Title: Dietary Glycaemic Carbohydrate, Physical Activity and Cardiometabolic Health in Postpubertal Adolescents

Name: Ben Rhys Davies

Dietary Glycaemic Carbohydrate, Physical Activity and Cardiometabolic Health in Postpubertal Adolescents.

by

Ben Rhys Davies

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DECLARATION

I declare that this thesis is my own unaided work. It is being submitted for the degree of *Doctor of Philosophy* at the University of Bedfordshire.

It has not been submitted before for any degree or examination in any other University.

Name of candidate: Ben Davies Signature:

Date:

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ABSTRACT

The principle aims of this work were two fold; firstly to identify the current dietary intakes (specifically dietary glycaemic carbohydrate (CHO)) and physical activity (PA) and cardiorespiratory fitness (CRF) levels of a UK, postpubertal, adolescent population (n = 105) and assess the relationship between these factors, adiposity and cardiometabolic health. Diet and health relationships were assessed whilst accounting for energy misreporting and controlling for levels of PA and CRF. The effect of excluding dietary misreporters on the associations between glycaemic CHO and health was assessed whilst comparing an established technique (the Goldberg equation) to a novel approach (the ratio of energy intake (EI) to energy expenditure (EE)), which utilised RT3 accelerometry data (EI:EE(RT3)). Associations of PA and metabolic risk factors were also assessed whilst comparing two child specific PA thresholds for the assessment of PA subcomponents. Secondly, the impact of a flexible, *ad libitum*, low GI dietary intervention on cardiometabolic health was examined in an 'at risk', overweight, postpubertal, adolescent population.

Glycaemic index (GI) but not glycaemic load (GL) was shown to be associated significantly with anthropometric measures (body mass index (BMI), waist circumference (WC)) and adiposity (body fat percentage (BF%)) in this general group of post-pubertal adolescents from Bedfordshire. When adjusting for dietary intake, CRF was also associated with adiposity but PA was not. The prevalence of misreporting varied depending on the method used to assess the validity of dietary intakes; between 23% and 31% increasing to 62.1% (in overweight) of adolescents under reported energy intakes and up to 11.1% over reported. The novel application of a triaxial accelerometer to measure EE resulted in more under and over reporters being identified than when compared to the widely used Goldberg equation. Increased dietary GI was associated with increased odds of having a high WC; however, associations between GL and other risk factors were less clear; no associations with risk were observed. Excluding dietary misreporters from analysis had important implications for these associations. Only after removal of misreporters by EI:EE(RT3) was a borderline significant positive association between GL and blood glucose (BG) revealed using multiple analysis of covariance (MANCOVA), that was not present in prior analyses. Increased GI (moderate vs low GI intake)

was significantly associated with reduced high density lipoprotein cholesterol (HDL) and increased triglyceride (TG) levels (borderline significant) after removal of misreporters. In addition, using different PA thresholds to assess PA intensity resulted in different relationships between PA subcomponents and metabolic risk factors. Regardless of the threshold used, evidence suggested that limiting sedentary (SED) behaviour and engaging in moderate to vigorous PA (MVPA) is beneficial for blood pressure (BP) in this adolescent population. Additionally, irrespective of the threshold utilised, higher levels of vigorous PA (VPA) were associated with reduced odds of having a high clustered risk score and the associations observed between CRF and risk factors were stronger than those observed with PA.

Despite a lack of significant improvement in individual metabolic risk factors as a result of the low GI (LGI) dietary intervention, there was a significant reduction in clustered risk score for the LGI group at week 12. A borderline significant improvement in glycated haemoglobin ($HbA1_c$) was also observed as a result of the LGI intervention compared to those in the control group. Conversely, there appeared to be an unfavourable effect of the LGI diet on fasting insulin levels and thus the diet's impact on health overall is unclear. The small sample size of this randomised controlled trial (RCT) means that caution is required when interpreting the results.

These data suggest that future research in this age group should target improvements in CRF and a lower dietary GI to reduce adiposity. Controlling for dietary misreporting appears to have a significant impact on associations of glycaemic CHO and cardiometabolic health and should be an important consideration of future research. The low GI intervention may be an effective approach for reducing glycaemic CHO, whilst maintaining a healthy macronutrient intake, in comparison to more restricted dietary regimens published in the literature. However, the impact of this regime needs to be confirmed utilising a larger sample of adolescents. This may provide a useful approach for future research aiming to assess the impact of reduced GI and GL.

LIST OF ABBREVIATIONS

	DESCRIPTION
NHS	National Health Service
ALP	Alkaline phosphatase
ALT	Aminotransferase
ANOVA	Analysis of variance
ATP	Adult Treatment Panel
BF	Body fat
BG	Blood glucose
BIA	Bioelectrical impedance
BMI	body mass index
BMR	Basal metabolic rate
BP	Blood ressure
CHO	Carbohydrate
CI	Confidence interval
CL	Confidence limits
cm	Centimeters
COR	Centre for Obesity Research
CPM	Counts per minute
CRF	Cardiorespiratory fitness
Crisk	Clustered risk
CV	Coefficient of variation
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DLW	Doubly labelled water
EI	Energy intake
FFM	Fat free mass
FM	Fat mass
g	Grams
GGT	Gamma-glutamyltransferase
GI	Glycaemic index
GL	Glycaemic load
HbA1c	Glycated haemoglobin

	DESCRIPTION
HDL	High density lipoprotein
hhs-CRP	High sensitivity C-reactive protein
IDF	International Diabetes Federation
IL-6	Interleukin-6
Kg	kilograms
L&DH	Luton and Dunstable Hospital
LDL	Low density lipoprotein
LPA	Light physical activity
m	Meters
MANCOVA	Multiple analysis of covariance
MPA	Moderate physical activity
MUFA	Monounsaturated fatty acid
MVPA	Moderat to vigerous physical activity
PA	Physical activity
PAL	Physical activity level
PARQ	Physical activity readiness questionnaire
PRO	Protein
PUFA	Polyunsaturated fatty acids
RES	Residual
SBP	Systolic blood pressure
SED	Sedentary
SES	Socio economic status
SFA	Saturated fatty acid
TG	Triglyceride
TNF	Tumour necrosis factor alpha
VLDL	Very low density liporotein
VO2 max	Maximal oxygen uptake
VO2 peak	Peak oxygen uptake
W	Watts
WC	Wasit circumference
WK	Week

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Chapter One: General introduction

1.0 Introduction

The metabolic syndrome represents a clustering of health risk factors (adverse lipid profile, hypertension, hyperglycaemia and insulin resistance) (Despres and Lemieux, 2006, Schindler, 2007). It is recognised as an important marker of ill health; adults with the metabolic syndrome, particularly those with increased adiposity, are at greatly increased risk of developing CVD and type II diabetes (Jolliffe and Janssen, 2007). Obesity (excessive adiposity) is regarded as an important determinant of metabolic risk factors (Mathieu et al., 2009). Overweight and obese individuals, particularly those with centrally stored, abdominal obesity, are at a markedly increased risk of developing the metabolic syndrome and subsequent CVD and type II diabetes (Despres and Lemieux, 2006). This evidence is concerning given that childhood and adolescent obesity rates in England increased from 3.1% to 6.9% and 5.2% to 7.4% in boys and girls, respectively between 1995 and 2007. (Stamatakis et al., 2010). The prevalence of overweight and obesity in the UK is 17.9% and 21.8% for boys and girls, respectively (Stamatakis et al., 2010). The prevalence of the metabolic syndrome is also rising in youngsters (Eckel et al., 2005) and has been observed in 4.5% of US (Ford et al., 2008) and 4.1 % of European (Vissers et al., 2007) adolescents. Young people with the metabolic syndrome are more likely to express these risk factors in adulthood (Camhi and Katzmarzyk, 2010); highlighting the importance of developing appropriate strategies for improving metabolic health in youths.

Obesity has been associated with ‘unhealthy’ eating behaviours (Phillips et al., 2010, Galgani et al., 2008), physical inactivity (Ekelund et al., 2012) and leading a sedentary lifestyle (Hussey et al., 2007), and there is also evidence that genetics can play an important role in its development (Stunkard et al., 1990, Allison et al., 1996).

Recently, the suitability of the traditional ‘healthy’ diet (restricted in energy and fat but high in carbohydrate (CHO)) has been questioned (Summerbell et al., 2008). Where energy restricted, low fat diets have proven successful in the short term, evidence suggests that longer term adherence is poor (Astrup, 2008). In the USA, obesity rates have risen at the same time as dietary fat consumption has decreased

(Weinberg, 2004), providing some support for the notion that low fat, high CHO diets may not be the most appropriate way to target metabolic health. Subsequent to this notion, more recent evidence suggests that a reduced CHO diet elicits greater improvements in adiposity and lipid profile (Esposito et al., 2007).

Within the last 20 years, the speed at which CHO foods release their energy has been identified as a potential factor linking CHO consumption to ill health (Ludwig, 2000). This led to the development of a CHO classification system know as the glycaemic index (GI), which ranks CHO containing foods by the speed at which they release glucose into the bloodstream (Jenkins et al., 1981). Because the quantity of carbohydrate consumed also affects the postprandial glucose response, in the 1990s, this concept was furthered to include the glycaemic load (GL) which combines the quality (GI) and quantity of CHO in a food or meal (Salmerón et al., 1997). Dietary GI and GL will collectively be referred to as 'glycaemic CHO' for the purpose of this thesis.

Observational studies, supported by controlled intervention studies have provided evidence that modifying the quality (GI) of dietary CHO (Rizkalla et al., 2004, Jebb et al., 2010) can beneficially alter a number of risk factors linked to the development of type II diabetes and CVD, in healthy and 'metabolically at risk' adults and youths. Although a number of dietary approaches have proved successful in improving health outcomes (Jebb et al., 2010, Pereira, 2004, Fajcsak et al., 2008), including low GI and GL interventions, they tend to be heavily controlled or 'restrictive' which may account for the lack of evidence for their efficacy in the longer term (Astrup, 2008). The feasibility of dietary interventions and long term adherence are issues which warrant further investigation, particularly in youths. The lack of evidence in younger populations, particularly those from the UK, has made it difficult to ascertain whether reducing dietary glycaemic CHO is an effective approach to improving metabolic health. Furthermore, there is a lack of intervention evidence in adolescents with existing metabolic risk factors and therefore the effect of a low GI dietary intervention in 'at risk' youths is unknown.

A further limitation of much of the previous literature in adolescents is that it does not account for the impact that puberty can have on adiposity, insulin resistance and subsequent metabolic complications (Hannon et al., 2006). Thus, the previous

research in adolescents assessing associations between diet and health may be methodologically flawed.

A common issue when assessing dietary intake is that of misreporting, which is described as a discrepancy between reported energy intake (EI) and measured energy expenditure (EE) (Posluna et al., 2009). Misreporting may distort intakes of energy as well as nutrients and if unaccounted for, it is likely to result in spurious associations between diet and health markers (Rosell et al., 2003). It has been evidenced that associations between dietary GI and GL with type II diabetes risk were strengthened following removal of dietary misreporters from analyses, and thus should be an important consideration when assessing these relationships. Moreover, little is known of the impact of dietary misreporting on these associations in an adolescent population.

An established method of assessing misreporting is the application of the Goldberg equation which involves calculating the cut-off of EI:basal metabolic rate (BMR) to distinguish if EI represents habitual or random low intake (Black et al., 1997). This equation requires knowledge of physical activity (PA) engagement and this is often estimated from PA questionnaires, an approach which can be flawed in terms of its validity (Sirard and Pate, 2001, Trost, 2007). Very few studies have utilised accelerometry to objectively measure PA in the assessment of misreporting in youngsters and none have used triaxial accelerometry to directly estimate EE in comparison to EI, which may provide a more accurate representation of energy misreporting. Thus, the potential for this technique in the assessment of dietary misreporting, with a more valid measure of PA and its ease of application may be an attractive prospect for future studies assessing diet and health relationships.

Additionally, another limitation of previous research in the area of diet and health has been the failure to adjust statistical analysis for PA and CRF (CRF). Both variables have been evidenced to attenuate associations between diet and health in adults (Héroux et al., 2010), thus, making the previous research methodologically flawed.

Furthermore, the evidence is unclear with regards to the most effective approach recommended for improving metabolic health through manipulation of PA and sedentary behaviours (Healey e al., 2008, Ekelund et al., 2012). There are

inconsistencies in the way in which PA behaviours are measured and quantified in youths which may be a contributing factor to the equivocal findings in the literature (Ekelund., 2011). For example, there are now various different thresholds utilised to derive PA subcomponents (such as sedentary, light, moderate and vigorous PA) from accelerometry data in children and adolescents (Chu et al., 2007a, Rowlands et al., 2004a, Vanhelst et al., 2010b) yet there is no consensus as to which is most effective for assessing PA in youths. Bailey et al (2013) identified that the use of different thresholds for assessing PA levels resulted in markedly different classification of the same accelerometry data in children. No studies have compared the use of different accelerometry thresholds when assessing the associations between physical activity and metabolic health in postpubertal adolescents.

Moreover, there is a general lack of research assessing associations between diet, PA and metabolic health in adolescents, yet this is a group who are making more independent lifestyle choices about diet and PA in comparison to children; therefore understanding their PA and dietary behaviours and how these relate to metabolic health is important (Ebbeling et al., 2003). Furthermore, there appears to be no research in youths which accounts for relative macronutrient intake when assessing the associations between PA and metabolic health. Furthermore, because metabolic aberrations in this group track into adulthood (Camhi and Katzmarzyk, 2010) identifying an effective intervention programme which can be adhered to by an adolescent population needs to be identified.

To this end, this thesis will explore the current dietary glycaemic CHO intakes and PA levels of a postpubertal population of UK adolescents and how they relate to markers of the metabolic syndrome. Associations between glycaemic CHO and metabolic health will be assessed whilst accounting for PA and CRF. Additionally the impact of dietary misreporting on these associations will be considered, whilst comparing the novel application of EI:EE(accelerometry), to an established technique (Goldberg equation). Furthermore, the associations between metabolic health, PA and sedentary behaviour will be assessed using two different youth-specific thresholds for quantifying PA engagement whilst accounting for macronutrient intake. Finally, the impact of a flexible, *ad libitum*, low GI dietary intervention, compared to a control group on markers of metabolic health will also be assessed in a sample of specifically ‘at risk’ (expressing risk factors for the metabolic syndrome) overweight or obese postpubertal adolescents.

1.1 Aims and objectives

The principle aim of this work is to identify the current dietary intakes and PA and CRF levels of a UK, postpubertal, adolescent population and to assess the relationship between these factors, adiposity and cardiometabolic health. Diet and health relationships will be assessed whilst accounting for misreporting and controlling for levels of PA and CRF. The impact of a flexible, *ad libitum*, low GI dietary intervention on metabolic health will be assessed in an 'at risk' postpubertal overweight adolescent population.

