest plot of PWV by anatomical region measured and overall pooled estimate. Note : Weighted Mean difference (ms^{-1}) between obese and non-obese subjects. Subgroup analysis was done for the studies that of low/medium and high potential for bias (Differences in mean change in PWV are calculated by inverse variance statistical method of random effects model)

Augmentation index

Figure 1.6 shows a forest plot for studies measuring AI. No significant difference was found in AI for obese versus non-obese participants (WMD 4.75 (-3.95 to13.45)). As

all but one of the included AI studies were rated high risk of bias, sub-analyses risk of bias were not performed.

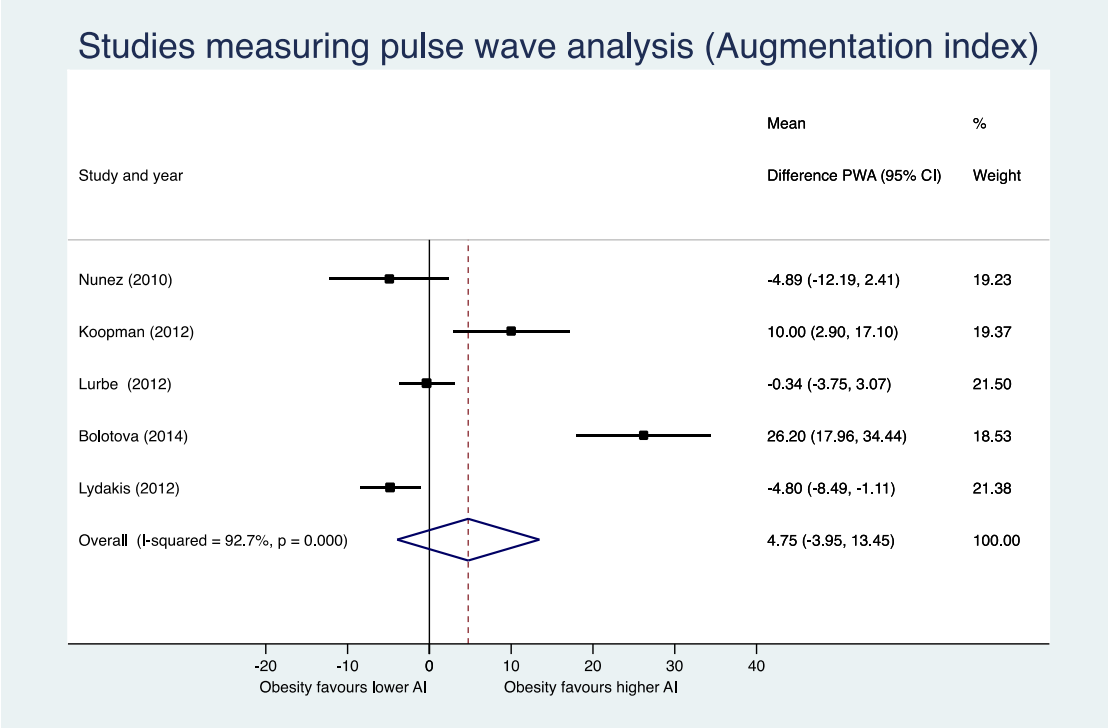


Figure 1.6 : Forest plot of Augmentation Index studies

Forest plot of AI and overall pooled estimate. *Note : Weighted Mean difference (ms^{-1}) between obese and non-obese subjects. Subgroup analysis was done for the studies that of low/medium and high potential for bias (Differences in mean change in PWV are calculated by inverse variance statistical method of random effects model)*

Quality measures for meta-analysis

Heterogeneity was high (I^2 >90% in all meta-analyses). A funnel plot was not perceived to demonstrate asymmetry (see figure 1.7). Low significance in both Begg's test (p=0.6) and Egger's test (p=0.14) supported this perception. There were an insufficient number of studies measuring PWA to assess publication bias adequately.

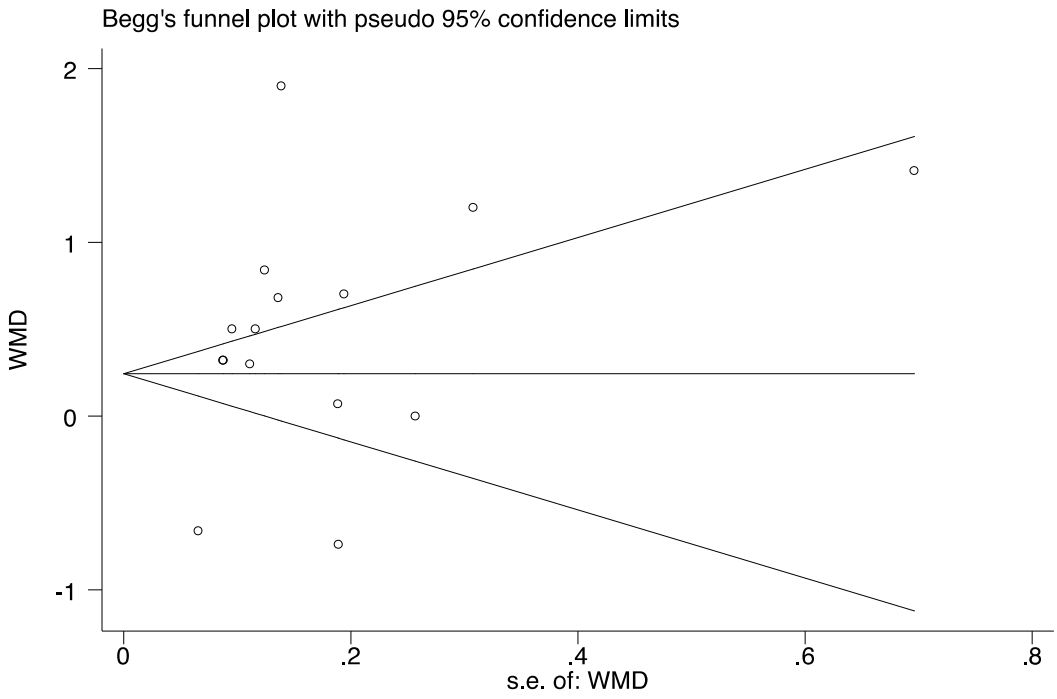


Figure 1.7 : Funnel plot of included PWV studies

A funnel plot of included studies in the PWV meta-analyses. Whilst spread was wide, the appearance of scattering is symmetrical, and this perception is backed by non- significant Begg's and Egger's testing.

Table 1.6: Summary of included studies, individual study details, findings and overall risk assessment.

Author (year)	**PWV or AI measurement site and method**	**Sample characteristics**				**PWV obese versus non-obese**	**Obesity definition**	**Healthy controls include overweight? (>85th centile <95 centile)**	**Sex , age and pubertal differences between obese and healthy controls?**	**Other cardio-metabolic differences**	**Overall bias assessment**
		Country, setting and recruitment.	**Number of subjects***	**Age (years) Mean (SD) or median (IQR)**	**Sex**						
Bolotova et al. (2014)[71]	Aortic PWV and AI (using aorta directly).	Single centre, Russia. Unclear where obese or control subjects were recruited from.	25 obese 20 non-obese subjects	Whole group median 12 (range 11-16)	Unclear	PWV ↑ in obese subjects	≥ 95th BMI centile **	Unclear	Unclear sex differences. wide variety of pubertal stage across all subjects	SBP and DBP was greater in obese subjects. Triglycerides, cholesterol and HOMA were greater in obese subjects.	High

										Ethnicities not reported.	
Cabrera-Rego et al. (2014)[72]	Carotid PWV (Carotid echo tracking :left and right common carotids averaged)	Single centre, Cuba. Unclear recruitment methods	66 (69%) obese 30 (31%) non-obese	Obese : 11.3 (2.7) Non-obese: 12.0(2.9)	Obese : 62.1% male Non-obese: % male	PWV ↑ in obese subjects	≥ 97th BMI centile **	Unclear	No differences in age and sex between obese and non-obese Puberty status not reported	SBP (but not DBP) was greater in obese subjects. Triglycerides (but not cholesterol) and HOMA were greater in obese subjects. Ethnicities not reported.	H
Çelik et al (2011)[73]	Aortic PWV. (Directly between aortic valve	Single centre, Turkey. Sample from a clinic	30 (50%) obese.	Obese : Mean 13.2(SD 2)	Obese : 40% male Non-	PWV ↑ in obese subjects.	≥ 95th BMI centile **	Yes	No differences in age and sex between obese and non-obese	SBP and DBP were greater in obese subjects. No differences in other blood cardio-	H

	and diaphragm: Echocardiography machine using doppler)	separated into obese and non-obese	30 (50%) non-obese	Non-obese: Mean 12.5(SD 1.7)	obese: 43.3% male				Puberty status not reported.	metabolic markers measured (fasting glucose and lipids). Ethnicities not reported.	
Charakida et al. (2012)[74]	Carotid-Radial PWV. two site carotid artery and radial artery tonometry.	Multi-centre, United Kingdom. Obese and non obese recruited from community birth cohort sample (ALSPAC);	PWV data available on 6293 subjects 252 (4%) obese 5034 (80%) healthy	Only reported age by whole sample and by sex. Males: median 10.6 (IQR 10.5–10.8)	Whole sample only reported 49% male.	PWV ↓ in obese subjects	IOTF	No	Sex, puberty and age information by weight status grouping not reported. Pubertal range wide for whole group.	SBP and DBP were greater in obese subjects. Higher lipids and in obese subjects. Ethnicities not reported.	L/M

			weight.	Females Median 10.6 (IQR 10.5–10.8)							
Dangardt et al. (2013)[75]	Carotid-Radial PWV. two site carotid artery and radial artery tonometry.	Single centre, Sweden. Obese from obesity clinic. Non-obese from regional schools).	28 (67%) obese 14 (33%) non-obese	Obese : Median 13.8 (Range 10.2–17.6) Non-obese: median 13.8 (Range 11.5–16.1)	Obese : 39% male Non-obese: 36% male	No difference in PWV between obese and non-obese subjects.	IOTF	No	No differences in age and sex between obese and non-obese subjects. Puberty status not reported.	DBP (but not SBP) was greater in obese subjects. Differences in blood lipids and cholesterol markers between groups not reported. Ethnicities not reported.	H

Hacıhamdioğlu et al. (2014)[76]	Carotid-Femoral PWV two site carotid artery and femoral artery tonometry.	Single centre, Turkey Obese recruited endocrine clinic. Non-obese unclear.	61 (51%) obese 58 (49%) non-obese	Obese : 13.2 (1.8) Non-obese: Mean 13.2 (2.1)	Obese : 49% male Non-obese: 48% male	PWV ↑ in obese subjects	≥ 95th BMI centile **	No	No differences in age and sex between obese and non-obese subjects. All children were reported as being in puberty, but staging not reported All subjects reported as Turkish.	SBP and DBP greater in obese subjects. Differences in blood lipids and cholesterol markers between groups not reported.	H
Harris et al. (2012)[77]	Aortic PWV (directly between the ascending and	Single centre, Canada. Obese referred from an obesity program.	61 (53%) obese 55 (47%) non-obese.	Obese : Mean 13.8 (2.3) Non-obese: Mean 13.8	Obese : 56% male Non-obese: 40% male	PWV ↑ in obese subjects	≥ 95th BMI centile **	Unclear	No differences in age and sex between obese and non-obese subjects. Puberty status not reported.	SBP (but not DBP) was greater in obese subject, but no correlation found between PWV and systolic BP in all subjects.	H

	descending aorta : Echocardiography machine using doppler)	Non obese unclear subjects were recruited from.		(4.0)						Triglycerides were abnormally high in obese subjects using adult reference data; lipid levels in non-obese subjects not reported. Ethnicities not reported.	
Jin et al. (2013)[78]	Carotid PWV (Both common carotids averaged	Single centre, China. Obese subjects recruited from a paediatric centre (?clinic)	71 (60%) obese 47 (40%) non-	Obese : Mean 10 (1.6) Non-obese:	Obese : 73% male Non-obese:	PWV ↑ in obese subjects	≥ 95th BMI centile **	Unclear	No statistical differences in age and sex between obese and non-obese subjects. Puberty status not	SBP and DBP were greater in obese subjects. Differences in blood lipids and cholesterol	L/M

	:Carotid dopplers)	Non-obese subjects recruited from children in the same department enrolled into an obesity prevention program.	obese.	Mean 9 (1.6)	72% male				reported.	markers between groups not reported. Ethnicities not reported.	
Koopman et al. (2012)[79]	Carotid-Femoral PWV & AI (radially derived) two site carotid artery	Single centre, Canada. Obese subjects from lipid clinic.	21 (44%) obese (but see methodological comments	Obese : 14.2(2.0) Non-obese: Mean 13.9 (2.3)	Obese : 81% male Non-obese: 81% male	PWV ↑ in obese subjects	≥ 95th BMI centile **	No	No statistical differences in age and sex between obese and non-obese subjects. Puberty status not reported.	Ethnicities not reported. SBP (but not DBP) was greater in obese subjects. Differences between other cardio-	H

	and femoral artery tonometry; radial tonometry for AI	Non-obese subjects from existing normal control group.	) 27(56%) non-obese							metabolic markers not reported, however all of the obese subjects had lipid abnormalities (with two patient on lipid lowering medical treatment).	
Lurbe et al. (2012)[80]	Carotid-Radial PWV & AI (radially derived) two site carotid artery and femoral artery tonometry and cAI generated	Single centre, Spain Obese and non obese subjects recruited from a clinic of referrals for vascular phenotype assessment.	284(57%) obese 79 (16%) healthy weight	Obese : 12.0 (2.2) Healthy weight: Mean 13.4 (2.6)	Obese : 56% male Healthy: 44% male	PWV ↓ in obese subjects	≥ 95th BMI centile **	No	Differences between age and sex presented by ANOVA across the 3 groups (healthy weight, overweight, obese). No statistical differences in sexes across the three groups, but obese children younger.	There was no difference between peripheral SBP and DBP across different weight status groups, however central DBP (but not SBP) was greater in obese subjects. were greater in obese subjects. Differences in blood lipids and cholesterol	H

	from radial tonometry.								Puberty status not reported.	markers between groups not reported. All subjects were Caucasian.	
Lydakis et al. (2012)[81]	AI (brachial pulse derived)	Single centre, Greece All subjects recruited from 3 local schools.	36(13%) obese 157 (57%) healthy weight Further 84 subjects (30%) were overweigh	All children aged 12.	Obese : 61% male Healthy: 48% male	No difference in AI across obese, overweigh t and healthy subjects	IOTF	No	All children aged 12. Puberty status not reported.	Peripheral and central SBP and DBP greater across groups with increasing degree of overweight using ANOVA. Differences in blood lipids and cholesterol markers between groups not reported.	L/M

			t not described or included here.							Differences in blood lipids and cholesterol markers between groups not reported. All subjects were Caucasian.	
Martínez-Costa (2014)[82]	Carotid PWV (right common carotid Carotid echo-tracking)	Single centre, Cuba. All subjects recruited from paediatric clinic.	66 (48%) obese 42 (31%) healthy weight	Obese : 11.3 (3.0) Healthy weight: 12.4 (2.4)	Sex data only presented for all 137 subjects, and not by weight category: 64% male	PWV ↑ in obese subjects	≥ 95th BMI centile ***	No	No differences in age between obese and non-obese subjects. Sex proportions by weight status not reported. Puberty status not	SBP and DBP were greater in obese subjects. Blood triglycerides and HOMA were greater in obese subjects. All subjects were Caucasian.	H

									reported		
Montero et al. (2013)[83]	Upper and lower limb PWV (whole limb from clavicle to finger and groin to toe). Continuous wave Doppler and photoplethysmography	Single centre, France. Obese subjects from an obesity clinic. Controls unclear	15 (45%) obese 18 non-obese (55%)	Obese: 15.20 (1.27) Non obese: 13.38 (1.19)	Obese : 33 % male Non-obese: 39 % male	Baseline upper limb : No significant difference between PWV in obese subjects and non obese Baseline lower limb:	IOTF, but all severe > 3 SD BMI	No	No differences between proportions of sex between obese and non-obese subjects. Obese children were younger. Puberty status same across both groups Tanner 3 or 4.	No differences in BP between obese and non-obese subjects. Differences in blood lipids and cholesterol markers between groups not reported. Ethnicities not reported.	H

						PWV ↓ in obese subjects					
Nunez et al. (2010)[84]	Carotid PWV and AI (carotid derived, Right common carotid artery). Carotid dopplers	Single centre, Spain. Obese subjects from an obesity clinic. Non-obese subjects from a cardiac murmur clinic.	45 (57%) obese 34 (43 %) non-obese	Obese : 12.4 (2.2) Non-obese: Mean 11.6 (1.9)	Obese : 71 % male Non-obese: 62 % male	PWV ↓ in obese subjects with PWV.	≥ 2 SD BMI ***	No	No differences in age and sex between obese and non-obese subjects. Puberty status not reported	SBP (but not DBP) was greater in obese subjects. Blood triglycerides and cholesterol were greater in obese group. Ethnicities not reported.	H

Pandit et al. (2011)[85]	Carotid PWV (Right common carotid artery, Carotid echo-tracking)	Single centre, India All subjects recruited from Multiple centres (health clinics and schools).	95 obese (46%) 69 (33%) healthy weight	Ages reported by sex Males Obese : 11.31 (2.9) Healthy weight: 12.35(2.7) Females Obese : 10.73 (3.2)	Obese : 54% male Healthy weight: 42 % male	PWV ↑ in obese subjects in both males and females	≥ 95th BMI centile **	No	No differences in age between obese and non-obese subjects in males and females; sexes analysed separately. Puberty status not reported	SBP and DBP were greater in obese subjects in both sexes – and relationship between BP and PWV reported. Blood triglycerides and cholesterol were greater in obese group in both sexes. Ethnicities not reported – but likely all South East Asian.	L/M

				Healthy weight: 11.53 (2.4)							

*Proportions of overweight (i.e. not healthy and not obese proportions not included). ** Used regional data to establish centile/z-score *** Used CDC/WHO data to derive CDC/WHO data to establish centile/z-score. BP =Blood pressure; SBP = systolic blood pressure, DBP = diastolic blood pressure. PWV = pulse wave velocity, AI = augmentation index. BMI = body mass index. BMI z = body mass index z-score. SD = standard deviation. IOTF = international obesity task force. HOMA. CDC = centre for disease control, WHO = world health organization. cAI = central augmentation index.

1.9.4 Systematic review discussion

The systematic review found moderate evidence that obese children had greater arterial stiffness than healthy weight children as assessed by PWV. There was no evidence for a difference in AI. This may be because AI is a poorer marker of arterial stiffening, with published findings on AI in obese inconsistent[86-88] but moreover, in contrast to PWV, AI appears to be a poorer marker of future cardiovascular risk from adult studies.[64 65] I also think it is likely that AI has drawbacks because as a proxy of arterial stiffening, it is calculated from a constructed arterial pulse form not directly measured whereas PWV directly measures wave velocity between two points.

There was also evidence of variation in stiffness (PWV) between regions; with increased stiffness in obese children found only at the carotid and direct measures at the aorta. This is concerning given the central location within the vascular system. Atherosclerotic changes in the carotid are recognized as a proxy for global CVD risk, [89] and stiffness of the aorta may increase strain on the left ventricle, perhaps explaining thicker and more dilated left ventricles reported in obese children in two studies in our review,[73 77] and reports of left ventricular changes published elsewhere in children with obesity.[90-92]

The systematic review also showed that there was a paucity of longitudinal data looking at longer -term changes in PWV in children and adolescents, with only one study identified.

The systematic review had a number of strengths and limitations. Independent raters assessed papers using agreed bias criteria. Included sample size of studies pooled was also large. Repeated analyses excluding studies with high risk of bias were performed and presented with findings. Variation between studies (e.g. age, sex, pubertal status, ethnicity, co-existing cardio-metabolic risk and measurement techniques and sex) likely contributed to bias when pooled. Indeed, most studies mixed pubertal stage, and even within studies where age grouping was in a similar, or tight range, there still would have been pubertal variation within groups. Such variation was expected and hence our a priori decision to use a random effects model. A small number of studies combined overweight and healthy weight together

as controls (this was evident for 1 study and unclear for 4 others – see table 1.6), potentially blunting differences between obese and non-obese control stiffness. Four included studies that defined obesity using the more conservative Internal Obesity Task Force (IOTF) cut-offs, whilst the others used less conservative cut-offs. In a subgroup analysis no significant differences in WMD for PWV was found between obese and non-obese in studies using IOTF but significant differences found in studies using less conservative cut-offs (data not shown). However given that studies using lower centiles would have included obese children above IOTF centiles, grouping of studies in this way mixed bias ratings, and importantly studies using IOTF for PWV were all studies measuring upper limb PWV evident differences were most likely driven by regional variation rather than differences in cut-offs. Different measuring techniques in studies, ranging from direct regional echocardiographic methods to between sites using tonometers, were found. Regional variations in PWV within vessels have been demonstrated in magnetic resonance imaging (MRI) in adults,[93] and it is possible that the echocardiographic techniques would have been subject to less regional variation – and this may explain the differences found between direct measures of PWV at the aorta compared to those measuring between the carotid and the femoral, as well as the findings in the carotid which all measured the carotid directly.

Many studies in our review found greater average blood pressures in the obese and a number found associations between PWV and blood pressure, yet few controlled for blood pressure. The natural history of arterial stiffening in children and how it may relate to blood pressure or obesity is still limited. Normative data for PWV show a steady rise with age as discussed above,[59] however one longitudinal study of aortic PWV in healthy children found a plateauing of PWV in early childhood independent of increases in BP, [94] with resumption of increases at 10 in females and 12 in boys. In our review, Charikida et al[74] (studying 10 year olds), found greater artery diameter and lower carotid-radial PWV in obese subjects. Dangardt et al[75] (studying 10-17 year olds) also found greater arterial diameters in obese versus non-obese participants but no difference in carotid-radial PWV at baseline; and at 5 year follow up there was a greater increase in PWV in obese participants whilst radial diameter

continued to increase at similar rates in both obese and non-obese. The authors in both studies hypothesized for a compensatory vessel compliance in obesity in earlier childhood, preventing stiffening. Monero et al[83] reported no differences in artery diameter between obese and non-obese participants aged 12-17 years, and Koopman et al[79] found higher PWV in obese children with lower distension coefficients.

It was evident to me from the systematic review that more research was needed on longitudinal changes in PWV in obesity, to see how PWV changes with BMI over time. Also research was needed to examine the differential interaction between overweight and blood pressure on PWV. Further study should also investigate the influence of puberty on PWV in obesity, which was absent in most studies that were found in the systematic review.

1.10 Implications of findings presented in chapter one for the thesis

In this chapter I have outlined what is known about the long term cardiovascular risk associated with obesity in children, and questioned the validity of cardio-metabolic risk markers like blood pressure and lipids as predictors of increased risk. I have summarized the evidence that currently exists, and shown that current evidence of arterial stiffness is a potential way of understanding risk by identifying evidence of contemporaneous arterial pathology. From the results of the systematic review, I felt that that the use of PWV is promising. I decided not to use augmentation index. I decided to study a group of adolescents with obesity from the HELP trial to investigate PWV:

1. What cross-sectional and longitudinal relationships exist between PWV, obesity, blood pressure and other conventionally cited cardio-metabolic risk measures such as lipids and acanthosis nigricans within a group of adolescents with obesity? In particular how is change in PWV over time affected by changes in adiposity, blood pressure and cardio-metabolic risk factors?

2. What difference does partitioning degree of obesity make to PWV within partitioned groups, as a way of testing the validity of current definitions of severe obesity?
3. Given the findings of the systematic review, do individual factors such as puberty, ethnicity and sex change the relationship between PWV, obesity, blood pressure and cardio-metabolic risk?

Chapter 2 : Relationship between stress, obesity and cardiovascular risk.

2.1 Introduction

In chapter one I explained that my interest in long-term cardiovascular risk in adolescents with obesity stemmed from my experiences and questions which emerged whilst working in a clinical obesity service for adolescents. I also explained that the opportunity as medical fellow for the HELP trial allowed me to study this concept which I present here in this thesis. In this chapter I will outline another area for the thesis which interested me and emerged whilst working in the obesity service, which relates to the aetiology of obesity and its long-term complications, in how stress may play a part in this. Thus in this chapter I will address research questions 4-6 : 4)how measures of stress are associated with degree of adiposity at a cross-sectional and longitudinal level; 5) how measures of stress are associated with blood pressure at a cross-sectional and longitudinal level; and 6) how measures of stress are associated with pulse wave velocity at a cross-sectional and longitudinal level).

Although an imbalance between energy intake and expenditure is a widely accepted explanation for the main process in the aetiology of overweight and obesity in children and adolescents,[95-97] I was struck by the variation in development of degree of obesity and acquisition of components of MetS between individuals. Whilst on the one hand this led me to consider what the long-term implications of these findings meant for young people, I also wondered what other factors might be involved which might differentially affect young people I saw in the clinic. There are a number of potential factors which are reviewed well by Proctor.[98] However, I was interested in the potential involvement of stress – and in particular in how it might influence degree of obesity, cardiovascular co-morbidities such as blood pressure and lipids; and moreover how stress might be associated with long-term cardiovascular risk within groups of obese children. As the fellow in The HELP trial I used the opportunity to investigate this question as part of the thesis. In this chapter I will explore the concept of stress and examine current knowledge on its association with obesity in children and adolescents; and then finally provide the rationale for further investigation presented in this thesis.

2.2 Stress and a potential relationship between stress, obesity and the metabolic syndrome

The concept of stress comes originally from the physical sciences, with a stressor being a force that can act upon a system and cause change. The maintenance of biological systems within certain conditions against a stressor was first recognized and referred to by Cannon in 1929 as "homeostasis" which is now accepted as a fundamental principle in biology.[99] Seyle was the first to use the word "stress" in this biological context and developed the idea that rather than stress responses being simple relationships in single systems, the number of stressors, environmental changes and number of systems involved in maintaining the physiology due to a stressor within an animal is more complex – with a number of systems potentially involved.[100] The ability for an animal to control its physiology through complex changing environments using multiple systems was later referred to as "allostasis".[101] This is characterized by complex systemic responses to multiple factors, importantly with the overall aim to maintain systems, like many biological systems, in the short term, ultimately with the aim of securing short term survival. The consequence of response and adaptation to stress therefore may be helpful in the short term but might lead to the development of longer-term consequences, which are potentially deleterious to health. [101 102] The concept that by adapting to stressors in the short term can lead to deleterious effects in the long term has produced a large body of research,[100] and several journals dedicated to the study of stress in medicine now exist. In humans, stressors can be physical, behavioral and psychological; and the responses to them can be physical, physiological and behavioral.[100] Stressors are appraised by the CNS, processed and translated into both behaviours and via the physiological "stress response" through the hypothalamic-pituitary axis (HPA) and sympathetic nervous system (this is conceptualized graphically in figure 2.1). It has been hypothesized that stress may be a causal factor in obesity, and I examine this hypothesis further here.

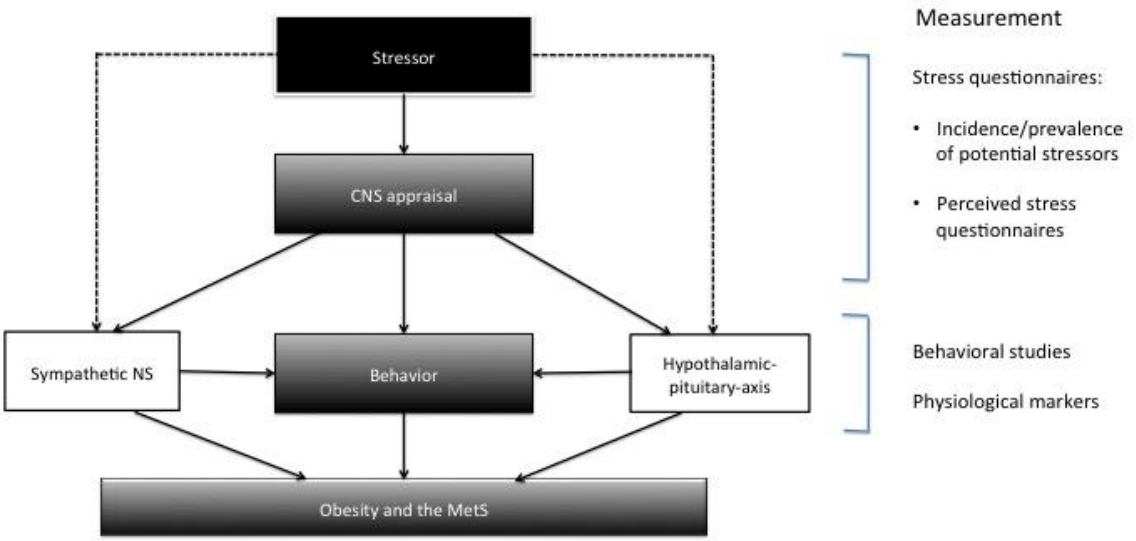


Figure 2.1 : Conceptualization of how stressors can interact with physiological processes and lead to obesity and the metabolic syndrome

Diagram illustrating how stressors can affect behaviour, and interact with the HPA axis and sympathetic nervous systems. The diagram also shows how these interactions can be measured at each level.

One mechanism by which stress might influence body habitus is by influencing eating behaviour. This topic was recently systematically reviewed and meta-analysed by Hill et al. They pooled 13 studies including 28,070 participants aged 8-18 years and found that overall there was no association between stress and change in eating behaviour.[103] However when they separated their analyses to look at younger and older children they found that stress was negatively associated with healthy eating in older children. Interestingly, the impact of stress was independent of type of stress measure or stressor. Groups of children without obesity undergoing stress testing have shown higher levels of cortisol, and those with such cortisol responses have been shown to eat more in the absence of hunger,[104] a finding which has also been demonstrated on stress testing in adults.[105]

It has also been postulated that chronic stress, mediated by prolonged stimulation of the hypothalamic-pituitary-axis (HPA), may also be an important factor in the development of obesity and associated cardiovascular co-morbidity,[106-109] with the example of Cushing's syndrome (where pathological excess of cortisol causes central adiposity, hypertension, insulin resistance and dyslipidemia) being used as an analogy.[106 110] Peckett et al[111] performed a review of the literature looking at the effects of glucocorticoids on lipid metabolism, in particular the effects on adipose tissue, and highlighted that glucocorticoids stimulate the conversion of pre-adipocytes to mature adipocytes, thus having a role in adipose hypertrophy. Evidence from an animal study involving rats have shown that rats subjected to stress for a month subsequently developed larger adipocytes and tended to place fat centrally.[112] Increased sympathetic nervous system activity might independently contribute to hypertension, insulin resistance and dyslipidaemia in obesity as part of the stress response,[109] as well as influencing production of inflammatory cytokines.[113] Mechanisms linking the HPA-axis and sympathetic nervous system have also been proposed.[114]

In research, stress has been measured by objective exposure to potential stressors or more subjective perceptions of stress in questionnaires by individuals,[115 116] and also by measuring physiological markers such as cortisol[117] and catecholamines.[109] Of particular interest in health research is the cortisol awakening response (CAR). Cortisol secretion is known to be diurnal, with greater levels in the morning and lowest in the late evening.[118] However, in most individuals, waking from sleep is accompanied by an increase in cortisol secretion 30-45 minutes after waking see in Cortisol secretion.[119] The CAR has been used to examine cortisol secretion using salivary cortisol techniques in stress research by measurement of rate of increase and area under the curve, and this has been used as a proxy for cortisol reactivity in stress, in particular in obesity.[119 120]

2.3 Key adult reviews on stress and obesity

There have been two important systematic reviews in adults of high relevance to this thesis looking at the potential relationship between stress and obesity:

1. Wardle et al[121] systematically reviewed and performed a meta-analysis of 14 longitudinal studies in adults looking for a relationship between stress and adiposity. Stressors were measured in studies using self-report questionnaires (for example perceived stress) and also objective stress exposure (for example job stress). Though there were variable findings between studies, on pooling all 14 studies, they found that stress was positively associated with obesity, though the effect size was small. Interestingly they also found no differential relationships between stress and location of obesity (central adiposity versus total body obesity); they also found an interaction between stress, sex and obesity such that the association was greater in men compared to females.
2. Incollingo Rodriguez et al[120] systematically reviewed the adult literature on the HPA axis in obesity to look for evidence of dysregulation. They identified 34 relevant papers. They reported that for the cortisol awakening response, there were differing findings. 5 studies reported a negative relationship between obesity and the CAR, 2 an exaggerated response and 2 studies with no relationship. They highlighted that all of these studies were cross-sectional and so did not examine for longitudinal relationships, which are important when trying to understand interactions and possible causal relationships. They also found 9 studies which looked at total daily cortisol production (using urine, blood and salivary cortisol). Again the findings were conflicting with around half showing raised cortisol and half normal or low. One key, consistent finding was that cortisol reactivity – in this case a spike in measured cortisol during stress – was greater in studies which investigated obese versus non obese responses; in particular abdominal adiposity seemed to be linked with cortisol reactivity. The overall conclusion of this paper was that the findings were inconsistent, and largely limited by quality of published data (given the paucity of longitudinal data, control for sex and chronicity of stress).

As presented above, much of the published literature examining a relationship between stress, obesity and the MetS has been conducted in adults, or outcomes of childhood stress in adulthood.[122-124] However, data for associations within childhood have begun to emerge, but have not yet been systematically reviewed. As part of my exploration of the topic and

to plan investigation in this thesis, I led a systematic review of the literature for associations between measures of stress and obesity with or without the MetS specifically in childhood and adolescence (<18 years).

2.4 Stress, obesity and the metabolic syndrome in children and young people: systematic review

2.4.1 Systematic Review Methods

Data sources and search strategy

An initial systematic search (round one), was performed in July 2011 by myself with (Dr) Leena Zhou who was a visiting medical student to our department. This was updated without a second researcher by myself alone in September 2017 (round two) from 2011 where the first round had left off. In both cases Professor Viner adjudicated inclusion of studies. For the purpose of the thesis, the results are presented together, however in the methodology and search results I will separate by search round.

Searches used MEDLINE, PubMed, PyschINFO and Web-of-science databases to identify studies in childhood and adolescents under 18-years of age which investigated associations between stress and obesity +/- one or more components of the MetS (central adiposity, hypertension, dyslipidaemia and insulin resistance/type 2 diabetes). Databases were searched from inception date to date of search. Search terms used are summarized in table 1. Searches consisted of database specific terms (e.g. MESH) relating to stress (psychological and physiological) AND (obesity/ body mass index / body fat) OR (components of the metabolic syndrome) AND children and adolescents (or where available, age restrictions <18 were applied to searches). They are summarized in table 2.1.

Table 2.1: Summary of search terms and strategy for each database searched

Database	Stress	Obesity and MetS	Age
PubMed (n =1713)	"Stress, Physiological"[Mesh] OR "Stress Disorders, Post-Traumatic"[Mesh] OR "Stress, Psychological"[Mesh] OR "Stress Disorders, Traumatic, Acute"[Mesh] OR "Stress Disorders, Traumatic"[Mesh] OR "Combat Disorders"[Mesh] OR “Cortisol”[Mesh] OR “Hydrocortisone”[Mesh] OR “Life Event”[Mesh]	"Abdominal obesity metabolic syndrome"[Supplementary Concept] OR "Obesity"[Mesh] OR "Obesity, Abdominal"[Mesh] OR "Obesity, Morbid"[Mesh] OR “Hyperlipidemias"[Mesh] OR “Type 2 Diabetes Mellitus"[Mesh] OR “Cardiovascular Diseases"[Mesh] OR “Dyslipidemias"[Mesh] OR “Hypertension"[Mesh] OR “Body Mass Index"[Mesh] OR “BMI"[Mesh]	"Child"[Mesh] OR "Disabled Children"[Mesh] OR "Adult Children"[Mesh] OR "Homeless Youth"[Mesh] OR "Child of Impaired Parents"[Mesh] OR "Child, Orphaned"[Mesh] OR "Only Child"[Mesh] OR "Child, Unwanted"[Mesh] OR "Child, Preschool"[Mesh] OR "Child, Institutionalized"[Mesh] OR "Child, Hospitalized"[Mesh] OR "Child, Abandoned"[Mesh] OR "Adolescent"[Mesh] OR "Adolescent, Institutionalized"[Mesh] OR "Adolescent,

Database	Stress	Obesity and MetS	Age
			Hospitalized"[Mesh]
PsychINFO (n= 53)	Cortisol or Hydrocortisone or Physiological Stress or Post-Traumatic Stress Disorders or Psychological Stress or Acute Traumatic Stress Disorders or Traumatic Stress Disorders or Combat Disorders or Life Event	Abdominal obesity metabolic syndrome or Obesity or Abdominal Obesity or Morbid Obesity or Hyperlipidemias or Type 2 Diabetes Mellitus or Cardiovascular Diseases or Dyslipidemias or Hypertension or Body Mass Index or BMI	*Limit to:* childhood <birth to 12 years> or adolescence <13 to 17 years> (Also limited humans and English language)
Medline (n= 944)	Cortisol or Hydrocortisone or Physiological Stress or Post-Traumatic Stress Disorders or Psychological Stress or Acute Traumatic Stress Disorders or Traumatic Stress Disorders or Combat Disorders or Life Event	Abdominal obesity metabolic syndrome or Obesity or Abdominal Obesity or Morbid Obesity or Hyperlipidemias or Type 2 Diabetes Mellitus or Cardiovascular Diseases or Dyslipidemias or Hypertension or Body	Limit to : all child 0-18 years (Also limited humans and English language)

Database	Stress	Obesity and MetS	Age
		Mass Index or BMI	
Web Of Science (n = 231)	TS=(Cortisol) OR TS=(Hydrocortisone) OR TS= (Physiological Stress) OR TS=(Post-Traumatic Stress Disorders) OR TS=(Psychological Stress) OR TS=(Acute Traumatic Stress Disorders) OR TS=(Traumatic Stress Disorders) OR TS=(Combat Disorders) OR TS=(Life Event)	TS=(Abdominal obesity metabolic syndrome) OR TS=(Obesity) OR TS=(Abdominal Obesity) OR TS=(Morbid Obesity) OR TS=(Hyperlipidemias) OR TS=(Type 2 Diabetes Mellitus) OR TS=(Cardiovascular Diseases) OR TS=(Dyslipidemias) OR TS=(Hypertension) OR TS=(Body Mass Index) OR TS=(BMI)	TS=(Child) OR TS= (Disabled Children) OR TS=(Adult Children) OR TS=(Homeless Youth) OR TS=(Child of Impaired Parents) OR TS=(Orphaned Child) OR TS=(Only Child) OR TS=(Unwanted Child) OR TS=(Preschool Child) OR TS=(Institutionalized Child) OR TS=(Hospitalized Child) OR TS=(Abandoned Child) OR TS=(Adolescent) OR TS=(Institutionalized Adolescent) OR TS=(Hospitalized Adolescent)

*MeSH terms

Selection of studies

In the first round (up to 2011) two reviewers (LH and LZ) screened abstracts identified in searches simultaneously to select studies meeting inclusion criteria for retrieval. In round 2, I alone selected studies but Professor Viner adjudicated in both rounds.

Inclusion criteria were: 1) any study design except protocol papers, opinion papers or reviews (unless a systematic review); 2) studies investigating associations of stress and obesity (+/- MetS components) outcomes only in childhood and adolescents under 18 years of age (thus studies mixing children and adults in analyses or studies looking at the association of childhood stress and outcomes in adulthood were excluded); 3) studies measuring either psychological (by validated questionnaire or laboratory induced stress) or physiological (non-oxidative) stress involving the HPA or sympathetic nervous system; 4) studies using currently accepted criteria for obesity in childhood, i.e. centile (or equivalent z-scores) for body mass index (BMI) at least ≥95th centile; 5) in studies investigating MetS components, participants must also have had obesity; 6) studies in English language and involving human subjects only.

Exclusion criteria were: 1) Studies involving participants with genetic, syndromic or endocrine causes of obesity; 2) Studies which used self-reported rather than actual measured values to calculate BMI; 3) Psychological studies which reported perceptions of stress by a parent or guardian or exposure assessed as single events, not using a validated life event scale.

Selected studies were then retrieved and independently reviewed by two reviewers (LH and LZ) in the first round, and just by myself in the 2nd round to: i) ensure inclusion criteria were met; ii) identify outcomes in each study and iii) critically appraise each paper. Information extraction and appraisal for each paper was done using a proforma as shown in tables 2-3, with reference to published guidelines on systematic reviews in etiological questions.[125] Reviews were also searched for additional eligible studies, as were reference lists of retrieved studies. Agreed final studies were selected for inclusion and findings were adjudicated by a third researcher (RV) for both rounds.

2.4.2 Systematic review results

In the first round initial database searches yielded 2941 abstracts; 179 were retrieved for further review; 13 studies were identified for inclusion (see figure 2.1). In the second round database searches yielded 1285 abstracts; 63 were retrieved for further review and 8 were selected for inclusion (see figure 2.2). Studies are summarized in tables 2.2 and 2.3. Thus a total of 21 studies were included in the systematic review after both rounds. No additional studies were identified from reviews or hand-searching of retrieved papers. No randomized controlled trials were identified. 18 of the 21 studies included were cross-sectional and 3 had some longitudinal component (though most also presented cross sectional data in addition at baseline): 12 used controls, or at least a control group within a cohort (with healthy weight). Thirteen of the included papers were from the USA and 8 were from Europe (Netherlands x 2, Germany, Sweden, Greece, Italy, Turkey and one paper was joint Anglo-French). Papers were published between 1991-2015.

Findings are presented narratively with studies split into those looking for associations between stress and obesity and 1) physiological measures of stress and; 2) psychological measures of stress. All studies are summarized in tables 2.2 and 2.3.

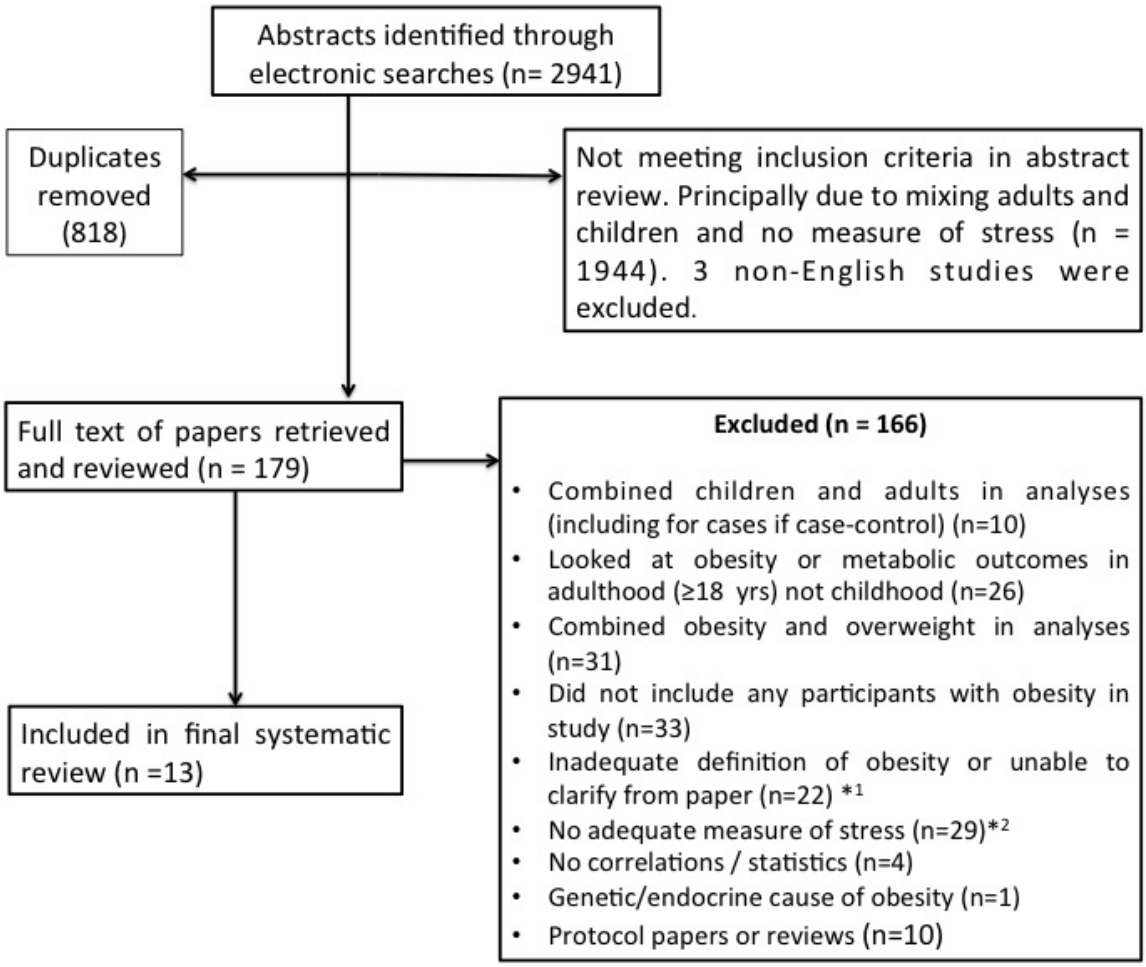
1. Physiological measures of stress

Seventeen studies reported various measures of human cortisol[126-142] (split below into blood, urine and saliva) with one study also investigating the sympathetic nervous system axis.[133] Seven papers compared obese children to healthy weight children, two investigated within groups of obese children. All but two of the studies were of very small numbers; most did not analyze males and females separately, nor stratified by pubertal status. In 6 studies obese participants were recruited from patients of clinical obesity/medical services; in 1 study participants were from a community cohort; in 3 studies it was unclear how patients had been recruited. Age ranges of children were from 5 years to 15 years, and would therefore have included a range of pubertal stages.

Cortisol levels from blood samples

Five studies examined blood cortisol and obesity. In one of two papers identified, Chalew et al (1991)[126] found that in a small group (n=16) of 5-16 year olds with obesity, 24-hour total integrated plasma levels of cortisol (IC-F) measured by repeated sampling were lower compared to data from lean children reported in a previous study. There was however no correlation between actual BMI and IC-F in those with obesity, nor was there any significant change in IC-F in a subgroup who were placed on a low caloric dietary regimen (though there were no measures of either compliance with the diet or effect on body mass index). In a second paper by the same author, Chalew et al (1997)[127] reported that both integrated 24-hour cortisol and cortisone levels (IC-E: a less active metabolite of cortisol) in a small sample of 10-15 year olds with obesity was lower than a small group of similarly aged lean controls ($p<0.04$).

Misra et al[131] reported no differences in mean overnight cortisol levels in 15 obese females aged between 12-17 years and 30 sex and bone-age matched healthy weight controls. Eliakim et al[133] looked for differences between a small group of obese male and female children all aged between 12-13 years and similarly aged non-obese controls in fasting morning blood cortisol and blood cortisol levels immediately and again, 180 minutes post a 30-minute exercise



*1 : weight not BMI, weight for height% not centile (13),adult definitions(2), unclear how defined (3), self-reported measures BMI (2).

*2 *No stress measure in paper (5), Oxidative stress (1), Mental health not stress (6), Exposure to potential stressor but no reported / measured stress (7), Psychological aspects of weight interventions no specific stress measures (3),Parenting stress / parental reported stress or parent reporting stress in child (5), Quality of life measures not stress (2)*

Figure 2:1 Summary of searches for 1st round systematic review

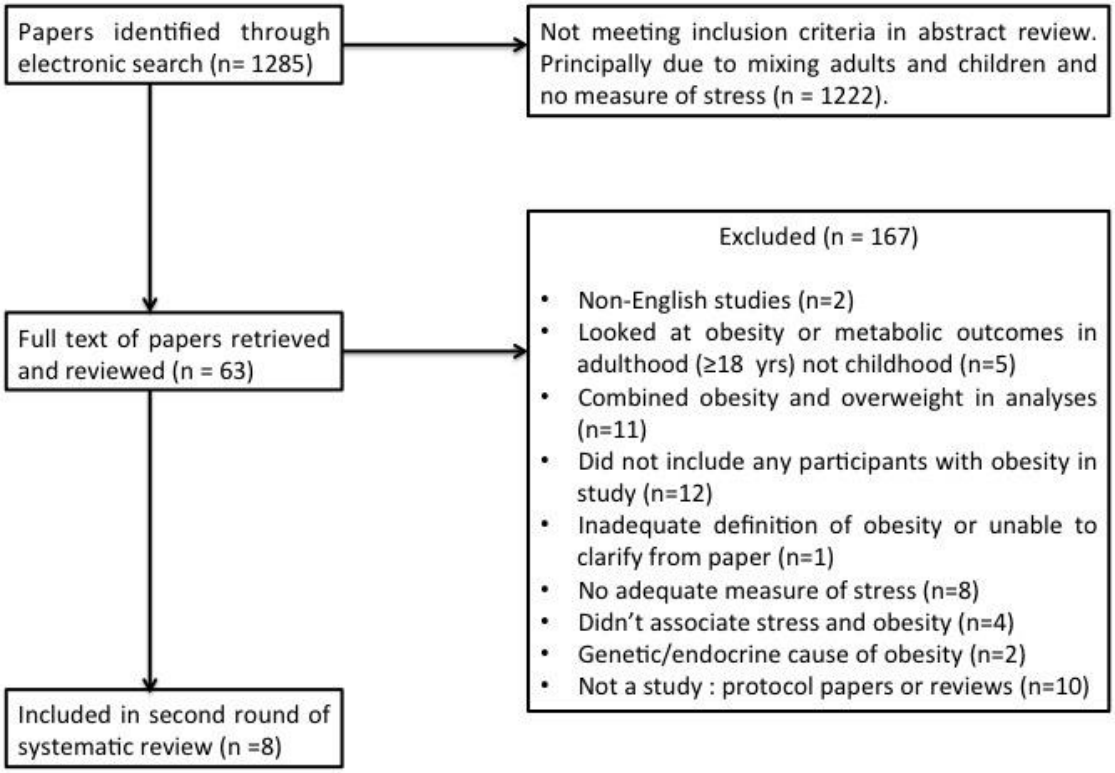


Figure 2:2 Summary of searches for 2nd round systematic review

regime. No differences were found in either fasting or post exercise cortisol between groups. Reinehr et al studied a small number of obese versus healthy weight children matched for age, sex, and puberty in a community lifestyle intervention; children were aged 8.5 +/- 2.1 years.[142] They measured serum glucocorticoids at baseline and then at follow up. They found that glucocorticoids were significantly higher in the obese versus controls at baseline, including cortisol and cortisone; and interestingly the children with obesity who lost weight at follow up showed a significant drop in cortisol levels compared to baseline.

Four studies looked for associations between blood cortisol values and components of the MetS in the context of obesity. Misra et al[131] reported a positive association between the log mean overnight cortisol and amount of visceral adipose tissue (on MRI) (r=0.53,p=0.01) in obese and non-obese children combined, but this was not significant in the obese children alone. In a regression model controlling for BMI-SDS, log mean cortisol was independently associated with subcutaneous adipose tissue (p=0.02) in all children but not visceral adipose tissue. The log mean cortisol also correlated positively with HOMA-IR (r=0.56,p=0.004) and fasting triglycerides (r=0.41,p=0.046) in all children. Barat et al[134] studied a group of pre-pubertal obese children from 2 centres and found no correlation between first morning blood (fasting) cortisol and Adrenocorticotropic hormone (ACTH) and whole body fat mass (as measured by DEXA), but when adjusted for measures of whole body fat mass, first morning cortisol was positively correlated with truncal fat mass (r=0.38, p<0.05), fasting cholesterol (r=0.41, p<0.05) and triglycerides (r=0.44,p<0.05) but not HOMA. Sen et al[132] measured fasting morning blood cortisol and ACTH in 241 obese 2-17.6 year old males and females and found that both mean fasting cortisol and ACTH were higher (p=0.023 and p=0.04s respectively) in the 44% of participants who met criteria for the MetS (using contemporaneous WHO and National Cholesterol Education Program Adult Treatment Panel-III guidelines). There was a positive correlation between ACTH and weight, blood pressure and fasting glucose in all children. However, only a weak positive correlation was found between fasting cortisol and systolic BP(r=0.12, p=0.05). Guzzetti et al[141] studied 1027 children and adolescents with obesity in an endocrine clinic. They divided their subjects into 3 groups by age: (1) 6 – 10 years, (2) 10-16 years and (3) 16-18 years. Looking at all subjects, they found blood cortisol was weakly associated with both diastolic and systolic blood pressure (when controlled for age, gender, puberty and BMI z). In the younger and middle age groups, cortisol was positively associated with fasting glucose, blood pressure and HOMA (after adjustment for age, sex, puberty and BMI z). Associations were weak.

Urinary cortisol

Four studies explored urinary excretion of cortisol and obesity. Russell et al[128] reported higher levels of 24-hour urinary free cortisol (UFC) in a small group of obese 12-18 year old

females versus lean controls matched for pubertal stage and bone age (p=0.03). Misra et al[128] also found higher levels of UFC in obese females compared to controls (p = 0.02) and that log UFC correlated with BMI z-score in obese females (r=0.633, p =0.01) and in obese and non obese combined (r=0.54, p=0.002).

Three studies looked for associations between urinary cortisol and components of the MetS in the context of obesity. Russell et al[128] found that UFC levels within obese subjects predicted higher highly sensitive CRP and Il-6 levels (when controlled for fat mass). Misra et al[131] found no association between UFC and visceral fat distribution or cardio-metabolic blood components of the metabolic syndrome (HOMA-IR and fasting lipids). Barat et al[134] (see above) did not find any association between 24-hour UFC and %body fat (by DEXA), measures of fat distribution nor fasting cardio-metabolic bloods (lipids and HOMA) in a subgroup of the obese children in their study.

Salivary cortisol

Eight studies investigated salivary cortisol and obesity, with two studies reporting longitudinal investigation. Rosmalen et al[130] measured salivary cortisol at waking and 30 minutes later (the cortisol awakening response) in a large number of male and female children (1768) aged 10-12 and as a part of their study, looked for associations with body mass index, although the proportion of children with obesity was small (2.5%). On analysis of all participants, no association was found between any salivary cortisol measure and BMI. However on separate analysis of females, there was a very small correlation between BMI and Area under the curve (AUC) between time 0 and 30 min cortisol (r=0.02, p=0.04). Tukey HSD analysis also showed greater cortisol levels at 30 minutes after waking in obese children as a group compared to non-obese (p=0.02) and AUC between time 0 and 30 was greater in obese compared to normal BMI children (p=0.04). Hershberger et al[129] compared salivary cortisol levels throughout the day (including before and after meals and before and after exercise) in 10 obese male and female children (at either Tanner stage 1 and 2) with 11 non-obese Tanner matched controls. No differences in fasting morning salivary cortisol levels

were found between groups and a normal diurnal pattern was seen in both groups. However, a mean increase in cortisol post-lunch was greater in the obese group ($p<0.05$). Mean cortisol decreased post exercise in the obese group compared to a rise seen in the non-obese group ($p<0.05$). Barat et al[134] (see above) also measured salivary cortisol at 8am, 11:30 and 12:00 in a subgroup of the obese children in their study. No association between morning salivary cortisol values were found with %body fat (by DEXA), body distribution or fasting cardio-metabolic bloods. Interestingly, a negative association was found between the salivary cortisol response to lunch (change between 11:30 and 12:30) and truncal fat mass ($r=-0.43$, $p<0.05$) in all children, but more prominently in females ($r=-0.78$, $p<0.05$). Guseman et al[140] measured salivary cortisol levels across the day in a small number of adolescents with obesity and calculated total area under the curve (i.e total cortisol secretion through the day). They found that mean area under the curve cortisol did not vary by sex, or severity of obesity (comparing >95th centile BMI with >97th centile and >99th centile). There was no difference in area under the curve cortisol for those with the metabolic syndrome versus those who did not. Pervanidou et al [139] examined children in an obesity clinic and compared them to healthy weight controls. They measured salivary cortisol throughout the day (5 times per day) and created an area under the curve for analysis. They found that AUC for cortisol was lower in the obese group – however this significance was lost when controlled for age, gender and BMI z. Kjolhede et[138] al measured salivary cortisol in 342 children aged 6-12 recruited from community schools at three time points, morning, afternoon and evening. They found that cortisol levels were lower in the obese than healthy weight when grouped by BMI category.

Two studies provided longitudinal investigations using salivary cortisol. Hill et al[137] measured awakening cortisol (single sample) in 649 children entered into a community based intervention to prevent weight gain, of which 12.8% had obesity at baseline. They compared means (adjusted for age) for morning cortisol between healthy weight and obese subjects (including separating by male and females) and found no statistical difference. Within the obese group at baseline, there was a weak, positive association between baseline salivary cortisol and both waist circumference and BMI z increase following the intervention. Ruttle et al[135] measured salivary cortisol for 3 consecutive days running in children with measures

after waking, mid afternoon and evening salivary cortisol. They repeated these measures in the same individuals at ages 11, 13, 15 and 18 years. They found that there was a negative association between cortisol measures (in terms of time of day and also changes through the day) at proceeding ages and BMI at the next age (e.g. cortisol at 11 years and impact on BMI at age 13), including longitudinal cortisol on 18 year olds. Whilst their analyses were across all BMI groups (only around 10% at each age were obese), they did repeat analyses within obese group and found the same findings. They also found that cortisol and pubertal stage was only associated at age 13, not other ages. This paper is important for the thesis because they used longitudinal, repeated measures to assess the relationship of cortisol with BMI, and used multi-level modeling – however they do not appear to have used longitudinal data with time as panel data, but rather as changes between groups between preceding and following year.

Hair cortisol.

Veldhorst [136]compared scalp hair cortisol levels in 20 obese children with sex matched controls of similar age and found greater cortisol levels in the hair of obese children.

Blood Catecholamines

In addition to measuring cortisol, Eliakim et al[133] (see above) measured blood catecholamines before and after a 30-minute exercise regime and found that catecholamines increased in greater amounts in non-obese children during exercise, though there were no differences in the baseline values.

2. Psychological studies

Only four studies meeting inclusion criteria used psychological assessments to measure stress and investigate for an association with obesity. Only one study looked at a component of the MetS in the context of obesity and stress. All studies investigated specific groups of obese patients involved in clinical obesity services. Only one study attempted to compare stress between a group of obese patients and healthy weight controls.

Stress measured using questionnaires

Kubiak et al[143] asked a small group of obese 14-17 year old females to record frequency of perceived daily hassles on electronic diaries whilst an inpatient for an obesity program and to also record frequency of associated rumination about eating. A mixed regression model showed a significant interaction between rumination and number of hassles ($p<0.05$) – the implication being that daily hassles was a measure of stress and thus its association with rumination would lead to increased eating with consequent impact on body mass. Zeller et al[144] measured self-reports in an outpatient setting (using appropriate child and adolescent stratified questionnaires), one component of which measures self-perceived social stress. Regression analysis showed no significant relationship between social stress scoring and BMI z-score in both children and adolescents. Porter et al[145] asked 135 children aged between 11-17 presenting to an obesity clinic to self-report if they felt a stressful event had triggered weight gain in their history – 66% reported an event. No further analysis or associations were made with regard to BMI or MetS.

Response to induced psychological stress

Ribeiro et al[146] studied the blood pressure and forearm vascular conductance responses during an induced mental stress test in 39 obese children compared to 10 healthy weight controls. Mean blood pressure increased in obese children, but not healthy controls ($p=0.01$); there were no changes in heart rate. Obese participants were then placed on a diet and exercise regime and responses re-measured 4 months later after which time there was no difference between BP changes in obese compared to healthy controls following a mental stress test.

2.4.3 Systematic review discussion

At the time of writing, I believe this is the first systematic review on the potential role of stress in childhood obesity and the MetS. The first key finding was that the findings in

studies were inconsistent, and secondly most studies were cross-sectional and of low quality.

A number of methods of measuring cortisol were used. Of the 5 studies investigating blood cortisol – 2 found lower levels in the obese, 2 no difference and 1 higher. However, results of analyses in studies examining the metabolic syndrome components within obese groups found positive associations. Of 4 studies investigating urinary cortisol levels, half found higher levels in obese participants and half found lower. Salivary cortisol measures were of particular interest to me as they allowed ambulatory measurements at different time points. Again findings were varied however, with most studies finding little difference cross-sectionally, or difference in metabolic syndrome components.

Within the psychological papers, only one study compared self-reported stress between an obese and non-obese group of children and found no differences. None of the papers included a measure of cortisol and psychological measures.

The studies identified in this systematic review possess a number of methodological issues that may potentially introduce bias, and are of relevance to the design of my study included in this thesis. Most studies involved small numbers and recruited subjects from clinical settings. Some studies provided no explanation of how they had recruited participants. Longitudinal studies were few, and even in those that were longitudinal, baseline or previous time point values of cortisol were used to predict the next time point obesity measure, or cardio-metabolic marker; there was no use of more complex multi-level modeling using time in models. This leads to weaknesses in analysis associated with missing data and by grouping all participants together at set time points does not allow for differential gaps in measuring points over time. In addition, presence of components of the MetS and weight status may change within individuals overtime during adolescence,[147] thus mathematical modeling needs to be more considerate of change in more complex ways.

Although some studies matched controls for sex, age and pubertal stage, a number of studies combined all three in analyses. This is problematic given the differential findings in some adult studies between sexes, and that cortisol levels may vary by both pubertal stage and age in the normal population.[130 148-150]

The search strategy aimed to identify associations within the context of obesity and therefore a number of studies were excluded which combined overweight with obesity, or inappropriately defined obesity, reducing the number of studies in our review. The search strategy deliberately looked for papers that measured only self-reports of stress or physiological measurement within individuals. We therefore excluded a number of papers that reported exposure to potential stressors, as well as papers using parental-stress or parental perception of child stress. Although it is likely that some children and adolescents experience e.g. abuse, poor quality of life or difficult socio-economic circumstances that may lead to stress, there is likely to be a number of other mechanisms in addition to stress at work.

A particular note here should be made on mental illness such as depression and anxiety. We did not include them as a measure of stress – and this is because whilst mental health problems can be stressors, they are not synonymous with stress. This is important to my thesis, because I measure and treat stress and mental health separately in analyses in this thesis (see below). Moreover, separate bodies of literature exist for the associations of obesity with mental health[151] and with child abuse.[152] Exclusion of parental report also limits the measurement of stress in very young children and children with disabilities. Physiological markers of stress may therefor be of particular value in measuring stress in such groups.

I will also highlight one study here that was found during searches, but was not included in the thesis because it combined overweight and obese participants; yet provides an important theme for the thesis. Toledo et al measured cortisol using saliva and blood through the day and measured carotid artery intima-media thickness in a group of black and Hispanic overweight adolescents, and found that some measures of cortisol were associated

with increased arterial thickening in this group.[153] As far as I am aware, at the time of writing, this is the only paper which examines a relationship of stress, either using physiological or psychological measures and arterial stiffening within the context of obesity in children and young people.

2.5 Implications of findings presented in chapter two for the thesis

This chapter has outlined the hypotheses linking stress to obesity, but also the existence of cardio-metabolic risk markers within the context of obesity and stress. I used salivary cortisol measures within the group of adolescents with obesity to measure possible relationships within this group and degree of obesity and co-existing cardio-metabolic risk markers. The use of salivary cortisol allowed ambulatory results from the participants, which provided information about cortisol profile throughout the day. I also decided to use a validated questionnaire for exposure to stressful events (the A-file : see 3.10.1) within the group. Importantly I wanted to study relationships not just at baseline, cross-sectionally, but also changes over time using more complex modeling of change over time. By using a questionnaire focused on eating, I was also interested in understanding how much stress might be associated with eating behaviour, in particular level of binge eating, within an obese group of adolescents. I therefore wanted to address the following questions:

1. Within the obese group of adolescents who took part in the HELP trial, what relationships existed between degree of obesity, conventional measures of cardiovascular risk such as blood pressure and lipids, pulse wave velocity and physiological measures of stress (salivary cortisol) and measures of exposure to stressful events at cross-sectional and longitudinal levels? In particular, how do changes in stress measures, relate to changes in PWV, adiposity, blood pressure and other blood measures over time.

2. What relationship, if any, was there between measures of cortisol and the validated questionnaire for stress exposure ?

3. What relationship, if any, was there between stress and binge eating behaviours?

4. What relationships, if any, were there between measures of stress and measures of mental health co-morbidities within the group of adolescents?

Table 2.2: summary of studies of physiological measures of stress and obesity

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
Chalew (1991)	CS:CC	16 obese (5.3-16.8yrs) recruited from hospital obesity clinic	30 (7-17 year olds) controls with BMI range SDS ≥ -2 ≤ 2 controls Described in previous studies but unclear how recruited.	No	No	Blood : 24 hour integrated cortisol(IC-F) (continuous 24 hour draw totalled)	Lower IC-F in obese versus lean (t-test p<0.0001). No correlation between BMI and IC-F. Low calorie diet in a small number re-measured later did not alter IC-F.	Commented on similar findings in adult studies. Speculated that low cortisol may be a response to hyperinsulinism found in obesity.
Chalew (1997)	CS: CC	9 obese (12.3yrs +/- 3.2) recruited from hospital obesity	15 non-obese with BMI range SDS ≥ -2 ≤ 2 (12.7 yrs +/-	Mixed but used sex as a variable in regression	No	Blood: 24 hour integrated cortisone (IC-E) i.e. continuous 24 hour	Mean IC-E in obese subjects lower (t-test p<0.04). IC-E levels highly correlated with the IC-F levels (p<0.0001).	Concluded that IC-F converted to cortisone thus levels of cortisone lower in obesity due to

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
		clinic.	2.2). Described in previous studies but unclear how recruited.	model		draw totalled.	Multiple regression model for total IC-E using BMI SD and sex showed lower IC-E levels with increasing BMI SD ($p<0.0054$); more prominent in boys ($p<0.043$)	lower IC-F.
Hershberger (2004)	CS:CC	10 obese (9.4yrs +/- 0.2;) Note controls <75th centile	11 non-obese (<75th centile BMI) (age 9.2 +/-0.7)	No	All cases and controls Tanner stage I or II and very close age.	Salivary cortisol fasting 0900, then post-breakfast; pre- and post 30 min exercise ; then pre- and post lunch (Enzyme immunoassay)	No difference between fasting morning cortisol values. Mean change post-breakfast greater in obese group (t-test, $p<0.05$); Mean salivary cortisol decreased in the obese group post exercise, but increased in non-obese ($p<0.05$). Similar increases after lunch. Normal diurnal variation observed in both groups	Paper's predominant investigation was relationship between lipolysis and obesity and if it was related to cortisol. No relationship found – additional differences in cortisol between obese and lean groups highlighted here.

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
Rosmalen (2005)	CS: CH	1768 total 2.9% obese. All children recruited from a population cohort study of children. This study at one assessment. Age range 11.08 yrs(SD 0.55)	84.9% <85th centile;12.2% >85th centile <95th	Differences for whole group looked at, but not for analyses shown here.	Each participant graded by Tanner pubertal stage. Looked at pubertal differences for whole group and no differences found within the group of cortisol findings by pubertal stage.	Salivary cortisol at waking (07:00) and then 30 mins later (07:30)(morning response) and then at 20:00. (immunoassay) Values used to produce changes between times and Area under curve (AUC).	Tukey HSD showed significantly higher salivary cortisol levels 30 mins after waking between normal and obese (p= 0.015) and between overweight and obesity (p=0.043). AUC was significantly greater between normal and obese (p=0.006) and between overweight and obese (p=0.046). (Note- Other than that presented here : most analyses looking at BMI and saliva included obese, overweight and normal weight together)	Main emphasis of trial was to look at HPA axis though salivary cortisol in cohort group and for influences. Analyses across all BMIs did not find significant relationship between any salivary cortisol values and BMI (no other metabolic factors measured); differences found at different BMI categories was not main focus and not much comment in the paper.

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
Eliakim (2005)	CC	25 obese (12.3 +/- 0.5) Obese and non-obese participants recruited by exercise research centre but no details on how or form where.	25 healthy weight : BMI 3.5-77.8th centile (12.8+/- 0.5; 12 male)	No	No	Fasting blood cortisol taken in the morning, and then again immediately post, and 120 mins post, using an exercise protocol and Blood catecholamines (epinephrine, norepinephrine, dopamine). Fasting first morning pre- and then immediately post and 120 mins post an exercise protocol	No differences between fasting, or post exercise cortisol levels between obese and non-obese groups No statistically significant differences in baseline catecholamines between obese and non-obese. Increases in all three catecholamines were smaller in obese children during exercise versus controls (mixed model ANOVA $p<0.05$).A negative correlation was found in all children between change in all three catecholamines during	Study also looked at insulin and growth hormone changes during exercise but stress hormone results are presented here.The authors conclude that blunted catecholamine response in obese children during exercise may represent an underlying central attenuation of the stress response.

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
						by indwelling venous catheter inserted 30 mins prior to exercise.	exercise and BMI centile (Dop r=-0.28, p=0.048;Epi r=-0.31, p=0.03 ; NA r=-0.29, p=0.043); as well as body fat% (measured by DEXA) (Dop r=-0.46,p=0.0006;Epi r=-0.49, p=0.0004; NA -0.43, p=0.002)	
Barat (2007)	CS	45 children with obesity (6-13 years). Recruited from 2 hospital obesity clinics.	None	Looked at mixed and individually	Inclusion criteria pre-pubertal (Tanner stage 1)	All children : Fasting blood cortisol and ACTH; 29 also had 24 hour urinary cortisol and 0800, 11:30 and 12:00 salivary cortisol.	No correlation between any HPA investigation and whole body fat mass (DEXA). When adjusted for whole body fat mass %, truncal fat mass was positively correlated with first morning cortisol in all boys and girls total (not separately) r = 0.38 ($p<0.05$). In all participants, adjusted for WBFM and TBFM, positive	Noted that salivary cortisol did not show same relationship as blood cortisol, but that smaller numbers had been used. Lunch response to cortisol opposite to what the authors have found in post-menopausal obese women (where response

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
							correlation between morning cortisol and fasting total cholesterol (r=0.41, p<0.05), and fasting triglycerides (r=0.44, p<0.05). A negative correlation was found between TBFM and change in salivary cortisol post lunch (r= -0.43, p < 0.05) . In girls a positive correlation was found between glucocorticoid metabolites and Truncal fat (r=0.92, p<0.05) but not in males or the whole group. Salivary cortisol response to lunch not associated with other cardiometabolic risk in total group, but in boys negative correlation with	same in those with abdominal versus peripheral fat).

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
							HOMA (r=-0.56, p<0.05).	
Sen (2008)	CS	241 obese (2-17.6yrs). Unclear where recruited from	None	No	No	blood cortisol (fasting) 08:00 and blood ACTH.	All participants had obesity. Those with MetS (43.9% of participants) *3 had higher 8am cortisol and ACTH (t-test p=0.023;p=0.042). ACTH correlated positively with weight (r=0.13, p=0.02), systolic (r=0.21,p=0.002) and diastolic (r=0.17,p=0.01) pressure, fasting glucose (r=0.17,p=0.01). Cortisol weakly correlated with systolic pressure (r=0.12,p=0.05).	Suggested an association between HPA activity and metabolic syndrome in children with obesity.
Misra (2008)	CS: CC	15 obese (12-18yrs)	30 non-overweight :15-18th	All females	Bone age matched (and race	Overnight blood cortisol taken every 20 minutes from	Blood: No significant difference between obese and control group in overnight	Higher cortisol measures associated with higher metabolic risk, but in all

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
		Unclear how recruited – either cases or controls.	centiles BMI (12-18yrs)		ethnicity) Tanner 2 and 3 stages of puberty	23:00-08:00 (RIA). 24 hour urinary cortisol measured same time (Gamma-coat I-RIA). Fasting cardiometabolic markers also measured	mean cortisol. Log UFC greater in obese versus controls (t-test, p=0.02). Positive correlation between logUFC and BMI in obese girls (r=0.63, p =0.01) and in whole group (r=0.54, p =0.002). Association between visceral adipose tissue log mean cortisol (r=0.53, p=0.01) in whole group. Regression model: controlling for BMI-SDS association with log mean cortisol with subcutaneous adipose tissue (p=0.02). Log mean cortisol positively associated with HOMA-IR in all (r=0.56, p =0.004). UFC was not. Fasting triglycerides	participants not just obese (however small numbers when separated).

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
							positively associated with log mean cortisol in all group	
Russell (2009)	CS: CC	15 obese (12-18yrs old) Unclear where cases or controls recruited from	15 healthy weight BMI between 15-85th centile (15-18yrs)	All females	By bone age (within 1 year) and pubertal stage and race & ethnicity	24 hour free urinary cortisol	Mean UFC greater in obese (t-test p=0.03) versus non-obese; In all participants, those with higher CRP (>3mg/l) had greater UFC (t-test p=0.004). UFC was positively correlated (r=0.51,p=0.05) with IL-6 levels. In a regression model, controlling for regional fat mass, UFC was positive correlated with log hsCRP (r^2 =0.24, p =0.03) in all subjects; and logIL-6 in obese (r^2 =0.03, p =0.04) and all subjects (r^2 =0.35, p =0.0008)	Concluded that urinary cortisol levels associated with markers of long term cardiovascular risk. This paper also looked at growth hormone levels. Stipulates GH def may impact on 11 beta hydroxysteroid dehydrogenase type 1 activity cortisol to cortisone

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
Ruttle (2012)	Longitudinal Repeated measures at 11,13, 15 and 18 years cohort	11-18, 323 at baseline and 303 followed by age 18. BMI stable	**10% obese approximately throughout**	Yes, sex was not associated with BMI at next age	Yes, greater pubertal stage associated with BMI. Only associated with cortisol at age 13	Salivary cortisol over 3 days – morning, mid day and evening.	Negative association between cortisol indices pre-ceding age with next age (eg 13 to 15). Most were not obese but was repeated in obese participants alone and same finding.	Good example of longitudinal measures, however each year used as predictor of next not using time in multilevel model. Not clear how many altered from obese or into obese over time.
Hill et al (2011)	CS cortisol measures but longit	649, of whom 12% had obesity at baseline. Age 6.1-12.0 at baseline	**82% were overweight or healthy weight.**	Yes. In comparisons of cortisol between obese and healthy,	Mean cortisol at baseline controlled for age, but not puberty.	Salivary cortisol. Single awakening measure.	No difference between salivary cortisol in obese versus healthy weight. Within the obese group, there was weak association positive with cortisol and weight change in	Single measures of cortisol used as a predictor of subsequent weight gain. Longitudinal therefore. Obese proportion were

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
	udinal BMI and waist measurements. Obesity intervention.			done by sex.			the intervention.	small.
Kjolhede et al. (2013)	CS from schools in Sweden	Total of 342 children of whom 8.4% were obese. Age 6-12 years	**77.3% were healthy weight**	Yes, no differences between cortisol by sex	Yes, no differences between cortisol by age.	Three samples of salivary cortisol measured at am, afternoon and evening.	Salivary cortisol levels were lower in the obese at all time points compared to healthy weight.	No pubertal controls, and most girls in study would have been in puberty, likely not an issue for boys.

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
Veldhorst et al. (2012)	CS from obesity clinic.	20 obese children 10-13 years	**20 healthy weight controls**	Sex matched controls	Of similar age but not pubertally assessed	Scalp hair cortisol levels	Hair cortisol concentration was greater in obese children	Not pubertally matched.
Reinehr et al (2013)	Longitudinal study following children (some obese, some healthy weight	40 obese participants, 7-10	**40 healthy weight controls, matched for gender, age and pubertal stage**	Controls matched for age, sex and pubertal stage	Controls matched for age, sex and pubertal stage	Serum steroid hormones	Obese children had higher glucocorticoids in serum at baseline, and the obese children who lost weight showed a drop in the serum glucocorticoids.	Longitudinal change in cortisol with weight loss. Small numbers, well matched controls.

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
	) in community lifestyle intervention							
Guzzetti et al (2014)	CS.Patients from an endocrine clinic	1027 children and adolescents (6-18 split into groups for analysis)	**None**	Yes analyses controlled for sex	Age and puberty both adjusted for in analyses	Blood cortisol	Blood cortisol associated with a number of metabolic syndrome components – HOMA, blood pressure and fasting glucose after adjusting for age, gender and pubertal status	Cross sectional, split into ages. 16-18 no associations but numbers larger for other age groups so likely power issue.
Pervanidou et al (2014)	CS salivary cortiso	110 obese participants, age 11-15	**31 healthy controls. Same age group**	Yes in analyses	Age considered in analyses not puberty,	Salivary cortisol, 5 samples through the day used to create AUC for all	Obese participants had lower salivary cortisol, but differences no longer significant when adjusted for	Small numbers and so power likely to have been an issue for small effect sizes in adjusted

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
	l in an obese group from an obesity clinic versus healthy controls				though puberty measured	day cortisol	age and gender.	analyses.
Guseman et al (2015)	CS. Patients from a weight manag	50 obese patients aged 12.3-18.9 mean 14.8	**None**	Yes, no gender differences. 30% male	Yes. Considered in analyses, and no differences.	Salivary cortisol - measured through the day at time intervals, then area under curve derived from samples.	Mean AUC cortisol was not associated with presence versus not of metabolic syndrome. Severity of obesity not associated with AUC cortisol.	Small numbers. 69% had an initial increase after waking.

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Type of stress measurement	Findings / other MetS features measured/ control for other confounders	Comments
	ement clinic							

KeyCC: case-control study; CS: cross-sectional study; CH: Cohort study ; UFC = Urinary free cortisol; HPA : Hypothalamic-pituitary-adrenal axis. BMI = Body Mass Index. DEXA = dual-energy X-ray absorptiometry; SDS = standard deviation score; GH = growth hormone. WBTM = whole body fat mass. TBFM = Truncal body fat mass. ACTH = adrenocorticotropic hormone. RIA = radio-immunoassay cortisol measuring technique

Table 2.3: summary of psychological papers

Author	Design	Numbers (ages); cases with obesity*	Numbers (ages) non-obese (controls)	Sex considered separately in analyses?	Age or Puberty considered in analyses?	Measure of stress	Findings / other MetS features measured/ control for other confounders	Comments

Zeller (2004)	CS: Recruited from a pediatric interdisciplinary weight management clinic -69% of potentially eligible recruited	121 children (8-17 yrs)	None	No	Separated into two groups: children 8-11 and adolescents 12-17. No pubertal consideration	Behaviour assessment system for children questionnaire – self-report (SRP-C and SRP-A) which includes a measure of social stress	Intercorrelations and hierarchical regression analysis between z-BMI and self-report of social stress not significant in both children and adolescents.	A number of other psychological domains examined in this paper – including depression, anxiety and reported parental psychological distress. These were not included in the systematic review.
Riberio (2005)	CC: consecutive patients obesity recruited from a hospital obesity clinic.	39 children (mean 10yrs +/0.2yrs)	10 aged matched lean controls: BMI <85th centile Unclear how controls recruited (mean 10+/-0.3 yrs).	No	Age matched but no pubertal consideration	Mental stress test using a Stroop colour word test – identify colour not the word. Mean BP during test	Mean BP increased in obese children but not in lean (graphically represented lean flat). Two-way ANOVA for changes between groups significant p=0.01. No difference in heart rate changes. Both diet alone and diet + exercise (4 months) reduced rise in BP in obese children (p<0.05) both cases, and were comparable to lean controls.	Obese children have a higher BP response to stress event; this reduces after weight loss and weight loss with exercise. No exercise alone group included.

Kubiak (2008)	CS. Participants on a 6-week inpatient weight management program. (Known to binge eat.)	16 obese females (14-17, mean 15.5 yrs).	None	All female	No though most likely to be late puberty given age.	Electronic diaries with prompt to complete 4 times per day for 7 days: i) daily hassle yes or no. ii) momentary negative mood (scale by Positive Negative Affect Schedule). iii) rumination about eating yes or no iv) current desire to eat (visual analogue scale 0 – 30)	Random-intercept mixed regression model found significant interaction ($p<0.05$) between rumination and number of hassles as well as rumination and negative affect.	Subjective report of what a daily hassle was. Small sample size. Was in a setting where food intake was restricted – may not represent normal life.
Porter (2010)	CS Initial intake interview into a weight management program	135 aged 11-18 yrs	**None**	Yes No gender differences were yielded among factors assoc. with psychological	No	30-60 minute Interview questioning of perceived stressful events leading to weight gain.	66%? (where is this value from) reported an event triggering weight gain (19% family event; medical issues 13%, relocation 8%; interpersonal issues with peers 3%).	No associations made between degree of obesity

				well-being				

KeyCC: case-control study; CS: cross-sectional study; CH: Cohort study ; UFC = Urinary free cortisol; HPA : Hypothalamic-pituitary-adrenal axis. BMI = body mass index

AUC = area under the curve.

Chapter 3 : Methods

Here I present the methodology of the thesis which was used to address all 6 research questions.

3.1 The Healthy Eating And Lifestyle Trial (HELP): the source of data for the thesis.

The main HELP trial has now been published in detail and the reader is directed to the publication.[2] However, I will outline key details here. As discussed in Chapter 1, I was employed as the medical research fellow for the HELP trial for the entirety of the trial.

The HELP trial was a randomized controlled trial of an obesity intervention aimed at weight loss in adolescents with obesity. The intervention consisted of a ten session program using a manual, which worked with young people, and where possible their families, using motivational interviewing techniques and solution focused methods to help achieve weight loss. This was compared to control which was a single once off session with a General Practice Nurse. In the HELP trial, the intervention was not successful in supporting weight loss with no difference at 6 months between intervention and control for change in BMI (effect of the intervention at 6 months after controlling for age, sex and BMI at entry in the trial was -0.11 (95% confidence intervals -0.62 to 0.40, p = 0.7).[2] There were also no differences in any other cardio-metabolic marker or adiposity measure (waist circumference or fat mass).

Participants of the HELP trial were recruited from the community sources such as GPs, schools, youth groups and self-referrals. Participants were recruited from the Greater London region. This was a study of adolescents with obesity (>95th centile BMI) with age group 12-19. Information on baseline characteristics is presented in Chapter 4.

The following participants were excluded from the HELP trial:

1. Individuals with chronic illnesses (excluding asthma, unless they had had more than 3 courses of oral steroid in the preceding year; and note

participants on inhaled steroids were included; or more minor chronic issues such as eczema)

2. Individuals with known or suspected genetic or endocrine causes of obesity.
3. Individuals with diagnosed learning difficulties and mental health diagnoses such as depression or anxiety

Recruitment ran from January 2011- July 2013, and (as explained below in section 4.2) participants were repeatedly measured at assessments at 4 discrete intervals :

1) Time of entry into the trial/ baseline (henceforth referred to as time 0/t0)
2) 3 months (henceforth referred to as time 1/t1) post first assessment
3) 6 months (henceforth referred to as time 2/t2) post first assessment
4) 1 year (henceforth referred to as time 3/t3) post first assessment.