Primary objectives:

Study 1: Physical activity levels and nutritional intake of postpubertal Bedfordshire adolescents: associations with adiposity.

a) To investigate the current dietary intakes, PA and CRF levels of postpubertal adolescents from the UK.

b) To assess the relationship between dietary CHO, PA and CRF and metabolic risk factors

Study 2: The use of objective physical activity monitoring to assess the accuracy of recorded energy intake in adolescents.

a) To compare the novel application of EI:EE (triaxial accelerometry) versus the Goldberg equation in the assessment of dietary misreporting in postpubertal adolescents from the UK

Study 3: Associations between glycaemic index, glycaemic load and other dietary factors with the metabolic syndrome in postpubertal UK adolescents.

a) To explore the associations between dietary glycaemic CHO with metabolic risk factors whilst accounting for PA and CRF in postpubertal adolescents.

b) To compare the associations between glycaemic CHO and health markers whilst accounting for dietary misreporting and to compare how a novel technique for identifying misreporters impacts on these associations.

Study 4: Associations between physical activity and fitness with the metabolic syndrome in postpubertal UK adolescents: the impact of different PA thresholds.

a) To explore the associations between PA, CRF and metabolic risk factors whilst accounting for energy and relative macronutrient intake in postpubertal adolescents.

b) To compare the associations between PA and health markers whilst utilising two different child specific PA thresholds.

Study 5: The impact of a low glycaemic index diet on the metabolic health of postpubertal adolescents with features of the metabolic syndrome.

a) To assess the impact of a flexible, *ad libitum*, low glycaemic index dietary intervention on metabolic syndrome risk factors in a group of 'at risk' postpubertal adolescents from the UK.

Chapter Two: Literature Review

2.0 Obesity and the Metabolic Syndrome

2.0.1 Obesity

Obesity is an excessive amount of total body fat (adipose tissue) relative to body weight that may impair health, classified by the body mass index (BMI) as a ratio of body weight (kilograms) to height (meters) of greater than 30 kg/m^2 and above 25 kg/m^2 for overweight (World Health Organisation [WHO], 2011). Overweight and obesity is the 5th leading mortality risk globally and according to the WHO (2012), it is responsible for at least 2.8 million adult deaths each year. Forty four percent of burdens associated with diabetes, ischemic heart disease (23%) and certain cancers (7% and 4%) are attributable to overweight and obesity (WHO, 2012). In 2008, the WHO reported that more than 1 in 10 of the worlds adult population was obese, however, according to recent estimations, by 2015, this figure is projected to rise to 1 in 3 (WHO, 2012 overweight & obesity fact sheet, accessed July 2012). Previously overweight and obesity were considered to be a problem for developed countries, however, obesity rates have increased in middle and low-income countries, particularly in urban environments (WHO, 2012). Importantly, obesity is not isolated to adult populations, according to the WHO (2012), in 2010 over 40 million children under 5 years old were overweight.

2.0.2 Aetiology of Obesity

The proximate cause of increased adiposity is energy imbalance, when food EI exceeds that of total energy expenditure (TEE) (Popkin, 2005). This imbalance is thought to be a consequence of the environmental changes that have shaped the modern western lifestyle (Swinburn et al., 2011). Altered eating habits and expanded food options have led to increased production of readily available, high fat, energy dense (Hill et al., 2000), and more recently sugar rich foods (Prentice and Jebb, 2003). Furthermore, people are less physically active as a result of advances in technology, such as mechanisation and automation (Hill et

al., 2000). These factors combined have increased the likelihood of consuming more whilst expending less energy; leading to weight gain and obesity.

Obesity, however, isn't just a result of overeating or lack of exercise; it is affected by the interaction of physiological, behavioural, environmental and genetic factors. Studies of twins reared apart suggest that approximately two-thirds of the variability in BMI is due to genetics (Stunkard et al., 1990, Allison et al., 1996). The study of genomics has advanced current knowledge of the role that genes play in the development of obesity. These advances have identified gene mutations, such as those affecting the central pathways of food intake regulation, for example, the 'ob' gene may result in improper coding of leptin (a satiety hormone) leading to overeating and obesity (Andersson, 1996). The interaction between genes and the environment is important, as it seems that some individuals are genetically predisposed to develop obesity, but only under certain adverse environmental conditions (sedentary lifestyle and high fat diet), may that genotype be expressed (Stunkard, 1988).

2.0.3 Implications of obesity for disease: the metabolic syndrome

Adipose tissue is now regarded as a complex organ understood to play an important role in the regulation of energy transfer, interacting with the inflammatory system and vascular wall (Mathieu et al, 2009). Of particular concern is centrally stored adipose tissue, located amongst internal organs of the abdomen, known as visceral fat. Abdominal obesity is associated with insulin resistance and the development of type II diabetes, atherogenic dyslipidemia and subsequent cardiovascular disease (CVD) (Despres and Lemieux, 2006). The metabolic syndrome represents a cluster of these obesity related cardiovascular risk determinants, such as central obesity, hypertension, insulin resistance and an abnormal lipid profile. For example, low high-density lipoprotein cholesterol (HDL), raised low-density lipoprotein cholesterol (LDL) and hypertriglyceridemia (high levels of circulating triglycerides [TG] in the blood) (Schindler, 2007). An individual is identified as having the metabolic syndrome when three or more of these determinants coexist (Chen et al., 2006). Those demonstrating factors associated with the metabolic syndrome are at greatly increased risk of CVD and Type 2 diabetes (Joliffe & Janssen, 2007). Furthermore, clustering of these risk factors may present cumulative risk

exceeding that from individual risk combined (Golden et al., 2002). According to the International Diabetes Federation (IDF, 2007) one quarter of the world's adult population suffer from the metabolic syndrome and the prevalence is increasing in children and adolescents due to the growing obesity epidemic within this population (Weiss and Kaufman, 2008). As Clustered risk in children and adolescents has been observed to track into adulthood (Camhi and Katzmarzyk, 2010), identifying and treating clustered metabolic risk in adolescents is of great importance.

2.1 Pathogenesis of the metabolic syndrome

2.1.1 Insulin resistance

Insulin resistance occurs when insulin sensitive tissues (muscle, liver and adipose tissue) are less responsive to the action of insulin (Eckel et al., 2005). An insulin resistant state is associated with cardiometabolic aberrations, the development of CVD and type II diabetes (DeFronzo and Ferrannini, 1991). Obesity is understood to contribute to insulin resistance via an increased supply of non-esterified fatty acids or free fatty acids (FFA), derived from the lipolysis of triglyceride (TG) stored in expanded adipose tissue deposits (particularly visceral adipose tissue) (Grundy, 2004). In an insulin resistant state the antilipolytic action of insulin on adipose tissue is suppressed, leading to continued breakdown and release of TG stored in adipose tissue, resulting in excessive levels of FFA. FFA are the main source of nutrient energy in the fasted state. When FFA levels exceed that of tissue needs, they are taken up by muscle tissue and the liver (Grundy et al., 2004). Randle et al (1963) postulated that an influx of FFA into muscle tissue inhibits glucose oxidation (glucose-fatty acid cycle). More recently, Shulman (2000) suggested that FFA within muscle tissue leads to an increase of intracellular fatty acid metabolites (diacylglycerol, fatty acyl-CoA and ceremides). These metabolites activate a serine/threonine kinase cascade which results in serine/threonine phosphorylation of insulin receptors and thus inhibiting insulin signalling. The subsequent insulin resistance results in hyperglycaemia, as glucose uptake into the muscle is impaired (Shulman, 2000).

At the liver, insulin's primary role is to regulate glucose production (Pereira and Maahs, 2009). An increased supply of FFA to the liver increases hepatic

glucose output through the inhibition of insulin-mediated suppression of glycogenolysis (Boden et al, 2002). It is suggested that this effect occurs via similar mechanisms as those described in muscle tissue (Schinner et al, 2005; Schulman, 2000). Additionally, FFAs stimulate the production of glucose by the liver (gluconeogenesis) through the formation of acyl-CoA and consequent synthesis of intermediate substrates during FFA oxidation. Under normal circumstances, hepatic autoregulation prevents FFA-induced increases in gluconeogenesis from increasing glucose production (Clore et al, 1991). In those who exhibit insulin resistance, however, hepatic autoregulation is impaired and hepatic glucose production is increased as a result of of FAA elevations (Boden et al., 2002).

2.1.2 Glucose intolerance

The defective action of insulin, subsequent to insulin resistance, results in a decreased ability of insulin to mediate the uptake and metabolism of glucose in skeletal muscle and adipose tissue, and to suppress gluconeogenesis (Eckel et al, 2005). In order to maintain euglycaemia, in the presence of insulin resistance, insulin secretions (from pancreatic β cells) must be increased resulting in hyperinsulinemia, however, this compensatory mechanism will maintain normal blood glucose (BG) levels (Rao et al., 2004). In those who will develop type II diabetes, on the other hand, the compensatory hyperinsulinemia will result in a defect in insulin secretion and an inability to maintain normal postprandial glucose levels (impaired glucose tolerance), and eventually to impaired fasting glucose. Ultimately, persistently high levels of circulating insulin leads to β cell dysfunction (Kahn, 2001). A number of different mechanisms have been postulated as explanations for the development of β cell dysfunction. These include β cell exhaustion, as a result of the increased secretary demand for insulin (DeFronzo et al, 1991) and desensitisation of the β cell due to the progressive elevation of BG as impaired glucose tolerance develops (Robertson et al., 1994, Yki-Järvinen, 2003). Furthermore, excessive supply of FFA to the pancreas may create a state of lipotoxicity within β cells, having a deleterious effect on insulin secretions (Unger, 1995, Joseph et al., 2004).

2.1.3 Dyslipidemia

FFA accumulation at the liver is understood to impact on lipid metabolism resulting in an abnormal lipid profile (low HDL, raised LDL and

hypertriglyceridemia (Schindler, 2007). Low-density lipoproteins transport FFA (packaged as TG) to cells, including those lining arterial walls, when oxidised they form atherosclerotic plaques via the stimulation of monocyte-macrophage infiltration and lipoprotein deposition (McArdle et al, 2007). Alternatively, HDL facilitates the reverse transport of surplus cholesterol from peripheral tissues (including arterial walls) where they are eventually taken up by the liver, catabolised and excreted (McArdle et al, 2007).

At the liver, raised FFA flux is associated with increased synthesis of triglyceride (TG)-rich very low-density lipoproteins (VLDL) (Eckel et al, 2005). After cholesteryl ester transfer protein promotes exchange of TG's from VLDLs to less dense LDLs and the reverse transport of cholesteryl ester transfer protein to VLDLs, TG enriched LDL particles are produced in large amounts (Mauriege et al., 1993) contributing to hypertriglyceridemia (Eckel et al, 2005). By the action of hepatic lipase, TG enriched LDL become smaller and denser; studies have shown that small dense LDL particles are potentially highly atherogenic (Despres and Lemieux, 2006).

In addition to LDL, the composition of HDL is altered in a similar way, resulting in a reduction in HDL levels. In the presence of hypertriglyceridemia, a decrease in the cholesterol content of HDL is a consequence of reduced cholesteryl ester content of the lipoprotein core, in combination with cholesteryl ester protein-mediated alteration in TG (Eckel et al, 2005). This makes HDL particles small and dense and results in their increased clearance from the circulation (Eckel et al, 2005).

An atherogenic state brought on by dyslipidemia, increases the likelihood of CVD, by means of hardening arterial walls and the formation of atherosclerotic plaques. An increase in circulating small, dense-LDL is associated with formation of fatty streaks within the lining of arterial walls (Grundy, 2004). These are characterised by an accumulation of lipid-filled macrophages within the intima of the artery, which continue to accumulate and eventually form lesions known as fibrous plaques (Stary et al., 1995). Fibrous plaques can be responsible for obstructing blood vessels and potentially leading to myocardial infarction, furthermore, these plaques can rupture, releasing thrombotic substances that can lead to ischemic stroke (Daniels et al., 2008).

2.1.4 Hypertension

An elevated blood pressure is more prevalent in obese compared to lean individuals (Grundy, 2004), and is a strong risk factor for CVD (Chobanian et al., 2003). The notion that insulin resistance is associated with hypertension is well established (Ferrannini et al., 1987). Under normal conditions, insulin is a vasodilator with secondary effects on sodium reabsorption by the kidney (Steinberg et al., 1994, DeFronzo et al., 1975). In the presence of insulin resistance the vasodilatory effects of insulin can be lost as the mediating role of the endothelium-derived nitric oxide can become impaired, whilst at the same time, sodium reabsorption is maintained (Tooke and Hannemann, 2000, Kuroda et al., 1999). It has been observed that vasoconstriction may be further promoted by angiotensin, generated by angiotensinogen, released from an expanded adipose tissue mass (Egan and Julius, 2004). Moreover, relative vasoconstriction may be mediated by FFA (Tripathy et al., 2003).Together, this can lead to an increased extracellular volume in the blood, whilst blood vessels remain constricted, thus resulting in hypertension (Kuroda et al, 1999).

2.1.5 Further metabolic complications

Insulin resistance is often accompanied by a number of complications that do not comprise the diagnostic criteria for the metabolic syndrome. These include an increase in proinflammatory cytokines and the presence of non-alcoholic fatty liver disease (NAFLD) and or non-alcoholic steatohepatitis (NASH) (Eckel et al, 2005). The metabolic syndrome has been associated with inflammation (Sutherland et al, 2004) as a result of proinflammatory cytokines including interlukin 6 (IL-6), resistin, tumour necrosis factor α (TNF α) and C-reactive protein (CRP) (Fernandez-Real & Ricart, 2003). These cytokines mirror over production by the expanded adipose tissue mass (Trayhurn, 2004), possibly generated, in part, by monocyte-derived macrophages found within adipose tissue (Weisberg et al., 2003). Inflammatory cytokines are understood to produce more insulin resistance and the lipolysis of adipose tissue, resulting in further increased supply of FFA (Eckel, 2005). Circulating cytokines, including IL-6, may enhance hepatic glucose production, synthesis of VLDL by the liver, insulin resistance in muscle and a prothrombotic state (Eckel et al., 2005). Furthermore, an expanding adipose tissue mass reflects a reduced production of the anti-inflammatory and insulin sensitising cytokine adiponectin, enhancing

the proinflammatory state associated with insulin resistance and the metabolic syndrome.

2.2 Physical Activity, Fitness and the Metabolic Syndrome

PA is any bodily movement generated by skeletal muscles that requires energy to be expended (WHO, 2012). Lack of PA has been identified as responsible for 27% of diabetes; 25% of breast and colon cancers and approximately 30% of ischaemic heart disease burden (WHO, 2012). CRF can be defined as the ability of the cardiorespiratory system to adequately and safely meet the demand of bodily tissues and organs for blood, oxygen and other nutritional requirements, primarily for working muscles during PA (Keong, 1981). A review of literature evidenced that those with a higher CRF had at least a 50% lower mortality rate compared with less fit individuals (Blair et al., 2001b). Moreover, it has been reported that mortality rates are three to four-fold higher in participants with the lowest CRF compared to those with the highest CRF (Blair et al., 1989, Blair et al., 1991). There is some debate surrounding PA and CRF with regard to which is most important for health. Lee et al (2010) compared metabolic risk factors and the prevalence of the metabolic syndrome across levels of CRF, assessed by a treadmill VO_2 max test, and observed that physical fitness was an independent predictor of the metabolic syndrome in 909 young Korean adults (24 yrs) (Lee et al., 2010). This is supported by (Carnethon et al., 2003) who found that those who demonstrate lower levels of CRF had an increased CVD risk than those who were classified as moderately to highly fit. (Karelis et al., 2008) assessed the associations of PA as well as CRF with the metabolic syndrome in overweight and obese postmenopausal women. Those classified as having the metabolic syndrome demonstrated significantly lower levers of PA energy expenditure (measured by doubly labelled water (DLW)) and CRF (measured by peak oxygen consumption).