Because of attrition, not all participants originally recruited had data collected at each time point, and totals at each time point are presented in chapter 6 which focuses on longitudinal results and analyses. Some participants also had home visits organized for subsequent data collection to maximize information for the outcomes measured in the HELP trial (this is discussed in more detail below). For a small group of participants data for time point 3 were chased some time after 1 year, but for most time point 3 was close to 1 year. This is evident and described in the longitudinal analyses in chapter 6.

Recruitment and retention was indeed a significant issue for the HELP trial, as it is for many trials.[154 155] Leading and providing input on recruitment, which included visiting GP surgeries, communicating with the media and leading a group of research assistants in recruitment, was key part of my role as the research fellow. Final power was reached for the study, but mid study it was possible that the trial would be closed down to the ethics of recruiting to a trial that was not expected to meet power. The experience of problems and solutions for recruiting was a key part of collecting information for this thesis. Concern about any additional burden on

participants in collecting the additional data for my thesis was an important factor in a number of decisions relating to the methodology in my thesis, which I will outline with each aspect of data description below.

Ethics permission for the HELP study was provided by the regional, central London ethics committee, with all extra data collected as presented below for this thesis were accepted as amendments by the ethics committee.

3.2 Medical assessments and outline of data collected at each visit

3.2.1 General background to the assessment and time points

A summary of data collected on participants relevant to this thesis at each visit over time is shown in table 3.1 below. As mentioned above, some participants did not attend all visits and some had more rudimentary data collected from a visit at an agreed location closer to the participant's home, which included visiting them in their home. Ethics permission was sought and achieved for this change in the original protocol.

Participants were initially invited to attend an initial visit at the Clinical Research Facility at Great Ormond Street Hospital (CRF-GOSH) at time 0. This visit served three purposes : 1) for face-face discussion of the trial and consent, 2) to collect information to allow screening for inclusion and exclusion criteria and 3) to collect data at baseline (before randomization into the trial). It was my responsibly to obtain consent from all participants at the first visit. We obtained signed consent from the parents of participants under the age of 16 and signed assent from participants aged under 16. Participants aged 16 or over were asked to sign consent forms themselves. I undertook good clinical practice training prior to the start of the trial to ensure appropriate skills and acknowledge around consent and conduct during research.

Data collection was the shared responsibility of the nursing staff at the CRF-GOSH and myself, though certain data collection tasks were unique to individuals, and this

is explained as individual data collection descriptions below. For the HELP trial, all individuals measuring any variables over time (thus myself and the nursing staff) were blinded to the intervention/control status of the participants. I was involved with coordination of the main data collection at time 0 and time 2; nursing staff were responsible for data collection at time 1 and 3: this was mostly handing out and collecting questionnaires, and measuring adiposity and blood pressure measurements. Nurses at all 4 time points measured adiposity and blood pressure measurements. The same equipment, technique and protocols were used for all visits and all participants throughout the trial. As mentioned above, whilst most of the assessment happened at the CRF-GOSH, some visits happened at home by a trained researcher, and not all participants had data at each time point.

Table 3.1: Summary of information collected at each time point of the HELP trial.

TIME	**Time 0** **(0)**	**Time 1** **(3 months)**	**Time 2** **(6 months)**	**Time 3** **(1 year)**
Function Of Assessment	Assessment for eligibility and for data collection.	Data collection (by now in the intervention)	Data collection	Data collection
Demographic data: **Ethnicity** **Smoking status** **Deprivation score (from postcode)**	 ✓ ✓ ✓			

TIME	Time 0 (0)	Time 1 (3 months)	Time 2 (6 months)	Time 3 (1 year)
Puberty	✓	✓	✓	✓
Anthropometry	✓	✓	✓	✓
Blood pressure	✓	✓	✓	✓
Pulse wave velocity	✓		✓	
Blood testing	✓		✓	
Salivary Cortisol collection	✓		✓	✓
Questionnaires	✓		✓	

3.2.2 Details on the medical assessments

I provided an initial medical assessment for all participants at baseline. The focus of this assessment was to assess whether participants met inclusion and exclusion criteria. For all participants the following was performed:

- Physical assessment for any signs of syndromes – in particular potential genetic syndromes and Cushing's syndrome.

- Details of parental heights were taken to establish predicted height to check that the participant did not have short stature, which would alert to short stature, which might point to an endocrinopathy.
- Previous and current medical history assessment to screen for chronic conditions and medicines use.
- Mental health screen for existing involvement with CAMHS. Screening questionnaire for suicidal thoughts.
- Assessment for presence and grade of acanthosis nigricans : see section 4.5 below.

3.2.3 Risk and safeguarding

As the medical lead for the trial I was responsible for safeguarding children. I provided a safeguarding course for the non-clinical research team members. There was a clear protocol for what would happen if safeguarding issues were raised amongst the research team. Whilst patients were at the CRF-GOSH, hospital safeguarding protocols were in place.

There were three areas of medical risk identified at screening and assessments in the trial:

1. Identification of potential medical problems such as endocrinopathy at assessments. This also included finding significant hypertension. The protocol here was for me to contact the participant's GP and request referral to local secondary care pathways. This was the case for two young people (not entered into the trial and not included in this thesis) one of whom was found to have Diabetes Mellitus Type 2 on screening with HbA1c (see below) and was successfully referred to a secondary diabetes service; and a second young person who was identified to have abnormal liver function testing and was referred to a regional liver unit for further investigation.
2. Identification of potential too rapid weight loss leading to medical instability[156] – see comments on blood pressure below.

3. Identification of significant and serious co-morbid mental health disorders. A questionnaire, the E-26, used in the trial (see 3.11.4 below) asked for suicidal ideation in the last 6 months.[157] The protocol here was for me to contact the GP directly and recommend referral urgently to local child and adolescent mental health services, as well as ensuring that the young person was leaving the CRF-GOSH to a place of safety (either home with parents or to accident and emergency). This was the case for two young people (not entered into the trial and not included in thesis) who were found to be actively suicidal and were successfully picked up by their GP urgently.

3.3 Demographic data collected

3.3.1 Ethnicity

Ethnicity was self-reported at baseline on questionnaire and then grouped into 4 groups – white, black, Asian (South Asian e.g. Indian, Pakistani, origin) or mixed/other (e.g. Chinese) for use in analyses.

3.3.3 Smoking status

Because of the possible relevance to arterial stiffening, information was also collected on cigarette smoking by written questionnaires at baseline. Participants grouped as either: 1) never smoked; 2) currently smoked; or 3) either currently smoked or had smoked in the past; with these groups used for analyses.

3.3.4 Index of Multiple Deprivation

Index of Multiple Deprivation (IMD) scores were calculated using postcode for usual place of residence (so in the case of subjects whose parents were separated, main place/most frequent place of residence was used) at baseline. IMD is the official measure of the UK government for relative deprivation for small regions in UK.[158] For each participant raw IMD score was used for analyses (with greater score meaning more deprivation). Greater IMD score implies greater deprivation. IMD information

was also available as quintile of the population in the UK and this was used for summary of participants at baseline.

3.4 Pubertal assessment

It was decided that direct measurement of puberty would have been of too higher burden on participants, and so therefore participants were asked to self-report their pubertal status by Tanner stage using a published pictures chart which used pubic hair for both sexes, genital appearance for males and breast appearance for females.[159] Puberty was recorded at all time collection points (time 0-3). Female participants were also asked to provide information on whether they had reached menarche. Once this information had been collected, pubertal status was then grouped into three stages – pre/early (Tanner 1 and 2), mid (Tanner 3 and 4), late/complete (5) for use in analyses. Any girl who reported that they had reached menarche was grouped as late/complete by default.

3.5 Assessment of acanthosis nigricans

I measured AN at the neck by clinical inspection for each participant using a previously reported grading system [160] as follows:

1. Absent
2. Mild (limited to base of skull not reaching lateral margins of the neck)
3. Moderate (extending to lateral margins of neck but not visible from front)
4. severe (visible from the front).

AN was recorded at all 4 time collection points. These findings were then dichotomized into two variables for analysis : 1) AN present or not and 2) severe grade or not (i.e. milder grade or not present).

3.6 Anthropometric data collected

All participants were measured for anthropometry by trained nurses at the Clincial Research Institute, Great Ormond Street Hospital using protocols.

3.6.1 Height

Heights were measured to the nearest 0.1cm using an electronic stadiometer (Seca 242 Electronic Measuring Rod, Seca GmbH & Co.KG, Germany). Participants were asked to remove shoes before having their height measured. Participants were instructed to stand underneath the head stop, with their back to measuring rod, with feet together and flat on the floor and heels touching the base plate. With legs straight, buttocks and scapula against the wall and arms loosely at their side, the head stop on stadiometer was lowered until it touched the participant's head. It was ensured that head was in the Frankfurt Plane (corner of the eyes horizontal to the middle of the ear). Participants were asked to breathe in normally and exhale, with the measurer exerting upward pressure on the mastoids as the measurement was read. Height was recorded at all 4 time points (t 0-3).

3.6.2 Weight

Weights were measured to the nearest 0.01 Kg on the Tanita BC 418MA (Tanita, UK) with scales on a solid surface. The participant was weighed bare foot, in loose clothing. Participants were asked to empty their pockets of mobile phones, change and wallets etc. Heavy jewelry was also removed. 1 kg was removed from each measurement and for each participant to allow for the weight of remaining over clothing. Height was recorded at all 4 time points (t 0-3)

3.6.3 Fat mass and fat mass index.

Total impedance from the Tanita BC 418MA (Tanita, UK) was used to generate a value for fat mass using a validated formula published for use in adolescents : Free fat mass = -2.211 + 1.115(height^2/impedance).[19] Fat mass was then converted into a fat-mass-index which was derived by fat mass / height^2, analogous therefore to BMI; and was used for analyses rather than raw fat mass. Fat mass was measured and

index derived at all 4 time points (t 0-3). Throughout this thesis, fat mass index is referred to as fat mass index or FMI in tables and graphs.

3.6.4 Measures of abdominal circumference

Waist circumference was measured midway between the 10th rib and iliac crest to the nearest mm using a non-elastic flexible tape in the standing position.[16] Waist circumference was measured three times and averaged. Waist circumference was recorded at all 4 time points (t 0-3).

Anterior-posterior sagittal abdominal dimension (SAD) was measured to the nearest 0.1cm by caliper (Holtain Kahn abdominal caliper, Holtan Ltd UK) at the centre of the abdomen following exhalation with participants in a supine position.[17 18 161] The caliper was initially slid to its fullest height. The participants were then asked to raise their hips so that the base of the caliper could be slid below the lumbar-sacral region of the back. The caliper was then adjusted via its upper arm down until it was just above the mid-abdomen. The caliper contains a bubble which acts as a spirit level. This was used to ensure that the vertical angle of the caliper was 90 degrees to the surface that participant was lying on. Participants were then instructed to inhale and then fully exhale and hold, whilst the top arm of the caliper was slid down to touch the skin of the abdomen, resting without compressing. The diameter of the abdomen was then read to the nearest 0.1cm. SAD was recorded at all 4 time points (t 0-3).

3.6.5 Derived variables from anthropometry measurements

BMI was calculated using weight and height (weight (Kg)/height (m)^2), and fat mass index was calculated from fat mass (Kg) / height (m)^2. BMI and waist circumference z-score (BMI z and waist z) were generated using the LMSgrowth program version 2.69 (Harlow Healthcare, UK) which utilizes UK 1990 population growth reference

data.[162 163] As discussed above (4.6.3), fat mass derived from the Tanita machine and validated formula was converted into fat mass index. As no validated standardized data is currently available for fat mass index and SAD, raw data were used for analyses. Participants were further grouped by BMI z into above/below 2.5 z [164] and 3.5z[1] as two conventionally referred to cut-offs for severe obesity as discussed in Chapter 1. Throughout this thesis, BMI z-score is referred to as BMI z and waist z-score as waist z.

3.7 Blood pressure measurement

Blood pressure (BP) was measured using an automated machine (Philiips IntelliVue MP30 Monitor, Koninklijke Phillips N.V, Holland). Blood pressure was measured using an appropriate sized cuff as per published guidelines for obesity.[1] Participants were rested, seated for 20 minutes then the BP was taken at the right arm three times serially, with the third value recorded as data. Tight or restrictive clothing was removed from the participant's arm and the participant's arm was positioned horizontally at the level of the mid-sternum and ensured that it was well supported. Systolic and diastolic blood pressure was recorded at all 4 time points (t 0-3).

Systolic and diastolic BP were then converted to z scores using LMSgrowth program version 2.69 (Harlow Healthcare, UK) which utilizes UK BP population data.[163 165] Throughout this thesis, systolic BP z-score is referred to as systolic z and diastolic BP z-score is referred to as diastolic z.

Hypertension was defined >98th centile hypertension (>2.06 SD above the mean) for either systolic or diastolic BP.[1]

A lying and standing blood pressure measurement for comparison, as well as resting pulse was taken as a screen for potentially rapid weight loss, as explained above; but were not used in analyses as part of the thesis.

3.8 Pulse wave velocity measurement

I was trained to measure PWV on participants by the Vascular Physiology Department at the UCL Institute of Child Health using Sphygmocor, (AtCor Medical, Sydney Australia) using carotid-radial method. Training included demonstrating sufficient accuracy and reproducibility to a trainer (Alicja Rapala – see acknowledgements). I performed all PWV measurements on participants. It was a decision made by the trial leads that the carotid-radial methodology be used rather than carotid-femoral to limit burden on participants for the main trial (in the context of the issues with recruitment and retention for the HELP trial: see 4.1). It was also decided that measurements should only be measured once at 0 and time 2.

How PWV is calculated and derived is explained in a theoretical sense in Chapter 1. Here I will describe the practical points for measuring PWV on participants for this thesis. Patients were in a fasted state and rested for 30 minutes prior to measurement with room temperature 20-22 degrees. Pulse waves were measured at carotid and the radial pulses using tonometry and equipment associated software (Sphygmocor, AtCor Medical, Sydney Australia). The distance between the carotid and sternal notch, and sternal notch to the radial point (via the mid shoulder) were also measured, and combined with waveforms, ECG readings from 3 applied leads, and peripheral blood pressures using the software to derived carotid-radial PWV values.

I used the standard quality control measures for Sphygmocor. Specifically this was to ensure a standard deviation of derived PWV < 10% of the PWV value, all PWV wave forms within window and that the timing SD < 6%. If time allowed I repeated measures at time points to reach quality control. Data was only recorded if it met quality control measures. PWV was only measured once per participant at time 0 and 2.

3.9 Blood testing

Blood was taken by venesection, the majority of the time by the nurses of the CRF-GOSH, however I also took bloods if there was difficulty obtaining blood samples or a nurse was not available.

Blood was drawn following a 10-12 hour fast. Bloods were analyzed for cholesterol (including HDL), triglycerides, glucose, % glycated haemoglobin (HbA1c), insulin and Alanine aminotransferase (ALT). Blood was processed at Great Ormond Street Hospital laboratory. HOMA-IR was derived by using formula (insulin x glucose/22.5). Additional abnormal binary variables were generated for individual blood variables for above/below abnormal cut-offs based on a UK consensus statement,[1] and as described in chapter one, as follows : "Abnormal HOMA-IR" above 4.4, "Raised insulin" for insulin levels above/below values based upon pubertal stage (>10 mU/L pre/early puberty, >30 mU/L mid puberty, mU/L>20 late and complete puberty), "Low HDL" below <0.9 mmol/L, "Raised triglycerides " above >1.47 mmol/L, "Raised cholesterol" above >5.2 mmol/L, "Abnormal HDL/C ratio" above 4.3.

Blood results were checked within the first 24 hours after processing to screen for diabetes mellitus. Any participant with fasting glucose or HbA1c suggestive of diabetes was excluded from the trial and referred on for medical follow-up as explained in the risk section above (4.2.3). Similarly, anyone who was found to have an ALT > 100 were excluded and referred on to a liver specialist. Though most raised ALT were expected to be due to fatty infiltration of the liver associated with obesity, it was decided that this was important for the well-being of participants.

3.10 Stress measures collected

3.10.1 Salivary cortisol

Salivary cortisol has been demonstrated to correlate well with free plasma cortisol,[166] and represents a less invasive mode of measuring cortisol and allows ambulatory measurement. This is valuable as multiple measures through the day allow more accurate measurement of daily cortisol profile.

Salivary cortisol samples were collected at time 0, 2 and 3 HELP visits. Morning salivary cortisol samples were collected at home using a swab before attending assessments (Salivette® cortisol swabs, Sarstedt, USA.) which participants brought with them. The use of such swabs have been validated for use in human experiments and have been shown to effectively correlate with blood cortisol levels. [166]Participants were instructed to put swabs in their mouth and chew for 45 seconds until the swab was completely wet, and then place it in a protective plastic shell which was provided. Participants were instructed to take a sample as soon as they woke and then 30 minutes later (they were asked to put samples by their bed the night before the assessment and were contacted the day before as a reminder), and also asked to record the time of the samples taken. On arrival at assessments, salivary cortisol samples were collected along with times of the samples which were recorded as data. Samples were then frozen and stored at -20 degrees Celsius at the CRF-GOSH.

Additional evening samples were added to the protocol after the study had started after discussion at the PhD upgrade with the aim of increasing the day cortisol profile available. Participants were asked to take an evening salivary sample as close to 22:00 as possible (on the same day as the assessment – see figure). Participants were then asked to record the exact time of sample collection and send in the post

to the research team, the research team providing a pre-paid envelope. Samples are known to be stable at room temperature for several weeks.[167] When samples were received by the research team they were frozen and stored at -20 degrees Celsius.

Samples were then sent in batches to Dr Clemens Kirschbaum, Technische Universität Dresden (TU Dresden) in Germany. Dr Kirschbaum provided the following information on the process: "*After thawing, salivettes were centrifuged at 3,000 rpm for 5 min, which resulted in a clear supernatant of low viscosity. Salivary concentrations were measured using commercially available chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany). Sample and reagent handling was semi-automated using a liquid handling robot (Genesis, Tecan, Switzerland) and quality control samples of low, medium, and high cortisol concentrations were run on each microtiter plate assayed. The intra and interassay coefficients for cortisol were both below 8%.*"

The unit for salivary cortisol was nmol/L. Salivary cortisol values for awakening, 30 minutes post awakening and evening were then used to generate 6 variables for analysis to measure different aspects of day cortisol levels (see figure 3.1). These were as follows : 1) Cortisol on waking (C-wake) 2) Cortisol awakening response rate of change (CAR-Rate) 3) Cortisol awakening response are under the curve (CAR-AUC) 4) Evening cortisol (C-evening) 5) Ratio of Cortisol evening to cortisol on waking (C-ratio) : evening:awake calculated by evening/cortisol on wakening values and 6) Total day cortisol area under the curve from awake to evening (C-DayAUC). Where 30 minute after waking cortisol values were less than the cortisol on waking value, it was likely that the CAR had been missed due to timing mismatch of collection, which is well recognized as a common phenomenon when collecting samples for CAR in an ambulatory setting.[119 168] Negative values of CAR-AUC and CAR-Rate were therefore not entered into analyses as they were not expected to be true measures of the CAR. All cortisol data were used for the other derived variables.

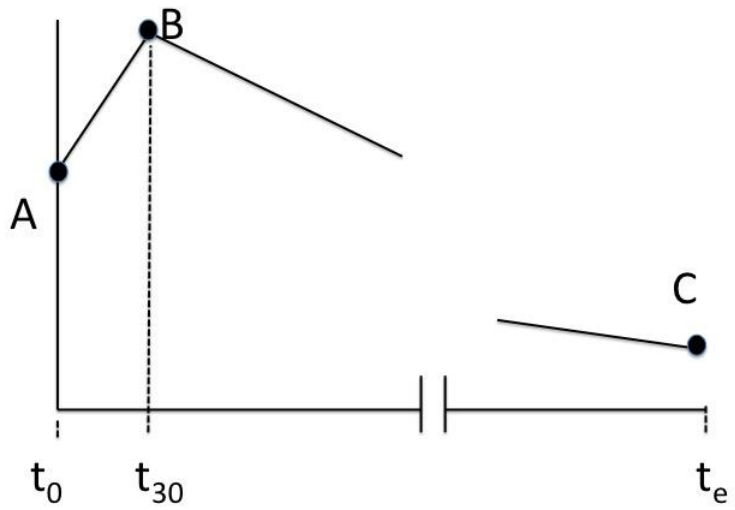


Figure 3.1. Cortisol variables and how they were derived

*Participants were asked to provide salivary cortisol samples at time of wakening (A, t_0), 30 minutes after waiting (B,t_{30}) and then an evening sample (C,t_e) : Six variables were then derived for analysis : 1) Raw value at waking, A (=C-wake); 2) rate of increase on wakening (B-A)/t_{30}) (=CAR-Rate); 3) Area under the curve A to B (0.5*B-A*t_{30} + t_{30} * A) (=CAR_AUC); 4) raw evening value, C (=C-evening;) 5) Ratio of evening to wakening C/A (=C-ratio) and 6) Area under the curve for the day (C * t_e – t_{30} +(0.5 * B-c) x (t_e – t_{30}) + Area of the curve A to B (=C-DayAUC). Note t abbreviations here refer to timing of cortisol measurements not HELP trial visits*

3.10.1 A-FILE questionnaire

The A-FILE questionnaire (Adolescent family inventory)[169] was used as a questionnaire to measure exposure to stressful life events affecting the participant individually and their family as measure of systemic stress in the preceding 12 months to assessment. Some of these events are normative (e.g. starting school), and others non-normative (experience of death of a parent). Designed for 12 to 18

year olds, The A-FILE consists of 50 items reported as yes or no, which are scored as 1 or 0 respectively. A number of sub scores by category are provided, but the recommendation (and as used in this thesis) is to give a total score out of 50, "called Total Recent Life Changes", with a greater score meaning greater perceived stressful events. McCubbin and Patterson have published validation of this score in 1981, which reported an internal reliability of alpha 0.69 using Cronbach's alpha, and a test-retest reliability of r =0.82.[170] The A-file was used at time 0 and 2. The questionnaire was delivered in paper form and scored by the data manager for the HELP trial. This questionnaire, along with the other questionnaires in this thesis are included as an appendix.

3.11 Other relevant questionnaires

3.11.1 Strengths and difficulties questionnaire (SDQ)

The SDQ is a validated questionnaire which looks at different domains of potential mental health problems (emotional difficulties, conduct difficulties, hyperactivity/inattention difficulties, peer relationship difficulties and prosocial behavioural difficulties.[171] [172] The SDQ provides a total score (with higher score meaning more difficulties) and then a graded high versus low risk binary score for any disorder and a disorder in the above listed domains. The questionnaire was delivered in paper form and scored by the data manager for the HELP trial at time 0 and time 2; the paper questionnaires are included in this thesis as an appendix. For analysis, participants were classified as probable mental health diagnosis or not.

3.11.2 Developmental and wellbeing assessment (DAWBA)

The DAWBA is a validated package of questionnaires,[173] which was delivered on the computer before arriving for the first assessment. Input was requested from young people and their parents, and the answers were then used by the DAWBA team to derive predictions for important mental health diagnosis based on DSM-IV. If participants and their families had not been able to complete the questionnaires at home then an opportunity to do so was provided at the CSF-GOSH. Predictions were

derived even if only a young person or parent completed the questionnaire alone. The DAWBA categorises young people into one of six probability bands for each mental health disorder (from >0.1% to >70% chance).[174] DAWBA is a well established and frequently used research tool, and is also used in clinical contexts as a predictor for mental health.[175] Because few young people had > 70% risk for any mental health disorder, a binary variable was created for >50% or below 50% risk.

3.11.3 Rosenberg's Self Esteem Score (RSE)

The RSE is a validated questionnaire, which was originally designed for use in adolescents in the 1960s.[176] Since then it has been used in many research settings and is established as a principle method for assessing esteem by self-report in research.[177] [178] A greater score implies higher self-esteem. The questionnaire was delivered in paper form and scored by the data manager for the HELP trial at time 0 and time 2; the paper questionnaires are included in this thesis as an appendix

3.11.4 The Eating Attitudes Test (EAT-26)

The EAT-26 is a widely used, standardized questionnaire for self-report of symptoms associated with eating disordered pathology.[157] It is mostly used to establish eating disorder risk and is used in clinical and research contexts. Here, the particular feature of this questionnaire that I was interested in was to look for evidence of binge eating, which is asked and coded in binary form as "*Have you gone on eating binges where you feel that you may not be able to stop? (Eating much more than most people would eat under the same circumstances)".* I was particularly interested in this as it allowed an opportunity to look for associations between stress measures (cortisol and A-FILE questionnaire) and eating behaviour that might be causal in degree of obesity, so to look for a potential mechanism how stress might influence cardiovascular risk (as discussed in chapter 2). Another question which was allied to the binary question about behaviours around binge eating was asking for number of binges in the last 6 month, which provided a continuous variable to look for associations between stress and degree of binging for mechanism. This

questionnaire was completed at time 0 and time 2. The questionnaire is shown, with other questionnaires, in the appendix

3.12 Analysis of HELP data in this thesis

3.12.1 General analysis methods

I collected and collated all data at each assessment apart from the measurements performed at home. Data were entered into the HELP trial database by a trained data manager. I performed all analyses using STATA version 13 (StataCorp, Texas, USA). Statistical significance level, as per convention was set at a $p < 0.05$ for type 1 errors in statistical analysis. In tables statistical significance is marked by the presence of an asterix "*". P values were rounded to 1 decimal place ≥ 0.2 and to decimal places < 0.2.

Where non normally distributed data were presented in tables, summaries were provided using median and inter-quartile ranges, and for normally distributed data means and standard deviations. Comparisons of normally distributed distributions where matching of pairs was not required (see longitudinal data analysis below) were done using t-tests, for non normally distributed data Mann-Whitney U.

At baseline, data were examined for normality by graphical assessment of distributions as well as application of STATA's normality testing function which uses the Jarque-Bera test, which looks for goodness-of-fit as to whether a sample variable has skewness and kurtosis in keeping with normally distributed data.[179] Where data were found to be not normally distributed, the ladder of powers function was used on STATA which provides 9 logarithmic transformations (cubic, square, identity, square root, log, 1/(square root), inverse, 1/square, 1/cubic) and provides Jarque-Bera testing for derived transformed data to allow selection of best goodness-to-fit for normality. As I will show in the results, some of the skewed data were unable to be normalized by transformation. Regression models were repeated for non-

transformed and transformed variables, with transformed variables used in models only if there was a significant difference.

3.12.2 Cross sectional regression analyses

To test hypotheses of possible associations at baseline, associations (in chapter four and five) between variables were examined by using linear regression. Univariable models were presented with numbers used in the model, coefficients, 95% confidence intervals for coefficients and corresponding p-value. Individual variables found to be associated with at $p < 0.10$ were entered into multivariable regression models. For binary outcomes (for example abnormal HOMA yes or no), logistic regression was used instead of linear regression, similarly using univariable regressions in the first instance and then building up models with variables found to be significant (with odds ratios presented instead of coefficients). As BMI z, waist z, FMI and SAD are recognized as proxies for adiposity (whilst also independently associated), each was entered into univariable and multivariable regression models discretely. Effect sizes of models were described using coefficients of determination (R^2) for univariable and multivariable models, with small, medium and large effects defined conventionally as $R^2 > 0.02$, >0.13 and >0.26 respectively.[180] Non normally distributed continuous variables were transformed for use in univariable regression analysis. If the transformation did not alter the models significantly, models using non-transformed variable were presented.

3.12.3 Longitudinal analysis of variables measured at two time points (t0 and t2)

Whilst standardized measures of adiposity such as BMI z and waist z are the optimal method for assessing adiposity in cross-sectional analyses, for longitudinal analysis looking for change in BMI, using a standardized score such as BMI z presents challenges. This is because most of the participants in the trial were at extreme ends of the BMI normal distribution, and as such it would take a large change in weight or BMI within an individual in order to effect quite a small change in BMI z. Cole et al have demonstrated that change in BMI z or centile in children and adolescents over

time are poor measures of change in body fat (DEXA), and have recommended using raw BMI rather than standardized BMI.[181 182] Thus analyses focusing on longitudinal changes used changes in raw scores of waist circumference and BMI rather than by z-score. Raw FMI and SAD were used, as they were not standardized anyway. For completeness, both raw blood pressures and z-scores of blood pressures were included in longitudinal analyses as it is less clear in children what the best measure of change in blood pressure would be (standardized or raw).

All participants would have been randomized to either an intervention or control at times 1-3, and therefore any longitudinal analyses were adjusted for control or intervention status in the HELP trial. Whilst the intervention in the HELP trial did not alter the outcomes measured, there were a number of factors measured and unmeasured in this thesis that might have potentially been affected by the intervention status so I felt it was important to control for it.

Variables with measures at time 0 and time 2 were compared for differences between time points using paired t-test. Whilst some of the data were not normally distributed, t-tests were applied consistently, and this is appropriate for larger sample sizes > 50; [183] however for completeness, for non-normally distributed variables at baseline, results from differences between time 0 and time 2 using t-tests were confirmed using the non-parametric test of the Wilcoxson signed rank test.

To look for associations between change in PWV between 0 and time 2 and changes in the adiposity measures, blood pressure and cardio-metabolic blood markers between time 0 and time2, multivariable linear regression models were used. Models were created to predict variables (e.g PWV) at time 2 as a dependent variable. In these models predictors were 1) the variable at time zero; 2) predictor variables (eg BMI) at time 0 and time 2; and 3) time between t0 and t2 (in years). So for example to examine for a potential relationship between change in BMI between time 0 and 2 and change in PWV the following model was used:

$$PWV2 = \alpha PWV0 + \beta time\_difference + \gamma BMI2 + \delta BMI0 + constant.$$

Similar models were used for the cardio-metabolic blood variables measured only at time 0 and time 2. Sensitivity analyses were also run to include intervention status in each of these models, and were presented in models if there was a significant change in the model.

3.12.3 Multi-level longitudinal analysis for variables measured at four time points (t0-3)

To investigate the change of variables over time, multi-level random effects models were used. This is a repeated-measures analysis method that allows for variation in the actual timing of repeated measures, allows for missing data at certain time points, and also takes account of how the individual changes over time within the group being studied. Firstly, the database was reshaped to long format so that each participant had a measure of variables which were measured at three or more time points (adiposity measures, blood pressure and cortisol).

To examine the change of variables over time, initially models were created using time as a predictor for each adiposity measure, each cortisol measure and systolic and diastolic blood pressure individually. Most individual trajectories in variables were parallel, so random intercept models were produced which provided fixed effects components (presented as coefficient, 95% confidence intervals for coefficients, constants and 95% confidence intervals for constants; and random effects information on the variance (reported as SD) for the constant (providing information about the between subject variation) and on residuals (providing information about the within subject variation). Models are presented in tables in Chapter 6. In these tables, coefficient describes the change in predicted variable by one unit increase in the predictor (so in the case of models predicting BMI over time, the increase in BMI in Kg/m^2); and constants represent average baseline value for the predicted variable (with 95% confidence intervals also presented).

Standard deviations reported for constant and residuals were then used to calculate the % of variance in the dependent variable in the model (e.g. BMI) due to

unobserved participant specific (so within the individual) characteristics that not explained by components in the model, as follows:[184]

$$\frac{\text{SD constant}^2}{\text{SD constant}^2 + \text{SD residual}^2}$$

Once univariable models for variables over time were created, additional variables including baseline demographic information were also added (as well as adiposity measures over time to models of systolic and diastolic blood pressure to examine the relationship between the two over time). Lastly, to assess the association of cortisol measures over time upon adiposity measures, and on BP and systolic BP, cortisol measures were added to individual models of adiposity and blood pressure measure over time.

For each model using time, time^2 was also added to examine whether non-linear models existed

3.12.4 A note on power in the thesis.

The data in this thesis were collected as part of the HELP trial, which was powered to detect an intervention effect of 0.5 standard deviation change in BMI at 0.8 power and 5% significance. The sample size was also inflated to account for the clustering effect of therapists using in the HELP trial. The trial was not powered to examine the outcomes examined in this thesis. Thus it was not possible to power the sample size used in this thesis for its hypotheses. This is discussed in detail in the discussion.

Chapter 4 : Results : cross sectional analyses cardio-metabolic factors and PWV.

Here I present the baseline data from the HELP trial and present analyses to address cross-sectional aspects of research questions 1-3 (namely 1) how degree of adiposity is associated with pulse wave velocity as a proxy of arterial stiffening at a cross-sectional level; 2) how blood pressure is associated with pulse wave velocity as a proxy of arterial stiffening at a cross-sectional level; and 3) how other conventional measures of cardio-metabolic risk such as blood testing and acanthosis nigricans are associated with pulse wave velocity as a proxy of arterial stiffening at a cross-sectional level).

4.1 Baseline characteristics of anthropometric, cardio-metabolic markers and pulse wave velocity data of participants entered into the HELP trial.

Of 519 contacts with young people or their families for potential eligibility into the HELP trial, 210 were invited for baseline assessment. 25 of these young people were found not to meet criteria at assessment, 9 declined to participate and 2 were lost before being entered into the trial. A total of 174 children were recruited and randomized into the HELP trial (65 (37%) male). Source of recruitment for these participants is shown in table 4.1 below.

Table 4.1 : Summary of recruitment routes for participants.

Origin of recruitment	Number (%)
Not eligible for other younger community weight loss programs	7 (4%)
Picked up from advertising in attending clinics in secondary care	12 (7%)
Contacted by GPs who know young person	98 (56%)

Origin of recruitment	Number (%)
had obesity or refereed by GP directly or primary care trust	
School referrals from teachers, school nurses	23 (13%)
Responded to newspaper adverts	5 (3%)
Word of mouth	14 (8%)
Community dieticians	2 (1%)
Pharmacists (advertisements in pharmacies and then referred on)	6 (3%)
Directly from website	4 (2%)
Advertisements within universities	3 (2%)
Unknown/unrecorded	1 (1%)

The participants were ethnically diverse. Proportions in each ethnic group were: white 66 (38%), black 53 (30%), South Asian 36 (21%), mixed/other 19 (11%). Proportions at each pubertal stage were pre/early (Tanner 1/2) 21 (12%), mid (3/4) 38 (22%), late/post (5) 115 (66%). There were more females in complete/late puberty than in other stages (89% versus 12%, $p < 0.01$). The sex ratio was similar in all ethnic groups. 14 (8%) of participants were current smokers and 48 (29%) were either current or ex-smokers. Deprivation level in the sample was high, with mean IMD score 29.80 (SD 13.50) which lies in the 4^{th} quintile nationally.

Characteristics of continuous variables for the study group are shown in Table 4.2. Age was not normally distributed, but the sample appeared evenly distributed across age groups. Males were taller than females but similar in height z-score, while females had greater waist z and FMI. Males had greater metabolic markers as

triglycerides, fasting glucose and ALT. 126 participants (72%) had BMI z > 2.5 and 17 (10%) exceeded 3.5.

Table 4.2 Summary of baseline variables at time 0.

	Males					Females					All subjects				
Variable	**n**	**Mean**	**Median**	**SD**	**IQR**	**n**	**Mean**	**Median**	**SD**	**IQR**	**n**	**Mean**	**Median**	**SD**	**IQR**
Age (years)	65		15.0		3.2	109		15.6		2.9	174		15.3		3.2
IMD score	65	32.0			14.3	109		28.5	12.83		174	29.81		13.5	
Weight (kg)	65		87.4		23	109		85.4		20.6	174		86.2		22
Height (cm)	65	168.4*		9.6	13.2	109	164.3		6.5		174	165.8		8.1	
Height z	65	0.39		0.88		109	0.52		1.00		174	0.47		0.96	
BMI ($kg/m^{2)}$	65		30.9		5.9	109		32.5		6.1	174		32		6.1
BMI z	65	2.83		0.47		109	2.78		0.59		174	2.80		0.55	
Waist (cm)	65		101		15	109		95.4		17	174		99		15.9
Waist z	65	2.97*		0.51		109	3.68		0.68		174	3.45		0.72	

	Males					Females					All subjects				
Variable	**n**	**Mean**	**Median**	**SD**	**IQR**	**n**	**Mean**	**Median**	**SD**	**IQR**	**n**	**Mean**	**Median**	**SD**	**IQR**
SAD (cm)	57	23.1		3.2		102	22.3		3.5		159	22.6		3.4	
FMI (kg/m^2)	63	12.5*		4.0		108	15.0		3.6		171	14.1		3.89	
Systolic BP (mmHg)	63	109		10		109	106		10.		172	107		10	
Systolic BP z-score	63	-1.05		1.03		109	-1.09		1.08		172	-1.07		1.06	
Diastolic BP (mmHg)	63	53		9		109	54		10		172	54		9	
Diastolic BP z-score	63	-0.60		1.10		109	-0.63		1.19		172	-0.61		1.15	
Cholesterol (mmol/L)	65	4.5		0.9		109	4.3		0.8		174	4.4		0.8	

	Males					Females					All subjects				
Variable	**n**	**Mean**	**Median**	**SD**	**IQR**	**n**	**Mean**	**Median**	**SD**	**IQR**	**n**	**Mean**	**Median**	**SD**	**IQR**
Triglycerides (mmol/L)	64		1.1*		0.6	109		0.9		0.6	173		1.0		0.6
ALT (mmol/L)	65		32*		27	109		24		13	174		26		16
HDL (mmol/L)	62		1.1		0.4	108		1.1		0.3	171		1.1		0.3
HbA1c (%)	62		5.5		0.5	105		5.4		0..4	167		5.4		0.5
HOMA	65		3.0		2.9	108		2.4		2.1	173		2.6		2.6
Fasting Insulin (mU/L)	65		15		14.1	109		12.1		10.5	174		13		11.1
Fasting Glucose (mmol/L)	65		4.6*		0.6	108		4.4		0.45	173		4.4		0.5
PWV (ms^{-1})	54	7.3		1.1		92	7.1		1.2		146	7.1		1.2	

4.2 Baseline Metabolic risk summary

4.2.1 Summary of metabolic risk factors

No participant had systolic hypertension or evidence of impaired fasting glucose. Four (2%) had diastolic hypertension, 35 (20%) raised HOMA, 34 (20%) raised insulin for pubertal stage, 29 (17%) elevated cholesterol, 29 (17%) raised triglycerides, 20 (12%) low HDL, and 66 (39%) had an elevated HDL/C ratio. As such only two participants met any of the criteria for the metabolic syndrome using criteria shown above (IDF criteria). Abnormal fasting insulin was associated with BMI z and pubertal stage : abnormal insulin: odds ratio 8.94, 95% CI 3.49 to 22.92, $p < 0.01$ for BMI z and odd's ratio 0.16, 95% CI 0.04 to 0.60, $p < 0.01$ for mid puberty versus pre/early; 0.30, 0.11 to 0.80, $p = 0.02$ for late/post versus pre/early. Abnormal HOMA-IR was associated with BMI z: odds ratio 5.02, 95% CI 2.20 to 11.46, $p < 0.01$.

4.2.2.Relationship between BMI and systolic BP and diastolic BP at baseline

Regression analyses for associations between adiposity measures and raw systolic, systolic-z, diastolic raw and diastolic z blood pressure measures are shown in tables 4.3-4.6. There was a positive association between waist circumference and raw systolic blood pressure (coefficient 0.15, 95& CI 0.01 -0.28, p= 0.03; with a small effect size of $r^2 = 0.03$), SAD and both raw diastolic BP (coefficient 0.48, 95% CI 0.04 to 0.92, $p = 0.03$, with a small effect size of $r^2 = 0.03$) and diastolic z BP (0.06, 95% CI 0.02-0.12, $p = 0.02$, with a small effect size of $r^2 = 0.04$); but no other adiposity measures.

Table 4.3 : Linear regression analyses of adiposity markers as predictors of raw systolic blood pressure.

Systolic BP	N	Coefficient	95% CI coefficient	p
BMI	172	0.14	-0.21 to 0.50	0.4
BMI z	172	1.00	-1.80 to 3.81	0.4
Fat mass index	169	0.09	-0.31 to 0.38	0.7
waist	172	0.15	0.01 to 0.28	0.03*
Waist z	172	0.75	-1.38 to 2.90	0.5
SAD	157	0.40	-0.07 to 0.88	0.09

Table 4.4 : Linear regression analyses of adiposity markers as predictors of systolic blood pressure z-score.

systolic z	N	Coefficient	95% CI coefficient	p
BMI	172	0.01	-0.04 to 0.04	0.9
BMI z	172	0.15	-0.14 to 0.44	0.3
Fat mass index	169	0.02	-0.02 to 0.06	0.4
waist	172	0.01	-0.01 to 0.02	0.19
Waist z	172	0.15	-0.07 to 0.37	0.19
SAD	157	0.03	-0.02 to 0.07	0.3

Table 4.5 : Linear regression analyses of adiposity markers as predictors of raw diastolic blood pressure.

diastolic	N	Coefficient	95% CI coefficient	p
BMI	172	0.01	-0.32 to 0.33	0.9
BMI z	172	-1.31	-3.90 to 1.28	0.3
Fat mass index	169	0.05	-0.32 to 0.42	0.8
waist	172	-0.01	-0.13 to 0.13	0.9
Waist z	172	0.34	-1.63 to 2.33	0.7
SAD	157	0.48	0.04 to 0.92	0.03 *

Table 4.6 : Linear regression analyses of adiposity markers as predictors of diastolic blood pressure z-score.

diastolic z	N	Coefficient	95% CI coefficient	p
BMI	172	-0.01	-0.05 to 0.03	0.6
BMI z	172	-0.12	-0.44 to 0.20	0.5
Fat mass index	169	-0.01	-0.05 to 0.04	0.8
waist	172	-0.00	-0.02 to 0.02	0.9
Waist z	172	0.04	-0.30 to 0.21	0.8
SAD	157	0.06	0.02 to 0.12	0.02*

4.3 Baseline Pulse Wave velocity regression analysis

Pulse wave data were available for 146 (84%) participants. Cases were missing mostly because of lack of time during HELP assessments to collect core data for the HELP trial, and some young people did not want to wait to have PWV measured. PWV was not associated with heart rate (coefficient 0.02, 95%CI 0.00 to 0.07, p = 0.8).