2.2.1 Assessment of CRF

Direct measurement of oxygen consumption during maximal exertion (VO_2 max), is regarded as the best single marker for the functional capacity of the cardiorespiratory system; often referred to as CRF (Dencker et al., 2008). It was previously outlined that CRF has been associated with markers of poor health in adults and youths. However, a vast range of techniques have been used to

assess CRF in children and adolescents. These methods include direct and indirect assessment of VO_2 max via sub-maximal and maximal cycle ergometer test, as well as treadmill tests and shuttle run tests (Rizzo et al., 2007, Boreham et al., 1997, Tomkinson and Olds, 2007). Indirect methods generally include prediction equations utilising workload data (Brage et al., 2004) and heart rate (Krekoukia et al., 2007). Although these methods may be regarded as less technical and require less specialist equipment (Dencker et al., 2008), indirect methods of estimating VO_2 max present prediction errors that may dilute any existing associations of CRF with the health marker or parameter under analysis (Rowland, 1996) limiting a study's ability to draw accurate conclusions from its data and make comparisons with other research. Research investigating associations of directly assessed CRF with health markers is limited in children (Parrett et al., 2010) and there appears to be a lack in adolescent populations.

2.2.2 Assessment of Physical Aactivity

A number of methods are available for quantifying PA, the DLW technique is regarded as a reference measure of energy expenditure (EE). DLW, however, is a time consuming and relatively expensive method. Other techniques for assessing EE include self reported physical activity questionnaires that are prone to reporting error (Sirard and Pate, 2001, Trost, 2007). Technology has allowed for the introduction of more practical objective measures of PA, such as pedometry and accelerometry. Accelerometry has been recommended over self report questionnaires and pedometers due to their greater accuracy when assessing PA as compared to the measurement of oxygen consumption (Adamo et al., 2009, Eston et al., 1998).

Accelerometry detects the acceleration of body movements which can be transformed into a measure referred to as activity 'counts' (Romanzini et al., 2012). Due to the arbitrary nature of accelerometer counts, EE calibration studies have been utilised in order to derive biological meaning to this information through the use of cut-points or thresholds corresponding to PA intensities (Rowlands et al., 2004b, Vanhelst et al., 2010a, Chu et al., 2007b). Following these developments, the accelerometer has become a popular tool for investigating the associations of health markers with total PA or accumulated minutes of PA at various intensities: time spent sedentary (SED);

in light PA (LPA); in moderate PA (MPA); in vigorous PA (VPA) and in moderate to vigorous PA (MVPA) (Ekelund et al., 2007a, Bailey et al., 2012a). Furthermore, this data can be used to estimate the proportions of a population meeting PA guidelines (Ekelund et al., 2011b). For instance, current UK guidelines recommend that youths should engage in a minimum of 60 minutes of MVPA per day and that SED time should be minimised (DOH, 2012). Recent accelerometry data suggests that only 24% of girls and 32% of boys are meeting these recommendations (DOH, 2008).

2.2.3 Physical Activity and CRF in Youths

In a study of adolescents aged 12-19 years; boys classed as 'not fit' had significantly increased levels of total cholesterol (TC), TG, insulin and CRP compared to those classified as 'fit' (Kwon et al., 2010). In the girls assessed, only TC was significantly increased in the 'not fit' group. This suggests that in adolescents, independent of fatness (as data were adjusted for WC and BMI), being fitter may be beneficial for lipid profile in girls and insulin metabolism, lipid profile and inflammation in males. However, fitness was assessed via a sub-maximal treadmill test, thus allowing for error in the estimation of maximal oxygen consumption. In 586 Danish children, PA independently shared a significant inverse association with metabolic risk, this association was weakened after adjustment for fitness which was significantly, positively associated with PA (Brage et al., 2004). This interaction between fitness and PA suggests that any benefits of PA will be seen most strongly in those with lower CRF. Ekelund et al (2007) investigated the independent associations of PA (measured via 3-4 day accelerometry) and CRF (via a maximal cycle ergometer test) with metabolic risk factors in 9-16 year olds. Both PA and CRF were separately and independently associated with individual risk factors (WC, BP, BG, insulin, TG and HDL) and clustered metabolic risk. However, after adjusting the analysis for WC, associations between clustered risk and CRF were attenuated and thus appeared to be mediated by adiposity, whilst associations with PA were unaltered. Although there are discrepancies between which is the most beneficial for health, both CRF and PA appear to be important predictors of the metabolic syndrome.

Despite widespread use of accelerometers there is no standardised procedure for the reduction of raw data, furthermore, there are variations in the derivation of PA data that can impact on outcome variables and the conclusions drawn from this information (Masse et al., 2005). Additionally the various thresholds

for deriving PA intensities when utilising accelerometers means that there are inconsistencies surrounding the analysis of accelerometry data (Ekelund et al, 2011). A recent review identified that the prevalence of youths (from studies of 9-19 year old males and females) meeting the recommended 60 minutes per day of MVPA ranged between 1% and 100%. This highlights the confounding amongst these types of studies, which has been predominantly attributed to differences in intensity thresholds (Ekelund et al, 2011).

Triaxial accelerometry, which combines the acceleration of body movements across three planes, has been utilised to assess free-living PA and its relationship with health in children and adolescents using the RT3 (Stayhealthy, Inc., CA, USA) accelerometer. For instance, (Krekoukia et al., 2007) assessed associations of PA (measured via RT3 over 4 consecutive days) and CRF (estimated from heart rate) with insulin resistance, lipid profile and inflammation in 110 lean and obese 9-11 year old children. Total daily PA (negatively associated) and WC (positively associated) explained 49% ($P < 0.1$) of the variance in insulin resistance (measured by the homeostatic model of insulin resistance (HOMA-IR)). CRF was negatively associated with insulin resistance but this disappeared following adjustment for age, sex and fat mass (FM). Additionally, the RT3 was used to assess the relationships between time spent in moderate and vigorous PA (measured over 4 days), CRF and body composition in 152 7-10 year olds (Hussey et al., 2007). CRF was assessed via a multistage shuttle run test to estimate oxygen consumption and PA intensities were determined using the cut points of Rowlands et al (2004) see Table 1. In boys, WC was significantly correlated (negatively) with minutes of VPA and positively correlated with SED time; in girls, however, these associations were not expressed. CRF, on the other hand was significantly negatively associated with BMI and WC in both sexes. Bailey et al (2012) identified, in 100 10-14 year old children that clustered metabolic risk was significantly lower in those identified as fit following a maximal cycle ergometer test that predicts oxygen uptake from a formula based on work rate. PA subcomponents were derived using the thresholds of Rowlands et al (2004); clustered risk was not associated with any PA subcomponents. Unlike previous studies utilising RT3 data in youths (Hussey et al., 2007, Krekoukia et al., 2007), Bailey et al., (2012) collected accelerometry data over 7 consecutive days and participants were only included if they had worn the accelerometer for 3 or more days. The authors explain that a minimum daily wear time of 9 hours for weekdays and 8

hours for weekend days was required; information which many authors neglect to note. The lack of uniformity of studies utilising accelerometry and CRF data may be attributable to the confounding associations of PA and CRF with metabolic health in youth. If associations of PA and CRF with health markers are to be accurately understood, the most valid tools available for gathering these data should be utilised across studies. There appears to be a lack of investigations in adults and particularly adolescents that make use of triaxial accelerometry and directly measured CRF when assessing associations with health, furthermore there is a lack of data on 'at risk' youths already exhibiting risk factors for the metabolic syndrome.

The Rowlands cut points have been utilised to derive PA subcomponents in a number of studies of youths; Rowlands et al (2004) derived thresholds for activity intensities (SED, LPA, MPA, VPA) whilst validating the RT3 in 19 boys (mean age 9 ±1 years). More recently, however, different cut points have been validated using the RT3 accelerometer which have also been validated against oxygen consumption (Chu et al., 2007, Vanhelst et al., 2010). Chu et al (2007) identified intensity thresholds using the RT3 in 35 8-12 year old Chinese children; thresholds were derived from receiver operator curves (ROC). ROC analyses showed sensitivity and specificity values ranging from 72-98%, indicating that the intensity thresholds gave an accurate distinction between intensity categories. Later, Vanhelst et al (2010) identified a lack of intensity thresholds for adolescent populations and thus validate the RT3 against oxygen consumption in 40 10-16 year old children and adolescents. The thresholds values obtained at various PA levels were not significantly different when compared to an independent sample of 20 10-16 year olds. ROC analyses revealed sensitivity and specificity values for intensity thresholds ranged from 0.86-0.99 indicating accurate distinction of intensity categories. See table 1 for PA intensity thresholds.

Table 1. Activity-intensity thresholds for the RT3 accelerometer

Variable (CPM)	Rowlands	Vanhelst	Chu
SED	<288	<41	<420
LPA	288-969	41-950	420-1859
MPA	970-2,332	951-3,410	1860-4109
VPA	≥2,333	>3,410	≥4110

cpm, counts per min; PA, physical activity

The thresholds of Vanhelst et al (2010) and Rowlands et al (2004) are comparable and the similarities between the threshold for MPA (915 and 970 cpm, respectively) would likely place a similar proportion of participants within the MVPA category of which 60 minutes per day is recommended as sufficiently active (DOH, 2011). However, the threshold for MPA according to Chu et al (2007) begins at 1860 cpm; this is a markedly higher activity level and thus fewer participants would fall into the MVPA category in comparison to the thresholds of Rowlands et al (2004) and Vanhelst et al (2007). Furthermore, Bailey et al (2013) has compared the thresholds of Rowlands et al (2004) and Chu et al (2007) in a general population of 104, 10-14 year old children from the UK. The authors identified that 97.6% of boys and 93.7% of girls achieved the recommended 60 minutes of MVPA, when using the Rowlands thresholds. This very high proportion of sufficiently active youths may not be an accurate representation due to the low threshold for MPA (personal communication). When using the Chu thresholds, however, Bailey et al (2013) observed that only 31.7% and 20.6% of boys and girls, respectively, were sufficiently active. To date, no published studies have assessed the impact of different PA intensity thresholds on associations of PA and health markers in adolescents; this is of great importance as the guidelines for sufficient PA are based on these associations with health (DOH, 2011). Furthermore, these thresholds have not been compared when investigating the associations of CRF with time accumulated in various PA intensities in adolescents.

2.3 Diet and the Metabolic Syndrome

2.3.1 Traditional dietary treatments

Poor dietary choices have been acknowledged to predispose to the metabolic syndrome. Energy dense foods are one of the primary determinants of obesity, CVD (Phillips et al., 2010) and metabolic complications (Druet and Ong, 2008). Energy restriction has played a fundamental role in the reduction of obesity (Abete et al., 2010) but macronutrient distribution is also viewed as a potential mediator of weight reduction and certain metabolic alterations(Muzio et al., 2007).

Traditionally, energy restricted diets focused on reducing the intake of energy dense fat (Hill, 2002) for the treatment and prevention of obesity and its

associated morbidities (Abete et al, 2010). In addition to reducing energy intake, the benefits of lowering fat intake on cardiovascular health, meant that a low fat and subsequent high carbohydrate (CHO) diet was the primary nutritional treatment of obesity related disorders since the second half of the last century (Giugliano et al., 2008)

According to various health organisations such as the British Diabetic Association (BDA), (1992), the American Diabetes Association (ADA), (2002) and the U.K. Scientific advisory committee on Nutrition (SACN), (2008), for the prevention and treatment of metabolic aberrations, dietary fat intake should not exceed 35% of total energy. Saturated fatty acids (SFA) should not exceed 11%, mono-unsaturated fatty acids (MUFA) 13% and poly-unsaturated fatty acids (PUFA) 6.5% (SACN, 2008), whilst trans fatty acids should not exceed 1% of total energy (American Heart Association scientific statement; Grundy et al, 2005). Increased dietary fat intake has been positively associated with body weight in large cross-sectional studies of males and females (Satia-About, 2002; Park et al, 2005) as well as children and adolescents (Ortega et al, 1995). Although the majority of these data were gathered via means of self report, the consistency of the findings supports the notion that increased dietary fat is associated with increased weight. However, in terms of health risk, the composition of dietary fat appears to be more important than the total amount consumed (Abete et al, 2011). Studies have shown that the consumption of SFAs is associated with insulin resistance independent of body fat (Marshall et al., 1997, Maron et al., 1991), whereas PUFA may have an inverse relationship with insulin resistance (Feskens et al., 1994). Epidemiological evidence suggests that SFAs are also positively associated with elevated blood pressure whilst MUFA consumption is associated with lower blood pressure (Trevisan et al., 1990, Stamler et al., 1997). Associations of dietary fats with weight gain were assessed in an 8 year prospective cohort study of 41,518 female nurses aged 41-68 years (Field et al., 2007). Per 1% increase, the percentage of energy from total fat intake was only modestly associated with weight gain (beta estimate (β) = 0.11)), whilst increases in SFA and trans fats were more strongly associated (β = 0.40 and 0.54, respectively). Furthermore, increases in MUFA and PUFA were not associated with weight gain. The consumption of Omega 3 long chain PUFA (Ω3FA) have been evidenced as beneficial for good metabolic health (Abete et al, 2011). Weight loss regimes promoting fatty fish intakes rich in Ω3FA have shown independent increased benefits on fasting insulin, lipid

profile and blood pressure from Ω3FA consumption (Sirtori et al., 2009, Cicero et al., 2010). Moreover, dietary Ω3FA via oily fish consumption were highly positively associated with plasma Ω3FA and HDL levels and negatively correlated with plasma insulin, insulin resistance (HOMA-IR), TG and DBP in 447 Alaskan Eskimos (Ebbesson et al., 2005).

Recommendations for dietary protein (PRO) intake are set at 15-20% of total energy (BDA, 1992; ADA, 2002; SACN, 2008). Recommended protein intakes have not vastly changed over time, however, it is important that adequate quality PRO is consumed to meet amino acid requirements (St. Jeor et al., 2001). Increased dietary protein is associated with reduced energy consumption and weight loss due to the greater satiety from PRO compared to fat and CHO (Eisenstein et al., 2002). Furthermore, overweight women on high protein, hypo-energetic diets, have been shown to maintain greater fat free mass during weight loss compared to a low protein hypo-energetic diet matched for total energy (Campbell and Kelly, 2012). This may result in a sustained basal metabolic rate which will aid weight loss via increased energy expenditure (Baba et al, 1999). Consumption of PRO above the requirements may not be sufficiently utilised resulting in additional burdens associated with the metabolism and excretion of waste products such as ammonia and urea by the liver and kidneys (St. Joer et al, 2001).

The traditionally 'low fat focused' recommendations for a healthy diet, leaves CHO typically ranging from 50-60% of total energy. This macronutrient composition proved successful at reducing energy density and inducing weight loss over short periods, but the lack of satiety it achieved, meant that adherence to such diets could be poor (Astrup, 2008). Observations of these types of diets for weight loss revealed that they were often not suitable over long durations, with weight gain occurring after 18 months (Summerbell et al., 2008). In fact, in the USA, since 1976 as dietary fat consumption has decreased, rates of obesity and associated morbidities have continued to rise (Weinberg, 2004), suggesting that a low fat high CHO diet may not necessarily be an appropriate way to target obesity and metabolic complications. Further to this, the impact of the Mediterranean style diet (consumption of fruits, vegetables, nuts whole grain and olive oil) on body weight, over a 2 year follow up was assessed in 190 overweight Italian women (Esposito et al., 2007). Of the sample, 115 participants had a CHO intake greater than 50% of energy

(mean macronutrient composition: 58% CHO; 28% fat; 14% PRO), compared to 75 participants with a CHO intake below 50% of energy (45% CHO; 36% fat; 19% PRO). Those consuming a lower CHO diet benefited from significantly greater weight loss (p=0.01); reductions in TG (p=0.003) and increases in adiponectin (p=0.02) than those consuming a high CHO diet. These data suggest that a lower CHO (<50% of energy) diet with greater fat intake may be better for targeting the metabolic syndrome. It is important to note that the increased intake of PUFA and vegetable fats (Salmerón et al., 2001, Halton et al., 2006), associated with the Mediterranean style diet (Esposito et al., 2007), are likely to have a protective effect against metabolic complications and are thus important factors to consider when increasing fat intake. This notion is highlighted by (de Koning et al., 2011) who conducted a prospective cohort study of males aged 40-75 years, over a 20 year follow up and found that consumption of a low CHO diet high in animal protein and fat was significantly associated with type II diabetes. However, consuming a low CHO diet high in vegetable protein and fat was not significantly associated with Type 2 diabetes and in those <65 years old, the latter dietary profile was inversely associated with type II diabetes.

Further investigation into dietary CHO has identified sugar consumption as a potential burden to metabolic health. As recommendations on reducing total fat and SFA were increasingly adopted, food industries substituted sugar, fructose and high fructose corn syrup in their place (Johnson et al., 2009). Evidence suggests that this shift towards increased sugar intake may be responsible for reduced HDL and increased TG levels (Hellerstein, 2002, Ma et al., 2006). Sugar-sweetened beverages have been identified as the primary source of added sugars in the diets of Americans including children and adolescents (Guthrie and Morton, 2000, Wang et al., 2008). High sugar beverages contribute 10-15% of total daily energy among US children (Wang et al., 2008). Further to this a meta-analysis by (Forshee et al., 2008) positively associated their consumption with weight gain in paediatric populations.

To this end, the potential hindrance to metabolic health associated with higher CHO diets has been, in part, attributed to the speed at which CHO release their energy into the bloodstream (Ludwig, 2002); this notion has lead to the development of the glycaemic index.

2.4 Glycaemic Index and Glycaemic Load

2.4.1 Glycaemic index and glycaemic load conceptualised

The glycaemic index (GI) was first conceptualised by Jenkins et al (1981), it classifies carbohydrate containing foods according to their impact on the body's postprandial glycaemic response (Du et al., 2006). GI is defined as "the incremental area under the BG response curve (AUC) of a test food containing 50g of available carbohydrate, expressed as a percentage of the response to the same amount of available carbohydrate from a reference food (traditionally white bread or glucose)" (Jenkins et al, 1981). A foods GI is measured by measuring the average BG response of 10 or more healthy individuals for 2 hours following the consumption of a test food and repeating this process for the reference food (glucose or white bread) at a different time (Brand-Miller et al, 2005).

Based upon the degree to which carbohydrate containing foods impact on BG levels, they are assigned a GI value, categorised as low ($\leq$ 55), medium (56-69) or high ($\geq$ 70), (against glucose, GI = 100) (Du et al, 2006). As the quantity of carbohydrate in a food and not just its GI impacts upon the postprandial glucose response, the concept of glycaemic load (GL) was introduced by Harvard researchers in the 1990s, it combines the quality (GI) and quantity of CHO (GL = GI x available carbohydrate (g)/100) in a food or meal (Salmeron, 1997). GL values are also categorised as low (<10); Medium (11-19) and High (>20) for individual foods. Some researchers suggest that the quantity of carbohydrate (Bouche et al., 2002), whilst others believe carbohydrate quality (GI) (Wolever and Bolognesi, 1996), is more important when determining the glycaemic impact a food has on the body. Table 2, demonstrates how the quantity and quality (GI) of carbohydrate relationship can cause variation in the GI and GL of a single food. Although brown rice has a low GI value and Charlotte potato a high GI value, the GL of both foods (150g serving) is moderate; this is due to the lower amount of available carbohydrate in the potato offsetting its considerably higher GI value.

Table 2. Effects of the GI/CHO relationship on GL.

	GI	Available carbohydrate per 150g serving	GL per 150g serving
Brown rice (USA)	50 (low)	33	16 (moderate)
Charlotte potato, boiled (UK)	81 (High)	23	19 (moderate)

(Adapted from Brand-Miller et al., 2005)

Low GI foods have a slow digestion and absorption rate, thereby causing a slow and gradual rise in postprandial plasma glucose. High GI foods have a fast digestion and absorption rate resulting in a large and rapid increase in postprandial plasma glucose levels, subsequently increasing insulin and reducing glucagon secretions to counter act the rise in plasma glucose (Ludwig, 2002); see figure 1.

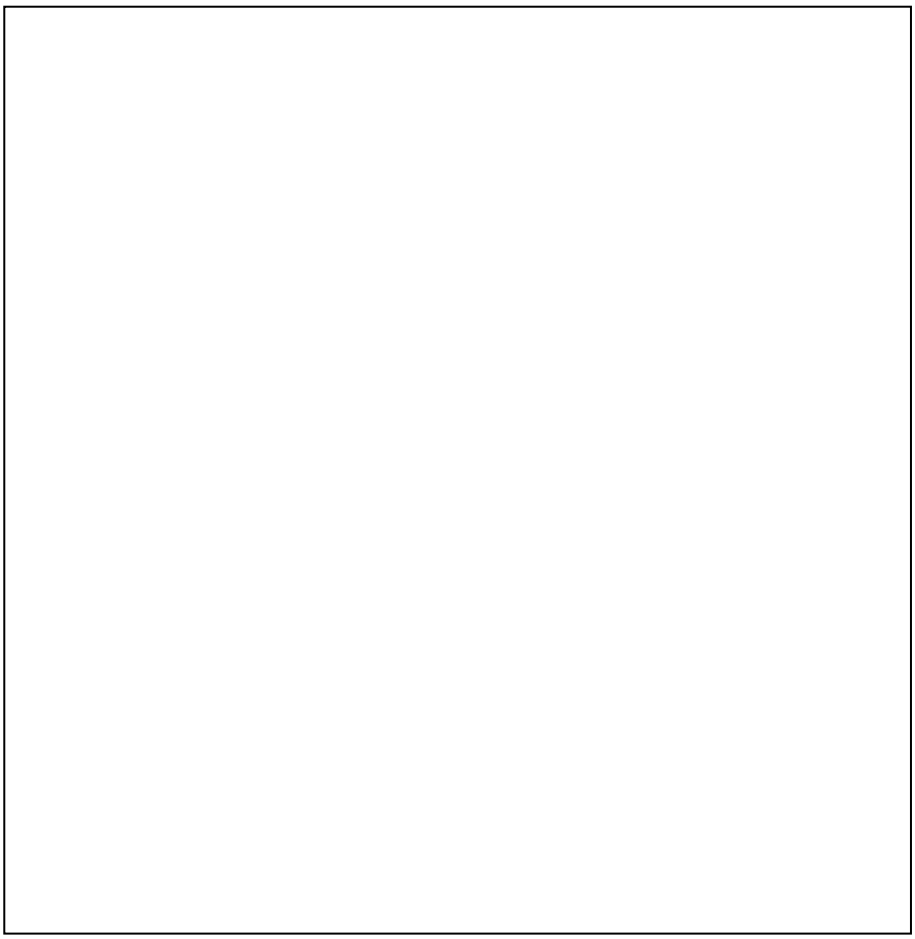


Figure 1. Glucose and insulin responses, following consumption of a low vs high GI food (Brand-Miller et al., 2005)

2.4.2 Effects of additional dietary variables on the GI of mixed meals

A number of factors can impact on the body's glycaemic response to a food or meal, including the method and or duration of cooking, the ripeness of a food, the method of food processing and the combination of foods eaten (Jenkins et al., 2002). Additionally a foods composition will alter glycaemic response, such as the amount of starch and sugar; the amount of protein, fat or fibre in a food or meal will reduce its GI through the slowing of digestion and rate of gastric emptying, as will acidity (Wolever and Bolognesi, 1996, Radulian et al., 2009). Low GL CHO foods are usually high in dietary fibre which contributes to delayed carbohydrate digestion and thus slows the release of glucose into the circulation (Riccardi and Rivellese, 2000). Furthermore, a foods structure, such as the presence of insoluble fibre found in whole intact grains will determine how quickly it is broken down and absorbed by the gut (Radulian et al, 2009). For example, raw and partially gelatinised starches, associated with low GI foods (such as legumes, pastas and whole grains) are more slowly digested

than the fully gelatinised starches associated with high GI foods (most breads and breakfast cereals) (Jenkins et al., 1987).

2.4.3 Application of the Glycaemic index

There is disagreement as to whether the GI should be promoted in the general public, as some regard it as too complex for public understanding (Pi-Sunyer, 2002, Franz, 2003). Despite an equivocal stand point on the concept of GI and its value to health at the time, in a Joint Expert Consultation, the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) endorsed the use of GI for categorising dietary carbohydrates and to guide food choices (FAO/WHO, 1997). Dietary GI can be lowered through the use of simple food swaps utilising GI food lists without the need for complex decision making or major dietary changes (Brand-Miller and Marsh, 2008). Studies of adults and children conducted in Australia have shown that a low GI diet was easier to follow than a conventional diet (Gilbertson et al., 2001, Moses et al., 2006). However, there is limited evidence supporting the application of low GI diets in the United Kingdom (UK). The majority of published GI data are based upon analysis conducted in Australia and the United States of America (USA) and many of the food brands published in GI tables are not accessible in the UK (Aston et al., 2008).

2.5 Associations of GI and GL with metabolic health

2.5.1 GI, GL and obesity

Evidence suggests that raised dietary GI and GL can play a role in the onset of obesity (Brand-Miller et al., 2002), as well as CVD and type II diabetes through the development of risk factors for the metabolic syndrome (Salmeron et al, 1997). However, as outlined below, in some of the literature there are equivocal data, regarding whether GI or GL is more important for metabolic health.

In healthy adults, dietary GI and GL have been positively associated with obesity (Brand-Miller et al., 2002); prospective cohort studies have identified that increases in GI appear to be associated with gains in body fat and WC (Du

et al, 2009; Hare-Bruun et al, 2006). Over a 6 year follow up of 89,432 males and females, a 10-unit increase in GI was significantly associated with a 0.19cm increase in WC per year, GL was not significantly associated with gains in WC (Du et al, 2009). Hare-Bruun et al (2006) identified that a 10-unit gain in GI, over 6 years, was significantly associated with gains in body weight (6%), body fat (3%) and WC (7cm) in sedentary women. In active women and all men associations with GI were not significant; GL shared no significant associations with adiposity variables. These data in adults suggest that reducing the GI of the diet rather than GL is more impactful on lowering adiposity and that this may be more important in females. Furthermore, these findings suggest that fitness may play a protective role in the development of diet-induced adiposity. Moreover, in 1354 healthy adult females from Japan, cross-sectional evidence suggests that GI but not GL is significantly associated with BMI, however body weight was self reported which may have implications for the validity of BMI calculations (Murakami et al, 2006). In children and adolescents, however, there is limited observational evidence of significant positive associations of GI or GL with adiposity, furthermore, evidence in UK populations is lacking. In studies of healthy, normal weight children (Scaglioni et al, 2005; Buyken et al, 2008) and pubertal adolescents (Cheng et al 2009) from Europe, neither GI nor GL were found to be associated with adiposity measures. Further to this, Hui and Nelson (2006) found that GL was not a significant independent factor associated with overweight in a sample of normal and overweight children from Hong Kong, however, intake from snacks was not considered when calculating GL. More recently, Joslowski et al (2011) suggested that postprandial increases in insulinemia, as estimated by the food insulin index, were associated with adiposity, whilst GI and GL where not associated with body fat % in 262 pubertal German participants aged 9-15 years. Conversely, in 10 and 16 year olds from Denmark, dietary GI and GL were significantly positively associated with body fatness as measured by the sum of four skinfolds but only in males aged 16 (Nielsen et al, 2005). Neither GI nor GL were associated with fatness in girls or in boys aged 10; associations of GI and GL with BMI were not significant. It was proposed that the greater degree of reporting bias by females may have been responsible for the differences in association observed for GI and GL with fatness. It appears that Neilsen et al (2005) are the only group to have reported association of GI and GL with adiposity in a group who are potentially entirely postpubertal (16 year old males; n = 181), however, pubertal

status was not assessed, highlighting a lack of evidence in postpubertal groups. Similarly to Nielsen et al, in 15,974 Japanese children (6-11 years old) and adolescents (12-15 years old) increasing dietary GL was significantly associated with greater risk of overweight (BMI) in male but not female adolescents, however, GL was also associated with overweight in male and female children (Murakami et al, 2011). A noteworthy limitation of this study is the use of self-reported weight and height and thus the calculations of BMI for categorisation of overweight may not be valid. To this end there appears to be limited evidence that glycaemic CHO are linked to increasing adiposity, but a common limitation of the above research is the potential confounding impact of puberty on adiposity which is rarely accounted for.

2.5.2 Impact of puberty on adiposity and metabolic health

During puberty, there is a transient increase in insulin resistance and associated metabolic health parameters (Hannon et al. 2006). During puberty hormonal changes are responsible for altered substrate metabolism and a transient increase in insulin resistance which return to pre-pubertal levels in the last stage of maturational growth (Staiano and Katzmarzyk, 2012, Moran et al., 1999) Furthermore, during puberty, fat mass, including visceral fat has been shown to increase in males and females, although, females are more susceptible to increased adiposity (Staiano and Katzmarzyk, 2012). Puberty begins at an average biological age of 13 years in boys and 11 years in girls (Tanner et al. 1975). The end of puberty is reached, on average, at the age of 14.9 and 14.4 years in boys and girls, respectively (Marshall and Tanner, 1969, Marshall and Tanner, 1970b). As such, the outcomes of observational research or interventional studies that aim to improve metabolic health might be flawed when including participants who fall within this age range. Indeed, many diet based intervention studies in children and adolescents fail to account for these pubertal changes and are thus methodologically flawed. Moreover, there appears to be a distinct lack of research investigating the effects of lowering dietary GI in postpubertal adolescents.