Univariable regression analyses between PWV and anthropometric, cardio-metabolic and demographic markers are shown in table 4.7. Significant positive associations were found between PWV and age, all adiposity markers (BMI z, FMI, waist z and SAD), and abnormal triglyceride grouping (effects sizes were however small, R^2 = 0.05, 0.04, 0.03, 0.03, 0.03, 0.02 respectively). Ethnic South Asians, and to a lesser extent blacks, had higher PWV compared with ethnic whites. PWV was lower in the low HDL group. Abnormal triglyceride grouping was no longer significant once adjusted for age, ethnicity and BMI z (data not shown). Low HDL grouping remained negatively associated with PWV when controlled for BMI z, age, ethnicity group (coefficient -0.60 (95% CI -1.17 to -0.03), beta -0.17, p = 0.04, R^2= 0.17). There were no differences in PWV by sex, pubertal stage, smoking or cardio-metabolic markers (including systolic and diastolic blood pressure). Scatterplots of PWV versus measures of adiposity, with fitted regression lines, are shown in in figure 4.1; In general the associations were weak.

Multivariable regression models for each adiposity measure adjusting for age, ethnicity, abnormal triglycerides and abnormally low HDL, are shown in Table 4.8. Although the significance of ALT in univariable regression was <0.1, it was not included in the final model as its statistical significance was poor on inclusion in models for each adiposity measure; and it also did not affect the quality of the overall models (data not shown). There was a positive association between PWV and all adiposity measures except SAD. Effect sizes for adjusted models for each adiposity measure were medium in size (R^2 in adjusted models for BMI z = 0.16, waist z = 0.13 and FMI = 0.15)

Participants with BMI z > 2.5 SD and > 3.5 SD had greater PWV on average than those below the cut-offs (>2.5 SD : 7.1 vs. 6.8, p =0.04; >3.5 SD: 7.6 vs. 7.0, p = 0.03), however the groups overlapped substantially (see Figure 4.2).

Elements of this analysis were published in: *Hudson L, Kinra S, Wong I, et al. Is arterial stiffening associated with adiposity, severity of obesity and other contemporary cardiometabolic markers in a community sample of adolescents with obesity in the UK? BMJ Paediatrics Open 2017;**1**:e000061. doi:10.1136/ bmjpo-2017-000061* and is included in appendix 9.3.

Table 4.7 : Univariable regression analyses of PWV with demographic, anthropometric and cardio-metabolic variables.

Variable predicting PWV	n	Coefficient	95% CI coefficient	P
Age	146	0.12	0.03 to 0.21	<0.01*
Female sex (reference male)	146	-0.19	-0.59 to 0.21	0.3
Pubertal stage (reference late/complete)	146			
pre/early (1&2)		-0.15	-0.84 to 0.54	0.7
mid (3/4)		-0.12	-0.58 to 0.34	0.7
Ethnicity (reference white)	146			
Black		0.42	-0.03 to 0.88	0.07
South Asian		0.67	0.15 to 1.19	0.01*
Mixed other		0.18		0.6
IMD	146	0.01	-0.01 to 0.02	0.6

Variable predicting PWV	n	Coefficient	95% CI coefficient	P
Current smoker	146	0.27	-0.41 to 0.96	0.4
Ever smoked	146	0.15	-0.27 to 0.59	0.5
Height z-score	146	-0.13	-0.34 to 0.06	0.18
BMI	146	0.08	0.03 to 0.12	<0.01*
BMI z	146	0.44	0.08 to 0.79	0.01*
FMI	144	0.05	0.00 to 0.10	0.03*
Waist z	146	0.27	0.00 to 0.53	0.04*
SAD	132	0.06	0.00 to 0.12	0.04*
Systolic BP	146	0.01	-0.01 to 0.03	0.17
Diastolic BP	146	0.01	-0.00 to 0.03	0.18
Systolic z-score	146	0.02	-0.17 to 0.22	0.8
Diastolic z-score	146	0.08	-0.09 to 0.25	0.3
Cholesterol	146	0.16	-0.06 to 0.39	0.16
High cholesterol versus low	146	-0.35	-0.87 to 0.18	0.2
HDL	142	0.16	-0.60 to 0.91	0.7
Low HDL versus high	142	- 0.63	-1.22 to 0.03	0.04*
Triglycerides	145	0.24	-0.07 to 0.56	0.13
Abnormal Triglycerides versus normal	145	0.52	0.02 to 1.03	0.04*
ALT	146	0.01	-0.00 to 0.02	0.08

Variable predicting PWV	n	Coefficient	95% CI coefficient	P
Fasting glucose	146	0.04	-0.40 to 0.48	0.9
HbA1c	146	0.23	-0.36 to 0.74	0.4
Fasting Insulin	146	0.01	-0.01 to -0.02	0.5
Abnormal insulin versus normal	146	0.17	-0.33 to 0.69	0.5
HOMA	146	0.01	-0.06 to 0.08	0.8
Abnormal HOMA versus normal	146	0.03	-0.45 to 0.51	0.9

Table 5.8 : Multivariable analyses of PWV on adiposity measures (adjusted for age, ethnicity, abnormal triglyceride, and low HDL).

	n	Coefficient (95% CI)	P
BMI z	145	0.49 (0.14 to 0.84)	<0.01*
Waist z	145	0.26 (0.01 to 0.52)	0.04*
FMI	144	0.05 (0.01 to 0.10)	<0.01*
SAD	131	0.05 (-0.13 to 0.10)	0.13

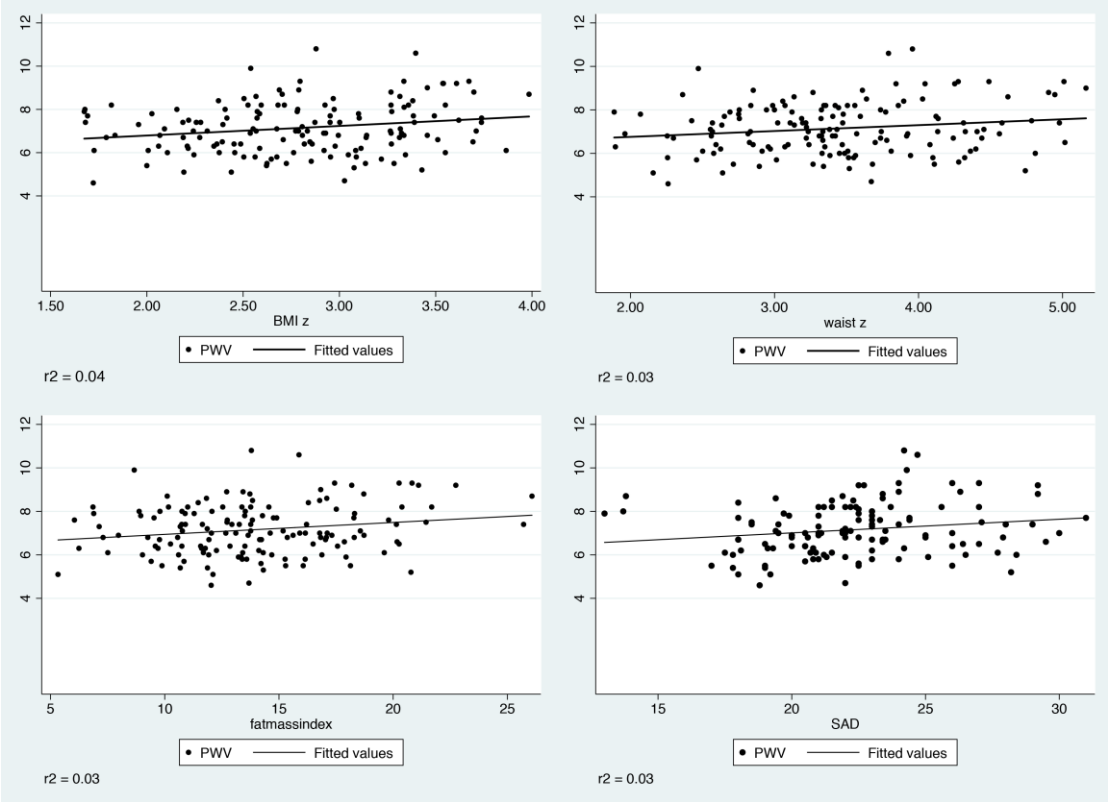


Figure 4.1 : Scatter with fitted regression lines for measures of adiposity against PWV (ms^{-1})

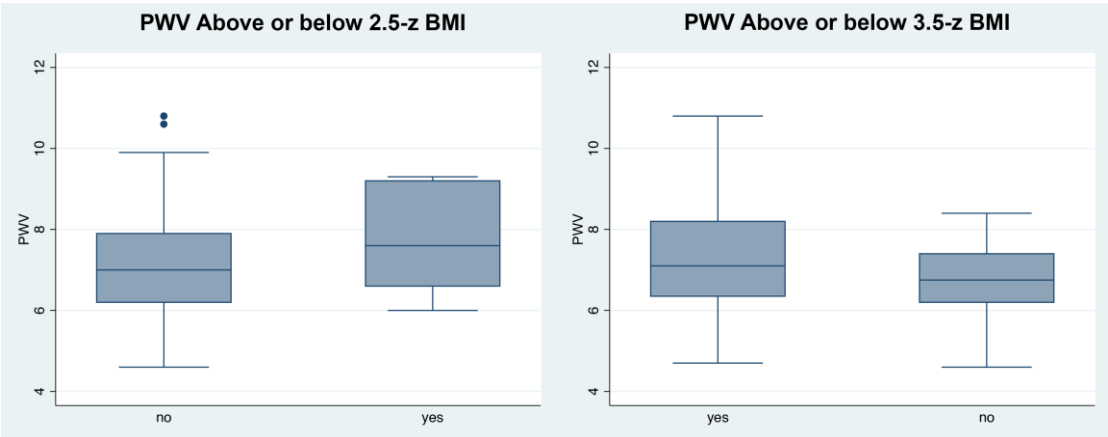


Figure 4.2 : Box plots of distribution of PWV (ms^{-1}) when grouped by presence or not of severe obesity (classified by >2.5z BMI and >3.5z respectively.). Central lines are median PWV.

4.4 Acanthosis Nigricans regression analyses

Assessment of AN was possible in 173 subjects, with AN present in 109 (63%) and 75 (43%) of the sample having severe grade AN. Presence of AN was associated with BMI z (OR 2.12, 95%CI 1.17 to 3.82, p =0.01); and was more common in all non-white ethnic groups (black OR 29.65, 95% CI 9.38 to 93.75, p <0.01; South Asian OR 8.47, 95%CI 3.27 to 21.92, p <0.01; mixed OR 5.24, 95%CI 1.73 to 15.85, p < 0.01).

There was no association between presence or severity of AN and sex, age or pubertal stage. Severe grade AN was associated with BMI z (OR 2.06, 95% CI 1.15 to 3.67, p = 0.02) and was more common in all non-white ethnic groups compared to white: (black – OR 21.92, 95% CI 8.31 to 57.78 p <0.001; South Asian OR 8.90, 95%CI 3.30 to 23.96, p <0.01; mixed OR 4.15, 95%CI 1.26 to 13.66, p = 0.02).

Presence of AN was positively associated with fasting cholesterol after adjustment for BMI z (OR 1.62, 95% CI 1.08 to 2.42, p = 0.01) but not fasting triglycerides, diastolic z or systolic z blood pressure. Severe AN was positively associated with diastolic z blood pressure after adjustment for BMI z (OR 1.36, 95% CI 1.03 to 1.81, p = 0.03) but no other cardio-metabolic marker.

Univariable models for associations of any or severe grade AN with fasting hyperinsulinaemia and abnormal HOMA-IR are shown in table 4.9. Presence of AN was associated with both fasting hyperinsulinaemia and abnormal HOMA-IR; however this association was attenuated after adjusting for BMI z and pubertal stage (table 4.10); with BMI z remaining strongly associated with insulin resistance in these adjusted models (OR 9.19, 95% CI 3.40 to 25.02, p <0.01, 4.91 for abnormal insulin, OR 4.56, 95% CI 1.91 to 10.87, p < 0.01)

Univariable models of associations between presence of any AN and severe AN and PWV is shown in table 4.11. Severe grade AN was associated with PWV, however, this association was attenuated in a multivariable model adjusting for BMI z, ethnic grouping and age, shown in table 4.12. BMI z remained associated with PWV in this multivariable model.

Thus the finding of any AN (or severity) in an obese group of adolescents does not provide additional information about individual cardio-metabolic risk beyond the degree of obesity itself. *Elements of this analysis were published (and presented in appendix 9.3) :Hudson L, Kinra S, Wong I, et al. Is arterial stiffening associated with adiposity, severity of obesity and other contemporary cardiometabolic markers in a community sample of adolescents with obesity in the UK? BMJ Paediatrics Open 2017;**1**:e000061. doi:10.1136/ bmjpo-2017-000061*

Table 4. 9 univariable models with presence of acanthosis nigricans and presence of severe acanthosis nigricans as predictors of insulin resistance

	Fasting hyperinsulinism			Abnormal HOMA		
	N	OR (95% CI)	p	N	OR (95% CI)	p
Any AN present	173	2.68 (1.09 to 6.58)	0.03*	173	1.88(0.88 to 4.00)	0.10
Severe AN present	173	3.44 (1.34 to 8.8)	0.01*	173	1.98 (0.94 to 4.21)	0.07

Table 4.10 a multivariable, adjusted model (for BMI z and pubertal stage) as predictors of acanthosis nigricans and insulin resistance. The relationships between AN and hyperinsulinism are no longer significant.

	Fasting hyperinsulinism			Abnormal HOMA		
	N	OR (95% CI)	p	N	OR (95% CI)	p
Any AN present	173	2.40 (0.85 to 6.76)	0.10	172	2.60 (0.96 to 7.01)	0.06

Table 4.11 univariable regression model of acanthosis nigricans and presence of severe AN as predictors of pulse wave velocity

PWV	N	Coefficient (95% CI)	p
Any AN present	145	0.36 (-0.03 to 0.76)	0.07
Severe AN present	145	0.51 (0.12 to 0.89)	0.01 *

Table 4.12 multivariable, adjusted model (for BMI z, ethnicity and age) of presence of severe acanthosis nigricans as a predictor of pulse wave velocity. The relationship between severe AN and PWV is no longer significant.

PWV	N	Coefficient (95% CI)	p
Severe AN present	145	0.37 (-0.07 to 0.80)	0.10

Chapter 5 : Results : cross-sectional stress, obesity, cardio-metabolic markers and PWV.

Here I present the baseline data from the HELP trial and present analyses to address cross-sectional components of research questions 4-6 (namely 4) how measures of stress are associated with degree of adiposity at a cross-sectional level; 5) how measures of stress are associated with blood pressure at a cross-sectional level; and 6) how measures of stress are associated with pulse wave velocity at a cross-sectional level).

5.1 Baseline characteristics of stress variables

Table 5.1 summarizes the stress variables measured at baseline for all participants, separated by male and female. There were no significant differences between the means and medians of all stress measures between sexes. None of the stress variables were normally distributed. Only rate of change awakening cortisol, evening cortisol, ratio of eve to am could be transformed to be normally distributed. Analyses with transformed variables made no difference. All participants exhibited a normal diurnal pattern of salivary cortisol with lower values in the evening than upon awakening. In 32 cases, the CAR as AUC and rate were negative and so were not included in analyses looking at rate and AUC for the CAR.

Table 5.1 Baseline characteristics of stress measures (cortisol and A-FILE questionnaire) presented as all subjects and by sex.

	Males			**Females**			**All subjects**		
Variable	**n**	**Median**	**IQR**	**n**	**Median**	**IQR**	**n**	**Median**	**IQR**
Awakening cortisol	60	12.71	7.75	103	14.23	9.4	163	13.56	8.8
Rate of change awakening	45	24.32	32.72	86	21.93	20.56	131	22.54	25.24

	Males			Females			All subjects		
Variable	**n**	**Median**	**IQR**	**n**	**Median**	**IQR**	**n**	**Median**	**IQR**
cortisol									
Evening cortisol	20	1.7	2.12	33	1.52	1.33	53	1.54	1.69
Ratio awakening to evening cortisol	20	0.15	0.14	33	0.10	0.06	53	0.11	0.11
Awakening cortisol AUC	45	9.69	4.35	86	10.29	5.34	131	10	4.97
Day cortisol AUC	20	188.16	136.39	33	193.76	98.65	**53**	193.76	137.42
A-File Total Recent life Changes	64	5	8	108	7	6.5	172	6.5	7.5

5.2 Associations of stress variables with baseline participant demographic characteristics

Regression analyses examining associations between the individual stress measures and participant demographic characteristics are show in tables 5.2 to 5.8. There were few associations found. CAR-Rate and C-DayAUC were both positively associated with deprivation (coefficient 0.28, 95% CI 0.03 to 0.53, p = 0.03; coefficient 1.84, 95%CI 0.31 to 3.36, p = 0.02 respectively), CAR-AUC was positively associated with black ethnicity compared to white (coefficient 2.55, 95% CI 0.16 to 4.73, p =0.04)

Table 5.2 Linear regression analyses of demographic associations with C-wake

C-wake	Univariable analyses			
	n	Coefficient	95% CI	p
Age	163	0.44	-0.24 to 1.14	0.2
Sex	163	1.32	-1.71 to 4.36	0.4
Pubertal stage	163			
Mid Puberty versus pre/early		0.81	-4.56 to 6.18	0.8
Late/post versus pre/early		2.31	-2.35 to 6.96	0.3
Ethnicity	163			
Black versus white		0.37	-3.21 to 3.95	0.8
Asian versus white		1.26	-2.78 to 5.30	0.5
Mixed versus white		-2.05	-7.07 to 2.97	0.4
Ever smoked	158	3.10	-0.18 to 6.36	0.06
IMD score	163	0.05	-0.06 to 0.16	0.3

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.3 Linear regression analyses of demographic associations CAR-Rate

CAR-Rate	Univariable analyses			
	n	Coefficient	95% CI	P

CAR-Rate	Univariable analyses			
	n	Coefficient	95% CI	P
Age	131	-0.85	-2.46 to 0.75	0.3
Sex	131	-5.07	-12.12 to 1.97	0.16
Pubertal stage	131			
Mid Puberty versus pre/early		1.78	-11.15 to 14.70	0.8
Late/post versus pre/early		0.72	-10.38 to 11.84	0.9
Ethnicity	131			
Black versus white		1.96	-6.33 to 10.25	0.6
Asian versus white		7.43	-1.49 to 16.36	0.10
Mixed versus white		-4.22	-15.48 to 7.04	0.5
Ever smoked	128	-1.08	-8.72 to 6.56	0.8
IMD score	131	0.28	0.03 to 0.53	0.03*

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.4 Linear regression analyses of demographic associations with C-evening

C-evening	Univariable analyses			
	n	Coefficient	95% CI	P

C-evening	Univariable analyses			
	n	Coefficient	95% CI	P
Age	53	-0.08	-0.31 to 0.15	0.5
Sex	53	-0.44	-1.51 to 0.63	0.4
Pubertal stage	53			
Mid Puberty versus pre/early		-0.91	-2.67 to 0.84	0.3
Late/post versus pre/early		-0.70	-2.22 to 0.81	0.4
Ethnicity	53			
Black versus white		-0.13	-1.42 to 1.17	0.8
Asian versus white		0.32	-1.06 to 1.70	0.7
Mixed versus white		0.68	-1.45 to 2.81	0.5
Ever smoked	50	0.12	-1.27 to 1.50	0.9
IMD score	53	0.01	-0.02 to 0.04	0.5

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.5 Linear regression analyses of demographic associations with C-Ratio

C-Ratio	Univariable analyses			
	n	Coefficient	95% CI	P
Age	53	-0.12	-0.6 to 0.03	0.6
Sex	53	-0.02	-0.22 to 0.19	0.8

C-Ratio	Univariable analyses			
	n	Coefficient	95% CI	P
Pubertal stage	53			
Mid Puberty versus pre/early		-0.08	-0.42 to 0.26	0.6
Late/post versus pre/early		0.05	-0.24 to 0.35	0.7
Ethnicity	53			
Black versus white		-0.06	-0.30 to 0.17	0.6
Asian versus white		0.09	-0.16 to 0.34	0.5
Mixed versus white		0.32	-0.06 to 0.71	0.10
Ever smoked	50	-0.11	-0.37 to 0.15	0.4

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.6 Linear regression analyses of demographic associations with CAR-AUC

CAR-AUC	Univariable model components			
	n	Coefficient	95% CI	P
Age	131	0.15	-0.30 to 0.59	0.5
Sex	131	1.16	-0.81 to 3.11	0.3
Pubertal stage	131			
Mid Puberty versus pre/early		-0.05	-3.58 to 3.47	0.9

CAR-AUC	Univariable model components			
	n	Coefficient	95% CI	P
Late/post versus pre/early		1.74	-1.29 to 4.77	0.2
Ethnicity	131			
Black versus white		2.55	0.16 to 4.73	0.04
Asian versus white		1.67	-0.76 to 4.11	0.18
Mixed versus white		-0.92	-4.07 to 2.25	0.6
Ever smoked	126	1.53	-0.61 to 3.67	0.16
IMD score	131	0.06	-0.01 to 0.13	0.07

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.7 Linear regression analyses of demographic associations with C-DayAUC

C-DayAUC	Univariable model components			
	n	Coefficient	95% CI	P
Age	53	0.69	-10.36 to 11.75	0.9
Sex	53	16.33	-35.43 to 68.09	0.5
Pubertal stage	53			
Mid Puberty versus pre/early		9.37	-77.33 to 96.08	0.8
Late/post versus pre/early		38.84	-36.83 to 114.51	0.2

C-DayAUC	Univariable model components			
	n	Coefficient	95% CI	P
Ethnicity	53			
Black versus white		16.17	-44.47 to 76.81	0.6
Asian versus white		50.58	-12.72 to 113.88	0.12
Mixed versus white		-44.19	-142.05 to 53.66	0.4
Ever smoked	50	40.67	-23.27 to 104.56	0.2
IMD score	53	1.84	0.31 to 3.36	0.02

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.8 Linear regression analyses of demographic associations with A-File questionnaire

A-File Total Recent life Changes	Univariable model components			
	n	Coefficient	95% CI	P
Age	172	0.21	-0.18 to 0.61	0.3
Sex	172	0.98	-0.71 to 2.68	0.3
Pubertal stage	172			
Mid Puberty versus pre/early		0.62	-2.30 to 3.55	0.7
Late/post versus pre/early		1.97	-0.57 to 4.52	0.13

Ethnicity	172			
Black versus white		1.94	0.0 to 3.88	0.05
Asian versus white		-1.60	-3.79 to 0.60	0.8
Mixed versus white		2.15	-0.59 to 4.88	0.6
Ever smoked	168	0.85	-1.00 t0 2.71	0.4
IMD score	172	0.04	-0.02 to 0.10	0.2

5.3 Associations of cortisol variables and A-file questionnaire.

Regression analyses for possible associations between the A-FILE questionnaire at baseline and cortisol measures are shown in table 5.9. There were no associations found.

Table 5.9 Linear regression analyses of cortisol measures associations with A-File questionnaire.

A-File Total Recent life Changes	Univariable models			
	n	Coefficient	95% CI	P
C-wake	162	0.02	-0.07 to 0.11	0.7
CAR-Rate	131	-0.01	-0.06 to 0.04	0.7
C-evening	53	-0.01	-0.19 to 0.17	0.8
C-Ratio	52	0.14	-3.59 to 3.62	0.9
CAR-AUC	131	-0.12	-0.19 to 0.17	0.8
C-Day AUC	52	0.01	-0.01 to 0.02	0.8

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening :

evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

5.4 Associations of stress variables with adiposity and cardio-metabolic measures.

To test if adiposity and cardiovascular risk markers were associated with the stress measures, linear regressions were performed with each stress measure in univariable analyses as predictors of the adiposity measures and other cardiovascular risk measures at baseline. These are shown in tables 5.10- 6.22. The only association found was a negative association between C-Ratio and systolic blood pressure (coefficient -0.94, 95% CI -1.82 to -0.06, p = 0.04).

Table 5.10 Univariable linear regression analyses of stress measures as predictors of BMI z.

BMI z	Univariable regression models			
	n	coefficient	95% CI	P
C-wake	163	0.001	-0.01 to 0.01	0.8
CAR-Rate	131	0.001	-0.005 to 0.005	0.9
C-evening	53	-0.03	-0.10 to 0.05	0.5
C-Ratio	53	-0.36	-0.79 to 0.07	0.10
CAR-AUC	131	-0.01	-0.02 to 0.02	0.8
C-DayAUC	53	0.001	-0.00 to 0.003	0.13
A-File Total Recent life Changes	172	0.02	0.00 to 0.03	0.05

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.11 Univariable linear regression analyses of stress measures as predictors of fatmass index.

Fat massindex	Univariable regression models			
	n	coefficient	95% CI	P
C-wake	160	0.03	-0.05 to 0.07	0.8
CAR-Rate	131	-0.003	-0.04 to 0.03	0.8
C-evening	53	-0.38	-0.91 to 0.16	0.16
C-Ratio	53	-2.55	-5.42 to 0.32	0.08
CAR-AUC	130	0.02	-0.11 to 0.14	0.8
C-DayAUC	53	0.01	-0.003 to 0.02	0.14
A-File Total Recent life Changes	169	0.09	-0.02 to 0.19	0.12

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.12 Univariable linear regression analyses of stress measures as predictors of waist z.

waist z	Univariable regression models			
	n	coefficient	95% CI	p
C-wake	163	0.01	-0.01 to 0.02	0.40
CAR-Rate	131	-0.002	-0.01 to 0.004	0.5
C-evening	53	-0.04	-0.13 to 0.05	0.4
C-Ratio	53	-0.35	-0.84 to 0.14	0.16
CAR-AUC	131	0.01	-0.01 to 0.03	0.4
C-DayAUC	53	0.002	-0.000 to 0.004	0.05
A-File Total Recent life Changes	172	0.02	-0.003 to 0.04	0.10

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.13 Univariable linear regression analyses of stress measures as predictors of SAD.

SAD	Univariable regression models			
	n	coefficient	95% CI	P
C-wake	148	-0.01	-0.06 to 0.05	0.8

SAD	Univariable regression models			
	n	coefficient	95% CI	P
CAR-Rate	121	0.01	-0.04 to 0.02	0.5
C-evening	50	0.05	-0.41 to 0.51	0.8
C-Ratio	50	-1.01	-3.36 to 1.34	0.4
CAR-AUC	121	-0.03	-0.12 to 0.07	0.6
C-DayAUC	50	0.002	-0.01 to 0.01	0.7
A-File Total Recent life Changes	158	0.05	-0.05 to 0.15	0.3

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.14 Univariable linear regression analyses of stress measures as predictors of systolic z.

systolic z	Univariable regression models			
	n	coefficient	95% CI	p
C-wake	161	0.003	-0.01 to 0.02	0.7
CAR-Rate	129	0.001	-0.01 to 0.01	0.7
C-evening	51	-0.07	-0.24 to 0.10	0.4
C-Ratio	51	-0.94	-1.82 to -0.06	0.04*

systolic z	Univariable regression models			
	n	coefficient	95% CI	p
CAR-AUC	129	0.004	-0.03 to 0.04	0.8
C-DayAUC	51	0.002	-0.002 to 0.005	0.4
A-File Total Recent life Changes	170	-0.01	-0.04 to 0.02	0.50

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.15 Univariable linear regression analyses of stress measures as predictors of diastolic z

diastolic z	Univariable regression models			
	n	coefficient	95% CI	p
C-wake	161	0.00	-0.01 to 0.02	0.9
CAR-Rate	129	-0.01	-0.02 to 0.00	0.3
C-evening	51	-0.03	-0.18 to 0.12	0.7
C-Ratio	51	-0.51	-1.32 to 0.30	0.2
CAR-AUC	129	-0.01	-0.05 to 0.03	0.6
C-DayAUC	51	0.00	-0.004 to 0.003	0.6
A-File Total Recent life Changes	170	-0.02	-0.05 to 0.01	0.19

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.16 Univariable linear regression analyses of stress measures as predictors of PWV.

PWV	Univariable regression models			
	n	coefficient	95% CI	P
C-wake	136	0.002	-0.02 to 0.02	0.8
CAR-Rate	109	0.001	-0.01 to 0.01	0.8
C-evening	38	-0.05	-0.21 to 0.12	0.6
C-Ratio	38	-0.12	-1.15 to 0.90	0.8
CAR-AUC	109	0.004	-0.03 to 0.05	0.9
C-DayAUC	38	-0.00	-0.01 to 0.00	0.6
A-File Total Recent life Changes	145	0.03	-0.01 to 0.07	0.14

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.17 Univariable linear regression analyses of stress measures as predictors of cholesterol.

Cholesterol	Univariable regression models			
	n	coefficient	95% CI	P
C-wake	163	-0.002	-0.02 to 0.01	0.8
CAR-Rate	131	-0.01	-0.01 to 0.001	0.09
C-evening	53	0.03	-0.10 to 0.15	0.7
C-Ratio	53	-0.09	-0.76 to 0.58	0.8
CAR-AUC	131	-0.03	-0.05 to 0.001	0.06
C-DayAUC	53	-0.000	-0.003 to 0.003	0.9
A-File Total Recent life Changes	172	-0.01	-0.03 to 0.01	0.3

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.18 Univariable linear regression analyses of stress measures as predictors of HDL.

HDL	Univariable regression models			
	n	coefficient	95% CI	P
C-wake	160	0.000	-0.003 to 0.004	0.9
CAR-Rate	129	-0.00	-0.002 to 0.002	0.6
C-evening	52	0.01	-0.03 to 0.05	0.8

HDL	Univariable regression models			
	n	coefficient	95% CI	P
C-Ratio	52	0.00	-0.21 to 0.21	0.9
CAR-AUC	129	0.000	-0.01 to 0.01	0.9
C-DayAUC	52	-0.00	-0.00 to 0.00	0.8
A-File Total Recent life Changes	168	-0.002	-0.01 to 0.01	0.6

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.19 Univariable linear regression analyses of stress measures as predictors of triglycerides.

Triglycerides	Univariable regression models			
	n	coefficient	95% CI	P
C-wake	163	0.01	-0.01 to 0.02	0.6
CAR-Rate	131	-.00	-0.01 to 0.00	0.8
C-evening	53	0.01	-0.09 to 0.08	0.8
C-Ratio	53	0.70	-0.53 to 0.36	0.7
CAR-AUC	131	-0.00	-0.02 to 0.02	0.9
C-DayAUC	53	0.00	-0.00 to 0.00	0.3

Triglycerides	Univariable regression models			
	n	coefficient	95% CI	P
A-File Total Recent life Changes	171	-0.06	-0.02 to 0.01	0.4

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.20 Univariable linear regression analyses of stress measures as predictors of fasting insulin.

Fasting insulin	Univariable regression models			
	n	coefficient	95% CI	P
C-wake	163	-0.04	-0.18 to 0.17	0.9
CAR-Rate	131	-0.01	-0.11 to 0.08	0.7
C-evening	53	-0.13	-1.18 to 0.92	0.8
C-Ratio	53	-3.86	-9.47 to 1.76	0.17
CAR-AUC	131	-0.21	-0.57 to 0.15	0.3
C-DayAUC	53	0.02	-0.00 to 0.04	0.10
A-File Total Recent life Changes	172	0.15	-0.15 to 0.46	0.3

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening :

evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.21 Univariable linear regression analyses of stress measures as predictors of HOMA.

HOMA	Univariable regression models			
	n	Coefficient	95% CI	p
C-wake	162	0.01	-0.05 to 0.04	0.8
CAR-Rate	130	-0.01	-0.02 to 0.02	0.9
C-evening	52	0.01	-0.25 to 0.24	0.9
C-Ratio	52	-0.72	-2.04 to 0.58	0.3
CAR-AUC	130	-0.04	-0.13 to 0.04	0.3
C-DayAUC	52	0.03	-0.01 to 0.01	0.13

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.22 Univariable linear regression analyses of stress measures as predictors of HbA1c.

HbA1c	Univariable regression models			
	n	coefficient	95% CI	P
C-wake	157	0.01	-0.01 to 0.01	0.14

HbA1c	Univariable regression models			
	n	coefficient	95% CI	P
CAR-Rate	126	-0.02	-0.00 to 0.01	0.13
C-evening	53	-0.02	-0.07 to 0.04	0.5
C-Ratio	53	-0.09	-0.38 to 0.21	0.6
CAR-AUC	126	0.00	-0.01 to 0.01	0.9
C-DayAUC	53	-0.00	-0.00 to 0.00	0.5
A-File Total Recent life Changes	166	0.01	-0.01 to 0.02	0.4

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

5.5 Associations between mental health screening, stress and cardiovascular risk.

5.5.1 The DAWBA

The DAWBA was completed by 106 young people completely and 24 partially; and was completed by 116 parents completely and 20 partially completed. DAWBA scores were able to be determined for 133 young people, of whom 45 (33%) were scored as having > 50 % chance of a mental health disorder. Logistic regression models for stress measures and adiposity and blood pressure measures as predictors of <50% chance of mental health disorder or not are shown in table 5.23 and 5.24 respectively. There were no associations.

Table 5.23. Univariable logistic regression analyses of stress measures as predictors of >50% chance of any disorder from DAWBA outputs

DAWBA any disorder	Univariable logistic regression models			
	n	Odd's ratio	95% CI	p
C-wake	124	0.97	0.92 to 1.01	0.19
CAR-Rate	103	0.99	0.97 to 1.01	0.7
C-evening	48	104	0.77 to 1.42	0.8
C-Ratio	48	1.10	0.16 to 7.51	0.9
CAR-AUC	103	0.96	0.89 to 1.04	0.3
C-DayAUC	48	1.00	0.00 to 1.01	0.3
A-File Total Recent life Changes	131	1.06	0.99 to 1.14	0.06

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.24. Univariable logistic regression analyses of adiposity and blood pressure measures as predictors of >50% chance of any disorder from DAWBA outputs

DAWBA any disorder	Univariable logistic regression models			
	n	Odd's ratio	95% CI	p
BMI z	133	1.05	0.55 to 1.99	0.9

DAWBA any disorder	Univariable logistic regression models			
	n	Odd's ratio	95% CI	p
FMI	132	1.02	0.93 to 1.12	0.7
waist	133	0.99	0.97 to 1.03	0.8
Waist z	133	1.05	0.64 to 1.75	0.8
SAD	124	0.98	0.88 to 1.09	0.7
Systolic z	131	0.82	0.59 to 1.15	0.3
Diastolic z	131	0.86	0.63 to 1.17	0.3

5.5.2 The Strengths and difficulties questionnaire (SDQ)

137 young people completed the SDQ. Of these 34 (25.8%) were estimated that a mental health disorder was probable. Logistic regressions of stress measures and adiposity and blood pressure measures as predictor of probable diagnosis or not are shown in tables 5.25 and 5.26 respectively. There was a small, positive association between risk of probable disorder and greater A-FILE score : OR 1.08 (1.01 to 1.16, p =0.02). There was no association between probable disorder and age, deprivation score, sex or puberty, though participants of Asian ethnicity were less likely to be categorised as probable mental health disorder compared to white participants (OR 0.15, 95% CI 0.03 to 0.72, p = 0.02), Both the relationship of AFILE score and ethnicity and SDQ prediction remained significant after combining in a multivariable model. Further analysis looking at potential diagnosis provided by the SDQ : emotional, behavioural or hyperactivity did not show significant associations with the A-FILE.

Table 5.25. Univariable logistic regression analyses of stress measures as predictors of probable any mental health disorder from SDQ.

SDQ any disorder probable	Univariable logistic regression models			
	n	Odd's ratio	95% CI	P
C-wake	128	0.99	0.95 to 1.03	0.7
CAR-Rate	105	0.99	0.97 to 1.01	0.4
C-evening	48	1.28	0.92 to 1.78	0.15
C-Ratio	48	0.81	0.09	0.6
CAR-AUC	105	0.99	0.92 to 1.07	0.8
C-DayAUC	48	1.00	0.99 to 1.01	0.12
A-File Total Recent life Changes	135	1.08	1.01 to 1.16	0.02

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.26. Univariable logistic regression analyses of adiposity and blood pressure measures as predictors of probable any mental health disorder from SDQ.

SDQ	Univariable logistic regression models			
	n	Odd's ratio	95% CI	P
BMI z	137	0.98	0.50 to 1.94	0.95

SDQ	Univariable logistic regression models			
	n	Odd's ratio	95% CI	P
FMI	135	0.95	0.85 to 1.06	0.4
Waist	137	0.98	0.95 to 1.02	0.4
Waist z	137	0.93	0.53 to 1.61	0.8
SAD	127	0.95	0.84 to 1.06	0.4
Systolic z	135	0.95	0.66 to 1.36	0.8
Diastolic z	135	0.91	0.65 to 1.26	0.5

5.5.3 Rosenberg's Self Esteem Score (RSE)

It is important to point out here that a greater RSE score means greater self esteem. Mean score for males was greater than females : 20.10 (SD 4.74) vs. 17.27 (SD 5.73), t-test : t = 3.24, df 163, p <0.01; i.e. females had lower self esteem than males in the sample. RSE was not associated with ethnicity, pubertal stage or age. There was a positive relationship with greater IMD score (i.e greater self esteem associated with greater degree of deprivation) : coefficient 0.07, 95% CI 0.01 to 0.13, p = 0.04. Regression analyses examining relationships between the RSE with stress measures as predictors and adiposity and blood pressure are shown in table 5.27 and 5.28 respectively. The A-FILE was associated negatively with RSE, i.e greater stressor exposures was associated with lower self esteem, and this was robust to a multivariable model adjusting for sex (as was the relationship with sex): coefficient for A-FILE as predictor for RSE score, adjusted for sex : -0.21 (-0.36 to 0.05, p <0.01) however $r^2 = 0.08$, which was small.

Fat mass index and waist circumference were negatively associated with RSE score, however they were not significant when adjusted for sex.

Table 5.27. Univariable regression analyses of stress measures and the RSE score

Rosenberg's	Univariable regression models			
	n	Co-efficient	95% CI	P
C-wake	156	-0.01	-0.10 to 0.09	0.9
CAR-Rate	126	0.04	-0.01 to 0.09	0.08
C-evening	50	0.04	-0.76 to 0.85	0.9
C-Ratio	50	1.45	-2.91 to 5.82	0.5
CAR-AUC	126	0.09	-0.08 to 0.28	0.3
C-DayAUC	50	-0.01	-0.02 to 0.01	0.5
A-File Total Recent life Changes	164	-0.22	-0.37 to -0.07	<0.01 *

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.28. Univariable regression analyses of adiposity and blood pressure measures and the RSE score

Rosenberg's	Univariable regression models			
	n	Coefficient	95% CI	P

Rosenberg's	Univariable regression models			
	n	Coefficient	95% CI	P
BMI z	165	-0.39	-1.98 TO 1.20	0.6
Fat mass index	162	-0.30	-50 TO -0.05	0.01 *
waist	165	-0.01	-0.08 TO 0.06	0.8
Waist z	165	-1.26	-2.42 to -0.10	0.03 *
SAD	152	-0.02	-0.29 to 0.25	0.9
Systolic z	163	-0.56	-1.36 to 0.23	0.17
Diastolic z	163	0.32	-0.43 to 1.09	0.4

5.5.4 EAT-26 questionnaire : binge eating

50 participants (28.9%) reported that they had previously binge eaten (and unable to stop eating) at baseline. 47 of those who had reported binge eating reported the number of times they had done so in the last 6 months. Median number of binge eating episodes in the previous 6 months was 3 episodes (IQR 2 to 10). Risk of bingeing was less for Asian ethnicity compared to white (Odd's ratio 0.31, 95% CI 0.11 to 0.91, p = 0.03), but there were no associations with other ethnic groups. Risk of bingeing was not significantly associated with age, sex, pubertal stage or deprivation score. Univariable logistic regression models with stress measures as predictors of self-reported bingeing is shown in table 5.29. There were no associations between any of the cortisol measures and risk of binge eating, however there was a positive association between A-FILE score (i.e. exposure to stressors in the last 12 months) and risk of binge eating. The effect size was however small, r^2 = 0.05.