2.5.3 GI and GL with metabolic health

Observational evidence suggests that increasing dietary GI and GL are associated with risk factors for the metabolic syndrome in healthy adults, as

outlined below. In 1,354 healthy Japanese female farmers, aged 21-64 years, GI was significantly positively correlated with fasting TG and BG (as was GL), BMI and glycated haemoglobin ($HbA1_c$); a marker of long term glucose control. GL however was inversely associated with fasting HDL levels (Murakami et al, 2006). Further to this, in healthy American (Culberson et al., 2009) and Mexican (Denova-Gutiérrez et al., 2010) adults, increasing dietary GI and GL have been associated with an adverse lipid profile and greater relative risk of CHD. In 1420 British adults (mean age 39 years old), (Frost et al., 1999) identified that GI was inversely associated with HDL levels; interestingly GI was the only dietary variable to be associated with any lipoprotein subfractions. It therefore appears that GI and GL may be associated with an adverse lipid profile. Furthermore In 37,846, healthy Dutch adults (21-70 years old), a 10 year prospective cohort study found that greater GI, GL and CHO intakes were associated with the incidence of type II diabetes; associations with GL were stronger than GI (Sluijs et al., 2010). This study also identified a reduced risk of type II diabetes with increasing fibre intakes. Moreover, in a prospective cohort (11.9 year follow up) of 19, 608, healthy (at baseline) Dutch adults, increased GL was associated with greater risk of CHD in men whilst GI was not associated with CHD in either sex (Burger et al, 2011). Additionally, greater GI was associated with an increased risk of stroke but also only in males. Conversely, in a general population of American males and females, although increased dietary GL was associated with lower HDL levels in all participants and males, no association was observed between GL and the prevalence of metabolic syndrome in either sex (Culberson et al, 2009). Additionally, TG and glucose levels, BP and WC were not associated with dietary GL. Dietary GI has also been associated with liver steatosis (measured by liver echography) in 247 apparently healthy adults; associations were strengthened in those exhibiting insulin resistance (Valtuena et al, 2006) suggesting that high GI foods are a greater burden to those who have difficulty controlling BG levels.

There is also evidence in “metabolically at risk” adult populations that GI and GL are associated with factors linked with the metabolic syndrome as well as mortality rates. In 780 adult males from America with type II diabetes, diets lower in GI and GL and higher in cereal fibre were associated with increased adiponectin levels (Qi et al, 2005). Moreover, Burger et al (2012) conducted a 4.4 year prospective cohort study, of 6,192 subjects with diabetes; the

investigation involved 10 European countries (not including the UK). Associations of dietary fibre, GI, GL, and CHO intakes with mortality risk were assessed; fibre intakes were significantly negatively associated with mortality, yet exposures of GL, GI and CHO intakes were not significantly associated with mortality risk. Interestingly, when analysed according to weight status, GL, CHO and total sugar were significantly associated with greater mortality risk in normal weight (according to BMI) adults. Moreover, following further analysis, associations were strengthened after exclusion of dietary under-reporters.

There is limited and equivocal observational research into associations of GI and GL with factors of the metabolic syndrome in children and adolescents. Davis et al (2007) investigated the relationships of dietary sugar and GI with insulin dynamics (insulin sensitivity, insulin response and disposition index) and adiposity in 120 overweight Latino youths (10-17 years old) with a family history of type II diabetes. Dietary fibre, GI and GL were not associated with adiposity or insulin sensitivity, however, total sugar intake was positively associated with BMI, fat mass and negatively associated with insulin sensitivity and disposition index (Davis et al, 2007). In a prospective cohort of 769 healthy 13-15 year old Australian adolescents, O'Sullivan et al (2010) investigated the associations of CHO, GI and GL with the presence of the metabolic syndrome as defined by two internationally recognised adolescent definitions; the international diabetes federation (IDF) and the Adult Treatment Panel III (ATPIII). Additionally, associations of insulin resistance (HOMA-IR) and a sample defined metabolic cluster (based on BMI, fasting insulin, glucose, TG and blood pressure values ('high risk' or 'low risk')) were assessed. The prevalence of the metabolic syndrome was 3.6% (IDF), and 4% (ATPIII); 25.9% of participants were in the high risk cluster. A 20 unit increase in GL and a 30g increase in CHO were associated with significantly increased odds of having metabolic syndrome (IDF). No significant associations were observed when utilising the ATPIII, cluster-defined or HOMA-IR definitions. The IDF criteria is the only definition that requires the presence of a high WC, thus, WC may be a mediating factor for associations of GL with the prevalence of the metabolic syndrome in adolescents (O'Sullivan et al, 2010).

The above observational evidence highlights the potential risk posed by a high GI and or GL diet. Both GI and GL have been associated with obesity

measures including WC, an adverse lipid profile and risk of CVD, as well as markers of inflammation, raised fasting glucose and $HbA1_c$. Furthermore, GI and GL have been associated with prevalence of the metabolic syndrome and mortality rates. In some cases, however, total CHO, sugar and fibre intakes have been associated with factors of the metabolic syndrome when GI and GL have not. There is a lack of evidence in child and adolescent populations, particularly for adolescent from the UK. There is also evidence that associations may be mediated by fitness or physical activity, weight status and sex.

2.6 Nutritional Assessment

2.6.1 Nutritional assessment techniques

Due to the association between diet and metabolic health (Giugliano et al., 2008), dietary intake measurement has become an important tool to assess the impact of nutritional interventions and associations in observational research. There are methodological challenges relating to the accuracy of dietary assessment, with additional difficulty in the assessment of children, due to their lesser ability to remember and record their diets as well as a limited knowledge of food and food preparation (Rockett and Colditz, 1997). Several different techniques are available for dietary assessment and those commonly used in the literature include: 24 hour dietary recall interviewing, food frequency questionnaires, and food diaries/records which can require participants to estimate or weigh portion sizes (Hu, 2008). Seven day weighed dietary records are considered a reference tool for nutritional assessment (Kipnis et al 2001). This is because participants are not required to remember and recall intakes as the diary can be completed immediately after consuming a meal. Furthermore, the fact that food can be weighed reduces the need to make estimations and increases accuracy of recorded food quantities (MRC DAPA toolkit). Weighed food diaries have been deemed valid and utilised for dietary assessment in U.K. national surveys (Gregory et al., 2000, Henderson et al., 2002) and studies of children and adolescents (Robinson et al., 1999, Tuomilehto et al., 2001, Reinehr et al., 2003, Balagopal et al., 2005). Robinson et al (1999) used weighed dietary records, completed on 7 consecutive days as a reference tool for dietary assessment in adolescents. Reinehr et al (2003) used weighed dietary records to assess dietary changes from a lifestyle intervention in

children and adolescents aged 7-15 years, however, the researchers used 3 day food diaries, as they are less onerous than 7 day records and are thus likely to be more accurately completed. Three day weighed records are often utilised in observational studies and to assess dietary changes of lifestyle interventions on adults and children expressing metabolic complications (Tuomilehto et al, 2001; Balagopal et al, 2005).

Errors in self reporting of nutritional intakes (misreporting) can lead to inaccurate assessment of dietary intakes, either through failure to recall all foods consumed or by underestimating the amount eaten; underreporting is more common, but overreporting of intakes is also a source of error. It is therefore important to identify and control for reporting error. It is important to identify and attempt to control for reporting error when assessing associations of diet and health as measurement error can attenuate correlations between nutrients and outcome variables (Poslusna et al, 2009). Furthermore this may result in error when calculating GI based on carbohydrate intakes (Collins et al, 2010).

2.7 Dietary Misreporting

Dietary misreporting is a discrepancy between reported energy intake (EI) and measured energy expenditure (EE) where body weight is assumed constant during the reference period (Poslusna et al, 2009). Misreporting increases error in the estimation of EI but also that of other nutrients. It is evidenced that population characteristics can affect the degree of misreporting, such as age, sex and adiposity (Collins et al., 2010). Ascertaining accurate intakes in overweight and obese can be more challenging than in normal weight individuals, as under reporting generally increases with BMI (Poslusna et al, 2009). According to (Subar et al., 2003) and (Mahabir et al., 2006) BMI was a significant predictor of underreporting in adults. Additionally, studies have identified that a higher proportion of females and older participants underreport nutritional intakes (de Vries et al., 1994, Hirvonen et al., 1997).

During the 1980's, the validity of self reported nutritional intakes was assessed utilising biomarkers, namely, 24 hour urinary nitrogen excretions to assess

validity of protein intakes (Isaksson, 1980) and measurement of EE by doubly labelled water (DLW) (Schoeller and van Santen, 1982). Comparison of reported EI with EE (DLW) allows for bias in reported intakes to be assessed, based on the principle that in weight stable individuals EI and EE are equal (Rosenbaum et al., 1996). These principles were furthered by Goldberg et al (1991) who introduced the assessment of reported EI by comparison with estimated energy requirement (ER). Through the use of measured or estimated BMR and thus accounting for individual variations in minimal ER, mean EI can be compared with the presumed mean ER of the population, both expressed as a multiple of BMR (EI:BMR and EE:BMR). The ratio of EE:BMR represents the component of total EE from physical activity (PA) known as the physical activity level (PAL) (Black et al, 1997). Goldberg devised an equation (Goldberg et al, 1991) for calculating the confidence limit or cut-off of EI:BMR below which it is unlikely that mean EI is representative of habitual intake or a random low intake. This technique assumes a PAL value, relative to BMR, for the population. The Goldberg equation has become a useful tool for the assessment of underreporting (Black et al, 1976), and is widely used in the literature. The calculation accounts for the errors associated with sample size, length of dietary assessment as well as variation in food intakes, physical activity and BMR. Studies employing this technique have demonstrated that underreporting of reported EI is common. Black et al (1991) identified that two thirds of 37 studies had a mean reported EI (at group level) below the Goldberg cut-off, these studies compared EI:BMR with a group PAL value of 1.55 x BMR, based on the value for 'light activity' defined by the WHO (1985). This value was commonly used based on calorimetry and early DLW studies confirming that 1.55 x BMR as a likely minimum ER for healthy sedentary individuals (Goldberg et al, 1991). If EE is unknown and PAL is therefore assumed, only the lower confidence limit can be calculated and reported EI is classified as low or non-low. However, if a population's PA is known such that an appropriate mean PAL is assigned, the upper confidence limit can also be calculated (Black, 2000). The Goldberg cut-off has also been used to identify underreporters at the individual level (n=1), allowing for the characteristics of underreporters to be assessed (Pryer et al, 1997; Rotishauser et al, 1994), utilising the 1.55 x BMR PAL value. However, a presumed PAL value representative of 'light' activity can only identify bias in reported intakes of those leading a sedentary lifestyle (Black et al, 1997; Black, 2000). Furthermore, the

extent and degree of reporting bias has likely been under estimated in those with a higher PAL value than the traditional 1.55 x BMR. As many dietary surveys include participants with a broad range of EE, a PAL value should be estimated for stratified sub-groups of the population based on physical activity habits, or to be more specific, an individual PAL value can be applied when calculating a cut-off for EI:BMI. The utility of the Goldberg cut-off in identifying underreporters compared to direct measurement of EE (DLW) was assessed in 45 adults who completed a 16 day weighed food diary (Black et al., 1997). Underreporting was present at all levels of EE highlighting that misreporting is likely regardless of a groups PA engagement. Furthermore, the Goldberg equation was shown to be a good predictor of misreporting compared to DLW ($r = 0.65$, $P = <0.0001$), but only when information regarding PA was known, moreover, when a specific PAL was calculated from DLW data the validity of the Goldberg equation improved further (Black et al., 1997). Thus if an individual PAL can be ascertained, misreporting can be quantified with greater validity. A PA questionnaire can be used to assign a sub-group (e.g. sedentary, low active, moderately active or highly active) or individual PAL. Self reported PA, however, can be prone to reporting error (Sirard and Pate, 2001:Trost, 2007) and may lack the accuracy required to gain valid insight into an individual's PA habits. For instance, most PA questionnaires are designed to gather information on high-intensity activities, yet much of the variation between participants comes from sitting and standing activities of which can be hard to quantify in self report (Poslusna et al, 2009). Therefore, more recently the use of accelerometry has allowed for an objective assessment of PA that can be applied to the assessment of misreporting, however, it seems only a few studies have utilised accelerometry in this manner (Samuel-Hodge et al, 2004; Noel et al, 2010).

If individual EE is known it can be directly compared with EI and the bias of these values represents the degree of misreporting (assuming body weight is constant) (Rosenbaum et al, 1996). DLW provides an objective and independent measure of EE, however, its expensive and time consuming nature as well as the requirement of a sophisticated laboratory set up means that it cannot be routinely used to validate EI. Although accelerometry has been used to assign a PAL when estimating the Goldberg cut-off, very few studies have directly compared accelerometry obtained EE with EI for the assessment

of misreporting. Samuel-Hodge et al, (2004) assessed the validity of dietary data by comparing EI (3 x 24 hour recalls) with EE estimated by 7 day uniaxial accelerometry in 185 African females (mean age 59 years) with type II diabetes. Underreporting was assessed by direct comparison of EI and EE and by calculating a participant specific PAL for use in the Goldberg cut-off; derived from measured EE. Participants were classified as underreporting if the ratio of EI:EE was <0.79 (where EI:EE should equal 1 in weight stable individuals). This cut-off is representative of the lower 95% confidence limit of EI:EE, based on studies utilising DLW data (Black and Cole, 2001). According to EI:BMR, 58% of the population underreported compared to 81% as assessed by EI:EE. This suggests that using EE data from accelerometry compared directly to EI yields a greater proportion of underreporters than the Goldberg cut-off utilising an individual PAL derived from the same EE data. However, this study employed the use of uniaxial accelerometry which is likely to underestimate the measurement of activities with limited vertical accelerations. Moreover, accelerometer data was included if 4 days consisting of >4 hours wear time per day was achieved. A low wear time criteria such as the four hour minimum could result in an underestimation of PA, which in turn would result in underestimation of energy underreporting (Samuel-Hodge et al, 2004).

It appears that the objective nature and accuracy of triaxial accelerometry has not yet been exploited when measuring TEE to identify misreported EI, furthermore, there appears to be a distinct lack of research assessing EI:EE using accelerometry in a U.K. adolescent population.

2.7.1 Impact of dietary misreporting: Associations of diet and health

Misreporting can have important implications for the outcome of studies investigating diet and health relationships (Poslusna et al, 2009). Amelioration of these distorted associations between diet and health as a consequence of underreporting is hindered by the evidence that misreporting is not random and is selective for different macronutrients. In 301 healthy males, 7 day food diaries were used to investigate the effects of underreporting on associations with the metabolic syndrome (Rosell et al, 2003). The prevalence of overweight, a raised WC, raised systolic BP and the metabolic syndrome were significantly higher among underreporters. Furthermore, associations of fasting insulin with

PUFA and fats from milk were significantly stronger in underreporters compared to non-underreporters, highlighting that underreporting could introduce spurious associations between diet and health. However, Rosell et al (2003) did not identify and account for overreporting in the non-underreporting group, and therefore intakes from this group may not represent valid reporting. In terms of macronutrients, evidence suggests that underreporters tend to report higher intakes of PRO than non-underreporters, however, data for FAT and CHO are inconsistent (Poslusna et al, 2009).