Univariable logistic regression models with adiposity and blood pressure and risk of binge eating are shown in table 5.30. There were positive associations between most adiposity markers and risk of binge eating, and between systolic blood pressure and risk of binge eating. Effects sizes for all of these univariable models were small with all $r^2 < 0.05$.

Multivariable logistic regression models were created to predict risk of binge eating with the A-FILE, ethnicity, systolic z and individual adiposity measures (added in individually as per analysis plan : so BMI z, waist z for example not included in models together) as predictors. The A-FILE remained robust to adjustment in all models, as did systolic-z, and Asian ethnicity. The only adiposity marker that remained significant in multivariable modeling was SAD, with components of this model shown in table 5.31. R^2 for this model was 0.16.

There were no associations between any stress measure (cortisol and A-FILE), adiposity measure or blood pressure measure and number of times binged in univariable regression models (data not shown).

There were no associations between binge eating and any of the blood markers at baseline, or PWV.

Table 5.29. Univariable logistic regression analyses of stress measures and risk of binging from self-report (EAT-26)

Risk of binge eating	Univariable regression models			
	n	Odd's ratio	95% CI	P
C-wake	156	0.99	0.95 to 1.03	0.8
CAR-Rate	126	1.00	-.99 to 1.02	0.6
C-evening	50	1.04	0.77 to1.41	0.8
C-Ratio	50	0.40	0.04 to 4.20	0.4

Risk of binge eating	Univariable regression models			
	n	Odd's ratio	95% CI	P
CAR-AUC	126	0.97	0.89 to 1.05	0.5
C-DayAUC	50	1.00	0.99 to 1.01	0.3
A-File Total Recent life Changes	172	1.11	1.04 to 1.18	<0.01*

C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 5.30. Univariable logistic regression analyses of adiposity and blood pressure measures and risk of binging from self-report (EAT-26)

Risk of binge eating	Univariable regression models			
	N	Odd's ratio	95%CI	P
BMI z	173	1.89	1.00 to 3.54	0.05
Fat mass index	170	1.10	1.01 to 1.20	0.03*
waist	173	1.04	1.01 to 1.06	0.03*
Waist z	173	1.87	1.15 to 3.00	0.01*
SAD	158	1.16	1.04 to 1.29	<0.01*
Systolic z	171	1.45	1.04 to 2.01	0.03*

Risk of binge eating	Univariable regression models			
	N	Odd's ratio	95%CI	P
Diastolic z	171	0.92	0.69 to 1.23	0.6

Table 5.31. Multivariable logistic regression analyses of A-FILE score, SAD, Asian ethnic group and systolic z blood pressure to predict risk of bingeing from self-report (EAT-26)

Variable in model				
	N	Odd's ratio	95%CI	P
A-FILE score	156	1.12	1.04 to 1.20	<0.01*
SAD		1.18	1.05 to 1.33	0.01*
Asian ethnicity versus white		0.20	0.06 to0.72	0.01*
Systolic z		1.59	1.09 to 2.33	0.02*

Chapter 6: Longitudinal data

Here I present the longitudinal data from the HELP trial and present analyses to address longitudinal aspects of all 6 research questions (namely 1) how degree of adiposity is associated with pulse wave velocity as a proxy of arterial stiffening at a longitudinal level; 2) how blood pressure is associated with pulse wave velocity as a proxy of arterial stiffening at a longitudinal level; and 3) how other conventional measures of cardio-metabolic risk such as blood testing and acanthosis nigricans are associated with pulse wave velocity as a proxy of arterial stiffening at a longitudinal level) 4) how measures of stress are associated with degree of adiposity at a longitudinal level; 5) how measures of stress are associated with blood pressure at a longitudinal level; and 6) how measures of stress are associated with pulse wave velocity at a longitudinal level).

6.1 Analysis of change for variables between time points t0 and t2

Of the 174 young people entered into the HELP trial, 87 were randomized to the intervention and 87 to the control. As highlighted in chapter 3 in the summary of the HELP trial, there were no differences in change in BMI at t2 between control and intervention. There were also no differences in change in any of the anthropometry measures, blood tests or psychological measures between control and intervention at time 2.

Differences in matched pairs between time 0 and 2 are shown in table 6.1 below using paired t-testing. Though some of these variables were non normally distributed, changes were confirmed using non parametric testing and results were consistent (see methods). A number of variables changed between time point 0 and 2, however the particular variable of interest, PWV, did not change between times 0 and 2; which was the only time it was measured. The sample distributions of PWV at time 0 and 2 are illustrated in figure 6.1, which also demonstrates the stability of PWW between the two time points.

Table 6.1 Summary of changes in key variables measured at time 0 and 2 with paired t-testing.

Variable	n	Mean time 0 (SD)	Mean time 2 (SD)	Mean difference (95% CI)	t	p
PWV	72	7.26 (1.25)	7.03 (1.25)	-1.29 (-0.07 GO 0.52)	1.46	0.15
BMI	145	32.44 (4.45)	32.66 (4.74)	0.22 (-0.47 to 0.02)	-1.78	0.08
waist	125	99.68 (11.33)	99.49 (12.13)	-0.19 (-098 to 1.36)	0.32	0.8
Fat mass index	115	13.85 (3.94)	14.28 (4.08)	0.42 (-0.67 to -0.17)	-3.31	<0.01 *
SAD	130	22.59 (3.52)	22.38 (3.60)	-0.21 (-0.49 to 0.91)	0.58	0.6
Systolic BP	141	106.98 (10.34)	109.38 (10.47)	2.40 (-4.34 to -0.45)	-2.43	0.02 *
Systolic z	141	-1.06 (1.08)	-0.88 (1.03)	0.17 (-0.38 to 0.02)	-1.74	0.08
Diastolic BP	141	54.36 (9.60)	57.01 (11.19)	2.65 (-4.81 to -0.50)	-2.42	0.02 *
Diastolic z	141	-0.56 (1.17)	-0.29 (1.29)	0.27 (-0.53 to -0.01)	-2.00	0.04 *
Cholesterol	117	4.36 (0.91)	4.15 (0.79)	-0.22 (0.12 to 0.32)	4.49	<0.01 *
HDL	113	1.12 (0.23)	1.13 (0.24)	0.03 (-0.06 to	-2.08	0.04

Variable	n	Mean time 0 (SD)	Mean time 2 (SD)	Mean difference (95% CI)	t	p
				-0.01)		
Triglycerides	116	1.13 (0.64)	1.08 (0.56)	-0.06 (-0.05 to 0.16)	1.09	0.3
Fasting insulin	117	14.88 (11.92)	18.75 (12.41)	3.88 (-5.61 to -2.15)	-4.45	<0.01 *
HOMA-IR	116	3.06 (0.26)	3.84 (0.24)	0.79 (-1.18 to -0.39)	-3.90	<0.01 *
HbA1c	111	5.46 (0.38)	5.44 (0.98)	-0.02 (-0.17 to 0.21)	0.24	0.8
C-wake	121	15.74 (10.05)	17.21 (9.10)	1.47 (-4.04 to 1.10)	-1.13	0.3
CAR-Rate	72	23.54 (16.54)	24.00 (28.43)	0.46 (-8.37 to 7.45)	-0.11	0.9
C-evening	31	1.92 (1.87)	4.03 (5.16)	2.10 (-4.01 to -0.19)	-2.24	0.03 *
C-Ratio	31	0.19 (0.32)	0.21 (0.21)	0.02 (-0.16 to 0.10)	-0.45	0.7
CAR-AUC	72	10.52 (4.73)	10.79 (6.52)	0.26 (-2.11 to 1.58)	-0.28	0.8
C-DayAUC	31	194.85 (86.9)	216.13 (102.01)	21.28 (-64.51 to 21.95)	-1.01	0.3
A-FILE	132	7.66 (5.21)	5.39 (4.63)	-2.28 (1.56 to 3.00)	6.23	<0.01*

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

Figure 6.1 Box plot of distribution of PWV at time 0 and time 2.

6.2 Regression analyses using change in variables between t0 and t2 to predict pulse wave velocity at t2

Regression analyses using the models described in 3.12.3 are shown in tables 6.2-6.10. Sensitivity analyses including intervention status in models made no difference to models, and as explained above, there were no differences in any of the measures used in models between intervention and control groups in the HELP trial.

Table 6.2 Regression models to predict PWV at time 2 using demographic, adiposity, blood pressure, blood risk measures, and stress measures as predictors, adjusted for the same variable at time 0, PWV at time 0 and time between measurements for time 2 and 0 in years.

Pulse Wave Velocity Time 2.	Multivariable model components			
	n	Coefficient	95% CI	P
PWV time 0	72	0.49	0.28 to 0.71	<0.01 *
Sex		-0.31	-0.86 0.23	0.3
Time from 0 to 2		-1.86	-4.75 to 1.03	0.20
PWV time 0	72	0.46	0.24 to 0.68	<0.01 *
Ethnicity				
Black versus white		0.32	-0.29 to 0.91	0.3
Asian versus white		0.68	-0.11 to 1.47	0.3
Mixed versus white		0.14	-0.87 to 1.13	0.8
Time from 0 to 2		-1.44	-4.39 to 1.50	0.3
PWV time 0	70	0.48	0.26 to 0.71	<0.01 *
Pubertal stage time 0				
Mid Puberty versus pre/early		0.70	-0.48 to 1.87	0.2
Late/post versus pre/early		0.99	-1.04 to 3.02	0.3
Pubertal stage time 2				
Mid Puberty versus pre/early		-0.38	-1.91 to 1.15	0.6
Late/post versus pre/early		-1.03	-3.33 to 1.25	0.4

Pulse Wave Velocity Time 2.	Multivariable model components			
	n	Coefficient	95% CI	P
Time from 0 to 2		-2.02	-5.05 to 1.01	0.19
PWV time 0 Intervention Time from 0 to 2	72	0.48 0.04 -1.75	0.27 to 0.70 -0.48 to 0.57 -4.66 TO 1.16	<0.01 * 0.8 0.2
PWV time 0 IMD Time from 0 to 2	72	0.49 0.02 -1.94	0.28 to 0.69 0.01 to 0.04 -4.73 TO 0.84	<0.01 * 0.01 * 0.17
PWV time 0 Ever smoked Time from 0 to 2	70	0.49 0.04 -1.81	0.27 to 0.70 -0.61 to 0.70 -4.83 to 1.22	<0.01 * 0.9 0.2
PWV time 0 BMI time 0 BMI time 2 Time from 0 to 2	72	0.54 -0.02 -0.02 -1.72	0.31 to 0.78 -0.24 to 0.19 -0.21 to 0.18 -4.63 to 1.18	<0.01 * 0.8 0.9 0.2
PWV time 0 Fat mass index time 0 Fat mass index time 2 Time from 0 to 2	69	0.64 -0.10 -0.02 -1.81	0.42 to 0.85 -0.29 to 0.10 -0.211 to 0.16 -4.48 to 0.84	<0.01 * 0.3 0.8 0.18

Pulse Wave Velocity Time 2.	Multivariable model components			
	n	Coefficient	95% CI	P
PWV time 0	72	0.56	0.34 to 0.78	<0.01 *
waist time 0		-0.01	-0.05 to 0.05	0.9
waist time 2		-0.02	-0.07 to 0.02	0.3
Time from 0 to 2		-1.68	-4.55 to 1.19	0.3
PWV time 0	60	0.45	0.22 to 0.69	<0.01 *
SAD time 0		0.05	-0.04 to 0.15	0.3
SAD time 2		0.03	-0.06 to 0.12	0.5
Time from 0 to 2		-0.89	-3.96 to 2.17	0.6
PWV time 0	72	0.47	0.26 to 0.69	<0.01 *
Systolic BP time 0		0.0003	-0.03 to 0.03	0.9
Systolic BP time 2		0.02	-0.01 to 0.05	0.3
Time from 0 to 2		-1.92	-4.86 to 1.01	0.2
PWV time 0	72	0.49	0.27 to 0.71	<0.01 *
Systolic z BP time 0		-0.04	-0.34 to 0.26	0.8
Systolic z BP time 2		0.11	-0.18to 0.40	0.5
Time from 0 to 2		-1.86	-4.82 to 1.10	0.2
PWV time 0	72	0.48	0.25 to 0.70	<0.01 *
Diastolic BP time 0		0.01	-0.01 to 0.04	0.5
Diastolic BP time 2		0.001	-0.03 to 0.03	0.94

Pulse Wave Velocity Time 2.	Multivariable model components			
	n	Coefficient	95% CI	P
Time from 0 to 2		-1.62	-4.72 to 1.58	0.3
PWV time 0 Diastolic z BP time 0 Diastolic z BP time 2 Time from 0 to 2	72	0.48 0.09 -0.003 -1.56	0.26 to 0.70 -0.14 to 0.32 -0.25 to 0.25 -4.66 to 1.54	<0.01 * 0.5 0.9 0.3
PWV time 0 Fasting insulin time 0 Fasting insulin time 2 Time from 0 to 2	72	0.46 -0.002 0.012 -1.56	0.24 to 0.68 -0.03 to 0.02 -0.01 to 0.04 -4.50 to 1.37	<0.01 * 0.9 0.4 0.3
PWV time 0 HOMA -IR time 0 HOMA-IR time 2 Time from 0 to 2	72	0.47 0.01 0.04 -1.53	0.25 to 0.69 -0.11 to 0.12 -0.08 to 0.17 -4.48 to 1.41	<0.01 * 0.9 0.5 0.3
PWV time 0 Abnormal fasting insulin time 0 Abnormal fasting insulin time 2 Time from 0 to 2	70	0.49 0.24 0.11 -1.45	0.26 to 0.70 -0.51 to 0.99 -0.52 to 0.73 -4.47 to 1.56	<0.01 * 0.5 0.7 0.3
PWV time 0 Abnormal HOMA-IR time 0 Abnormal HOMA-IR time 2	72	0.49 0.22 -0.02	0.27 to 0.71 -0.44 to 0.87 -0.63 to 0.59	<0.01 * 0.5 0.9

Pulse Wave Velocity Time 2.	Multivariable model components			
	n	Coefficient	95% CI	P
Time from 0 to 2		-1.61	-4.56 to 1.34	0.3
PWV time 0 HbA1c time 0 HbA1c time 2 Time from 0 to 2	69	0.46 0.16 0.05 -1.89	0.23 to 0.68 -0.55 to 0.88 -0.17 to 0.28 -4.91 to 1.13	<0.01 * 0.7 0.6 0.2
PWV time 0 Cholesterol time 0 Cholesterol time 2 Time from 0 to 2	72	0.52 0.71 -0.63 -1.39	0.31 to 0.72 0.25 to 1.17 -1.15 to -0.12 -4.15 to 1.36	<0.01 * 0.03* 0.02* 0.3
PWV time 0 HDL time 0 HDL time 2 Time from 0 to 2	70	0.48 2.15 -2.01 -1.81	0.27 to 0.69 0.64 to 3.66 -3.49 to -0.54 -4.62 to 0.98	<0.001 * 0.01 * 0.01 * 0.2
PWV time 0 Triglycerides time 0 Triglycerides time 2 Time from 0 to 2	71	0.47 0.02 0.23 -1.57	0.25 to 0.69 -0.66 to 0.71 -0.46 to 0.91 -4.53 to 1.37	<0.001 * 0.9 0.5 0.3

Pulse Wave Velocity Time 2.	Multivariable model components			
	n	Coefficient	95% CI	P
PWV time 0	64	0.44	0.22 to 0.65	<0.01 *
C-wake 0		0.02	-0.01 to 0.04	0.17
C-wake 2		0.03	-0.01 to 0.08	0.09
Time from 0 to 2		-2.20	-4.98 to 0.57	0.12
PWV time 0	45	0.51	0.23 to 0.78	<0.01*
CAR-Rate 0		0.01	-0.01 to 0.03	0.4
CAR-Rate 2		0.01	-0.02 to 0.03	0.9
Cholesterol time 0		-2.67	-6.62 to 1.27	0.18
PWV time 0	20	0.13	-0.48 to 0.74	0.7
C-evening 0		0.20	-0.25 to 0.65	0.4
C-evening 2		0.05	-0.10 to 0.22	0.4
Time from 0 to 2		-0.53	-19.4 to 18.34	0.9
PWV time 0	20	0.14	-0.51 to 0.78	0.7
C-Ratio 0		2.29	-5.35 to 9.94	0.5
C-Ratio 2		-0.70	-3.81 to 2.42	0.6
Time from 0 to 2		-3.77	-28.52 to 20.98	0.8
PWV time 0	45	0.53	0.27 to 0.80	<0.01 *
CAR-AUC 0		0.08	0.01 to 0.15	0.03
CAR-AUC 2		0.03	-0.04 to 0.11	0.4
Time from 0 to 2		-3.11	-6.73 to 0.50	0.09
PWV time 0	20	0.06	-0.61 to 0.72	0.9
C-DayAUC time 0		0.004	-0.001 to 0.01	0.08

Pulse Wave Velocity Time 2.	Multivariable model components			
	n	Coefficient	95% CI	P
C-DayAUC 2		0.001	-0.004 to 0.006	0.6
Time from 0 to 2		8.13	-18.45 to 34.71	0.5
PWV time 0	70	0.49	0.27 to 0.72	<0.01 *
A-File Total recent life changes time 0		-0.03	-0.10 to 0.04	0.4
A-File Total recent life changes time 2		0.03	-0.06 to 0.11	0.5
Time from 0 to 2		-1.88	-4.86 to 1.09	0.2

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

Table 6.3 Regression models to predict Cholesterol at time 2 using demographic, adiposity, blood pressure, blood risk measures, and stress measures as predictors, adjusted for the same variable at time 0, PWV at time 0 and time between measurements for time 2 and 0 in years.

Cholesterol Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Cholesterol time 0	117	0.71	0.62 to 0.81	<0.01 *
Sex		0.04	-0.14 to 0.22	0.6
Time from 0 to 2		-0.34	-1.03 to 0.35	0.3

Cholesterol Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Cholesterol time 0	117			
Ethnicity				
Black versus white		0.01	-0.19 to 0.22	0.8
Asian versus white		0.03	-0.21 to 0.27	0.7
Mixed versus white		0.01	-0.30 to 0.31	0.9
Time from 0 to 2		-0.35	-1.07 to 0.37	0.3
Cholesterol time 0	115	0.73	0.64 to 0.83	<0.01 *
Pubertal stage time 0				
Mid Puberty versus pre/early		-0.12	-0.46 to 0.22	0.4
Late/post versus pre/early		-0.03	-0.57 to 0.50	0.9
Pubertal stage time 2				
Mid Puberty versus pre/early		0.25	-0.23 to 0.73	0.3
Late/post versus pre/early		0.25	-0.41 to 0.92	0.4
Time from 0 to 2		-0.41	-1.09 to 0.28	0.2
Cholesterol time 0	117	0.71	0.62 to 0.81	<0.01 *
Intervention		0.12	-0.05 to 0.28	0.17
Time from 0 to 2		-0.37	-1.05 to 0.31	0.3
Cholesterol time 0	117	0.71	0.62 to 0.80	<0.01 *

Cholesterol Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
IMD		0.001	-0.01 to 0.01	0.8
Time from 0 to 2		-0.36	-1.05 to 0.32	0.3
Cholesterol time 0	114	0.72	0.62 to 0.81	<0.01*
Eversmoked		0.02	-0.18 to 0.22	0.8
Time from 0 to 2		-0.36	-1.06 to 0.33	0.3
Cholesterol time 0	117	0.69	-0.60 to 0.79	<0.01 *
BMI time 0		0.03	-0.04 to 0.10	0.4
BMI time 2		-0.01	-0.07 to 0.06	0.8
Time from 0 to 2		-0.34	-1.02 to 0.33	0.3
Cholesterol time 0	113	0.71	0.62 to 0.80	<0.01 *
Fat mass index time 0		0.01	-0.05 to 0.08	0.6
Fat mass index time 2		0.01	-0.05 to 0.08	0.6
Time from 0 to 2		-0.30	-0.99 to 0.39	0.4
Cholesterol time 0	117	0.70	0.61 to 0.80	<0.01 *
waist time 0		0.00	-0.02 to 0.01	0.7
waist time 2		0.01	-0.01 to 0.02	0.2
Time from 0 to 2		-0.40	-1.09 to 0.28	0.3
Cholesterol time 0	104	0.71	0.61 to 0.80	<0.01 *
SAD time 0		<0.001 *	-0.03 to 0.03	0.9
SAD time 2		0.01	-0.02 to 0.03	0.7

Cholesterol Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Time from 0 to 2		-0.28	-0.98 to 0.42	0.4
Cholesterol time 0	116	0.70	0.61 to 0.80	<0.01 *
Systolic BP time 0		0.001	-0.01 to 0.01	0.9
Systolic BP time 2		0.004	-0.01 to 0.01	0.4
Time from 0 to 2		-0.37	-1.07 to 0.33	0.3
Cholesterol time 0	116	0.71	0.61 to 0.80	<0.01 *
Systolic z BP time 0		<0.001 *	-0.08 to 0.09	0.9
Systolic z BP time 2		0.02	-0.07 to 0.12	0.6
Time from 0 to 2		-0.35	-1.05 to 0.36	0.3
Cholesterol time 0	116	0.71	0.62 to 0.81	<0.01 *
Diastolic BP time 0		<0.001 *	-0.01 to 0.01	0.9
Diastolic BP time 2		<0.001 *	-0.01 to 0.01	0.9
Time from 0 to 2		-0.37	-1.07 to 0.33	0.3
Cholesterol time 0	116	0.71	0.62 to 0.81	<0.01 *
Diastolic z BP time 0		-0.01	-0.09 to 0.06	0.7
Diastolic z BP time 2		-0.002	-0.08 to 0.07	0.9
Time from 0 to 2		-0.37	-1.07 to 0.33	0.3
Cholesterol time 0	103	0.68	0.57 to 0.79	<0.01 *
C-wake 0		-0.003	-0.01 to 0.01	0.5

Cholesterol Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
C-wake 2		-0.002	-0.01 to 0.01	0.6
Time from 0 to 2		-0.31	-1.03 to 0.42	0.4
Cholesterol time 0	62	0.67	0.52 to 0.81	<0.01 *
CAR-Rate 0		-0.005	-0.01 to 0.002	0.18
CAR-Rate 2		-0.005	-0.01 to 0.003	0.2
Cholesterol time 0		0.57	-0.67 to 1.81	0.3
Cholesterol time 0	26	0.52	0.27 to 0.77	<0.01 *
CAR-evening time 0		-0.003	-0.11 to 0.11	0.9
CAR-evening 2		0.001	-0.05 to 0.05	0.9
Time from 0 to 2		1.83	-3.76 to 7.42	0.5
Cholesterol time 0	26	0.51	0.23 to 0.8	0.01
C-ratio 0		-0.1	-0.82 to 0.63	0.7
C-ratio 2		-0.13	-1.49 to 1.23	0.8
Time from 0 to 2		2.53	-4.1 to 9.16	0.4
Cholesterol time 0	62	0.69	0.54 to 0.83	<0.01 *
CAR-AUC 0		-0.01	-0.03 to 0.02	0.6
CAR-AUC time 2		-0.003	-0.03 to 0.02	0.7
Time from 0 to 2		0.32	-0.94 to 1.58	0.6
Cholesterol time 0	26	0.51	0.26 to 0.77	<0.01 *

Cholesterol Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
C-DayAUC time 0		-0.001	-0.004 to 0.002	0.4
C-DayAUC time 2		<0.001 *	-0.002 to 0.003	0.7
Time from 0 to 2		2.63	-3.69 to 8.94	0.4
Cholesterol time 0	115	0.72	0.62 to 0.81	<0.01 *
A-File Total recent life changes time 0		<0.001 *	-0.02 to 0.02	0.9
A-File Total recent life changes time 2		0.01	-0.02 to 0.03	0.6
Time from 0 to 2		-0.39	-1.09 to 0.3	0.3

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

Table 6.4 Regression models to predict triglycerides at time 2 using demographic, adiposity, blood pressure, blood risk measures, and stress measures as predictors, adjusted for the same variable at time 0, PWV at time 0 and time between measurements for time 2 and 0 in years.

Triglycerides Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Triglycerides time 0	116	0.51	0.38 to 0.64	<0.01 *

Triglycerides Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Sex		-0.08	-0.26 to 0.1	0.4
Time from 0 to 2		0.11	-0.57 to 0.79	0.7
Triglycerides time 0	116	0.46	0.32 to 0.6	<0.01 *
Ethnicity				
Black versus white		-0.22	-0.42 to -0.02	0.03
Asian versus white		0.08	-0.16 to 0.32	0.5
Mixed versus white		-0.05	-0.34 to 0.24	0.7
Time from 0 to 2		0.09	-0.6 to 0.78	0.8
Triglycerides time 0	114	0.5	0.37 to 0.64	<0.01 *
Pubertal stage time 0				
Mid Puberty versus pre/early		-0.06	-0.4 to 0.27	0.7
Late/post versus pre/early		-0.37	-0.91 to 0.17	0.17
Pubertal stage time 2				
Mid Puberty versus pre/early		-0.05	-0.58 to 0.48	0.8
Late/post versus pre/early		0.29	-0.41 to 0.99	0.4
Time from 0 to 2		0.1	-0.59 to 0.8	0.7
Triglycerides time 0	116	0.51	0.38 to 0.64	<0.01
Intervention		0.03	-0.14 to 0.20	0.7

Triglycerides Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Time from 0 to 2		0.14	-0.53 to 0.82	0.6
Triglycerides time 0	116	0.52	0.39 to 0.65	<0.01 *
IMD		-0.01	-0.01 to 0.001	0.01
Time from 0 to 2		0.16	-0.51 to 0.83	0.6
Triglycerides time 0	113	0.49	0.36 to 0.63	<0.01*
Eversmoked		-0.13	-0.33 to 0.06	0.18
Time from 0 to 2		0.20	-0.47 to 0.88	0.5
Triglycerides time 0	116	0.52	0.38 to 0.65	<0.01 *
BMI time 0		-0.35	-0.81 to 0.10	0.13
BMI time 2		0.40	-0.005 to 0.80	0.05
Time from 0 to 2		0.10	-0.57 to 0.77	0.7
Triglycerides time 0	112	0.51	0.38 to 0.64	<0.01 *
Fat mass index time 0		-0.03	-0.10 to 0.03	0.3
Fat mass index time 2		0.04	-0.02 to 0.11	0.2
Time from 0 to 2		0.08	-0.62 to 0.78	0.8
Triglycerides time 0	116	0.50	0.37 to 0.63	<0.01 *
waist time 0		0.00	-0.01 to 0.02	0.8
waist time 2		0.01	-0.01 to 0.02	0.3
Time from 0 to 2		0.11	-0.56 to 0.77	0.7

Triglycerides Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Triglycerides time 0	77	0.65	0.47 to 0.82	<0.01 *
waist z time 0		-0.08	-0.39 to 0.23	0.6
waist z time 2		0.12	-0.15 to 0.40	0.4
Time from 0 to 2		0.41	-0.71 to 1.54	0.5
Triglycerides time 0	103	0.51	0.37 to 0.64	<0.01 *
SAD time 0		0.01	-0.02 to 0.03	0.7
SAD time 2		0.02	0.004 to 0.05	0.09
Time from 0 to 2		0.24	-0.43 to 0.91	0.5
Triglycerides time 0	115	0.52	0.38 to 0.65	<0.01 *
Systolic BP time 0		0.00	-0.01 to 0.01	0.5
Systolic BP time 2		0.01	-0.004 to 0.02	0.2
Time from 0 to 2		0.09	-0.60 to 0.78	0.8
Triglycerides time 0	115	0.52	0.38 to 0.65	<0.01 *
Systolic z BP time 0		-0.03	-0.12 to 0.05	0.5
Systolic z BP time 2		0.04	-0.05 to 0.13	0.4
Time from 0 to 2		0.10	-0.60 to 0.80	0.8
Triglycerides time 0	115	0.52	0.39 to 0.65	<0.01 *
Diastolic BP time 0		-0.005	-0.01 to 0.005	0.3
Diastolic BP time 2		0.001	-0.01 to 0.01	0.9

Triglycerides Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Time from 0 to 2		0.12	-0.57 to 0.81	0.7
Triglycerides time 0	115	0.52	0.39 to 0.65	<0.01 *
Diastolic z BP time 0		-0.03	-0.11 to 0.04	0.4
Diastolic z BP time 2		0.01	-0.07 to 0.08	0.8
Time from 0 to 2		0.12	-0.57 to 0.81	0.7
Triglycerides time 0	103	0.49	0.35 to 0.64	<0.01 *
C-wake time 0		0.01	-0.004 to 0.01	0.3
C-wake time 2		0	-0.01 to 0.01	1.0
Time from 0 to 2		0.18	-0.54 to 0.9	0.6
Triglycerides time 0	64	0.54	0.38 to 0.7	<0.01 *
C-Rate time 0		-0.004	-0.01 to 0.003	0.2
C-Rate time 2		0.001	-0.01 to 0.01	0.8
Cholesterol time 0		0.47	-0.67 to 1.62	0.4
Triglycerides time 0	26	0.08	-0.2 to 0.36	0.6
Evening cortisol time 0		-0.01	-0.1 to 0.08	0.8
Evening cortisol time 2		-0.003	-0.04 to 0.04	0.9
Time from 0 to 2		3.45	-1.6 to 8.5	0.17
Triglycerides time 0	26	0.06	-0.22 to 0.34	0.6
C-ratio time 0		0.02	-0.51 to 0.54	1.0

Triglycerides Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
C-ratio time 2		-0.42	-1.41 to 0.56	0.4
Time from 0 to 2		3.06	-2.03 to 8.16	0.2
Triglycerides time 0	64	0.55	0.39 to 0.7	<0.01 *
CAR-AUC time 0		0.01	-0.01 to 0.03	0.5
CAR-AUC time 2		0.01	-0.01 to 0.03	0.15
Time from 0 to 2		0.26	-0.86 to 1.38	0.7
Triglycerides time 0	26	0.06	-0.23 to 0.35	0.7
C-DayAUC time 0		0	-0.002 to 0.002	0.8
C-DayAUC time 2		0.001	-0.001 to 0.003	0.16
Time from 0 to 2		3.38	-1.75 to 8.51	0.19
Triglycerides time 0	114	0.51	0.37 to 0.64	<0.01 *
A-File Total recent life changes time 0		-0.01	-0.03 to 0.01	0.3
A-File Total recent life changes time 2		0.01	-0.02 to 0.03	0.5
Time from 0 to 2		0.16	-0.53 to 0.84	0.6

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

Table 6.5 Regression models to predict HDL at time 2 using demographic, adiposity, blood pressure, blood risk measures, and stress measures as predictors, adjusted for the same variable at time 0, PWV at time 0 and time between measurements for time 2 and 0 in years.

HDL Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
HDL time 0	113	0.69	0.57 to 0.82	<0.01 *
Sex		0.03	-0.03 to 0.09	0.3
Time from 0 to 2		-0.11	-0.35 to 0.14	0.3
HDL time 0	113	0.68	0.55 to 0.81	<0.01 *
Ethnicity				
Black versus white		0.06	-0.01 to 0.13	0.1
Asian versus white		-0.01	-0.09 to 0.08	0.9
Mixed versus white		0.01	-0.1 to 0.11	0.9
Time from 0 to 2		-0.1	-0.35 to 0.15	0.4
HDL time 0	111	0.7	0.56 to 0.83	<0.01 *
Pubertal stage time 0				
Mid Puberty versus pre/early		-0.01	-0.13 to 0.11	0.8
Late/post versus pre/early		-0.01	-0.21 to 0.18	0.9
Pubertal stage time 2				
Mid Puberty versus pre/early		0.05	-0.14 to 0.25	0.6

HDL Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Late/post versus pre/early		0.09	-0.16 to 0.34	0.5
Time from 0 to 2		-0.14	-0.39 to 0.11	0.3
HDL time 0	113	0.70	0.57 to 0.83	<0.01 *
Intervention		0.01	-0.06 to 0.07	0.8
Time from 0 to 2		-0.12	-0.37 to 0.12	0.3
HDL time 0	113	0.70	0.57 to 0.83	<0.01 *
IMD		0.001	-0.002 to 0.003	0.6
Time from 0 to 2		-0.12	-0.37 to 0.12	0.3
HDL time 0	110	0.70	0.57 to 0.83	<0.01 *
Eversmoked		-0.002	-0.07 to 0.07	0.9
Time from 0 to 2		-0.12	-0.37 to 0.12	0.3
HDL time 0	113	0.67	0.54 to 0.80	<0.01*
BMI time 0		-0.07	-0.23 to 0.10	0.4
BMI time 2		0.02	-0.13 to 0.16	0.8
Time from 0 to 2		-0.14	-0.38 to 0.11	0.3
HDL time 0	110	0.69	0.56 to 0.83	<0.01 *
Fat mass index time 0		0.00	-0.03 to 0.02	0.7
Fat mass index time 2		0.00	-0.02 to 0.03	0.8

HDL Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Time from 0 to 2		-0.14	-0.39 to 0.11	0.3
HDL time 0 waist time 0 waist time 2 Time from 0 to 2	113	0.65 -0.01 0.003 -0.15	0.52 to 0.77 -0.01 to -0.001 0.002 to 0.01 -0.38 to 0.09	<0.01 * 0.02* 0.2 0.2
HDL time 0 SAD time 0 SAD time 2 Time from 0 to 2	100	0.60 -0.01 0.00 -0.10	0.48 to 0.73 -0.02 to 0.004 -0.01 to 0.01 -0.33 to 0.12	<0.01 * 0.2 0.8 0.4
HDL time 0 Systolic BP time 0 Systolic BP time 2 Time from 0 to 2	112	0.70 0.001 -0.002 -0.10	0.58 to 0.83 -0.002 to 0.004 -0.01 to 0.001 -0.35 to 0.14	<0.01 * 0.5 0.2 0.4
HDL time 0 Systolic z BP time 0 Systolic z BP time 2 Time from 0 to 2	112	0.70 0.01 -0.02 -0.11	0.58 to 0.83 -0.02 to 0.04 -0.05 to 0.01 -0.36 to 0.13	<0.01 * 0.5 0.2 0.3
HDL time 0 Diastolic BP time 0	112	0.7 0	0.57 to 0.83 -0.004 to 0.003	<0.01 * 0.8

HDL Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Diastolic BP time 2		0	-0.003 to 0.004	0.8
Time from 0 to 2		-0.13	-0.38 to 0.12	0.3
HDL time 0	112	0.7	0.57 to 0.83	<0.01 *
Diastolic z BP time 0		-0.01	-0.03 to 0.02	0.6
Diastolic z BP time 2		0	-0.03 to 0.03	1.0
Time from 0 to 2		-0.12	-0.37 to 0.13	0.3
HDL time 0	100	0.71	0.57 to 0.85	<0.01 *
C-wake 0		-0.003	-0.01 to 0	0.04*
C-wake time 2		-0.001	-0.004 to 0.003	0.7
Time from 0 to 2		-0.12	-0.36 to 0.13	0.4
HDL time 0	62	0.7	0.53 to 0.87	<0.01 *
CAR-Rate time 0		-0.001	-0.003 to 0.002	0.6
CAR-Rate time 2		-0.001	-0.004 to 0.002	0.7
Cholesterol time 0		0.13	-0.36 to 0.62	0.6
HDL time 0	25	0.73	0.42 to 1.05	<0.01 *
C-evening time 0		0.01	-0.03 to 0.05	0.6
C-evening time 2		0.01	-0.01 to 0.03	0.18
Time from 0 to 2		0.86	-1.54 to 3.26	0.4
HDL time 0	25	0.77	0.45 to 1.09	<0.01 *

HDL Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
C-ratio 0		0.12	-0.14 to 0.38	0.3
C-ratio time 2		0.09	-0.47 to 0.66	0.7
Time from 0 to 2		0.81	-1.69 to 3.3	0.5
HDL time 0	62	0.71	0.53 to 0.88	<0.01 *
CAR-AUC time 0		-0.01	-0.02 to 0.002	0.11
CAR-AUC time 2		0.001	-0.01 to 0.01	0.8
Time from 0 to 2		0.19	-0.29 to 0.66	0.4
HDL time 0	25	0.69	0.36 to 1.03	<0.01 *
C-DayAUC time 0		-0.001	-0.002 to 0	0.16
C-DayAUC time 2		0.001	0 to 0.002	0.2
Time from 0 to 2		0.67	-1.92 to 3.25	0.5
HDL time 0	112	0.71	0.57 to 0.84	<0.01 *
A-File Total recent life changes time 0		0.003	-0.01 to 0.01	0.5
A-File Total recent life changes time 2		-0.003	-0.01 to 0.01	0.5
Time from 0 to 2		-0.12	-0.37 to 0.13	0.3

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

Table 6.6 Regression models to predict fasting insulin at time 2 using demographic, adiposity, blood pressure, blood risk measures, and stress measures as predictors, adjusted for the same variable at time 0, PWV at time 0 and time between measurements for time 2 and 0 in years.

Fasting insulin Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Fasting insulin time 0	117	0.73	0.59 to 0.87	<0.01 *
Sex		1.67	-1.81 to 5.15	0.3
Time from 0 to 2		-1.81	-15.24 to 11.62	0.8
Fasting insulin time 0	117	0.7	0.56 to 0.84	<0.01 *
Ethnicity				
Black versus white		1.09	-2.86 to 5.04	0.5
Asian versus white		5.05	0.51 to 9.6	0.03*
Mixed versus white		3.23	-2.47 to 8.92	0.2
Time from 0 to 2		-4.08	-17.82 to 9.67	0.5
Fasting insulin time 0	115	0.74	0.6 to 0.88	<0.01 *
Pubertal stage time 0				
Mid Puberty versus pre/early		5.43	-1.15 to 12.01	0.11
Late/post versus pre/early		5.98	-4.63 to 16.59	0.3
Pubertal stage time 2				

Fasting insulin Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Mid Puberty versus pre/early		0.38	-9.1 to 9.86	0.9
Late/post versus pre/early		-1.38	-14.52 to 11.76	0.8
Time from 0 to 2		-4.61	-18.22 to 8.99	0.5
Fasting insulin time 0	117	0.73	0.58 to 0.87	<0.01 *
Intervention		0.10	-3.22 to 3.43	0.9
Time from 0 to 2		-2.70	-16.07 to 10.66	0.6
Fasting insulin time 0	117	0.72	0.59 to 0.86	< 0.01 *
IMD		0.02	-0.10 to 0.15	0.7
Time from 0 to 2		-2.77	-16.12 to 10.59	0.7
Fasting insulin time 0	114	0.71	0.57 to 0.85	< 0.01 *
Eversmoked		0.31	-3.58 to 4.20	0.8
Time from 0 to 2		-2.63	-16.05 to 10.79	0.7
Fasting insulin time 0	117	0.69	0.54 to 0.83	<0.01 *
BMI time 0		-1.65	-10.50 to 7.20	0.7
BMI time 2		4.59	-3.28 to 12.46	0.3
Time from 0 to 2		-3.51	-16.64 to 9.62	0.6
Fasting insulin time 0	113	0.72	0.58 to 0.86	<0.01 *
Fat mass index time 0		-0.64	-1.93 to 0.66	0.3

Fasting insulin Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Fat mass index time 2		0.92	-0.32 to 2.17	0.14
Time from 0 to 2		-3.43	-16.77 to 9.91	0.6
Fasting insulin time 0	117	0.68	0.53 to 0.82	<0.01 *
waist time 0		0.12	-0.18 to 0.42	0.4
waist time 2		0.05	-0.22 to 0.31	0.7
Time from 0 to 2		-3.66	-16.87 to 9.56	0.5
Fasting insulin time 0	104	0.68	0.53 to 0.83	<0.01 *
SAD time 0		0.02	-0.54 to 0.58	0.9
SAD time 2		0.51	-0.04 to 1.05	0.07
Time from 0 to 2		-4.67	-18.17 to 8.83	0.4
Fasting insulin time 0	116	0.71	0.57 to 0.85	<0.01 *
Systolic BP time 0		<0.001 *	-0.17 to 0.17	1.0
Systolic BP time 2		0.23	0.04 to 0.41	0.02*
Time from 0 to 2		-3.60	-16.88 to 9.68	0.6
Fasting insulin time 0	116	0.71	0.57 to 0.85	<0.01 *
Systolic z BP time 0		-0.12	-1.77 to 1.52	0.9
Systolic z BP time 2		1.93	0.20 to 3.66	0.03*
Time from 0 to 2		-2.29	-15.76 to 11.17	0.7
Fasting insulin time 0	166	0.72	0.58 to 0.86	<0.01 *

Fasting insulin Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Diastolic BP time 0		0.11	-0.08 to 0.29	0.3
Diastolic BP time 2		0.02	-0.15 to 0.2	0.8
Time from 0 to 2		-2.85	-16.37 to 10.67	0.7
Fasting insulin time 0	116	0.72	0.58 to 0.86	<0.01 *
Diastolic z BP time 0		0.77	-0.71 to 2.24	0.3
Diastolic z BP time 2		0.17	-1.28 to 1.63	0.8
Time from 0 to 2		-2.85	-16.4 to 10.69	0.6
Fasting insulin time 0	103	0.71	0.55 to 0.87	<0.01 *
C-wake time 0		0.08	-0.1 to 0.27	0.4
C-wake time 2		-0.02	-0.23 to 0.19	0.9
Time from 0 to 2		-2.39	-16.67 to 11.9	0.7
Fasting insulin time 0	64	1.01	0.76 to 1.26	<0.01 *
CAR-Rate time 0		0.004	-0.14 to 0.15	0.9
CAR-Rate time 2		-0.04	-0.2 to 0.12	0.6
Cholesterol time 0		8.9	-17.26 to 35.06	0.5
Fasting insulin time 0	26	1.3	0.93 to 1.67	<0.01 *
C-evening 0		-0.67	-2.06 to 0.71	0.3
C-evening 2		-0.13	-0.76 to 0.5	0.6
Time from 0 to 2		11.52	-56.8 to 79.84	0.7

Fasting insulin Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Fasting insulin time 0	26	1.21	0.83 to 1.59	<0.01 *
C-Ratio 0		-4.79	-13.18 to 3.61	0.3
C-Ratio time 2		-5.7	-21.11 to 9.72	0.4
Time from 0 to 2		21.76	-54.67 to 98.19	0.5
Fasting insulin time 0	64	1	0.75 to 1.26	<0.01 *
CAR-AUC time 0		0.34	-0.16 to 0.84	0.18
CAR-AUC time 2		-0.09	-0.55 to 0.38	0.7
Time from 0 to 2		3.79	-21.97 to 29.55	0.7
Fasting insulin time 0	26	1.23	0.76 to 1.7	<0.01 *
C-DayAUC time 0		0.004	-0.04 to 0.04	0.8
C-DayAUC time 2		0.01	-0.02 to 0.04	0.6
Time from 0 to 2		6.58	-70.22 to 83.38	0.8
Fasting insulin time 0	115	0.73	0.59 to 0.87	<0.01 *
A-File Total recent life changes time 0		0.11	-0.31 to 0.52	0.6
A-File Total recent life changes time 2		0.09	-0.38 to 0.56	0.7
Time from 0 to 2		-3.57	-17.14 to 10	0.6

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the

curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

Table 6.7 Regression models to predict HOMA-IR at time 2 using demographic, adiposity, blood pressure, blood risk measures, and stress measures as predictors, adjusted for the same variable at time 0, PWV at time 0 and time between measurements for time 2 and 0 in years.

HOMA-IR Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
HOMA-IR time 0	116	0.64	0.51 to 0.78	<0.01 *
Sex		0.32	-0.45 to 1.09	0.4
Time from 0 to 2		-0.47	-3.41 to 2.47	0.7
HOMA-IR time 0	116	0.61	0.47 to 0.74	<0.01 *
Ethnicity				
Black versus white		0.13	-0.74 to 0.99	0.7
Asian versus white		1.09	0.09 to 2.08	0.03*
Mixed versus white		0.64	-0.61 to 1.88	0.3
Time from 0 to 2		-0.97	-3.97 to 2.03	0.5
HOMA-IR time 0	114	0.64	0.5 to 0.77	<0.01 *
Pubertal stage time 0				
Mid Puberty versus pre/early		1.19	-0.29 to 2.66	0.11
Late/post versus pre/early		0.89	-1.46 to 3.23	0.4

HOMA-IR Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Pubertal stage time 2				
Mid Puberty versus pre/early		0.34	-1.76 to 2.44	0.7
Late/post versus pre/early		0.28	-2.6 to 3.17	0.8
Time from 0 to 2		-1.15	-4.12 to 1.82	0.4
HOMA-IR time 0	116	0.63	0.50 to 0.76	<0.01 *
Intervention		0.08	-0.65 to 0.81	0.8
Time from 0 to 2		-0.66	-3.58 to 2.26	0.4
HOMA-IR time 0	116	0.63	0.50 to 0.76	<0.01 *
IMD		0.01	-0.02 to 0.03	0.6
Time from 0 to 2		-0.67	-3.59 to 2.24	0.6
HOMA-IR time 0	113	0.62	0.48 to 0.75	<0.01 *
Eversmoked		0.006	-0.85 to 0.86	1.0
Time from 0 to 2		-0.63	-3.57 to 2.30	0.6
HOMA-IR time 0	116	0.60	0.46 to 0.73	<0.01 *
BMI time 0		-0.11	-2.04 to 1.81	0.9
BMI time 2		0.87	-0.83 to 2.58	0.3
Time from 0 to 2		-0.82	-3.67 to 2.03	0.6
HOMA-IR time 0	112	0.64	0.51 to 0.77	<0.01 *

HOMA-IR Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Fat mass index time 0		-0.11	-0.39 to 0.17	0.4
Fat mass index time 2		0.19	-0.08 to 0.46	0.17
Time from 0 to 2		-0.73	-3.65 to -2.19	0.6
HOMA-IR time 0	116	0.58	0.45 to 0.71	<0.01 *
waist time 0		0.04	-0.03 to 0.10	0.3
waist time 2		0.01	-0.05 to 0.07	0.7
Time from 0 to 2		-0.91	-3.76 to 1.95	0.5
HOMA-IR time 0	103	0.59	0.45 to 0.72	<0.01 *
SAD time 0		0.01	-0.12 to 0.13	0.9
SAD time 2		0.15	0.03 to 0.26	0.02*
Time from 0 to 2		-1.07	-3.98 to 1.83	0.5
HOMA-IR time 0	115	0.61	0.48 to 0.74	<0.01 *
Systolic BP time 0		0.002	-0.04 to 0.04	0.9
Systolic BP time 2		0.05	0.01 to 0.09	0.01
Time from 0 to 2		-0.84	-3.73 to 2.05	0.6
HOMA-IR time 0	115	0.62	0.49 to 0.75	<0.01 *
Systolic z BP time 0		-0.01	-0.37 to 0.35	1.0
Systolic z BP time 2		0.43	0.05 to 0.81	0.03*
Time from 0 to 2		-0.55	-3.48 to 2.39	0.7

HOMA-IR Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
HOMA-IR time 0	115	0.62	0.49 to 0.76	<0.01 *
Diastolic BP time 0		0.03	-0.01 to 0.07	0.14
Diastolic BP time 2		0.002	-0.04 to 0.04	0.9
Time from 0 to 2		-0.63	-3.58 to 2.31	0.6
HOMA-IR time 0	115	0.62	0.49 to 0.75	<0.01 *
Diastolic z BP time 0		0.22	-0.1 to 0.54	0.18
Diastolic z BP time 2		0.01	-0.3 to 0.33	0.9
Time from 0 to 2		-0.65	-3.59 to 2.3	0.6
HOMA-IR time 0	102	0.61	0.47 to 0.76	<0.01 *
C-wake time 0		0.02	-0.02 to 0.06	0.4
C-wake time 2		-0.002	-0.05 to 0.04	0.9
Time from 0 to 2		-0.7	-3.8 to 2.4	0.6
HOMA-IR time 0	64	0.96	0.71 to 1.22	<0.01 *
CAR-Rate time 0		-0.002	-0.03 to 0.03	0.8
CAR-Rate time 2		-0.01	-0.04 to 0.03	0.7
Cholesterol time 0		1.91	-3.63 to 7.45	0.4
HOMA-IR time 0	26	1.21	0.84 to 1.58	<0.01 *
C-evening time 0		-0.18	-0.48 to 0.12	0.2
C-evening time 2		-0.03	-0.16 to 0.11	0.6

HOMA-IR Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Time from 0 to 2		3.98	-10.74 to 18.7	0.5
HOMA-IR time 0	26	1.11	0.73 to 1.48	<0.01 *
C-Ratio time 0		-1.17	-2.95 to 0.62	0.19
C-Ratio time 2		-1.17	-4.49 to 2.16	0.4
Time from 0 to 2		6.98	-9.35 to 23.32	0.3
HOMA-IR time 0	64	0.95	0.7 to 1.21	<0.01 *
CAR-AUC time 0		0.06	-0.04 to 0.17	0.2
CAR-AUC time 2		-0.01	-0.11 to 0.09	0.8
Time from 0 to 2		0.86	-4.61 to 6.33	0.7
HOMA-IR time 0	26	1.16	0.7 to 1.63	<0.01 *
C-DayAUC time 0		<0.001 *	-0.01 to 0.01	0.9
C-DayAUC AUC time 2		0.002	-0.005 to 0.01	0.5
Time from 0 to 2		3.46	-13.3 to 20.21	0.6
HOMA-IR time 0	114	0.63	0.5 to 0.77	<0.01 *
A-File Total recent life changes time 0		0.01	-0.08 to 0.1	0.8
A-File Total recent life changes time 2		0.01	-0.09 to 0.12	0.8
Time from 0 to 2		-0.77	-3.74 to 2.21	0.6

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

Table 6.8 Regression models to predict HbA1c at time 2 using demographic, adiposity, blood pressure, blood risk measures, and stress measures as predictors, adjusted for the same variable at time 0, PWV at time 0 and time between measurements for time 2 and 0 in years.

HbA1c Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
HbA1c time 0	111	0.33	-0.15 to 0.81	0.17
Sex		0.21	-0.17 to 0.6	0.3
Time from 0 to 2		-0.13	-1.6 to 1.34	0.8
HbA1c time 0	111	0.45	-0.07 to 0.97	0.09
Ethnicity				
Black versus white		0.1	-0.35 to 0.56	0.6
Asian versus white		-0.2	-0.73 to 0.34	0.5
Mixed versus white		0.48	-0.17 to 1.14	0.15
Time from 0 to 2		-0.52	-2.06 to 1.01	0.5
HbA1c time 0	109	0.29	-0.2 to 0.78	0.2
Pubertal stage time 0				

HbA1c Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Mid Puberty versus pre/early		0.85	0.13 to 1.57	0.02
Late/post versus pre/early		0.81	-0.34 to 1.96	0.16
Pubertal stage time 2				
Mid Puberty versus pre/early		-0.92	-1.95 to 0.11	0.08
Late/post versus pre/early		-0.93	-2.35 to 0.49	0.2
Time from 0 to 2		-0.43	-1.91 to 1.04	0.5
HbA1c time 0	111	0.35	-0.13 to 0.83	0.15
Intervention		-0.28	-0.65 to 0.08	0.13
Time from 0 to 2		-0.20	-1.65 to 1.25	0.7
HbA1c time 0	111	0.26	-0.23 to 0.75	0.3
IMD		0.01	-0.01 to 0.02	0.3
Time from 0 to 2		-0.23	-1.70 to 1.23	0.7
HbA1c time 0	108	0.36	-0.14 to 0.87	0.16
Eversmoked		0.26	-0.20 to 0.72	0.3
Time from 0 to 2		-0.25	-1.73 to 1.23	0.7
HbA1c time 0	111	0.32	-0.17 to 0.80	0.20
BMI time 0		-0.75	-1.75 to 0.25	0.14
BMI time 2		0.79	-0.11 to 1.68	0.09

HbA1c Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Time from 0 to 2		-0.31	-1.77 to 1.15	0.7
HbA1c time 0	107	0.30	-0.20 to 0.80	0.2
Fat mass index time 0		-0.01	-0.16 to 0.14	0.8
Fat mass index time 2		0.04	-0.11 to 0.18	0.6
Time from 0 to 2		-0.22	-1.75 to 1.31	0.7
HbA1c time 0	111	0.30	-0.18 to 0.79	0.2
waist time 0		0.002	-0.03 to 0.03	0.9
waist time 2		0.004	-0.03 to 0.03	0.8
Time from 0 to 2		-0.24	-1.72 to 1.24	0.8
HbA1c time 0	99	0.09	-0.47 to 0.65	0.7
SAD time 0		0.04	-0.02 to 0.11	0.19
SAD time 2		0.01	-0.05 to 0.08	0.7
Time from 0 to 2		-0.27	-1.83 to 1.30	0.7
HbA1c time 0	110	0.40	-0.09 to 0.89	0.11
Systolic BP time 0		0.01	-0.01 to 0.02	0.6
Systolic BP time 2		0.02	-0.003 to 0.04	0.10
Time from 0 to 2		-0.18	-1.67 to 1.30	0.8
HbA1c time 0	110	0.37	-0.11 to 0.86	0.13
Systolic z BP time 0		0.04	-0.15 to 0.22	0.7

HbA1c Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
Systolic z BP time 2		0.15	-0.04 to 0.35	0.13
Time from 0 to 2		-0.08	-1.58 to 1.42	0.9
HbA1c time 0	110	0.33	-0.17 to 0.82	0.19
Diastolic BP time 0		0.01	-0.01 to 0.03	0.5
Diastolic BP time 2		-0.004	-0.02 to 0.02	0.7
Time from 0 to 2		-0.16	-1.66 to 1.34	0.8
HbA1c time 0	110	0.32	-0.17 to 0.81	0.2
Diastolic z BP time 0		0.05	-0.12 to 0.21	0.5
Diastolic z BP time 2		-0.04	-0.2 to 0.12	0.6
Time from 0 to 2		-0.15	-1.65 to 1.35	0.8
HbA1c time 0	97	0.2	-0.35 to 0.75	0.5
Awakening cortisol time 0		0.01	-0.01 to 0.04	0.2
Awakening cortisol time 2		-0.02	-0.04 to 0.01	0.16
Time from 0 to 2		-0.12	-1.7 to 1.46	0.8
HbA1c time 0	61	0.03	-0.89 to 0.95	0.9
Rate of change awakening cortisol rate time 0		-0.01	-0.03 to 0.01	0.2
Rate of change awakening cortisol rate time 2		0.003	-0.02 to 0.03	0.7
Cholesterol time 0		0.08	-3.56 to 3.73	0.9

HbA1c Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
HbA1c time 0	26	1.05	0.79 to 1.31	<0.01 *
Evening cortisol time 0		-0.01	-0.05 to 0.03	0.6
Evening cortisol time 2		-0.01	-0.03 to 0.01	0.17
Time from 0 to 2		-0.58	-2.61 to 1.45	0.5
HbA1c time 0	26	1.07	0.81 to 1.33	<0.01 *
Ratio awakening to evening cortisol time 0		-0.19	-0.44 to 0.05	0.11
Ratio awakening to evening cortisol time 2		-0.17	-0.61 to 0.28	0.4
Time from 0 to 2		-0.32	-2.54 to 1.9	0.7
HbA1c time 0	61	0.1	-0.86 to 1.06	0.8
Awakening cortisol AUC time 0		0.02	-0.05 to 0.1	0.5
Awakening cortisol AUC time 2		-0.03	-0.1 to 0.04	0.3
Time from 0 to 2		-0.88	-4.53 to 2.78	0.6
HbA1c time 0	26	1.06	0.78 to 1.34	<0.01 *
Day cortisol AUC time 0		<0.001 *	-0.001 to 0.001	0.5
Day cortisol AUC time 2		<0.001 *	-0.001 to 0.001	0.7
Time from 0 to 2		-0.86	-3.13 to 1.4	0.4
HbA1c time 0	109	0.31	-0.18 to 0.8	0.2
A-File Total recent life changes		0.002	-0.05 to 0.05	0.9

HbA1c Time 2	Multivariable model components			
	n	Coefficient	95% CI	P
time 0				
A-File Total recent life changes time 2		0.02	-0.03 to 0.08	0.4
Time from 0 to 2		-0.29	-1.78 to 1.21	0.7

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

Table 6.9 Logistic regression models to predict abnormal fasting insulin at time 2 using demographic, adiposity, blood pressure, blood risk measures, and stress measures as predictors, adjusted for the same variable at time 0, PWV at time 0 and time between measurements for time 2 and 0 in years.

Abnormal insulin Time 2	Multivariable model components			
	n	Odds ratio	95% CI	p
Abnormal insulin time 0	115	12.56	4.23 to 37.27	<0.01 *
Sex		1.21	0.46 to 3.2	0.7
Time from 0 to 2		0.34	0.003 to 34.42	0.6
Abnormal insulin time 0	115	14.82	4.47 to 49.09	<0.01 *
Ethnicity				
Black versus white		2.03	0.59 to 0.76	0.2

Abnormal insulin Time 2	Multivariable model components			
	n	Odds ratio	95% CI	p
Asian versus white		2.68	0.71 to 10.09	0.15
Mixed versus white		7.42	1.59 to 34.65	0.01*
Time from 0 to 2		0.12	0.001 to 11.51	0.3
Abnormal insulin time 0	115	32.66	6.77 to 157.51	<0.01 *
Pubertal stage time 0				
Mid Puberty versus pre/early		21.5	1.85 to 250.48	0.01*
Late/post versus pre/early		3.81	0.17 to 85.29	0.4
Pubertal stage time 2				
Mid Puberty versus pre/early		0.01	<0.001 * to 0.16	<0.01 *
Late/post versus pre/early		0.03	0.001 to 1.45	0.08
Time from 0 to 2		0.09	<0.001 * to 37.02	0.4
Abnormal insulin time 0	115	13.27	4.40 to 39.99	<0.001 *
Intervention		0.69	0.27 to 1.76	0.4
Time from 0 to 2		0.35	0.01 to 29.29	0.6
Abnormal insulin time 0	115	12.01	4.16 to 35.18	<0.01 *
IMD		1.00	0.97 to 1.03	1.00
Time from 0 to 2		0.31	0.003 to 30.14	0.6
Abnormal insulin time 0	112	11.19	3.82 to 32.79	<0.001 *

Abnormal insulin Time 2	Multivariable model components			
	n	Odds ratio	95% CI	p
Eversmoked		1.05	0.36 to 3.02	0.9
Time from 0 to 2		0.30	0.003 to 28.96	0.6
Abnormal insulin time 0	115	10.13	3.30 to 31.03	<0.001 *
BMI time 0		0.60	0.04 to 8.63	0.7
BMI time 2		2.39	0.21 to 27.19	0.5
Time from 0 to 2		0.23	0.00 to 28.35	0.5
Abnormal insulin time 0	111	14.62	4.48 to 47.73	<0.01 *
Fat mass index time 0		0.93	0.64 to 1.34	0.7
Fat mass index time 2		1.06	0.74 to 1.52	0.7
Time from 0 to 2		0.29	0.00 to 30.16	0.6
Abnormal insulin time 0	115	11.11	3.45 to 35.72	<0.01 *
waist time 0		1.00	0.91 to 1.09	0.9
waist time 2		1.01	0.94 to 1.09	0.7
Time from 0 to 2		0.27	0.003 to 28.04	0.5
Abnormal insulin time 0	102	11.88	3.46 to 40.80	<0.01 *
SAD time 0		0.96	0.82 to 1.12	0.6
SAD time 2		1.06	0.91 to 1.23	0.5
Time from 0 to 2		0.21	0.002 to 29.68	0.5
Abnormal insulin time 0	114	11.79	4.01 to 34.69	<0.01 *

Abnormal insulin Time 2	Multivariable model components			
	n	Odds ratio	95% CI	p
Systolic BP time 0		1.01	0.96 to 1.06	0.7
Systolic BP time 2		1.03	0.98 to 1.08	0.3
Time from 0 to 2		0.29	0.003 to 32.37	0.6
Abnormal insulin time 0	114	11.46	3.93 to 33.44	<0.01 *
Systolic z BP time 0		1.02	0.65 to 1.59	0.9
Systolic z BP time 2		1.18	0.73 to 1.91	0.5
Time from 0 to 2		0.31	0.003 to 33.50	0.6
Abnormal insulin time 0	114	12.41	4.21 to 36.59	<0.01 *
Diastolic BP time 0		1.02	0.97 to 1.08	0.3
Diastolic BP time 2		1	0.95 to 1.04	0.8
Time from 0 to 2		0.31	0.003 to 34.96	0.6
Abnormal insulin time 0	114	12.15	4.15 to 35.54	<0.01 *
Diastolic z BP time 0		1.18	0.79 to 1.76	0.4
Diastolic z BP time 2		0.97	0.66 to 1.43	0.8
Time from 0 to 2		0.31	0.003 to 33.57	0.6
Abnormal insulin time 0	101	9.11	2.97 to 27.96	<0.01 *
Awakening cortisol time 0		1	0.96 to 1.05	0.9
Awakening cortisol time 2		0.97	0.91 to 1.02	0.2
Time from 0 to 2		0.22	0.001 to 36.24	0.5

Abnormal insulin Time 2	Multivariable model components			
	n	Odds ratio	95% CI	p
Abnormal insulin time 0	63	9.65	2.2 to 42.27	0.03*
Rate of change awakening cortisol rate time 0		0.98	0.94 to 1.02	0.4
Rate of change awakening cortisol rate time 2		1.01	0.97 to 1.05	0.6
Cholesterol time 0		0.89	0.001 to 859.21	1.0
Abnormal insulin time 0	25	11.07	1.17 to 104.45	0.04*
Evening cortisol time 0		1.04	0.67 to 1.63	0.8
Evening cortisol time 2		0.98	0.79 to 1.22	0.8
Time from 0 to 2		54600000	0.004 to 7.01E+17	0.13
Abnormal insulin time 0	25	9.91	0.89 to 109.93	0.06
Ratio awakening to evening cortisol time 0		0.25	0.002 to 30.37	0.5
Ratio awakening to evening cortisol time 2		0.06	<0.001 * to 18.39	0.3
Time from 0 to 2		1.23E+10	0.001 to 1.68E+23	0.13
Abnormal insulin time 0	63	6.88	1.67 to 28.28	0.01
Awakening cortisol AUC time 0		1.02	0.91 to 1.16	0.7
Awakening cortisol AUC time 2		1.02	0.91 to 1.14	0.7
Time from 0 to 2		0.3	<0.001 * to 331.06	0.7
Abnormal insulin time 0	25	23.02	0.62 to 853.96	0.09

Abnormal insulin Time 2	Multivariable model components			
	n	Odds ratio	95% CI	p
Day cortisol AUC time 0		1	0.98 to 1.01	0.7
Day cortisol AUC time 2		1.01	0.99 to 1.02	0.3
Time from 0 to 2		1.17E+12	0.002 to 5.67E+26	0.11
Abnormal insulin time 0	113	15.9	4.97 to 50.82	<0.01 *
A-File Total recent life changes time 0		1.1	0.98 to 1.23	0.12
A-File Total recent life changes time 2		0.93	0.81 to 1.07	0.3
Time from 0 to 2		0.21	0.001 to 29.18	0.5

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

Table 6.10 Logistic regression models to predict abnormal HOMA-IR at time 2 using demographic, adiposity, blood pressure, blood risk measures, and stress measures as predictors, adjusted for the same variable at time 0, PWV at time 0 and time between measurements for time 2 and 0 in years.

Abnormal HOMA-IR Time 2	Multivariable model components			
	n	Odds ratio	95% CI	P

Abnormal HOMA-IR Time 2	Multivariable model components			
	n	Odds ratio	95% CI	P
Abnormal HOMA-IR time 0	117	12.52	4.32 to 36.23	<0.001 *
Sex		1.89	0.67 to 5.28	0.2
Time from 0 to 2		2.4	0.06 to 90.9	0.6
Abnormal HOMA-IR time 0	117	10.28	3.61 to 29.23	<0.01 *
Ethnicity				
Black versus white		1.08	0.34 to 3.48	0.8
Asian versus white		2.06	0.6 to 7.06	0.2
Mixed versus white		2.24	0.48 to 10.36	0.3
Time from 0 to 2		1.04	0.03 to 40.76	0.9
Abnormal HOMA-IR time 0	110	15.75	4.74 to 52.36	<0.01 *
Pubertal stage time 0				
Mid Puberty versus pre/early		2.81	0.34 to 22.91	0.3
Late/post versus pre/early		2.08	0.09 to 47.8	0.6
Pubertal stage time 2				
Mid Puberty versus pre/early		0.77	0.06 to 9.43	0.8
Late/post versus pre/early				
Time from 0 to 2		0.91	0.02 to 44.24	0.9
Abnormal HOMA-IR time 0	117	10.31	3.75 to 28.36	<0.01 *

Abnormal HOMA-IR Time 2	Multivariable model components			
	n	Odds ratio	95% CI	P
Intervention		1.38	0.55 to 3.46	0.5
Time from 0 to 2		1.64	0.05 to 60.10	0.7
Abnormal HOMA-IR time 0	117	11.12	4.02 to 30.74	<0.01 *
IMD		1.02	0.98 to 1.05	0.3
Time from 0 to 2		1.65	0.05 to 57.42	0.7
Abnormal HOMA-IR time 0	114	9.43	3.40 to 26.19	<0.01*
Eversmoked		0.64	0.21 to 1.96	0.4
Time from 0 to 2		1.81	0.06 to 58.53	0.7
Abnormal HOMA-IR time 0	117	8.71	3.08 to 24.63	<0.01 *
BMI time 0		1.18	0.09 to 15.16	0.9
BMI time 2		2.40	0.24 to 23.88	0.4
Time from 0 to 2		1.65	0.04 to 69.41	0.7
Abnormal HOMA-IR time 0	113	13.51	4.60 to 39.65	4.6
Fat mass index time 0		0.97	0.67 to 1.41	0.6
Fat mass index time 2		1.12	0.78 to 1.60	0.7
Time from 0 to 2		2.34	0.06 to 97.98	0.06
Abnormal HOMA-IR time 0	117	8.64	3.08 to 24.21	<0.01 *
waist time 0		1.03	0.95 to 1.12	0.4
waist time 2		1.01	0.94 to 1.08	0.8

Abnormal HOMA-IR Time 2	Multivariable model components			
	n	Odds ratio	95% CI	P
Time from 0 to 2		1.38	0.04 to 53.06	0.8
Abnormal HOMA-IR time 0	104	10.02	3.35 to 29.97	<0.01 *
SAD time 0		1.03	0.88 to 1.20	0.7
SAD time 2		1.04	0.89 to 1.21	0.6
Time from 0 to 2		1.15	0.03 to 51.49	0.9
Abnormal HOMA-IR time 0	116	10.81	3.84 to 30.42	<0.01 *
Systolic BP time 0		1.00	0.95 to 1.05	0.9
Systolic BP time 2		1.05	1.00 to 1.11	0.07
Time from 0 to 2		1.48	0.04 to 56.78	0.8
Abnormal HOMA-IR time 0	116	10.85	3.87 to 30.43	<0.01 *
Systolic z BP time 0		0.99	0.62 to 1.57	0.9
Systolic z BP time 2		1.50	0.91 to 2.47	0.11
Time from 0 to 2		1.97	0.05 to 77.98	0.7
Abnormal HOMA-IR time 0	116	11.8	4.16 to 33.49	<0.01 *
Diastolic BP time 0		1.03	0.98 to 1.09	0.2
Diastolic BP time 2		1.01	0.96 to 1.06	0.7
Time from 0 to 2		1.65	0.04 to 62.77	0.7
Abnormal HOMA-IR time 0	116	11.44	4.07 to 32.15	<0.01 *
Diastolic z BP time 0		1.25	0.83 to 1.89	0.3

Abnormal HOMA-IR Time 2	Multivariable model components			
	n	Odds ratio	95% CI	P
Diastolic z BP time 2		1.07	0.72 to 1.59	0.7
Time from 0 to 2		1.64	0.04 to 60.39	0.7
Abnormal HOMA-IR time 0	103	9.52	3.26 to 27.8	<0.01 *
C-wake time 0		1	0.95 to 1.05	1.0
C-wake time 2		1	0.95 to 1.06	0.8
Time from 0 to 2		1.15	0.03 to 47.88	0.9
Abnormal HOMA-IR time 0	64	8.47	2.21 to 32.43	<0.01 *
CAR-Rate time 0		1	0.96 to 1.04	0.9
CAR-Rate time 2		0.98	0.94 to 1.02	0.3
Cholesterol time 0		1.87	0.002 to 2002.08	0.8
Abnormal HOMA-IR time 0	26	7.24	1 to 52.47	0.05
C-evening time 0		0.88	0.5 to 1.55	0.6
C-evening time 2		1	0.8 to 1.24	1.0
Time from 0 to 2		5749213	<0.001 * to 2.37E+17	0.21
Abnormal HOMA-IR time 0	26	4.28	0.52 to 35.07	0.18
C-Ratio 0		0.003	<0.001 * to 1132.45	0.4
C-Ratio time 2		0.02	<0.001 * to 18	0.3
Time from 0 to 2		1400000000	<0.001 * to 5.45E+22	0.19

Abnormal HOMA-IR Time 2	Multivariable model components			
	n	Odds ratio	95% CI	P
Abnormal HOMA-IR time 0	64	8.1	2.13 to 30.82	<0.01 *
CAR-AUC time 0		1.01	0.89 to 1.15	0.8
CAR-AUC time 2		1.05	0.93 to 1.17	0.4
Time from 0 to 2		1.18	0.001 to 1182.34	1.0
Abnormal HOMA-IR time 0	26	10.73	0.98 to 116.94	0.05
C-DayAUC time 0		1	0.99 to 1.01	0.5
C-DayAUC time 2		1.01	0.99 to 1.02	0.3
Time from 0 to 2		54900000	<0.001 * to 9.23E+21	0.29
Abnormal HOMA-IR time 0	115	10.59	3.73 to 30.04	<0.01 *
A-File Total recent life changes time 0		1.11	0.99 to 1.24	0.07
A-File Total recent life changes time 2		0.94	0.82 to 1.07	0.3
Time from 0 to 2		1.36	0.03 to 53.85	0.8

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

6.3 Multilevel modelling of anthropometry, blood pressure and cortisol measures over times t0-t4

6.3.1. Summaries of longitudinal data across time points

Where variables were measured three or more times during the trial, multi-level models were applied to examine change over time and relationships between variables over time. Summaries of sample sizes for adiposity and blood pressure at each time point (t0-3) are shown in table 6.11; and for cortisol measures in 6.12. Note that the A-FILE was only recorded at time 0 and 2 and is thus not included in these analyses. Data was incomplete for all adiposity, blood pressure and cortisol measures at time points 1, 2 and 3 with increasing levels of incomplete data as time progressed. BMI was the most consistently measured over time, and was measured for all participants who were repeatedly measured during the trial at all time points, with 66% complete at time 3 compared to time 0. At time 2, 10 participants had withdrawn from the HELP trial, and 19 had been lost to follow up; by time 3, 25 participants had withdrawn and 34 were lost to follow up. Note that for a small number of participants, data for time point 3 were chased some time after 1 year, but for most time point 3 was close to 1 year, and this is highlighted on the trajectory graphs for each measure shown below.

Table 6.11 Adiposity and blood pressure markers at each time point

Variable	N at time 0	N at time 1	N at time 2	N at time 3	TOTAL
BMI	174	136	145	115	570
Fat mass index	171	130	117	102	520
SAD	159	126	140	110	535
Waist	174	134	125	115	548
Systolic BP	172	134	143	113	562

Variable	N at time 0	N at time 1	N at time 2	N at time 3	TOTAL
Systolic z BP	172	134	143	111	560
Diastolic BP	172	134	143	113	562
Diastolic z BP	172	134	143	111	560

Table 6.12 Cortisol measures at each time point

Variable	N at time 0	N at time 1	N at time 2	N at time 3	TOTAL
C-wake	163	-	131	93	387
CAR-Rate	131	-	91	53	275
C-evening	53	-	73	53	179
C-Ratio	53	-	73	53	179
CAR-AUC	131	-	91	53	275
C-DayAUC	53	-	73	53	179

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

6.3.2 Change in adiposity measures over time

Figures 6.2-6.4 show the individual trajectories for adiposity measures for each participant over time plotted together, with time in years. Note that for a small number of participants, data for time point 3 were chased some time after 1 year, but for most time point 3 was close to 1 year. Multi-level models for each adiposity

measure predicted by time are then shown in table 6.13. There was a significant, positive change in both BMI and fat mass index over time during the time of study. BMI increased by 0.59 kg/m^2 per year (95% CI 0.21 – 0.96) from a baseline average of 32.38 (95% CI 31.73 to 33.02). Fat mass increased by 0.43 kg/m^2 per year (95% CI 0.07 -0.78). There was no relationship for waist or SAD with time. A considerable proportion of variation from the fixed components of the model was explained by non-time dependent, unaccounted for inter-participant variation, at 93% and 94% for fat mass index. For each individual adiposity model, models using t^2 and t^3 were created. In all cases non-linear models were not significant.

Figure 6.2 Graph of change in BMI for the participants over time (time in years) with all participant trajectories over time joined by a single line per participant.

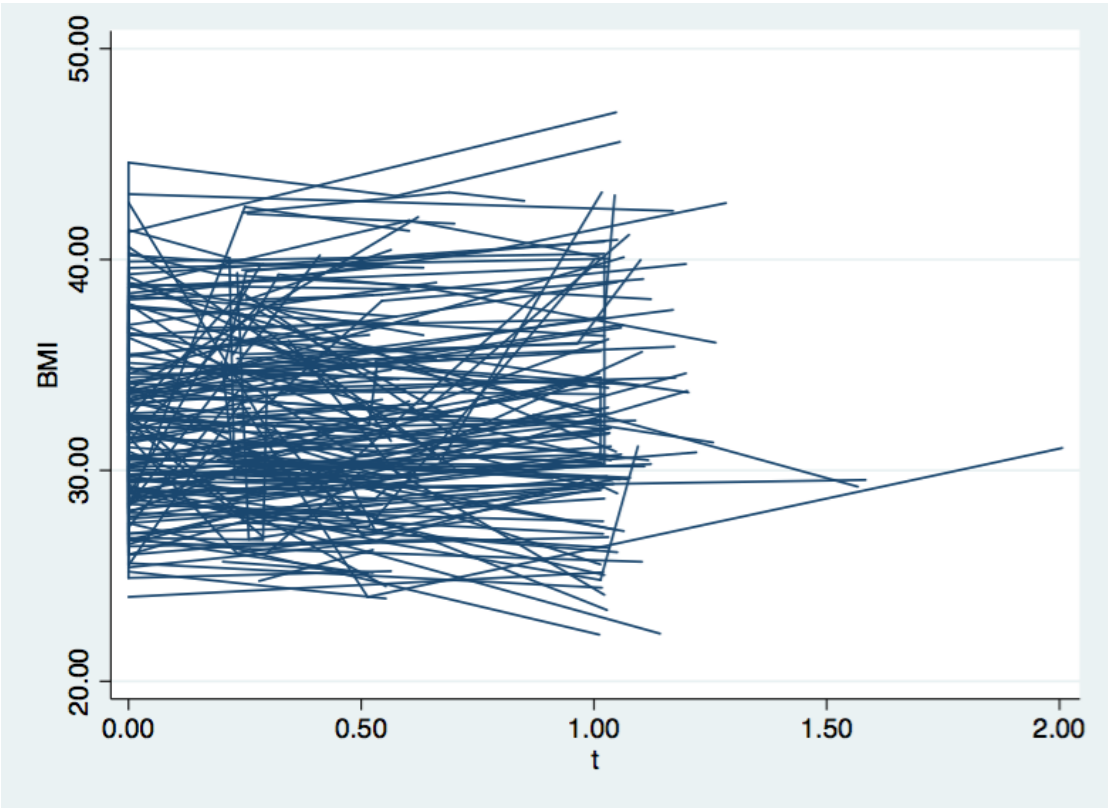


Figure 6.3 Graph of change in fat mass index for the participants over time (time in years) with all participant trajectories over time joined by a single line per participant

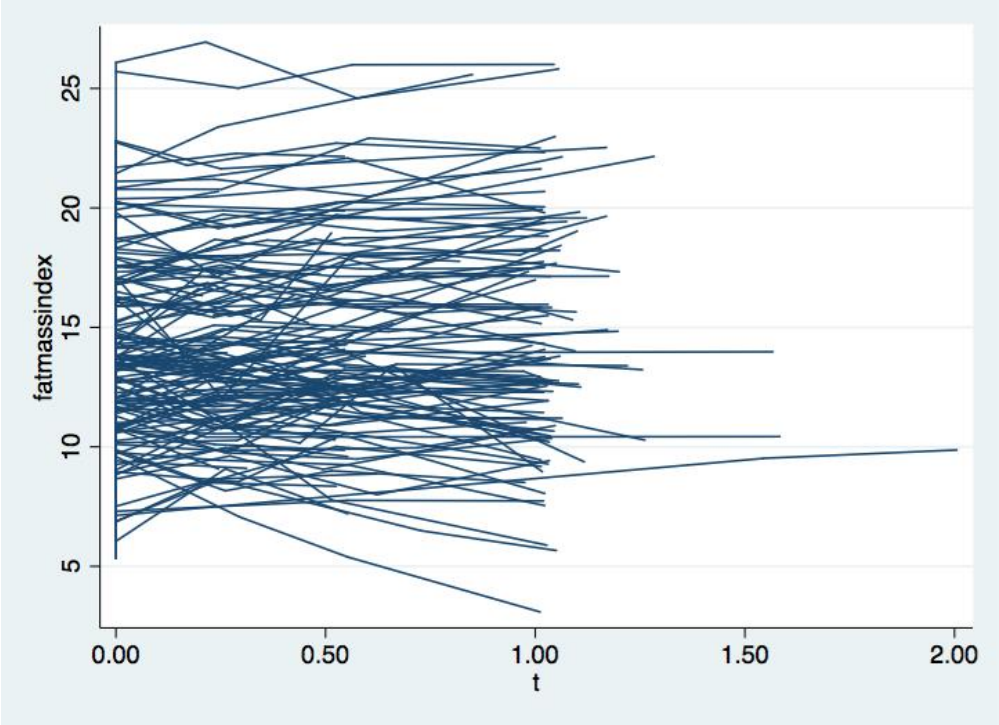


Figure 6.4 Graph of change in waist circumference for the participants over time(time in years) with all participant trajectories over time joined by a single line per participant

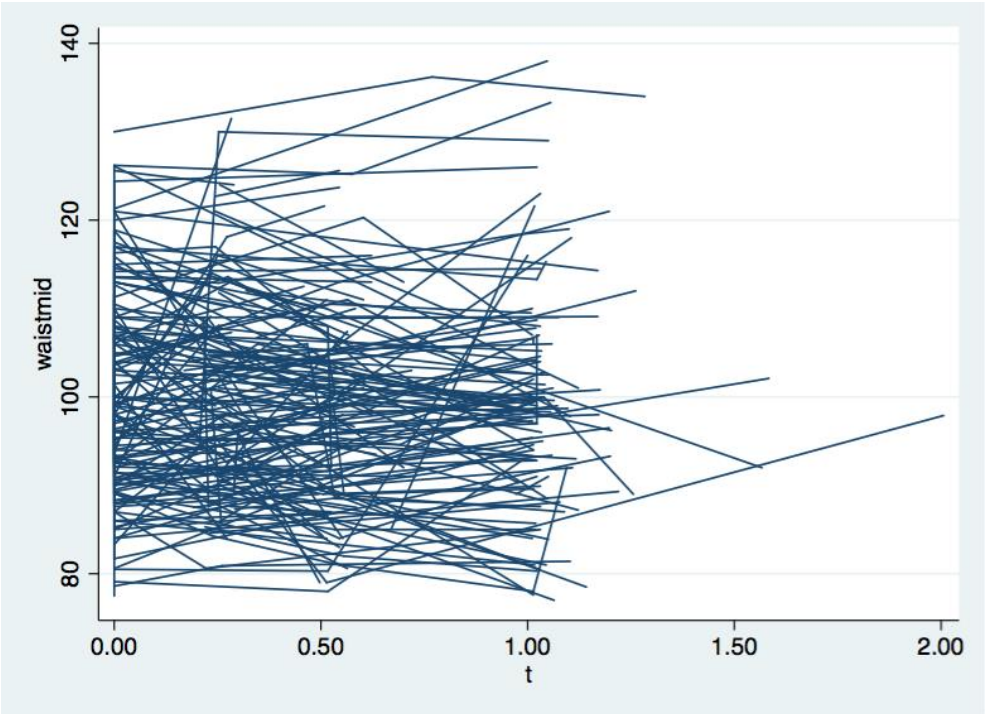


Figure 6.5 Graph of change in waist SAD for the participants over time (time in years) with all participant trajectories over time joined by a single line per participant.

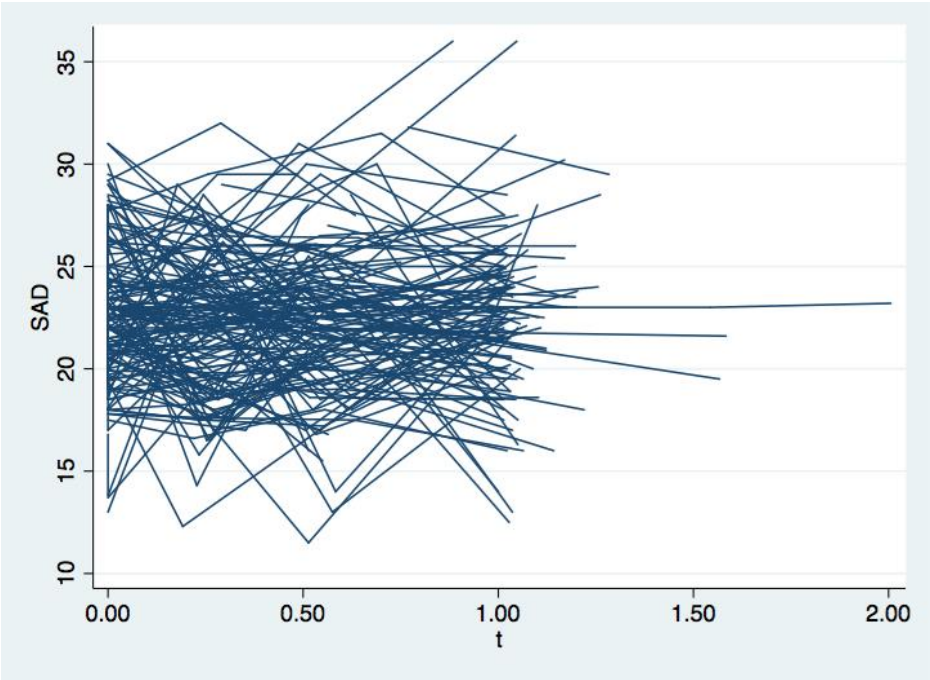


Table 6.13. Multi-level models (random intercepts) of each adiposity model with time.