It is noteworthy that some studies assessing associations of GI and GL with health found that dietary misreporting had minimal impact on associations. Excluding misreporters had a minimal impact on associations between glycaemic CHO (assessed by food frequency questionnaire (FFQ)) and BMI in 6334, 30 to 60 year old adults (Lau et al., 2006). Furthermore, Sluijs et al (2010) identified that removal of misreporters strengthened associations of GI and GL with type II diabetes risk, but the change was only slight and of minimal clinical importance. This was also observed in a study of British children (n= 818, 4-10 yrs old) and adolescents (n= 818, 11-18 yrs old) where there was no effect of removing or adjusting statistical analysis for misreporters on associations between adiposity and glycaemic CHO (Murakami et al., 2013). These data suggest that associations of glycaemic CHO with health markers may not be hindered by inaccurate food intakes. However, this will only be the case in circumstances where food intakes are merely underestimated, but if some foods are entirely omitted, this is likely to produce false values for GI and subsequently GL. It should be noted that Sluijs et al (2010), did not account for the PAL of the population when estimating misreporting through use of the Goldberg equation. Furthermore, Marukami et al (2013) calculated misreporting based on comparison of EI with estimated EE captured via PA diary and therefore may have underestimated EE and subsequently underreporting.

Removing individuals who misreport energy intakes from statistical analysis has not been recommended by some individuals, on the basis that it introduces unknown bias into the sample, being that only those participants reporting ‘normal’ intakes are examined (Gibson, 2005). A different approach, however, is to analyse all participants, but control for reported EI through the use of statistics. If dietary intakes can be observed relative to energy intake this may

attenuate the effect of over and underreporting (Poslusna et al., 2009), this will of course only be of value in those who misreport their entire diet as a whole rather than selectively for different macronutrients.

The nutrient density and residual method are the two most common techniques of energy adjustment (Mirmiran et al., 2006, Pryer et al., 1997). The nutrient density model is the sum of a total nutrient intake (e.g. total CHO intake) divided by total energy intake (Poslusna et al., 2009). This method is of course dependant on changes in reported EI and after adjustment nutrients will remain correlated with EI and thus, this technique may not be appropriate when assessing diet and health relationships. When utilising the residual method, however, adjusted nutrients are independent of EI (Mirmiran et al., 2006). The residual method utilises linear regression, with EI as the dependent variable and the intake of the nutrient being adjusted as the independent variable (Gibson, 2005). Each participant's energy adjusted nutrient intake is determined by calculating the residual difference between the observed nutrient value of each participant and their nutrient value predicted from the regression equation.

2.8 Glycaemic index and Glycaemic load Intervention studies

2.8.1 Intervention Studies: GI, GL and Obesity

Intervention studies have demonstrated that reducing dietary GI and GL can enhance weight loss over that of conventional energy restricted, high GI and low fat diets (Pereira et al, 2004; Speith et al, 2000), however, evidence suggests that low GI and GL may be more beneficial for individuals with features of the metabolic syndrome (Ebbeling et al, 2007; Pittas et al, 2005; Klemsdal et al, 2010). Overweight and obese adults have been shown to lose more weight when assigned to a low GI compared to a high GI (Pittas et al, 2005), and low fat diet (Ebbeling et al, 2007). However this was only observed in participants who at baseline had raised insulin responses to an oral glucose challenge, suggesting that low GI may not benefit those without reduced insulin sensitivity (Pittas et al, 2005; Ebbeling et al, 2007). This is in line with evidence demonstrating a more favourable effect on WC from a low GL diet in obese adults with the metabolic syndrome compared to those without (Klemsdal et al., 2010). However, in 11 moderately overweight (BMI 28 kg/m^2 ± 1), non-diabetic

men, who did not exhibit insulin resistance or other features of the metabolic syndrome, a 5 week low GI compared to high GI diet induced significantly greater fat loss (Bouche et al., 2002). In contrast to the findings of low GI and GL interventions in adults, greater fat loss has been observed in 16 obese adolescents (aged 13-21 years) consuming an *ad libitum* low GL versus a reduced fat diet (Ebbeling et al, 2003) over a 6 month period. Although no significant difference in calorie consumption was reported, the actual mean difference between baseline and intervention energy intake was 692 and 148 kcals in the low GI and reduced fat diet groups, respectively. Therefore it is unclear if the greater fat loss was a result of lower calorie consumption or reducing dietary GI. In a study of healthy obese children (mean age 10 ± 4 yrs), Spieth et al (2000), demonstrated that significantly more participants in a low GI diet group reduced their BMI by 3 kg/m^2 compared to an energy restricted, low fat diet group.

Gains in adiposity associated with increased dietary GI and GL have been explained in the literature by two potential mechanisms: increased satiety and increased fat oxidation (Mcmillan-Price and Bran-Miller, 2006). Low GI compared to high GI foods, matched for appearance and nutrient content, have been shown to induce greater satiety and are proceeded by less energy intake at subsequent meals (Ludwig, 2000). Furthermore, the consumption of a low GI breakfast has been shown to significantly reduce food intake at lunch in 37 normal and overweight children age 9-12 (Warren et al., 2003). Additionally, Holt et al (1992), showed that low GI mixed meals induced a greater secretion of the gut peptide cholecystokinin, which is associated with satiety and fullness, over a 180 minute period in healthy adult males. Satiety may be increased due to the slower rates of digestion and absorption in the small intestine associated with low GI foods (Lavin et al., 1998). This could result in extended feedback, via gut peptides such as cholecystokinin, ghrelin, glucagons and glucagons-like peptide-1, to the satiety centre in the brain as nutrient receptors are stimulated for a greater period of time (Lavin et al., 1998; Radulian et al., 2009). Furthermore, at the late postprandial stage following a high GI meal, both BG and fatty acid levels decline, often below fasting levels, a state that could be interpreted by the central nervous system as “low fuel status” (Ludwig 2002). Moreover, transient declines in BG during the post-absorptive state have been shown to correspond with spontaneous meal requests (Melanson et al., 1999).

Stevenson et al (2009) observed significantly greater fat oxidation over a 3 hour rest period following a low compared to high GI breakfast and lunch in recreationally active males, subsequent plasma glucose and insulin levels were significantly lower following the low GI meals. This group also reported significantly greater fat oxidation during exercise following a low compared to high GI breakfast (Wu et al., 2003). Alterations in fuel oxidation related to differing GI have been explained by postprandial increases in glycaemia and insulinaemia, which result in the rapid activation of key rate-limiting enzymes that increase CHO oxidation (Wolfe, 1998). An intermediate of CHO oxidation, malonyl-CoA, strongly inhibits fatty acid transport into the mitochondria, and thus, consumption of high GI foods can decrease fat oxidation (Wolf, 1998). Furthermore, the improved fasting insulin observed after low GL diets, and subsequent reduction in lipoprotein lipase enzyme (LPL) expression in adipose tissue may induce a decreased fat depot (Bouche et al, 2002). LPL stimulates the breakdown of triglyceride in the circulation into fatty acids to be stored in adipose tissue (Fielding and Frayn, 1998) and is correlated with insulinaemia (Boivin et al, 1994).

Further to this, habitual intake of high GI foods has been suggested to have a proteolytic effect, reducing muscle mass over time (McMillan-Price and Brand-Miller, 2006), as a result of the counter regulatory hormone response during the late postprandial hypoglycaemia (Zurlo et al., 1990, Weyer et al., 2000). This may result in a reduced BMR increasing the potential for a positive energy balance and weight gain. Low vs high GI (Agus et al., 2000) and low GI vs low fat diets that induce weight loss have reported significantly reduced declines in resting energy expenditure despite comparable body composition changes.

Together these data highlight the potential for low GI diets in body weight regulation through improved satiety and better maintenance of lean tissue, and thus greater potential for negative energy balance as well as an increased propensity to utilise fat stores in favour of CHO.

2.8.2 Intervention Studies: GI, GL and the Metabolic Syndrome

In adults with insulin resistance, the implementation of low GI and GL diets has improved a number of factors associated with the metabolic syndrome (Rizkalla et al, 2004; Jenkins et al, 2008). A low GI diet regime was found to significantly

reduce insulin resistance, improve lipid profile and long term glucose control ($HbA1_C$), when compared to a high GI diet, in a 4 week cross over intervention of 12 overweight men (Rizkalla et al., 2004) and in 210 men and women with type II diabetes (Jenkins et al, 2008). It is important to note that there were no significant changes in body weight and body composition, and that total daily calorie and macronutrient intakes were equal. Improved glucose utilisation and oxidation, as a result of the low GL intervention, may relate to the significant ($P < 0.01$) reduction in plasma FFAs observed (Rizkalla et al, 2004). In one of the few published low GI dietary intervention conducted in the UK, Jebb et al (2010) compared 4, 24 week dietary interventions following a 4 week high SFA (HSFA)/high GI (HGI) run in diet: HMUFA/HGI; HMUFA/LGI; low fat/HGI and low fat /low GI, in 548 adults featuring metabolic syndrome components. Reducing SFA improved lipid profile via reduced TC, LDL and apoB concentrations, however, lowering total fat content reduced HDL levels. Reducing dietary GI further enhanced TC and LDL improvements. All groups failed to significantly improve insulin sensitivity, however the low fat/LGI group improved insulin sensitivity to the greatest extent. Due to the HGI 4 week run in and a lack of data on its health impact, the effect of raising dietary GI was not elucidated. In healthy overweight and obese adults (n=39), a low GI diet has been shown to significantly reduce blood TG levels and insulin resistance compared to a conventional low fat, high carbohydrate diet (the intervention duration was the time taken to achieve 10% of weight loss) (Pereira et al, 2004). Both diets were calorie restricted to 60% of the energy requirements of each participant. Health improvements were observed despite no significant difference between weight and fat mass reductions in both groups, suggesting that there may be benefits of a low GI diet over and above that (or independent) of fat loss by conventional means (Pereira et al, 2004). Similarly, despite comparable reductions in BF%, between a low GL and low fat diet in overweight adults, BP was significantly reduced in the low GI group, (Klemsdal et al., 2010). Also in overweight but otherwise healthy men and women (Melanson et al., 2012) investigated the effects of 3 variations of a weight loss programme (weight watchers) over a 12 week period: 1) focused on portion control (restricted by energy and total fat with a minimum fibre intake), 2) low energy density (*ad libitum* consumption of wholesome low energy foods) 3) low GI (*ad libitum* consumption of foods based on the low GI Pyramid (Ludwig, 2000). All diets, however, induced significant reductions in FM and BF% as well

as factors associated with the metabolic syndrome (WC, systolic and diastolic BP, TG, insulin, HOMA-IR, glucose tolerance and CRP) with no significant difference between groups. All diets resulted in significantly increased PRO intakes and reduced total energy, % energy from fat and SFA with no between groups difference. Therefore, metabolic changes could be result of similar macronutrient and energy alterations. Although all 3 groups improved their metabolic risk components this could have been a result of a reduced GI and GL of which there was no significant difference between the low energy density, low GI and portion controlled diets after 12 weeks (GI: 30.04, 30.71 and 31.59; GL 54.39, 44.75 and 41.58, respectively). This highlights the importance of controlling for changes in dietary variable when comparing different dietary interventions.

A limited number of studies have investigated the impact of low GI diets on factors associated with the metabolic syndrome in healthy children and adolescents (Fajcsak et al, 2008; Ebbeleing et al, 2003), however, the evidence is ambiguous. Fajcsak et al (2008) found a significant reduction in body fat and metabolic risk factors following a 6 week *ad libitum* (which defines a diet with no restriction on consumption amount) low GL diet intervention in healthy, obese and overweight, prepubertal 11 year olds. The intervention focused on exchanging 50% of the high GI foods consumed with low GI foods. There was no significant difference between the calories consumed between baseline and intervention diets; dietary glycaemic changes were not reported however. This study also failed to include a control group for comparison, and furthermore, by attempting to control the glycaemic load of the diet, portion sizes were indirectly restricted, therefore failing to make the diet truly *ad libitum*. *Ad libitum* diets may be especially beneficial for adolescents, due to their flexibility. The desire to make choices and the lack of adherence to energy restricted diets that has been observed in adolescent populations may be better suited of the flexibility of *ad libitum* diets (Ebbeling et al, 2005). In contrast to Fajcsak et al (2008), a study of 16 obese adolescents assessing the impact of a 6 month low GI compared to low fat diet, Ebbeling et al (2003) found insulin resistance increased in both the low GI (non significant increase) and reduced fat diet groups. The authors suggested that this increase was associated with hormonal

changes during puberty, highlighting the importance of controlling for hormonal changes in studies of childhood and early adolescence.

Additionally, the impact of an unrestricted low GI diet compared to a standard reduced fat diet on obesity was examined in 190 children (10 yrs old) from the USA (Spieth et al., 2000). With the low GI diet children were advised to consume low GI CHO, PRO and fat at every meal and snack. The low fat diet emphasised consumption of low energy (approximate energy restriction of 250-500 kcals per day), low fat and low sugar and foods. The low fat group showed no change in BMI, contrastingly, a significant decrease in BMI of 1.15 kg/m^2 was observed in the low GI group. The low GI intervention was not restricted, whereas the low fat diet was heavily energy restricted. The difference in macronutrient intake of these diets makes it difficult to attribute the effect solely to GI. This evidence provides support for a flexible low GI diet in the reduction of obesity in children. Unfortunately, however, the authors did not monitor diet during the intervention and thus little is known of its impact on dietary intakes. Furthermore, there are no studies exploring the effect of a flexible, *ad libitum,* low GI diet on metabolic risk factors.

The following mechanisms have been suggested to promote the development of an adverse lipid profile, CVD, insulin resistance and type II diabetes as a result of a high GI diet. During the middle postprandial stage, following a high GI meal, the rise in circulating insulin leads to a rapid down regulation of BG; often to below fasting level. At the late postprandial stage, hypoglycaemia stimulates the release of hormones, such as adrenaline and glucagon to restore euglycaemia and activate fat oxidation to meet the energy demands of the body. The promotion of raised levels of circulating FFAs at rest, however, is associated with an adverse lipid profile and CVD as well as peripheral insulin resistance (in insulin sensitive non-adipose tissue such as the liver and skeletal muscle) (Weiss & Kaufman, 2008). Whereas, following a low GI meal, postprandial rises in gut hormones and insulin are reduced and the prolonged absorption of CHO suppresses the counteregulatory response and FFA release (Jenkins et al, 2002). Moreover, lipid profile improvements could be associated with a lower insulin response following a low GI resulting in reduced activity of insulin–stimulated 5-hydroxi-3-methylglutoryl-CoA reductase (the rate-limiting enzyme in cholesterol synthesis) (Rodwell, 1976). A low GI diet has been shown to benefit thrombolytic function, by reducing levels of plasminogen

activator inhibitor 1 (PAI-1), a protein involved with plaque and blood clot formation and thus reducing CVD risk in type II diabetic adults, (Järvi et al., 1999).