	Fixed effects							Random effect components of models	
Adiposity	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
BMI	570	174	0.59	0.21 to 0.96	<0.01*	32.38	31.73 to 33.02	4.23	1.08
Fat mass index	520	173	0.43	0.09 to 0.78	0.01 *	14.10	13.53 to 14.68	3.75	0.93
SAD	535	170	0.08	-0.56 to 0.72	0.8	22.49	22.01 to 22.97	2.06	2.89
Waist	548	174	-1.07	-2.63 to 0.15	0.09	100.20	98.58 to 101.82	10.10	4.92

6.3.3 Change in blood pressure measures over time.

As stated in chapter 4, at baseline no participants had systolic hypertension, and 4 had diastolic hypertension. At t3, 1 participant had developed systolic hypertension. At t3, only 2 participants had diastolic hypertension, however 2 of the baseline participants with diastolic hypertension were lost to follow-up.

Figures 6.5-6.8 show the individual trajectories for blood pressure measures for each participant over time, for systolic, systolic-z score, diastolic, and diastolic z-score, plotted together. Note that as for adiposity, for a small number of participants, data for time point 3 were chased some time after 1 year, but for most time point 3 was close to 1 year. Multi-level models for each blood pressure measure predicted by time are then shown in table 6.14. There was a significant, positive change in all blood pressure measurements over time during the time of study. Raw systolic BP increased by 4mm Hg per year (95% CI 2.02 to 5.98), systolic z score by 0.28 per year (95% CI 0.08 to 0.48), raw diastolic increased by 3.36 mmHg per year (95% CI 1.39 to 5.32) and diastolic z score by 0.31 (95%CI 0.07 to 0.55).

Random effect outputs from models showed that proportions of variation from the fixed models explained by non time-variant within participant factors were 28%, 25%, 23% and 17% for raw systolic BP, z-systolic, diastolic BP and diastolic z respectively. For each individual blood pressure model, models using t^2 and t^3 were created. In all cases non-linear models were not significant.

Figure 6.5 Graph of change in systolic blood pressure for the participants over time with all participant trajectories over time joined by a single line per participant.

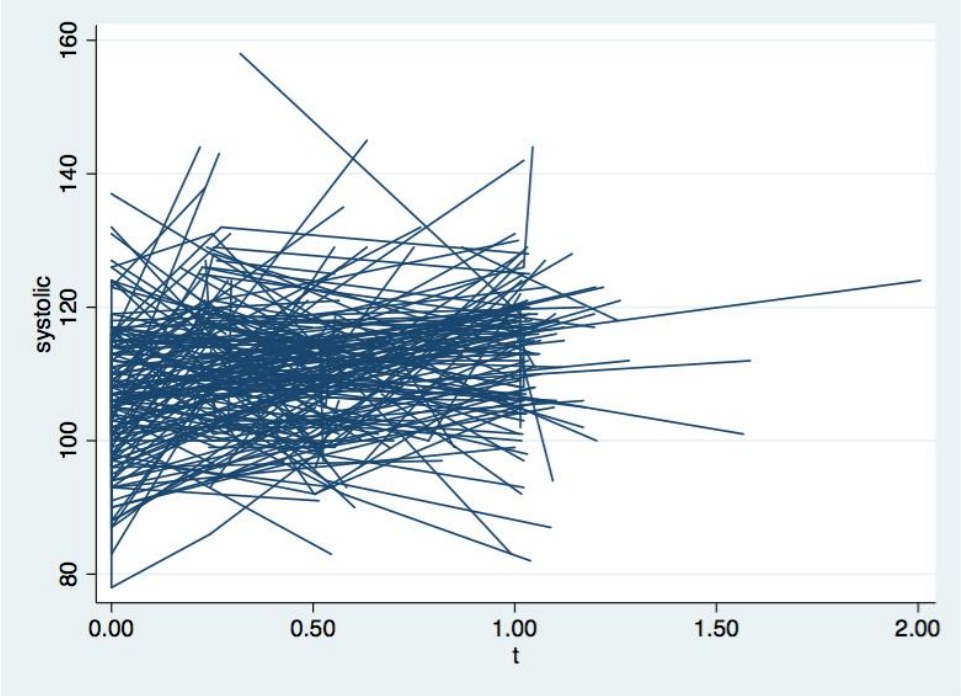


Figure 6.6 Graph of change in systolic z-score blood pressure for the participants over time with all participant trajectories over time joined by a single line per participant.

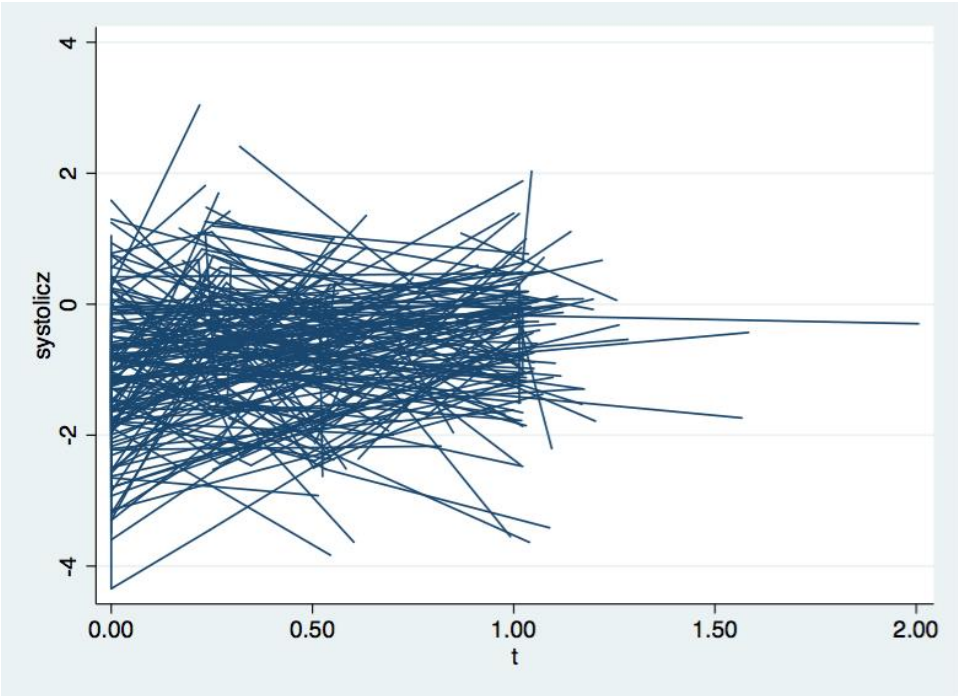


Figure 6.7 Graph of change in diastolic blood pressure for the participants over time with all participant trajectories over time joined by a single line per participant.

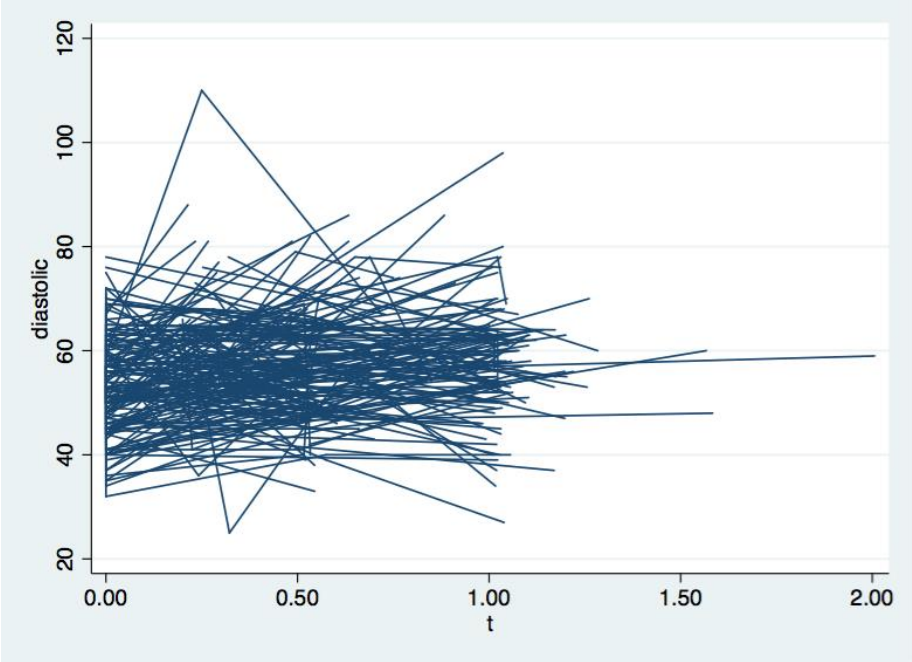


Figure 6.8 Graph of change in diastolic z-score blood pressure for the participants over time with all participant trajectories over time joined by a single line per participant

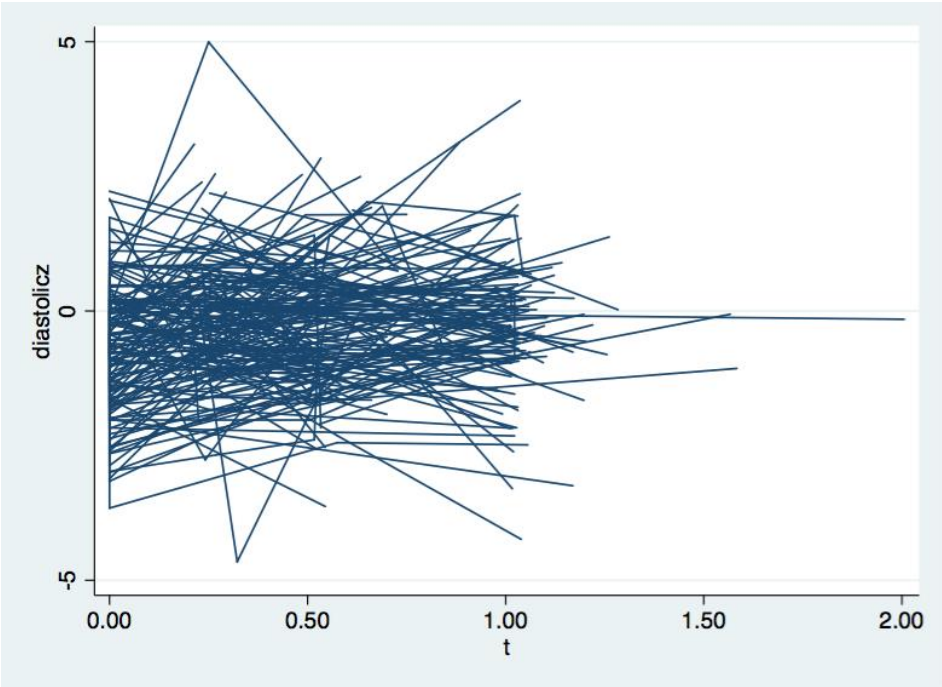


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Table 6.14. Multi-level models (random intercepts) of each blood pressure model with time.

	Fixed effects components							Random effect components of models	
Blood pressure	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Systolic	562	174	4.00	2.02 to 5.98	<0.01*	108.97	107.52 to 110.40	5.87	9.34
Systolic z	560	174	0.28	0.08 to 0.48	<0.01*	-0.86	-1.01 to -0.72	0.55	0.94
Diastolic	562	174	3.36	1.39 to 5.32	<0.01*	55.12	53.77 to 56.47	5.04	9.20
Diastolic z	560	174	0.31	0.07 to 0.55	<0.01*	-0.45	-0.61 to -0.30	0.51	1.12

6.3.4 Change in cortisol measures over time

Figures 6.9-6.14 show the individual trajectories for cortisol measures for each participant over time plotted together, with time in years. Multi-level models for each cortisol measure predicted by time are then shown in table 6.14. There was a significant, positive change in cortisol at awakening value (C-wake) over time during the time of study, with an increase of 3.88 nmol/L per year (95% CI 1.67 to 6.08). No associations were found in change in other measures of cortisol over time.

Random effects for C-wake showed that variation from the fixed model accounted for by only 4% within individual non time variant factors unaccounted for in the multi-level model. This implied that more simple modeling (eg. simple linear regression would have been appropriate), and indeed a linear regression of time on BMI produced a similar model : coefficient 3.89, 95%CI 1.64 to 6.14, $p < 0.001$). For each individual cortisol model, models using t^2 and t^3 were created. In all cases non-linear models were not significant.

Figure 6.9 Graph of change in C-wake for the participants over time with all participant trajectories over time joined by a single line per participant

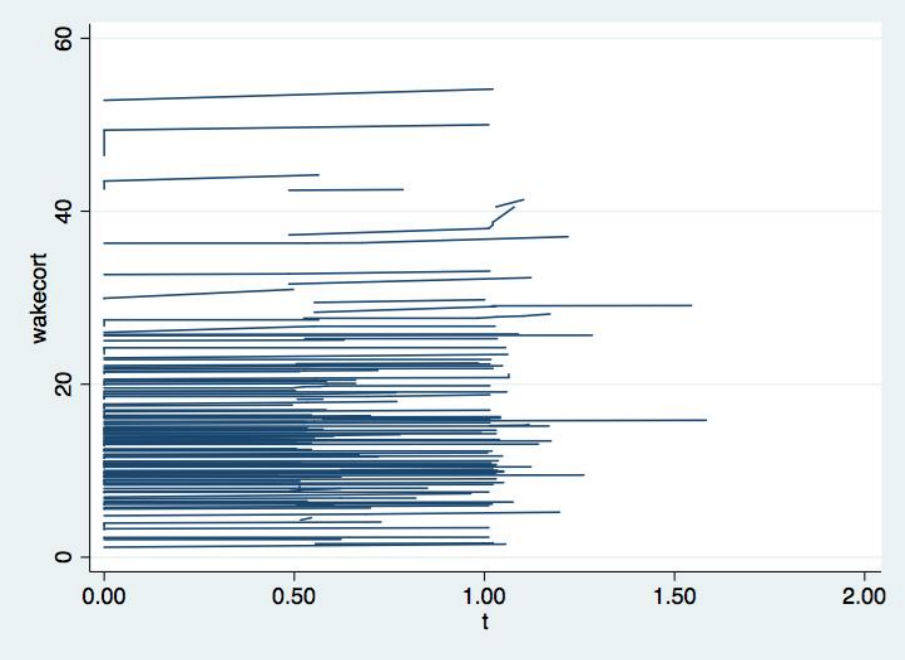

Figure 6.10 Graph of CAR-Rate for the participants over time with all participant trajectories over time joined by a single line per participant

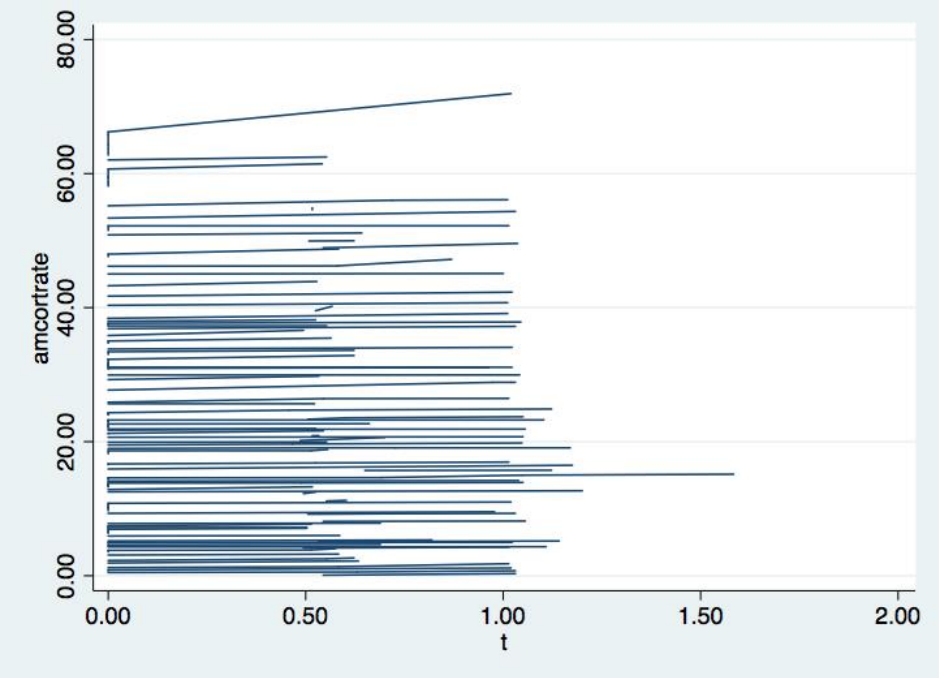

Figure 6.11 Graph of change in C-evening for the participants over time with all participant trajectories over time joined by a single line per participant

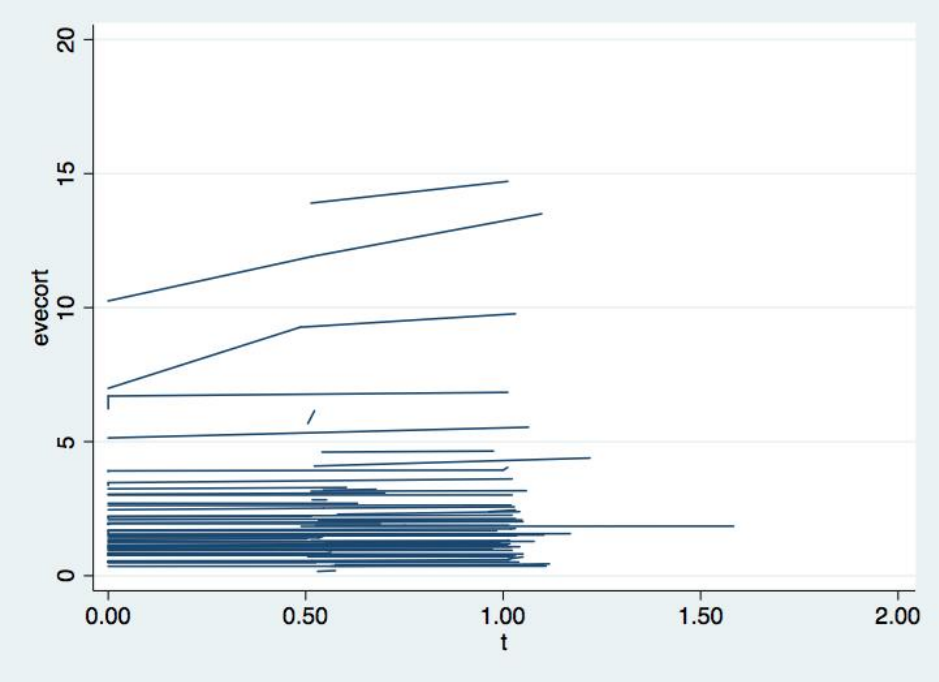

Figure 6.12 Graph of change in C-Ratio for the participants over time with all participant trajectories over time joined by a single line per participant

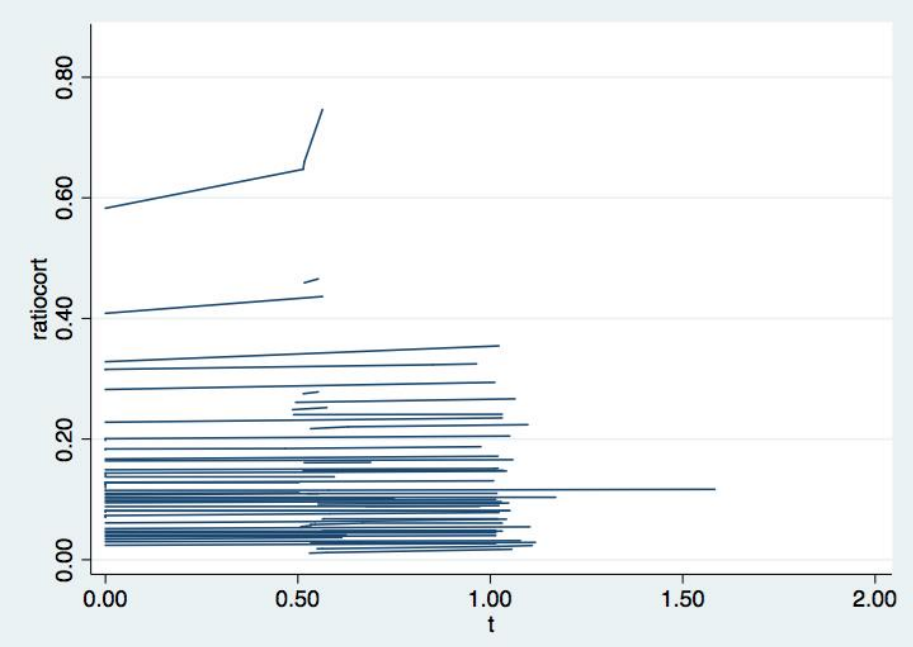

Figure 6.13 Graph of CAR-AUC for the participants over time with all participant trajectories over time joined by a single line per participant

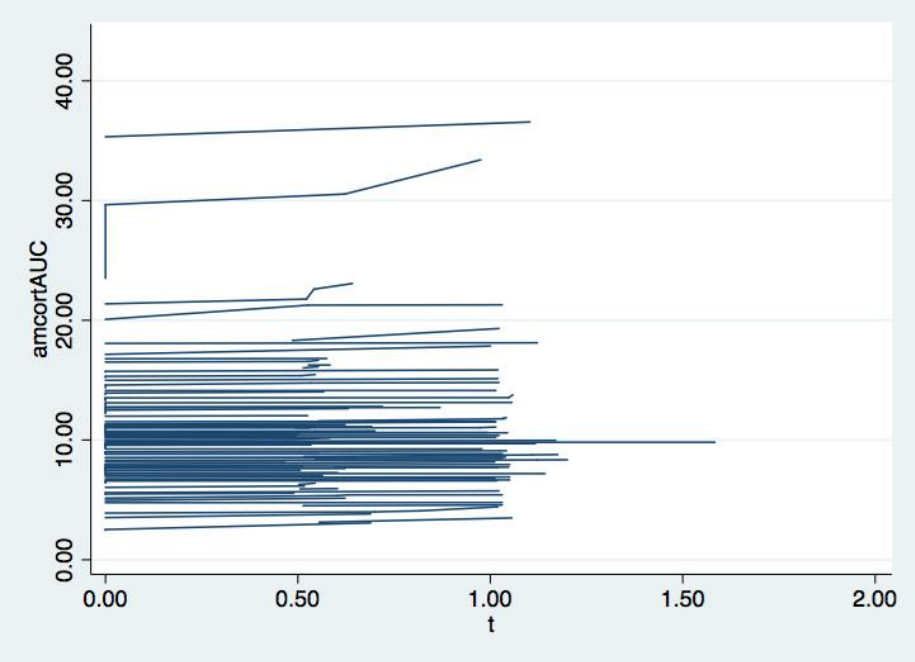

Figure 6.14 Graph of change C-DayAUC for the participants over time with all participant trajectories over time joined by a single line per participant

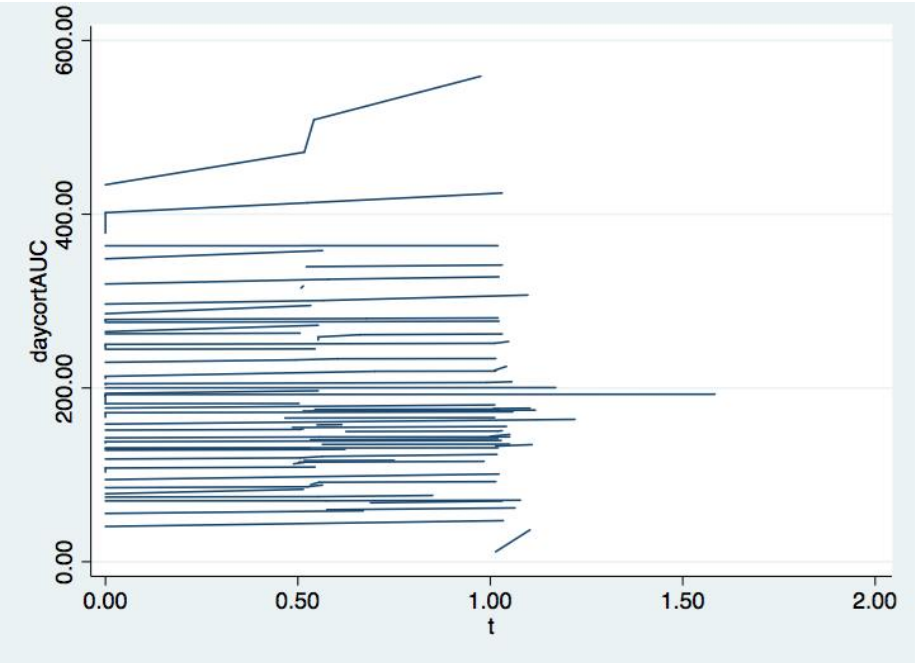

Table 6.14. Multi-level models (random intercepts) of each cortisol measure over time

	Fixed effects models							Random effect components of models	
	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
C-wake	387	160	3.88	1.67 to 6.08	<0.01*	15.03	13.63 to 16.42	1.92	9.42
CAR-Rate	275	157	-2.41	-7.15 to 2.32	0.3	24.91	22.17 to 27.65	3.10	16.50
C-evening	179	104	0.36	-0.55 to 1.28	0.4	2.21	1.55 to 2.86	1.01	2.50
C-Ratio	179	104	-0.03	-0.09 to 0.02	0.2	0.17	0.14 to 0.21	0.02	0.15
CAR-AUC	275	157	-0.13	-1.69 to 1.45	0.8	10.83	9.93 to 11.73	0.27	5.48

	Fixed effects models							Random effect components of models	
	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
C-DayAUC	179	104	-11.41	-43.87 to 21.05	0.5	198.64	175.26 to 222.02	47.43	84.08

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

6.3.5 Relationships between adiposity measures over time and baseline characteristics, pubertal change and stress measures

After individual models for adiposity and time were constructed, individual baseline characteristics – Sex, ethnicity, deprivation score and intervention status were added into models individually with time to predict adiposity measures. Next covariates that altered over time – pubertal status and cortisol measures - were added individually to models with time to predict adiposity measures. The addition of cortisol measures was performed to look for associations between change in cortisol measures over time and change in adiposity markers, to test one of the key hypotheses of the thesis.

These models are presented together in tables 6.15-6.18 below.

There were few associations found. For BMI, late/complete puberty compared to early/pre pubertal status was associated with BMI positively over time (and time remained significant in the model). There was a negative association between Sex and SAD over time (though time was no longer significant in this model).

There were no statistically significant associations found between any of the cortisol measures and any of the adiposity measures.

Table 6.15 Multi-level models (random intercepts) of individual baseline characteristics, pubertal change over time, and cortisol measures over time in models with time to predict BMI.

	Fixed effects components of models							Random effect components of model	
	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Time	570	174	0.58	0.21 to 0.96	<0.01 *	30.68	28.44 to 32.92	4.21	1.08
Sex			1.04	-0.28 to 2.36	0.12				
Time	425	174	0.43	0.07 to 0.80	0.02*	31.20	30.21 to 32.19	4.25	0.75
Pubertal stage									
Mid Puberty versus pre/early			0.26	-0.44 to 0.96	0.4				

	Fixed effects components of models							Random effect components of model	
	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Late/post versus pre/early			1.60	0.64 to 2.56	<0.01 *				
Time	570	174	0.59	0.21 to 0.98	<0.01 *	32.37	31.33 to 33.41	4.23	1.08
Ethnicity									
Black versus white			0.46	-1.09 to 2.01	0.5				
Asian versus white			-0.31	-2.06 to 1.44	0.7				
Mixed versus white			-0.57	-2.83 to 1.56	0.6				
Time	570	174	0.56	0.21 to 0.96	<0.01 *	32.17	31.26 to 33.08	4.23	1.08

	Fixed effects components of models							Random effect components of model	
	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Intervention			0.43	-0.86 to 1.71	0.5				
Time	387	173	0.72	0.35 to 1.09	<0.01 *	32.28	31.60 to 32.97	4.44	1.15
C-wake			-0.00	-0.02 to 0.12	0.9				
Time	275	157	0.60	0.11 to 1.07	0.02*	32.32	31.60 to 33.05	4.40	1.21
CAR-Rate			-0.00	-0.02 to 0.01	0.5				
Time	179	104	0.80	0.26 to 1.34	<0.01 *	31.38	30.46 to 32.28	4.38	0.98

	Fixed effects components of models							Random effect components of model	
	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
C-evening			-0.04	-0.11 to 0.04	0.2				
Time	179	104	0.62	0.09 to 1.16	0.02*	31.30	30.41 to 32.18	4.41	0.98
C-Ratio			-1.22	-2.72 to 0.27	0.11				
Time	275	157	0.60	0.12 to 1.07	0.02*	32.23	31.47 to 33.00	4.40	1.19
CAR-AUC			-0.01	-0.05 to 0.03	0.5				
Time	179	104	0.66	0.15 to 1.17	0.01*	31.61	30.62 to 32.61	4.41	0.96

	Fixed effects components of models							Random effect components of model	
	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
C-DayAUC			-0.00	-0.00 to 0.00	0.10				

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-DayAUC : total day cortisol from awakening to evening AUC

Table 6.16 Multi-level models (random intercepts) of individual baseline characteristics, pubertal change over time, and cortisol measures over time in models with time to predict fat mass index.

	Fixed effects components of models							Random effect components of model	
Fat mass index	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Time	520	173	0.43	0.09 to 0.78	0.01*	9.95	8.04 to 11.85	3.55	0.93
Sex			2.54	1.42 to 3.67	<0.01 *				
Time	382	173	0.36	-0.00 to 0.73	0.06	13.56	12.58 to 14.53	3.76	0.84
Pubertal stage									
Mid Puberty versus pre/early			0.24	-0.50 to 0.98	0.5				

	Fixed effects components of models							Random effect components of model	
Fat mass index	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Late/post versus pre/early			0.74	-0.26 to 1.74	0.15				
Time	520	173	0.43	0.09 to 0.78	0.01*	14.25	13.32 to 15.18	3.75	0.93
Ethnicity									
Black versus white			-0.18	-1.58 to 1.21	0.8				
Asian versus white			-0.29	-1.84 to 1.27	0.7				
Mixed versus white			-0.35	-2.30 to 1.60	0.7				
Time	520	173	0.43	0.08 to 0.78	0.01*	13.80	13.00 to 14.61	3.74	0.93

	Fixed effects components of models							Random effect components of model	
Fat mass index	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Intervention			0.60	-0.53 to 1.73	0.3				
Time	354	172	0.58	0.21 to 0.97	<0.01 *	14.00	13.3 to 14.62	3.76	1.14
C-wake			0.01	-0.01 to 0.02	0.3				
Time	255	151	0.42	-0.03 to 0.87	0.07	14.17	13.51 to 14.83	3.80	1.07
CAR-Rate			-0.00	-0.01 to 0.01	0.2				
Time	165	100	0.16	-0.43 to 0.77	0.5	13.76	12.89 to 14.62	3.95	1.08

	Fixed effects components of models							Random effect components of model	
Fat mass index	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
C-evening			0.03	-0.05 to 0.13	0.4				
Time	165	100	0.15	-0.50 to 0.78	0.7	13.92	13.06 to 14.77	3.91	1.12
C-Ratio			-0.76	12.87 to 1.36	0.4				
Time	255	151	0.65	-0.21 to 1.51	0.14	13.94	13.10 to 14.79	3.89	1.05
CAR-AUC			0.00	-0.03 to 0.04	0.8				
Time	165	100	0.17	-0.46 to 0.80	0.6	13.67	12.67 to 14.68	3.99	1.12

	Fixed effects components of models							Random effect components of model	
Fat mass index	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
C-DayAUC			0.00	-0.00 to 0.00	0.9				

Table 6.17 Multi-level models (random intercepts) of individual baseline characteristics, pubertal change over time, and cortisol measures over time in models with time to predict SAD.

	Fixed effects components of models							Random effect components of model	
SAD	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Time	535	170	0.09	-0.55 to	0.8	24.10	22.66 to	2.00	2.88

	Fixed effects components of models							Random effect components of model	
SAD	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Sex				0.73			25.54		
			-0.98	-1.81 to -0.15	0.02*				
Time	400	169	0.19	-0.66 to 0.71	0.9	22.26	20.89 to 23.63	2.14	2.78
Pubertal stage									
Mid Puberty versus pre/early			0.47	-1.03 to 1.98	0.5				
Late/post versus pre/early			0.30	-1.18 to 1.78	0.6				
Time	535	170	0.07	-0.58 to 0.71	0.8	22.26	21.56 to 22.95	2.03	2.89

	Fixed effects components of models							Random effect components of model	
SAD	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Ethnicity Black versus white			0.76	-0.21 to 1.74	0.12				
Asian versus white			-0.10	-1.21 to 1.01	0.8				
Mixed versus white			0.18	-1.17 to 1.54	0.8				
Time	535	170	0.08	-0.56 to 0.72	0.8	22.60	21.97 to 23.23	2.05	2.88
Intervention			-0.22	-1.03 to 0.59	0.6				
Time	364	168	-0.23	-0.94 to 0.49	0.5	22.95	22.24 to 23.65	2.03	2.72

	Fixed effects components of models							Random effect components of model	
SAD	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
C-wake			-0.02	-0.05 to 0.01	0.2				
Time	263	155	-0.08	-0.95 to 0.79	0.9	22.74	22.05 to 23.42	1.99	2.61
CAR-Rate			-0.00	-0.03 to 0.01	0.4				
Time	174	103	-1.53	-2.65 to -0.41	0.01*	23.51	22.65 to 24.37	1.48	2.92
C-evening			-0.07	-0.24 to 0.11	0.3				
Time	174	103	-1.52	-2.64 to -0.41	0.01*	23.32	22.46 to 24.18	1.52	2.84

	Fixed effects components of models							Random effect components of model	
SAD	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
C-Ratio			-0.74	-4.08 to 0.80	0.7				
Time	263	155	-0.07	-0.94 to 0.80	0.8	22.49	21.71 to 23.27	1.55	2.88
CAR-AUC			-0.74	-4.08 to 2.59	0.6				
Time	174	103	-1.52	-2.61 to -0.43	<0.01*	23.73	22.49 to 24.96	2.03	2.57
C-DayAUC			-0.00	-0.07 to 0.06	0.9				

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 6.18 Multi-level models (random intercepts) of individual baseline characteristics, pubertal change over time, and cortisol measures over time in models with time to predict waist circumference.

	Fixed effects components of models							Random effect components of model	
Waist	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals

	Fixed effects components of models							Random effect components of model	
Waist	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Time	548	174	-1.07	-2.62 to 0.48	0.18	10.6.40	100.88 to 111.92	9.93	4.93
Sex			-3.81	-7.06 to -0.56	0.02*				
Time			-0.82	-2.47 to 0.82	0.3	99.37	95.78 to 102.95	10.23	4.63
Pubertal stage									
Mid Puberty versus pre/early			0.37	-2.98 to 3.73	0.8				
Late/post versus pre/early			0.66	-3.22 to 4.56	0.7				
Time	548	174	-1.07	-2.62 to 0.48	0.18	100.98	98.38 to 103.57	10.06	4.93

	Fixed effects components of models							Random effect components of model	
Waist	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Ethnicity Black versus white			-1.04	-4.90 to 2.81	0.6				
Asian versus white			-0.83	-5.19 to 3.52	0.7				
Mixed versus white			-2.63	-8.08 to 2.81	0.3				
Time	405	174	-1.08	-2.63 to 0.48	0.17	100.16	97.88 to 102.43	10.10	4.92
Intervention			0.09	-3.09 to 3.28	1.0				
Time	375	173	-0.73	-2.47 to 1.02	0.4	100.4	98.41 to 102.42	10.47	5.81

	Fixed effects components of models							Random effect components of model	
Waist	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
C-wake			-0.77	-2.31 to 0.76	0.3				
Time	271	155	-0.51	-2.89 to 1.88	0.7	99.56	97.51 to 101.63	10.02	6.21
CAR-Rate			-0.01	-0.07 to 0.05	0.7				
Time	172	102	-1.44	-4.42 to 1.54	0.3	98.28	95.64 to 100.92	10.48	5.76
C-evening			-0.17	-0.62 to 0.28	0.5				
Time	172	102	-1.95	-4.91 to 1.02	0.2	98.21	95.60 to 100.83	10.40	6.04

	Fixed effects components of models							Random effect components of model	
Waist	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
C-Ratio			-2.11	-11.27 to 7.04	0.7				
Time	271	155	-0.50	-2.90 to 1.90	0.7	99.58	97.24 to 101.94	10.09	6.13
CAR-AUC			-0.12	-0.30 to 0.07	0.2				
Time	172	102	-1.90	-4.81 to 1.00	0.2	99.49	95.88 to 103.10	10.23	5.93
C-DayAUC			-0.01	-0.02 to 0.01	0.4				

6.3.6 Relationships between blood pressure measures over time and baseline characteristics, pubertal change and stress measures

After individual models for blood pressure and time were constructed, individual baseline characteristics – Sex, ethnicity, deprivation score and intervention status were added into models individually with time to predict blood pressure measures. Next covariates that altered over time – pubertal status and cortisol measures - were added individually to models with time to predict blood pressure measures. The addition of cortisol measures was performed to look for associations between change in cortisol measures over time and change in blood pressure markers, to test one of the key hypotheses of the thesis.

These models are presented together in tables 7.19-7.22 below. The only association found was a positive association between diastolic blood pressure and diastolic blood pressure z-score and Asian ethnicity over the time period studied versus white ethnicity. There were no statistically significant associations found between any of the cortisol measures and any of the blood pressure measures.

Table 6.19 Multi-level models (random intercepts) of individual baseline characteristics, pubertal change over time, and cortisol measures over time in models with time to predict systolic blood pressure.

	Fixed effects components of models							Random effect components of model	
Systolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Time	562	174	4.02	2.04 to 6.00	<0.01*	112.84	108.61 to 117.10	5.76	9.33
Sex			-2.38	-4.82 to 0.06	0.06				
Time	419	174	5.96	3.98 to 7.94	<0.01*	105.45	101.55 to 109.35	5.58	8.65
Pubertal stage									
Mid Puberty versus pre/early			1.33	-2.97 to 5.65	0.5				

	Fixed effects components of models							Random effect components of model	
Systolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Late/post versus pre/early			1.45	-2.76 to 5.67	0.5				
Time	562	174	3.96	1.98 to 5.94	<0.01*	108.63	106.56 to 110.71	5.78	9.34
Ethnicity									
Black versus white			1.60	-1.25 to 4.46	0.3				
Asian versus white			-0.89	-4.14 to 2.36	0.6				
Mixed versus white			0.26	-3.71 to 4.24	0.9				
Time	562	174	3.99	2.01 to 5.97	<0.01*	109.13	107.26 to 111.01	5.87	9.33

	Fixed effects components of models							Random effect components of model	
Systolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Intervention			-0.32	-2.70 to 2.70	0.8				
Time	382	172	5.26	3.22 to 7.30	<0.01*	106.13	104.11 to 108.13	5.29	8.24
C-wake			0.05	-0.04 to 0.14	0.3				
Time	271	155	5.23	2.73 to 7.72	<0.01*	105.85	103.73 to 107.98	5.73	8.04
CAR-Rate			0.03	-0.04 to 0.09	0.5				
Time	174	102	4.62	1.29 to 7.95	0.01*	106.75	104.15 to 109.35	5.18	8.56

	Fixed effects components of models							Random effect components of model	
Systolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
C-evening			-0.34	-0.87 to 0.21	0.2				
Time	174	102	4.75	1.52 to 7.99	<0.01	107.76	105.18 to 110.34	5.74	8.04
C-Ratio			-4.75	-14.36 to 4.86	0.3				
Time	271	155	5.31	2.79 to 7.83	<0.01	106.70	104.42 to 108.98	5.82	7.99
CAR-AUC			0.05	-0.15 to 0.25	0.7				
Time	174	102	4.63	1.25 to 8.00	<0.01*	106.7	102.95 to 110.57	5.45	8.45

	Fixed effects components of models							Random effect components of model	
Systolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
C-DayAUC			-0.00	-0.02 to 0.02	1.0				

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 6.20 Multi-level models (random intercepts) of individual baseline characteristics, pubertal change over time, and cortisol measures over time in models with time to predict systolic blood pressure z-score.