Furthermore, prolonged exposure to raised glucose and insulin levels can mimic a state of insulin resistance, which can lead to impairment or even failure of the β-cells of the pancreas in the longer term, and eventually to type II diabetes (Weiss and Kaufman, 2008). In insulin resistant individuals consuming a high GI diet, postprandial hyperglycaemia and insulinaemia are further magnified, possibly enhancing the likelihood of β-cell exhaustion and the development of type II diabetes (Salmeron et al, 1997 a; Salmeron et al, 2000). High GI foods may contribute to a state of glucotoxicity that can result in overproduction of reactive oxygen species by the mitochondrial electron transport chain leading to increased inflammation (Augustin et al., 2002). Furthermore, increased exposure to insulin has been associated with excessive amyloid deposits upon pancreatic β-cells leading to reduced β-cell function (Wolever, 2000).

In summary, there is a lack of low GI or GL intervention studies that include participants with poor metabolic health. Often, studies include overweight and or obese individuals that do not necessarily have poor metabolic health (Ebbeling et al, 2003; Fajcsak et al, 2008). This makes generalising the findings of such studies to metabolically 'at risk' populations difficult. There also appears to be an absence of dietary investigations and interventions, especially low GI interventions, in adolescents. Further to this, there is a lack of interventions that employ a control group. The most effective treatment for overweight young people with poor metabolic health is thus yet to be concluded. Therefore, in this population; an age group at risk of developing metabolic abnormalities that track into adulthood (Camhi and Katzmarzyk, 2010); a healthy diet that is well adhered to could be of great value, in order to promote a healthier lifestyle. To this end, nutritional intake, in relation to metabolic risk factors and the impact of a truly *ad libitum* dietary regime on metabolic health, compared to a control group, needs to be assessed. The proposed study therefore aims to provide knowledge on the current nutritional intakes and effectiveness of a low GI diet regime on improving metabolic health in overweight or obese, post-pubertal adolescents, exhibiting one risk factor of the metabolic syndrome, and thus help to inform future health-improving strategies.

Chapter Three: General Methodology

3.0 Introduction

This chapter describes the equipment and procedures used to conduct the investigations within this thesis.

The research is contextualised within 2 separate investigations:

1) A cross-sectional study examining the association of nutritional intake, fitness and physical activity with metabolic risk in postpubertal adolescents; entitled the CROSS- Sectional Study: Risk of Adolescent Disease (CROSSROADS);

2) An intervention study investigating the impact of a nutritional intervention upon the metabolic health of postpubertal adolescents who are overweight and obese; entitled The Study of Insulin Resistance Factors: Exercise and Nutritional Strategies (SIRENS).

3.1 CROSSROADS

Ethical approval was granted by the University of Bedfordshire research ethics committee. Participants were recruited from the University of Bedfordshire as well as schools and colleges within the Bedfordshire area. Schools and colleges were mixed sex, and the study was offered to those who expressed a willingness to take part. Data collection took place within the institution form which the respective participants were from. Participants were provided an information and consent form (see appendix 1) to be completed and signed by their parent or guardian if the participant was less than 16 years of age. The consent form included a physical activity readiness questionnaire (PARQ); participants were excluded if they indicated they could not safely take part in physical activity or if they had a known blood born disease that could be a hazard to the health of the researcher whilst acquiring a fingerpick blood sample. Participants could not take part if they were smokers.

To be classed as a postpubertal adolescent participant, the recruitment age was 15-19 in order to avoid the increased insulin resistance observed during puberty, which returns to prepubertal levels after completion of puberty (13.8 ± 0.9 years of age) (Moran et al, 1999). Ethical approval was granted by the University of Bedfordshire ethics review board.

3.2 SIRENS

Ethical approval was granted by the eastern region ethics committee (EREC). The SIRENS study is a collaborative project with the Centre for Obesity Research (COR) conducted entirely within the Luton and Dunstable Hospital (L&DH) NHS Trust. Collaborators from the L&DH included paediatricians, diabetologists, dieticians and pathologists, who had input in the experimental design of the investigation.

Participants consisted of postpubertal adolescents aged 14-19 years old. Participants were recruited through posters, local newspaper advertisements, local radio, school and college newsletters as well as via referral through local general practitioners (GP) and collaborating dieticians and paediatricians. Interested participants contacted the research team who subsequently sent a sex-specific consent and information form (see appendix 2) to be completed and signed by the participant. The consent form included a physical activity readiness questionnaire (PARQ); participants were excluded if they indicated they could not safely participate in exercise. Participants were informed (on the consent form) that they could not take part if they had any of the following conditions: Type 1 or Type 2 diabetes mellitus, heart dysfunction, thyroid problems, or if they: were receiving long term treatment for a health condition, were pregnant, had an eating disorder, had experienced recent dramatic weight changes, use medications containing steroids, were an alcohol or drug abuser, or had a known family history of: cardiac disease, renal disease, hypercholesterolemia or haemoglobinopathy. Participants could also not take part if they were smokers.

The application for ethical approval, recruitment process, data collection and intervention sessions were all conducted by the PhD candidate.

3.3 Data collection

For screening of metabolic health (blood samples, blood pressure and body composition); measures were taken in the morning and the participants were required to be fasted. Participants were advised to bring breakfast or a snack with them, to consume immediately after testing. Participants were also required to be well hydrated and thus were advised to sip water. Venepuncture was only conducted as part of the SIRENS study and thus was only carried out at the COR, L&DH. All other measures outlined below were taken from all participants at all research sites.

3.4 Haematology

3.4.1 Venepuncture Blood Sampling

Venous blood was collected at the L&DH only, by one of three trained technicians. Collection of venous blood samples adhered to the current L&DH clinical guidelines for Venepuncture.

Each participant was required to sit or assume a supine position on a medical bed before a tourniquet was applied above the distal region of the participant's bicep. The participants forearm was sterilised using a 70% isopropyl alcohol swab (Cutisoft® wipe, BSN medical, Hamburg, Germany) after the site had dried, blood samples were drawn using the S-Monovette system (Sarstedt AG and Co., Numbrecht, Germany). In order of draw, the following four blood collection tubes were filled to accommodate the various assays required (total blood sample of 17.6 ml): 2 x serum tube (brown tube - 4.7 ml) containing clot activator gel; 1 x glucose tube (yellow tube- 5.5 ml) containing 1.2 mg EDTA and 1.0 mg fluoride/ml; 1 x EDTA tube (pink tube- 2.7 ml) containing 1.6 mg EDTA/ml.

Once blood samples were collected, they were immediately delivered to the L&DH biochemistry laboratories where the samples were analysed by L&DH biochemistry staff and stored in multiple aliquots for further assessment at a later stage.

3.4.2 Fingerprick Blood Sampling

The fingerprick blood sampling procedures adhered to the University of Bedfordshire's guidelines for blood sampling. The participant's finger tip (usually index finger) was sterilised using a 70% isopropyl alcohol swab (Cutisoft® wipe, BSN medical, Hamburg, Germany) and then punctured using an autolancet. The first blood droplet was discarded, then, intermittent pressure was applied to the participant's finger (proximal to puncture site) using the thumb and index finger to promote the formation of a blood droplet. The tip of a heparinised capillary tube was placed into the droplet to draw a 40 µl blood sample. The sample was immediately transferred into a LDX® cassette sample well for the measurement of full lipid profile (total cholesterol, HDL, LDL, triglycerides) and glucose levels from arterialised capillary blood using the Alere Cholestech LDX® system, CA, USA.

3.5 Anthropometry

Waist circumference (mid axillary)

A tape measure was placed midway between the lower rib margin and the iliac crest of the participant whilst standing. When the iliac crest could not be identified the tape was placed at the level of the umbilicus. Following a gentle expiration by the participant waist circumference was measured to the nearest 0.1mm, whilst they were stood straight with feet together, arms by their side and looking directly ahead.

Standing height (stature)

Standing height was measured without shoes to the nearest 0.5 cm with a transportable stadiometer. During standing height measurements, participants were instructed to keep their shoulders in a relaxed position with their arms hanging freely and their head in the Frankfurt plane. They were then asked to take a deep inhaled breath prior to measurement whilst looking straight ahead. Participants were also required to maintain contact between their heels and the floor.

3.6 Weight and Body composition

Body mass and fat free mass (FFM) were measured to the nearest 0.1 kg and body fat % to the nearest 0.1 %, using the Tanita BC-418® Segmental Body Composition Analyser (Tanita Corp., Tokyo, Japan). The devise utilises bioelectrical impedance analyses (BIA) to estimate each participant's fat mass and FFM. Participant information (sex, age and height) was manually entered in to the device. Participants, in light clothing and bare feet, were then required to step onto the platform at the base of the device placing both feet on the electrode plates and stand steadily whilst their weight is measured. Then participants were required to grasp the handles (one in each hand), ensuring a firm grip around the electrode of each handle. Whilst stood still with arms held to the side, slightly abducted from the body, a current is passed through the hands and feet around the body for approximately 5 seconds whilst body composition is measured. After a tone is sounded the participant can replace the handles and step off the platform.

BIA assesses the impedance of the electrical current through body tissues, utilising the principle that the electrical conductivity of FFM is greater than that of fat tissue (Lukaski et al, 1985). The resistance to the current can be used to estimate body fat

and fat free tissues, (heyward et al, 2004). The BIA estimates resistive impedance and bioconductor volume (corrected for height) which are used with the manufacturer prediction equations to estimate total body water, fat and FFM.

3.7 Resting heart rate and blood pressure

Resting heart rate and BP was measured using an Omron M5-I automated oscillatory device (Omron Matsusaka Co. Ltd., Matsusaka, Japan). Following a 5 minute seated rest period, the cuff of the monitor is placed on the left arm, proximal to the anticubital fossa, at the brachial artery. With the participant sat still and their left arm supported on an adjacent table, at the level of their heart, three blood pressure readings were taken at 2 minute intervals. The mean of the lowest 2 readings was recorded (Chobanian et al, 2003)

3.8 CRF

CRF (CRF) was determined by a maximum cycle ergometer test as described by Hansen et al (1989) and employed in the European Youth Heart Study (Ekelund et al. 2007). Participants were required to cycle at a cadence between 50-70 revolutions per minute (rpm) on a Monark ergomedic 818e cycle ergometer (Monark, Varberg, Sweden). The test protocol was sex and age-specific in terms of the work load at each stage.

Following the initial work rate for 3-minutes (40 watts [W] for females, 50W for males), the incremental workload increased every 3 minutes (by 40W for females, 50W for males). Heart rate was recorded continuously throughout the test via a Polar heart rate monitor, worn on the chest of each participant. The test continued until the participant could no longer continue. An exhaustive effort was considered to have been achieved if the participant had a heart rate > 185 beats/minute when the researcher had observed that the participant could no longer continue exercising. If the participant's pedalling rate dropped below 30 rpm, the participant was considered to have stopped the test.

During the test, in order to assess CRF, each participant was required to breathe through a portable indirect calorimetry gas exchange system that is suitable for wearing during exercise (Cortex MetaMax 3B). Data was analysed using MetaSoft 3.9 software to determine peak oxygen uptake. A suitably sized facemask and base

jacket (in which the MetaMax is held) was selected for the participant prior to testing. Prior to each fitness test, the appropriate equipment was cleaned and calibrated following manufacturer guidelines.

3.9 Physical activity

Physical activity was assessed utilising the RT3 ® triaxial acclerometer (Stayhealthy, Inc., Monrovia, CA). Triaxial accelerometry measures accelerations and decelerations of movement on 3 planes (vertical (x), anterioposterior (y), and mediolateral (z)). Activity categories (SED, LPA, MPA, VPA, MVPA) were identified from the vector magnitude, calculated from the output of all three axes. From this data the average time spent in each activity category throughout the monitoring period was calculated.

Each participant was required to wear an accelerometer (clipped to their waist band) for the duration of their waking hours for 7 consecutive days (5 weekdays and 2 weekend days). Exceptional circumstances included: bathing, showering or swimming (as the monitor is not waterproof), during sleeping or whilst participating in contact sports that may result in injury to the participant or damage to the device. In these circumstances, participants were required to log the actives during which they removed their accelerometer. To be included participants were required to wear the activity monitor for a minimum of 3 days (2 week days and one weekend day); minimum wear time for a week day was 9 hours (Mattocks et al., 2008) and for a weekend day was 8 hours (Rowlands et al., 2004). Each activity monitor was programmed to capture activity counts in one minute time frames (epochs), a shorter epoch was not possible over a monitoring period of 7 days due to the memory capacity of the device. Prior to analysis, data was recoded and sustained 10 second periods of zero activity counts were removed as it was deemed that the monitor was not worn in these circumstances (Riddoch et al., 2004). Time accumulated in different PA intensities was averaged for week days and weekend days separately, the average of these values was then summed. The RT3 has been validated against oxygen uptake in free-living children and adolescents ($r = 0.87$, $P < 0.001$) (Vanhelst et al., 2010) and relative to body mass in children within a controlled laboratory setting ($r = 0.87$, $P < 0.01$) (Rowlands et al., 2004).

3.10 Nutritional assessment

Habitual nutritional intake was assessed via a 3 day weighed food diary based on a published example and accompanying material which was used in the National Diet and Nutrition Survey (NDNS) (Henderson et al., 2002). Participants were instructed in groups on how to complete, all participants were given an instruction sheet and example diary which clearly outlined how the diary should be completed (See appendix 3 for further details). Participants were required to detail, in an open diary, all of the food and drink they consumed over a 3 day period comprised of 2 week days and 1 weekend day consecutively. So that food items could be weighed, participants were provided with a set of dietary weighing scales and instructed on how to effectively use the scales to weigh mixed meals and individual food items. Where possible, participants were asked to provide food packaging and labels that provided specific nutritional information to aid analysis. Participants were encouraged to weigh all food items but in the circumstance where food could not be weighed, portion sizes had to be estimated and detailed within the diary. In order to accurately obtain the weight of food eaten, participants were required to record the weight of the container (e.g. cup or plate) that any food or drink items were eaten from and re-weigh the container after eating to provide a weight of food leftover. This method has been used by the NDNS and is suggested to minimise the burden of weighing leftover food items and increase adherence to this practice. All participants had a 10 minute one-to-one dietary follow up session, where the researcher and participant checked through the food diary to ensure all entries were legible and completed correctly. Where information appeared to be missing or erroneous, the researcher prompted the participant to recall this information in an attempt to minimise any unrecorded or inaccurately weighed items.

Food diaries were manually entered into a dietary analysis computer programme (CompEat Pro, Nutrition Systems, UK) which utilises McCane and Widdowson's composition of foods, 6^{th} edition (Krebs, 2002). Estimates of daily intake of selected nutrients and energy were averaged over the 3 day recording period.

Assignment of GI values

Assignment of individual food GI values from the weighed food records were based on the latest international tables of GI (Atkinson et al., 2008). Foods that contained

less than 0.5 grams (g) of CHO per 100g were assigned a GI value of zero. Where no specific GI value is available the most closely related food (based on macronutrient and fibre content) from the international tables of GI was assigned.