	Fixed effects components of models							Random effect components of model	
Systolic BPz	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Time	560	174	0.28	0.08 to 0.48	<0.01*	-0.92	-1.34 to -0.51	0.54	0.94
Sex			0.04	-0.21 to 0.28	0.7				
Time	417	174	0.50	0.29 to 0.70	<0.01*	-1.06	-1.46 to -0.67	0.53	0.88
Pubertal stage			-0.04	-0.47 to 0.40	0.8				

	Fixed effects components of models							Random effect components of model	
Systolic BPz	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Mid Puberty versus pre/early			-0.04	-0.45 to 0.38	0.8				
Late/post versus pre/early									
Time	560	174	0.28	0.08 to 0.48	<0.01*	-0.84	-1.04 to -0.64	0.54	0.94
Ethnicity									
Black versus white			0.06	-0.21 to 0.34	0.7				
Asian versus white			-0.17	-0.48 to 0.15	0.3				
			-0.09	-0.48 to	0.6				

	Fixed effects components of models							Random effect components of model	
Systolic BPz	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Mixed versus white				0.28					
Time	560	174	0.28	0.08 to 0.48	0.01*	-0.87	-1.05 to -0.68	0.54	0.94
Intervention			0.01	-0.22 to 0.24	1.0				
Time	382	172	0.43	0.25 to 0.65	<0.01*	-1.16	-1.37 to -0.96	0.55	0.85
C-wake			0.01	-0.01 to 0.02	0.2				
Time	270	155	0.42	0.17 to 0.68	<0.01*	-1.16	-1.39 to -0.94	0.59	0.82

	Fixed effects components of models							Random effect components of model	
Systolic BPz	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
CAR-Rate			0.01	-0.01 to 0.01	0.2				
Time C-evening	174	102	0.38	0.03 to 0.72	0.03*	-1.04	-1.31 to -0.78	0.51	0.88
			-0.04	-0.09 to 0.02	0.2				
Time	174	102	0.38	0.05 to 0.71	0.02*	-0.93	-1.19 to -0.67	0.55	0.82
C-Ratio			-0.54	-1.51 to 0.42	0.3				
Time	270	155	0.43	0.17 to 0.69	<0.01*	-1.13	-1.13 to-0.90	0.58	0.83

	Fixed effects components of models							Random effect components of model	
Systolic BPz	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
CAR-AUC			0.01	-0.01 to 0.03	0.3				
Time C-DayAUC	174	102	0.36	0.02 to 0.71	0.04*	-1.03	-1.42 to -0.65	0.54	0.82
			-0.01	-0.01 to 0.01	0.9				

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 6.21 Multi-level models (random intercepts) of individual baseline characteristics, pubertal change over time, and cortisol measures over time in models with time to predict diastolic blood pressure.

	Fixed effects components of models							Random effect components of model	
Diastolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Time	562	174	3.34	1.37 to 5.30	<0.01*	53.22	49.27 to 57.18	5.02	9.20
Sex			1.16	-1.11 to 3.43	0.3				
Time	419	174	4.33	2.29 to 6.37	<0.01*	53.42	49.64 to 57.19	4.85	8.70

	Fixed effects components of models							Random effect components of model	
Diastolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Pubertal stage Mid Puberty versus pre/early			-1.27	-5.51 to 2.96	0.6				
Late/post versus pre/early			1.21	-2.88 to 5.28	0.5				
Time	562	174	3.31	1.37 to 5.26	<0.01*	53.39	51.48 to 55.30	4.86	9.23
Ethnicity Black versus white			2.49	-0.10 to 5.08	0.06				
Asian versus white			3.52	0.56 to 6.49	0.02*				
Mixed versus white			2.29	-1.32 to 5.89	0.2				

	Fixed effects components of models							Random effect components of model	
Diastolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Time	562	174	3.36	1.39 to 5.32	<0.01*	54.64	52.90 to 56.39	5.02	9.19
Intervention			0.93	-1.25 to 3.11	0.4				
Time	382	172	3.78	1.62 to 5.93	<0.01*	53.45	51.42 to 55.47	4.70	8.45
C-wake			0.03	-0.07 to 0.13	0.5				
Time	273	156	2.96	0.01 to 5.92	0.05	54.02	51.88 to 56.16	4.29	8.17
CAR-Rate			-0.02	-0.09 to 0.05	0.5				

	Fixed effects components of models							Random effect components of model	
Diastolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Time	174	102	0.24	-2.76 to 3.25	0.8	55.86	53.46 to 58.25	5.31	7.60
C-evening			0.05	-0.44 to 0.53	0.8				
Time	174	102	0.62	-2.45 to 3.68	0.7	56.07	53.67 to 58.48	4.82	7.75
C-Ratio			3.83	-5.27 to 12.93	0.4				
Time	273	156	3.12	0.20 to 6.13	0.04*	55.10	52.73 to 57.48	4.27	8.23
CAR-AUC			-0.11	-0.33 to 0.10	0.2				

			0.88	-0.66 to 2.43	0.3				

	Fixed effects components of models							Random effect components of model	
Diastolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	P	Constant	95% CI constant	SD constant	SD residuals
Time	174	102	0.81	-2.27 to 3.89	0.6	55.44	51.96 to 58.92	4.92	7.74
C-DayAUC			0.01	-0.01 to 0.02	0.7				

Note table label : C-wake : cortisol on wakening. CAR-Rate : cortisol awakening response rate of change. CAR-AUC : cortisol awakening response area under the curve. C-evening : evening cortisol. C-ratio : ratio of evening to awakening cortisol. C-dayAUC : total day cortisol from awakening to evening AUC

Table 6.22 Multi-level models (random intercepts) of individual baseline characteristics, pubertal change over time, and cortisol measures over time in models with time to predict diasolic blood pressure. Z-sc0re

	Fixed effects components of models							Random effect components of model	
Diastolic BP z	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI SD(t)	SD constant	SD residuals
Time	560	174	0.31	0.07 to 0.55	0.01*	-0.34	0.00-0.00	0.50	1.12
Sex			-0.07	-0.32 to 0.18	0.6				
Time	417	174	0.81	-0.40 to 2.02	0.19	-0.50	0.00-0.00	0.53	1.07
Pubertal stage			-0.52	-1.87 to 0.83	0.5				

	Fixed effects components of models							Random effect components of model	
Diastolic BP z	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI SD(t)	SD constant	SD residuals
Mid Puberty versus pre/early			-0.32	-1.57 to 0.91	0.6				
Late/post versus pre/early									
Time	560	174	0.31	0.07 to 0.55	0.01*	-0.65	0.00-0.00	0.47	1.13
Ethnicity									
Black versus white			0.27	-0.02 to 0.55	0.07				
Asian versus white									
Mixed versus white			0.43	0.10 to 0.77	0.01*				
			0.23	-0.16 to 0.64	0.3				
Time	560	174	0.31	0.07 to 0.55	0.01*	-0.50	0.00-0.00	0.51	1.12

	Fixed effects components of models							Random effect components of model	
Diastolic BP z	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI SD(t)	SD constant	SD residuals
Intervention			0.09	0.45 to -0.15	0.3				
Time	382	172	0.39	0.13 to 0.65	<0.01*	-0.66	0.00-0.00	0.48	1.05
C-wake			0.01	-0.01 to 0.01	0.6				
Time	272	156	0.30	-0.05 to 0.65	0.10	-0.61	0.00-0.00	0.36	1.05
CAR-Rate			0.00	-0.01 to 0.01	0.8				
Time	174	102	0.01	-0.35 to 0.38	0.9	-0.33	0.00-0.00	0.54	0.95

	Fixed effects components of models							Random effect components of model	
Diastolic BP z	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI SD(t)	SD constant	SD residuals
C-evening			-0.01	-0.05 to 0.04	0.7				
Time	174	102	0.06	-0.32 to 0.43	0.7	-0.31	0.00-0.00	0.46	0.97
C-Ratio			-0.19	-0.71 to 0.32	0.5				
Time	272	156	0.32	-0.03 to 0.67	0.08	-0.49	0.00-0.00	1.06	1.06
CAR-AUC			-0.01	-0.03 to 0.10	0.3				
Time	174	102	0.07	-0.31 to 0.45	0.7	-0.31	0.00-0.00	0.46	0.98

	Fixed effects components of models							Random effect components of model	
Diastolic BP z	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI SD(t)	SD constant	SD residuals
C-DayAUC			-0.00	-0.01 to 0.01	0.7				

6.3.7 Relationships between change in blood pressure measures over time and adiposity measures.

To examine the association of changes in adiposity measures over time and blood pressure measures over time, multi-level models were constructed using time and adiposity measures as predictors of blood pressure measures. For all adiposity measures except for FMI, there was a positive relationship with systolic blood pressure, with time remaining significant in all models. With time in models, systolic blood pressure increased by 0.28 mmHg per 1 kg/m^2 of BMI (0.05 to 0.53), 0.41 mmHg per 1cm of SAD (0.15 to 0.67), and 0.10 mmHg per 1cm of waist circumference (0.02 to 0.2). BMI and SAD were associated with systolic.

Given the suggestion that there was an association with sex and systolic blood pressure in models predicting blood pressure with time, a model of BMI, time and sex was constructed to predict systolic BP, which is shown in table 7.27. The positive association of BMI over time and systolic BP remained robust to inclusion of sex (with girls having lower blood pressures compared to males). A similar model for waist and SAD were constructed, and in these models the positive relationship between systolic BP and SAD and waist remained robust, though associations with female sex did not (data not shown).

Diastolic blood pressure was not associated with adiposity measures in individual models of adiposity measures and time as predictors (tables 6.25 and 6.26).

Table 6.23 Multi-level models (random intercepts) of adiposity measures with time in models to predict systolic blood pressure.

	Fixed effects components of models							Random effect components of model	
Systolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Time	562	174	3.84	1.87 to 5.82	<0.01*	99.63	91.53 to 107.73	5.80	9.30
BMI			0.28	0.04 to 0.53	0.02*				
Time	514	172	3.72	1.62 to 5.81	<0.01*	106.91	102.50 to 111.34	5.97	9,92
Fat mass index			0.13	-0.16 to 0.42	0.4				
Time	530	170	4.40	2.37 to	<0.01*	99.27	93.18 to	5.81	9.22

SAD				6.41			105.37		
			0.41	0.15 to 0.67	<0.01*				
Time	543	173	3.72	1.71 to 5.70	<0.01*	98.21	89.13 to 107.30	5.60	9.32
Waist			0.10	0.02 to 0.20	0.02*				

Table 6.24 Multi-level models (random intercepts) of adiposity measures with time in models to predict systolic blood pressure z-score.

	Fixed effects components of models							Random effect components of model	
Systolic z	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Time	560	174	0.27	0.07 to	0.01*	-1.56	-2.35 to -	0.54	0.94

BMI				0.47			0.77		
			0.02	-0.01 to 0.05	0.08				
Time	512	172	0.29	0.04 to 0.47	0.02*	-1.21	-1.64 to -0.78	0.57	0.94
Fat mass index			0.02	-0.01 to 0.05	0.11				
Time	528	170	0.32	0.11 to 0.53	<0.01*	-1.59	02.21 to -0.97	0.56	0.93
SAD			0.03	0.00 to 0.06	0.02*				
Time	541	173	0.26	0.06 to 0.46	0.01*	-1.53	-2.44 to -0.62	0.55	0.94
Waist			0.01	-0.02 to 0.02	0.15				

Table 6.25 Multi-level models (random intercepts) of adiposity measures with time in models to predict diastolic blood pressure.

	Fixed effects components of models							Random effect components of model	
Diastolic BP and anthropometry	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Time	562	174	3.25	1.29 t0 5.21	<0.01*	49.39	41.84 to 56.95	5.02	9.19
BMI			0.17	-0.05 to 0.41	0.13				
Time	514	172	2.55	0.53 to 4.56	0.01*	52.29	48.23 to 56.35	5.27	8.96
Fat mass index			0.17	-0.10 to 0.44	0.2				

Time	530	170	3.65	1.62 to 5.67	<0.01*	49.76	43.83 to 55.68	4.85	9.30
SAD			0.23	-0.02 to 0.49	0.07				
Time	543	173	2.46	0.54 to 4.38	0.01*	52.65	44.11 to 61.20	5.09	8.95
Waist			0.02	-0.06 to 0.12	0.6				

Table 6.26 Multi-level models (random intercepts) of adiposity measures with time in models to predict diastolic blood pressure z-score.

	Fixed effects components of models							Random effect components of model	
Diastolic BPz	Total number of	Number of	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals

	observations	subjects							
Time	560	174	0.31	0.07 to 0.54	0.01*	-0.79	-1.64 to 0.05	0.51	1.13
BMI			0.01	-0.02 to 0.04	0.4				
Time	512	172	0.24	-0.01 to 0.41	0.06	-0.62	-1.08 to -0.16	0.53	1.10
Fat mass index			0.01	-0.02 to 0.04	0.6				
Time	528	170	0.36	0.11 to 0.60	0.01*	-1.08	-1.78 to -0.38	0.48	1.13
SAD			0.03	-0.01 to 0.02	0.08				
Time	541	173	0.22	-0.01 to 0.02	0.07	-0.7	-1.68 to 0.28	0.52	1.09
Waist			0.00	-0.01 to	0.6				

				0.01					

Table 6.27 Multi-level models (random intercepts) of BMI with time and Sex in models to predict systolic blood pressure

	Fixed effects components of models							Random effect components of model	
Systolic BP	Total number of observations	Number of subjects	Coefficient	95%CI	p	Constant	95% CI constant	SD constant	SD residuals
Time	419	174	5.44	3.45 to 7.43	<0.01*	96.75	88.41 to 105.07	5.14	8.67
BMI			0.30	0.05 to 0.54	0.02*				
Sex			-5.27	-8.51 to -2.02	<0.01*				

Chapter 7 : Discussion

In this last chapter, I will firstly summarize the findings of the thesis, contextualize them to the hypotheses of the thesis and existing literature, and also discuss in depth the limitations of the thesis. Lastly I will provide some suggestions for further research. I will not discuss the findings and limitations of the two systematic reviews from chapter 1 and chapter 2 as they are discussed in detail within those chapters themselves.

7.1 Key findings.

7.1.1 Validity of contemporary risk using PWV

Adiposity measures and PWV

At baseline I found a positive association between arterial stiffness and adiposity (BMI z, FMI) including central adiposity in the form of standardized waist circumference. This association was independent of age and ethnicity. The longitudinal measures of PWV showed no change from time 0 to time 2, and change in adiposity measures between time 0 and 2 were not associated with PWV at time 2. It is likely that longer period of time between measurements of PWV are required (see limitations section below) to answer the question of how changes in adiposity can affect arterial stiffening over time.

Partitioning subjects by levels of BMI z at baseline showed small differences in mean PWV by group, a finding also recently reported elsewhere.[185] However it is likely that this merely reflects a positive relationship between BMI z and PWV, and there was considerable overlap of PWV between groups (seen most clearly in figure 7.1). Thus our ability to classify and understand what constitutes “severe obesity” from the perspective of contemporary pathological processes remains limited.

At baseline in our adjusted models, a one SD increase in BMI (i.e a unit increase in BMI z) was associated with an increase of 0.5 ms^{-1} in PWV, equivalent to an 0.4 SD

change in PWV. For comparison, in the meta-analysis of adult studies Vlachopoulos et al[63] discussed in Chapter one, reported that a 1 SD increase in aortic PWV in adults led to around a 50% increase in total cardiovascular events and mortality. Similarly, Ben-Shlomo et al[186] reported hazard ratios of 1.35 and 1.5 for coronary heart disease and stroke respectively associated with a 1 SD increase in the log of aortic PWV. To be clear here, my data were collected by carotid-radial rather than aortic methodology, nonetheless these adult data suggest that the level of increased arterial stiffening seen in our sample is concerning, particularly for those with more extreme BMI z-scores. Encouragingly, a recent meta-analysis in adults concluded that weight loss by lifestyle change appears to reduce PWV[187].

Cardio-metabolic markers

I found few associations between PWV and conventional markers of cardio-metabolic risk, in particular standardized blood pressure, and this is discussed in more detail in section 8.1.2 below. As discussed above, the lack of change in PWV over time limits investigation of change in blood pressure and blood tests. There were few associations with any of the blood markers at baseline and no associations between change in blood markers and PWV at time 2 (although again, PWV did not significantly change). Combined with the evidence that conventional risk markers are often unstable across adolescence,[147] our findings highlight the need for larger, longitudinal studies to better understand the differential relationships between blood markers, blood pressure and arterial stiffening. However, I think that caution must be exercised in interpreting the significance of conventional cardio-metabolic markers such as hypertension and dyslipidaemia, especially when considering pharmacological treatments for them.

I also found no association between PWV and stage of puberty, although PWV increased with age. This is an important finding, as few of the studies identified in the systematic review in chapter one controlled for pubertal stage, and lack of knowledge about the effects of puberty on arterial stiffness has been identified as a key limitation of many published studies of PWV in children and adolescents with obesity.[53 188 189] The relationship between puberty, obesity and arterial stiffening also needs larger, longitudinal samples, as puberty is associated with the development of

sexual dimorphism in cardiovascular risk profiles that is not apparent earlier in childhood.

Lastly I found that South Asian adolescents had greater PWV compared to white, and our ability to describe differences in PWV between ethnicities typical of Northern European adolescent urban populations is I believe unique in the literature for the age group studied.

Acanthosis nigricans

AN was common in the study population, with 63 % and 43% found to have AN and Severe AN respectively. There was a positive association between BMI z and presence of AN (with an increase of 1SD in BMI leading to a doubling in the risk of AN) consistent with other published studies of AN in adolescent groups.[36-38] However both presence and severity of AN were poor markers of insulin resistance when adjusted for BMI z. There were no associations between PWV and AN, as a proxy for arterial stiffening, after adjusting for BMI z. These findings suggests that the finding of AN (or severity) in a group of adolescents with obesity does not provide additional information about individual cardio-metabolic risk beyond the degree of obesity itself. Kobaissi et al[39] have similarly reported that presence and severity of AN poorly predict insulin resistance in adolescent obesity after adjusting for BMI, albeit in an exclusively Hispanic group. However I believe that this is the first study to examine a relationship between the presence and severity of AN, and contemporary measures of arterial stiffness as a proxy for long-term cardio-metabolic risk.

7.1.2 The relationship between blood pressure, adiposity and arterial stiffening.

I will deal with the limitations of the thesis in detail below, however the first thing to acknowledge before any discussion about blood pressure here is that the presence of hypertension was low in the group of young people studied. This is in contrast to a number of publications, which have found greater blood pressure in groups of children and adolescents with obesity.[190 164 191 192] Whilst it is possible that the group

studied are atypical, as the baseline participant descriptions explain, the sample was representative of a cross-section of adolescents from an urban community in London in terms of ethnicity, age and deprivation level (see limitations below). Blood pressures were also averaged from three measures and used the same protocol for measurements used in large samples from the UK designed to provide representative centile charts.[165] The protocol for blood pressure measurement in my thesis gave emphasis to relaxation time before measure (and was measured at the same time as pulse wave velocity), and it may be that the allowed rest time provided a more representative, non-clinical measure of blood pressure. There is however evidence that single measures of blood pressures are not representative of the overall 24-hour pattern. Aguilar et al have recently published data on adolescents with obesity that in a clinic setting were found on spot measurements to be normotensive, on 24 hour ambulatory testing demonstrated elevated daytime systolic hypertension in around 10 percent and a third had elevated nighttime systolic blood pressure; and indeed it was those with the greater degree of obesity in this group who demonstrated the highest differences.[193]

Most of the published data, and most frequently cited studies on the association between blood pressure and obesity in children and adolescents, come from cross sectional data comparing large groups of children and adolescents with obesity versus healthy weight controls, or children of different degrees of obesity. For example, most recently Skinner et al,[190] reported a positive relationship between degree of obesity and systolic blood pressure from cross-sectional data on 8579 children from the NHANES study from the United States. In a large systematic review and meta-analysis of 63 studies, including 49, 2220 children, Friedemann et al reported around a 7mm Hg difference in systolic blood pressure, and children with obesity compared to healthy weight controls.[32] These studies, amongst others, have led to an agreed consensus that risk for hypertension is greater in children with obesity. My data is interesting because rather than cross sectional analyses alone, I followed change in BMI and blood pressure longitudinally over a period of time as adiposity changed, and I modeled this with multi-level analyses to examine the relationship between BMI and systolic blood pressure over time. I showed that in the

group of adolescents studied, systolic blood pressure increased by only a small amount of 0.28 mmHg per 1kg/m^2 per year (and this is all the more modest in the context that one standard deviation of systolic blood pressure for the whole group was 14mmHg at baseline, and reflected in the change in analyses of systolic z per 1 Kg/m^2 over the same time period was 0.2). I also found that increase in systolic blood pressure was associated with increase in waist circumference rather than fat mass index per se, fitting with other studies that have cross-sectionally reported that central adiposity is important in the development of hypertension, and its potential importance in definitions of the metabolic syndrome.[194] [195] [196]

The finding of a relatively low prevalence of hypertension combined with the finding at cross-sectional baseline that there were no associations between systolic blood pressure and BMI, highlights the importance of longitudinal studies; but may also mean that the participants, as a group, were observed to develop higher blood pressure as a result of acquisition of greater BMI and central adiposity and were potentially on a life time trajectory which has, for example in one study published recently demonstrated that adolescents with obesity are at greater risk in adult life of blood pressure related disease such as stroke.[197] There have been a number of studies published in the last decade which have investigated potential mechanisms for how adiposity may drive an increase in blood pressure,[198] and these have included production of aldosterone by adipose tissue which may directly affect the vasculature,[199] and also the effects that adiposity can have on the kidneys which in turn can effect hypertension.[198] It is possible that there are also genetic polymorphisms within individuals which lead to some individuals having blood pressure that are more sensitive to adipose changes that others,[200] and such genetic variation could be a reason for the 28% in variation in blood pressure not related to time variance and studied variables estimated from the random effect parameters in the multi level model constructed for systolic blood pressure and presented in chapter 6. Such evidence of genetics is however entirely limited, in the literature, to adults and the differential effects of genetics on blood pressure and in the context of obesity in children is an area for future study (see below).

Given that PWV did not change in the matched repeated measures analysis between time 0 and time 2, what I can say about temporal change in arterial stiffening from my data is clearly limited. However, I feel that the observation that at baseline PWV was associated with adiposity, but not blood pressure, coupled with the longitudinal findings of increased blood pressure with increased BMI and waist through the time of the study, has some potentially interesting links with two recent published studies looking at the temporal relationship of obesity, arterial stiffening and blood pressure (albeit not in children or adolescents). Weisbrod et al recently published their study of temporal changes in pulse wave velocity and blood pressure in relation to adiposity using an animal (mouse) model and experiment.[201] They compared blood pressure changes and arterial stiffening (using PWV) in a group of mice fed high fat/high sugar to a control group of mice on normal diet. They found that, in the obese group of mice, as body fat increased, arterial stiffening increased first, and was followed by a an increase in systolic blood pressure. They hypothesized that arterial stiffening may occur prior to onset of hypertension, and furthermore that arterial stiffening may be a mechanism whereby systolic blood pressure increased; backed further by the fact that PWV and blood pressure reduced with weight loss in the overweight mice. Kaess et al followed an existing cohort of adults (mean age 60 years, of mixed adiposity classifications) and investigated the temporal relationship between arterial stiffness and blood pressure.[202] They found that at baseline, there was no association between pulse wave velocity and systolic blood pressure, however over 7 years, baseline pulse wave velocity was predictive of future systolic blood pressure, and incident hypertension. Both of these studies have argued that abnormalities in blood pressure may develop secondarily, and further that arterial stiffening may be one of a number of mechanisms by which hypertension develops, in particular in obesity.[203] A number of studies have shown that blood pressure falls with weight loss[204] [205] (with weight reduction being a long established non pharmacological recommended intervention to reduce blood pressure in obesity) and a recent systematic review showed that weight loss is associated with a reduction in arterial stiffening;[187] though few have looked at links between the two. I am very careful here to be clear that I am not suggesting that my data adds weight to this argument, principally because of the lack of change in PWV. However it does

highlight the need for more prolonged longitudinal data on blood pressure and arterial stiffening such as investigated in my thesis, something I will discuss further in areas for further research, below.

7.1.3 Relationship between stress, adiposity, contemporary risk markers and pulse wave velocity.

There were no associations either cross-sectionally or longitudinally between stress measures and adiposity, conventional cardio-metabolic risk markers (for example blood pressure) or PWV. However, there was an association between a greater A-FILE score, i.e. exposure to stressors in the preceding 12 months, and the risk of binge eating. The effect size for this association was small, and baseline data, which found this association, was cross-sectional. However, the association is interesting and may represent a potential mechanism for how exposure to stressful events may influence eating behaviour itself, in terms of increased amount of food consumed which warrants further research; especially given similar findings in adult women.[206] These findings also fit alongside the recent systematic review by Hill et al,[103] which was discussed in chapter two, which looked exclusively at types of food consumed and eating habits like breakfast consumption, not explicitly evidence of amount of food consumed in relation to stress, such as binge eating. Hill et al's review also highlighted the need for investigation of how eating behaviour might be affected differentially by sex and pubertal stage. I have demonstrated in this thesis, that in the group of adolescents studied, binge eating was neither affected by sex nor pubertal stage (or age). From a clinical perspective, my findings highlight the need to identify binge eating in clinical settings, especially given how prominent it was amongst the group studied (around a quarter).

It is interesting that no associations were found between any of the cortisol measures and adiposity, cardiovascular risk or binge eating. This is in keeping with the inconsistency of findings reported in chapter 2. My study does have the additional strength of looking at longitudinal changes in cortisol and adiposity, and also I was also able to show no associations between cortisol levels and puberty

across the time points of study – longitudinal study and interaction of puberty were key limitations in the existing data presented in chapter two.

It is possible that one of the reasons that no associations were found was the limited sample size, and this is discussed in more detail below. One other potential reason for no association in the group studied is that stress exposure may have been low, for example the average A-FILE score was 6.5 out of a maximum of 50. Also measuring stress exposure over only one year might not have been enough. Given the degree of adiposity in participants, it is likely that obesity developed in a significant proportion some years before being enrolled into the trial, and thus looking at only 12 months of stressor exposure and the impact on adiposity may have been insufficiently long. The other drawback of the A-FILE is that although it measures stressors that young people were exposed to, it didn't measure the perceived impact of these stressors. There has also been some limited evidence that the response to stress in obesity may flatten over time, or "burn out"[137 207] and one possibility is that the absence of a cortisol association with obesity was no longer evident at the time studied. Against this is the relatively greater degree of salivary cortisol values evident in the group I studied compared to those published elsewhere. Mean C-wake but not C-evening was greater in my study compared to the Rosmalen data[130] (see chapter 2 : a study of a large number of children), and although the Rosmalen data was on younger children than in my study, our study showed no relationship between age, pubertal stage and cortisol levels. I also referred back to the normative data on adolescents from the original study on CAR by Pruessner et al,[208] and average baseline CAR-Rate and CAR-AUC appear equivalent to that original data, and CAR-Rate mean and median are consistent with the responses seen in other studies of the CAR in adolescents.[209 210]

7.2 Strengths and Limitations of data and analyses across the thesis

In this section I will explore strengths and limitations of the thesis – this will also cover the key bias issues in the bias assessment tool presented in table 1.4 in Chapter 1.

7.2.1 General considerations

There are a number of characteristics of the study sample making it likely to be representative of the background population of adolescents in London, and adolescents in urban areas of the United Kingdom in general. Firstly it was drawn from community sources rather than many other studies, which have used clinical samples. The sample had a representative spread across age groups and was ethnically diverse which is helpful for representing the background population and for applicability. However, against this, the HELP trial recruited young people and their families who were consenting for a trial to test an intervention for weight loss, and were therefore motivated (at least at baseline) to attempt to change for weight loss. As such the group studied may represent a biased group of community young people compared to the total background from which they came from.

An important consideration is the appropriateness of the HELP trial sample for use for the research questions I investigated in this thesis. The HELP trial was not originally designed, nor powered to investigate these questions. The benefit of the sample was that it allowed a community group of adolescents with obesity to be tracked over time. However it clearly did not allow for comparison with a control group. The other issue is that the young people studied over time were randomized to a control or intervention status. At baseline this would make no difference because they were not yet allocated an arm of the trial. For longitudinal data, I am reassured that there was no intervention effect in the trial at large, and to account for this I included the intervention status in longitudinal analyses (with no statistical associations found all such analyses). Issues with sample size are discussed in a separate section below.

7.2.2 Methodological limitations : pulse wave velocity

PWV was measured by single operator (me) for each participant, following the manufacturer's quality control measures and after training and supervision by the vascular physiology department. However there were a number of limitations in the measurement of PWV in this study. Firstly, to limit burden on participants to support

recruitment and continuation within the main HELP trial, there were two a priori decisions made which affect the validity of PWV data collected: 1) That PWV be measured by carotid:radial methodology rather than carotid:femoral and 2) That PWV be measured only once rather than repeated.

The location of measurement may be important because, as the systematic review in chapter one revealed, the carotid-femoral method appeared to have the most consistent findings between those with obesity and without in studies found. Carotid femoral is also generally considered to be the gold standard for PWV in adult studies used to predict cardiovascular disease outcomes. The reason to measure PWV carotid-radial was a pragmatic one as finding the femoral pulse in young people with obesity is technically challenging and was also perceived to be potentially embarrassing and less compassionate for young people; and this came down to ethical decisions about the over investigation and treatment of young people recruited into a main trial to support weight loss. My perception was that young people did not enjoy having PWV measured and it took time and perseverance; and unfortunately had to come last in priority in collecting data for the main HELP trial. I feel that this largely explains the 15% missing data at baseline and only 72 matched pairs of PWV at time 0 and 2 presented in the thesis. Since the data was collected for this thesis, a newer technology (Vicorder System) has been developed and validated which uses inflatable cuffs at the neck and the femoral region which measure carotid and femoral pulses to record PWV more quickly and without having to hold a tonometer over the actual pulse site.[211] The possible use of this in future studies is discussed below. The fact that PWV was only measured once per participant, again was a pragmatic decision to reduce burden on participants, however it would have been preferable to repeat measures at the same time point to allow averaging and to also measure repeatability analyses. In defense of the methodology and consistency of measurements however, both the fact that the SD of PWV at time 0 and time 2 were identical, and moreover the fact that there was no statistical difference between PWV at time 0 and 2, I feel are compelling as to the consistency of measurement quality.

As discussed above, one of the limitations of this thesis in investigating longitudinal impact of changes in variables such as adiposity on pulse wave velocity was the lack of change found at 6 months. Given the lack of longitudinal data found in the systematic review presented in chapter one, it was difficult to know what the correct time interval would be for PWV to find change, but the decision to measure it was pragmatic as time 0 and 2 were planned to be the timing for the bulk of repeated measures for the main HELP trial (with primary and secondary outcomes measured at 6 months), so PWV collection was protocolled within the main trial plan in a similar way. It would also have been likely challenging to measure PWV at 1 year in this group of young people as drop off by this point and need to visit on home visits would have made measuring PWV challenging, and indeed numbers at 1 year may well have been lower than those at 6 months.

Another important issue for measuring PWV, and indeed all measurements, was blinded status to intervention/control status of young people in the trial to avoid any inadvertent influence on measurement. There was careful effort to maintain blinding to reduce bias in the trial for both the nurses and myself involved in collecting data (including advising and requesting young people and their families to support this by preservation of blinding to measurers at repeat visits). That said, it is a recognized bias assessment criteria that measurers in observational trials (see table 1.4 in chapter 1) are blind to changes in important variables being measured (for example BMI and PWV in this thesis) that could have inadvertently influenced measurement at consecutive time points. The way that such information could influence measures is debatable in any case as measurements using protocols and the same equipment (such as the Tanita). Nevertheless, data on measurements at previous time points in weight and blood pressure were available at consecutive later measurement time points as they were transferred into patient clinic notes (generated for each participant as part of the CRF-GOSH standard protocols) and repeated measures put into safety protocols to monitor for safe weight loss; and theoretically young people and their families could have talked about weight changes during interactions at visits, especially given the fact that they were involved in a weight management trial.

7.2.3 Methodological issues: other measurements

I have already discussed limitations around measurement of blood pressure in the study above. A number of different nurses worked in the CRF-GOSH through the time of the study, and it is possible that this led to some inaccuracies in repeated measures of adiposity and blood pressure. In the HELP study, we tried to counter and limit this by providing clear training and protocols, however variation was still likely due to human error and inter-measurer variability. There were differences in the standard deviations of key adiposity measures such as waist circumference and SAD for example between distributions of measurements at time 0 and 2. From the longitudinal perspective, I think this was likely most evident in the measurement of SAD from the appearance of figure 7.5. In contrast to using the same standard scales for weight, height, machine for impedance and anatomical landmarks for waist circumference, SAD was a difficult measure in practical terms from my own observations as it required three-dimensional visualization in the holding of equipment, and for young people to exhale fully at the same time. Using averages were included in protocols to limit this.

Another limitation was that puberty was self-assessed rather than directly rated, and disparities between trained observer and self-report of puberty have been reported.[212] However we reduced misclassification bias by combining genital/breast measures with pubic hair and with menstruation in females. Whilst it would have been preferable in terms of accuracy to directly stage puberty by a trained clinician, this was not attempted to limit burden on participants, and I think it was the ethical thing to do for the young people in the HELP trial.

Because a number of young people appeared to have missed the 30 minute cortisol surge (CAR), and had negative cortisol at 30 minutes compared to awakening cortisol, I deleted a number of CAR-Rate and CAR-AUC values where this was the case. These inaccuracies are well established in obtaining ambulatory salivary cortisol in research,[119] and also around 25% of individuals have been reported to not demonstrate the CAR. There is debate about whether deleting such “non-

responders" or those with negative results, is appropriate or not.[213] [214] I decided to remove them from CAR analyses because I felt they were likely to introduce bias into analyses around CAR, but wanted to maximize power for the C-wake so kept all of the wakening sample values for C-wake analyses. Deleting data however does reduce the power and sample size as discussed above, which is a limitation. One other additional potential area of bias for measurement of cortisol is that participants might have been more stressed on days that they were coming to the hospital for measurements/assessments which coincided with the days that they provided salivary cortisol, even though a fundamental principle of measuring cortisol in home settings was to try to eliminate bias from cortisol measures within assessments.

In this study I did not investigate the sympathetic nervous system as one of the pathways of stress. I had originally planned at the formation of the methodology for the thesis to measure catecholamines in urine, however the technology and funding was not available for this as things developed. This is an important future area of study as there is a lack of published data on this branch of the stress response, as highlighted in chapter 2.

7.2.4 Sample, sample size and power issues

A key issue for the validity of results is sample size and the impact that this has on power in my analyses. In general, adiposity measures and blood pressure measurements were complete for most participants in the trial at baseline, but there was incomplete data for other key variables, in particular PWV, at baseline (as discussed above). As the HELP trial proceeded over time, there was drop out and the number of data available for repeated measures was limited. To some extent, the use of multi-level modeling for longitudinal analysis was a way of tackling missing data over time because of the benefits of this methodology in tackling incomplete data for all individuals, but clearly having more repeated measures would have improved the study and potentially increased detection of associations with small effect sizes (see below).

As discussed in the analysis section of chapter 3, the sample size was based on the sample size collected from the main HELP trial. The sample size required for this thesis should be based on the required sample to avoid a type 2 error by convention set at 80% power (allowing a 20% chance of a type II error), and the conventional measure of alpha (0.05).

For cross sectional analyses, the numbers required rely on the effect size. In this thesis, I have referred to and presented findings in regression models by co-efficient of determination (R^2), with size of effect sizes graded as per Cohen as R^2 = 0.02 – 0.13 as small, 0.13 -0.26 as medium and 0.26 or greater as large.[215] Miles and Shevlin have produced a helpful set of reference tables for cross-sectional regression models (a table per number of predictors) plotting sample number to achieved power for small, medium and large effect sizes;[216] these tables themselves being based on Faul et al's GPower sample size calculator.[217] Using these tables, the sample size of 174 was sufficient to capture associations with large and medium effects in univariable analyses; but only had a power of around 0.4 to identify associations with small effect sizes. Large numbers > 400 would be needed to detect small effect sizes at 80% power. It is possible therefore that no associations were found in regression analyses between for example the stress measures and adiposity measures because of inadequate power – and this likely more important given that most of the associations that were found cross sectionally were small in effect; and this is perhaps not surprising as complex variables such as BMI are likely to have a number of causes.

Sample size calculation in multi-level models of longitudinal data is more complicated than for single level cross-sectional analyses, in particular because these models allow for variation at the individual level (in the case of this thesis, individual participants). Tables have been produced to look at sample size for level 1 and level 2 variables, with the sample size in this study appropriate to capture large and medium effect sizes, however in multi-level modeling, many hundreds of participants are needed for 0.8 beta and 0.05 alpha to detect a small effect size;[218] thus it is possible that in multi-level models, small effects of, for example cortisol change on adiposity or blood pressure over time could have been missed in this

thesis. Multi-level modeling in this thesis did add the advantage of not being limited by missing data, and allowed an understanding of the individual participants, with calculation of the intra-class correlation to highlight what proportion of variation was due to non time variant included variables. [184]

7.3 Suggestions for future research

The experience of this thesis, the findings and, in particular, the limitations allow me to make some suggestions for further research which I will present here as a final section of this thesis.

7.3.1 General principles for further study

A key learning point for me in the HELP trial was how important recruitment and retention was, and this was a critical issue for data collection for the HELP trial at large, but also of course for the data in my thesis. In planning future research studies in obesity, concerted and multiple strategies must be applied to maximize recruitment – this was a key to success in finally reaching power for the HELP trial, as were strategies to avoid attrition (home visits, incentives). Following on from this thesis and the HELP trial I have supervised two other researchers to perform a systematic review of the literature for effective ways to increase recruitment and retention in trials of children and adolescents with obesity. We expect this to be valuable to others conducting research with young people and obesity, and of course for my own involvement in such research.

Another key learning point in this thesis was sample size and power. Larger numbers are needed to study the trajectories of young people with obesity than were recruited to the HELP trial (and as discussed above this represents the limitations of conducting research within another research study designed for a different reason). Finding large numbers can be challenging, but one way of doing this is to include PWV in existing or emerging large-scale population studies, such as they were cross-sectionally in the Avon Longitudinal Study.[74] This is likely to be expensive, with potential burden implications for participants and for planning studies with ethics

committees, and so the newer technologies discussed above for measuring PWV would be useful.

7.3.2 The group studied in the HELP trial

A future area of research that I am interested in is to follow up the group of young people studied in the HELP trial. When consented, consent was taken for permission to contact participants at a later stage; and this would allow me to examine the more long-term changes in PWV, blood pressure and adiposity. As outlined in 7.1.2, the hypothesis that adiposity is an important driver in arterial stiffening in adolescence independent of blood pressure is an important one (in particular because it assesses how important efforts to limit adiposity can impact on cardiovascular risk); and also how adiposity influences blood pressure. The HELP trial did not allow sufficient time to study this properly, and I would propose measuring BMI, blood pressure and PWV at further repeated time points – for example at 2 years, 5 years and if possible 10 years. The benefits of using the HELP trial group is that we have baseline data on the participants and have been recruited, thus designing and conducting research is already open (with appropriate ethics assessment). Key challenges here would be that there would likely be reduced numbers over time, reducing power and contacting/tracing individuals would likely be complex as young people became older and moved location. Additionally, more funding would need to be obtained to conduct this research with consideration of continuity of the research team and skills.

7.3.3 Further study of PWV and adiposity in other groups.

Beyond the original HELP group, as suggested above, larger cohorts could be set up particularly to study change in PWV, adiposity and blood pressure over time. A study, which followed up children pre-pubertal through puberty and into adulthood, measuring over 10 years with annual visits, would be favorable to understand the relationships between the two better. This could be made more effective if it formed part of cohort studies as discussed in 7.3.1. Such studies would need to analyze data with multi-level models as demonstrated in chapter 6, and control for sex, ethnicity

and pubertal stage; and should also look for other explanations for interparticipant variation such as genetic factors described above. A longer study, which tracked PWV, adiposity and blood pressure into adulthood and linked it to actual cardiovascular outcomes (e.g. myocardial infarction and stroke) would also be helpful to better identify how PWV predicts adult outcomes rather than stiffening per se. Because of the challenges discussed above, use of newer technology such as the Vicorder technology discussed above would allow measurement of carotid-femoral PWV quickly and likely of less burden to participants. Ideally such a study would use a group with different categories of obesity/non obesity status, which had sufficient numbers of each so as to form a case:control. One of the key issues with studies identified in the systematic review in chapter one was that there were only small proportions of obesity where cross-sectional studies of different weight status were used, few were community and few were longitudinal.

I also have an interest in the impact of underweight on PWV which at the time of writing, as far as I am aware, a study of which has never been published. It was not possible to investigate this in the thesis. I am interested in longitudinal study of PWV which would include underweight (for example in a population with Anorexia Nervosa).

7.3.4 Further study of stress, adiposity and cardiovascular risk.

My comments on further study of the role of obesity, cardiovascular risk and stress mirrors my comments above i.e. larger numbers, over a longer period of time, and preferably with control group are needed. In the HELP trial we used the A-FILE as a measure of stress exposure because we had access to this questionnaire, however in future research I would be interested in also using a stress measure that also examined perception of stress.[219] I would also measure cortisol over several days rather than just one day to get a more representative sample. Efforts to remind participants to take samples at the correct time (for example by text messaging)[220] should also be used to increase the validity of the awakening response which have been used elsewhere but where not available in the HELP trial.

7.4 Final conclusions

Despite the lack of long-term findings of PWV in this thesis, the cross-sectional findings are important and suggest that obesity as a public health issue is important, and there are important potential future implications for individuals and societies in terms of long-term cardiovascular health. The evidence for the validity of blood pressure, partitioning adolescents with obesity into groups and blood testing to indicate risk in adolescents with obesity still remains unclear and the evidence from this thesis questions their validity. There should remain a focus on managing obesity in children and adolescents; and research to understand how to support weight management in young people must be a priority, including the areas for further research I have identified above.

Chapter 8 : References

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