Calculation of dietary glycaemic index and glycaemic Load

Daily GI was calculated based on the procedures outlined by (Jenkins et al., 1981). The percentage contribution to total carbohydrate of each carbohydrate containing food (above 0.5g CHO per 100g) was quantified. The respective GI value of each food was then multiplied by its percentage contribution to total carbohydrate; these values were summed to give a dietary GI value for each participant's diet. To calculate daily GL, the amount of carbohydrate in each food was multiplied by its GI value/100, this was summed to give an overall daily GL.

3.11 Sample size calculations

CROSSROADS

The required sample size was calculated based on a cross-sectional study investigating the relationship between dietary GI/GL and metabolic health (O'Sullivan et al, 2010). The calculation was based on logistic regression analyses that revealed a significantly (p =0.03) increased likelihood of having the metabolic syndrome per unit increase in dietary GI (odds ratio: 1.89 95% CI; 1.06-3.36) and GL (odds ratio: 1.62 95% CI; 1.05-2.49). The calculation estimated that a minimum of 137 (based on the smaller effect size observed for GL) participants are required in order to reach sufficient statistical power in logistic regression analysis when assessing the relationship between dietary GI/GL and metabolic health (alpha set at 0.05, power of 0.85).

SIRENS

Sample size was calculated, based on a previous paper (Kadoglou et al. 2007) in which a significant decrease (P =0.023) in HOMA-IR (primary outcome measure) was found following treatment (aerobic exercise training) compared to a control group. To produce a significant effect size on HOMA-IR between treatment and non-treatment groups, a minimum sample size of 3 in each group is necessary

(alpha set at 0.05, power of 0.95). Moreover, a further power calculation was performed to determine the sample size required to detect a significant difference between two treatment types (e.g. aerobic exercise vs. diet), a sample size of 15 in each group was calculated (alpha set at 0.05, power of 0.80). Thus the required target sample size for the SIRENS study was 25 participants per group in order to achieve a minimum of 15 participants in each group (allowing for a 40% drop out rate).

Sample size was estimated using the computer software package (G*power 3.1.2)

Chapter Four: Study One

Physical activity levels and Nutritional intake of postpubertal Bedfordshire adolescents: associations with adiposity

4.0 Introduction

A healthy lifestyle can be characterised by one which consists of adequate PA and a 'healthy' diet, both of which have been positively associated with a lower incidence of overweight and obesity (Wareham et al., 2005). Obesity and particularly centrally located adiposity has been associated with a cluster of metabolic disorders associated with type II diabetes, atherogenic dyslipidemia and subsequent CVD (Despres and Lemieux, 2006).

Diet and Adiposity

Energy dense foods are considered a primary determinant of obesity and related diseases such as CVD (Phillips et al., 2010) and metabolic complications (Druet et al., 2007). Energy restriction has fundamentally been used to reduce obesity (Abete et al., 2011) but manipulation of macronutrient distribution has also been associated with weight reduction (Muzio et al, 2007). Traditionally, high fat diets were associated with increased adiposity and thus treatment of overweight and obesity was facilitated through lowering fat intake (Abete et al., 2010) which was subsequently compensated for by increasing CHO in place of fat. Increased dietary fat has been positively associated with body weight in large cross-sectional studies of males and females (Satia-About, 2002; Park et al., 2005) as well as in youth populations (Tucker et al., 1997; Ortega et al., 1995). This 'low fat focused' approach proved successful at reducing energy density and weight loss for a short period; however, these diets appeared to have poor adherence over a longer period due to a lack of satiety which is now associated with low fat diets (Atrup, 2008) and some studies have shown weight re-gains after 18 months of dieting (Summerbell et al., 2008). Since 1976, as dietary fat consumption has declined, rates of obesity and its co-morbidities have continued to rise (Weinburg, 2004). It thus appears that low fat, high CHO diets may not be the most appropriate technique for targeting adiposity. Over more recent years evidence has emerged that the composition of CHOs can alter the speed at which they are metabolised and this can have implications for satiety and fat metabolism leading to weight gain (Ludwig, 2000, Du

et al., 2006). To this end, research has assessed the associations of CHO consumption with adiposity, in terms of the speed at which CHOs are absorbed into the blood stream; ranked by the GI and GL. The GI classifies CHO containing foods according to their impact on the body's postprandial glycaemic response (Du et al., 2006), furthermore, as the quantity and not just the quality (GI) of CHO will impact on postprandial glucose levels the GL of the diet has also been considered. It has been evidenced that GI is positively associated with adiposity (Brand-Miller et al., 2002), in adults; prospective cohort studies of adults have shown BF% and WC significantly increase in relation to dietary GI (Du et al., 2009; Hare-Bruun et al., 2006). Furthermore, GI has been positively associated with BMI in 1354 Japanese females (20-78 yrs) (Murakami et al., 2006).

There is limited evidence supporting positive associations of GI and GL in youngsters; in normal weight children (Scaglioni et al., 2005; Buyken et a.l, 2008) and overweight youths (Hui and Nelson 2006) higher glycaemic CHO was not associated with fatness. Furthermore, Joslowski et al (2011) observed that postprandial insulinemia was associated with adiposity but that GI and GL shared no association in 262 9-15 year olds. However, in 486 children and adolescents from Denmark, both GI and GL were associated with body fatness, in 16 year old males but not children or females; the lack of association in girls was purported to be related to the increased reporting bias observed in the young females of this study Nielsen et al (2005). These findings (Neilsen et al., 2005) appear to be the only to report association of GI and GL with adiposity in a potentially entirely postpubertal group (16 year old males; n = 181), however, pubertal status was not assessed. One study has assessed associations of glycaemic CHO and adiposity in (818) British children (4-10 yr olds) and adolescents (11-18 yr olds) based on data from The National Diet and Nutrition Survey (NDNS) (Murakami et al., 2013). The authors observed that increasing GL was independently associated with increased risk of overweight (BMI) in children (P= 0.04) and central obesity (assessed by waist to height ratio) in adolescents (P= 0.02) but GI was not associated with adiposity (Murakami et al., 2013). Although this recent publication is a relatively large representative sample of UK youths, the NDNS data analysed is not current; data were collected in 1997 and therefore are unlikely to represent current glycaemic CHO consumption. A further limitation is that this investigation utilised an age range of adolescents (11-18 yrs) that is likely to encompass individuals of varying pubertal status and thus findings may be confounded by puberty (Moran et al., 1999).

During puberty hormonal changes are responsible for altered substrate metabolism and a transient increase in insulin resistance which return to pre-pubertal levels in the last stage of maturational growth (Staiano and Katzmarzyk, 2012, Moran et al., 1999); it is understood that these factors can influence metabolic health parameters (Hannon et al., 2006). Furthermore, during puberty, fat mass, including visceral fat has been shown to increase in males and females, although, females are more susceptible to increased adiposity (Staiano and Katzmarzyk, 2012). Therefore, puberty may confound associations of GI and GL with adiposity.

In summary, there is limited evidence of an association between glycaemic CHO and adiposity in youths. However, studies of children and adolescents tend to assess participants across a broad age range which encompasses pre-pubertal, pubertal and post-pubertal individuals. Therefore, it will be of benefit to assess these associations in a solely postpubertal adolescent population in an attempt to negate these confounding factors. The few studies investigating associations of adiposity with GI and GL in adolescents have only assessed adiposity according to BF% and BMI and not assessed associations with WC. Adolescents are at a stage in their lives where they are making more autonomous lifestyle choices regarding their eating behaviours (Ebbeling et al., 2003) and thus an understanding of their dietary GI and GL and the associations they share with adiposity important. However, little is known of the dietary GI and GL of adolescents from the UK. One reason for this could be that the lack of published GI data on foods commonly consumed in the UK and many of the food brands published in GI tables are not accessible in the UK (Aston et al., 2008). Currently the most comprehensive data are based upon analysis conducted in Australia and the USA and thus the application of dietary GI and GL in a health context is little understood in UK adolescents (Aston et al., 2008).

Physical Activity, CRF and Adiposity

It is well established that time spent being physically active shares an important association with obesity and associated co-morbidities (Wareham et al., 2005). Physical inactivity, or being sedentary may also play an equally important role in the development of adiposity (Healy et al., 2011). A cross-sectional study of European adults showed that being physically inactive (time spent sitting) shared a dose response relationship with obesity as assessed by BMI (Martínez-González et al.,

1999). Two studies, one of healthy American and one of diabetic Australian adults, provide evidence in adults that objectively assessed SED time may be a more important determinant of obesity than MVPA. All physical activity categories (negatively) including sedentary (positively) were associated with an increased WC (Healy et al., 2008b, Healy et al., 2011). SED time was shown to be independently associated with WC regardless of time in MVPA, however after adjustment for SED time MVPA was no longer associated with WC. Additionally, (Healy et al., 2008a) evidenced that breaks in SED time were associated with a lower WC suggesting that a transition from SED to LPA may be a useful strategy for weight reduction.

In children and adolescents PA engagement is negatively associated with adiposity (Deforche et al., 2003, Ekelund et al., 2012); however, compared to adults there is contrasting evidence in respect to which activity category is most important for adiposity. A study of school children identified that obese pupils, compared to non-obese, engaged in a similar amount of PA in leisure time (LTPA) but took part in significantly less higher intensity sporting activities (Deforche et al., 2003), although PA was assessed by questionnaire. In a study of 9-10 year old British males, SED time was associated with an increased WC and fat mass (as in adults), however, associations were attenuated after adjustment for time in MVPA (Steele et al., 2009). Furthermore, total PA and MVPA, were inversely associated with adiposity following adjustment for SED time (Steele et al., 2009). Similarly, MVPA was associated with WC independent of time spent SED in a large study of pooled data (n = 20871) in 4-18 year olds (Ekelund et al., 2012). As part of living a more independent lifestyle, adolescents are also making important decisions about their PA and exercise engagement. As children enter their adolescent years PA engagement begins to decline (Kimm et al., 2002) and a concomitant increase in weight status has been observed (Kimm et al., 2005). This may have important health implications as physical inactivity (measured by self assessed questionnaire) during adolescence (16-18 years old) has been shown to independently predict total and abdominal obesity levels in adulthood (25 years old) (Pietiläinen et al., 2008). In light of the evidence that MVPA is an important determinant of fatness and related health outcomes, the UK government recommend that children and adolescents should engage in >60 minutes of MVPA per day (DOH, 2011).

In addition to PA, CRF is an important factor associated with obesity and metabolic aberrations (Ekelund et al., 2007b). In a study of 366 SED males, those with moderate CRF had a lower average total fat mass, WC and visceral fat than those

categorised in to the low CRF group (Janssen et al., 2004). Similarly, in a sample of 3719 male and 3854 female adults (20-60 yrs), those with high CRF levels, demonstrated a lower WC and total fat mass compared to those with low CRF (Ross and Katzmarzyk, 2003)

In 1045 children aged 6-13 total and abdominal body fat (assessed by the skinfold method) were lower in those with a high, compared to low CRF (estimated via 20 metre shuttle run) (Nassis et al., 2005). In a large study of Spanish adolescents (13-19 year olds), BMI and WC were inversely associated with CRF (20 metre shuttle run) and positively with SED activities (assessed by self report questionnaire); however, no relationship was seen between LTPA and adiposity variables (Ortega et al., 2007). CRF (20 metre shuttle run) and PA were objectively assessed (RT3 accelerometer) in 7-10 year olds from Ireland; in girls fitness was also the only factor associated with body composition (inversely). In boys however body composition was negatively associated with both fitness and VPA (Hussey et al., 2007). Very few studies have assessed the association of adiposity with PA and CRF in children and adolescents and there is a distinct lack of evidence for objectively assessed PA and directly measured CRF (VO_2 uptake). Furthermore very few studies have assessed these relationships whilst controlling for dietary variables.

Therefore the following study investigated the current: 1) dietary GI and GL intakes; 2) objectively assessed physical activity levels; 3) directly measured CRF levels, of postpubertal adolescents from Bedfordshire. Due to the implications of overweight and obesity on cardio-metabolic health, the associations of dietary GI and GL, CRF and PA with adiposity were assessed in this group.

4.1 Methodology

Participants

The 105 males and female adolescents recruited for this cross-sectional research were part of the CROSS-Sectional Study: Risk of Adolescent Disease (CROSSROADS) or Study of Insulin Resistance Factors: Exercise and Nutritional Strategies (SIRENS) studies. Participants were recruited on a voluntary basis from Bedford and Luton schools, colleges, GP surgeries and through paediatric consultants from the Luton and Dunstable Hospital (SIRENS only). Participants were excluded from the study if they were smokers, could not safely participate in

PA or if they had any known blood born diseases that could be hazardous to the health of the researchers. Ethical approval was granted by the University of Bedfordshire ethics board (CROSSROADS) and the NHS EREC (SIRENS). Informed consent was gained from all participants over the age of 16 and parental consent was gained for all participants under 16.

Experimental Design

Data collection was conducted at multiple locations to facilitate the research: the University of Bedfordshire Sports Science Laboratory, schools and colleges (CROSSROADS) and the Luton and Dunstable Hospital Centre for Obesity Research (SIRENS). Measures were taken between 7 and 10 am in an allocated data collection room within the school or consultation room at the COR. Participants were required to arrive ready for testing after having fasted since 9pm the previous night; they were instructed to consume water during this period (up to 4 hours prior to testing) to ensure they were hydrated.

Measurements

Age, Ethnicity and Social Economic Status

The participants' date of birth was used to calculate age to two decimal places on the date of data collection. Ethnicity was recorded as non-white or white. Socioeconomic status (SES) was calculated for each participant based on home postcode converted into indices of multiple deprivation (IMD) using the 2007 IMD Geoconvert application (MIMAS 2008). A lower score represents greater deprivation.

Anthropometry and Body Composition

Suture and WC were recorded to the nearest 0.5 cm and 0.1 mm, respectively as outlined in section 3.5. Body mass, and fat mass were recorded to the nearest 0.1 kg using the Tanita BC-418 ® Segmental Body Composition Analyser (See section 3.6). BMI was calculated using the following equation: BMI =body mass (kg)/height^2 (m^2).

Dietary Intake

A three day (2 week days and 1 weekend day) weighed food diary was completed by each participant within 1 week of data collection as outlined in section 3.10. Intakes of nutrients and energy were averaged over the 3 day recording period.

Daily dietary GI and GL were calculated as outlined in section 3.10 of the 105 participants recruited 98 (93.3%) adequately completed the 3 day weighed food diary.

Physical Activity

PA engagement was monitored using the RT3 triaxial accelerometer (Stayhealthy, Inc., Monrovia, CA) for 7 consecutive days. To be included in the analysis participants had to wear the accelerometer for a minimum of 3 days (2 week days 1 weekend day), the minimum wear time was 9 hours per week day (Mattocks et al., 2008) and 8 hours for a weekend day (Rowlands et al., 2004). See section 3.9 for further details. Of the 105 participants assessed a total of 75 participants (71.42%) met the required wear time for accelerometry.

Time spent in different PA subcategories was assessed using the thresholds of Rowlands (Rowlands et al., 2004). The activity counts recorded each minute (CPM) were utilised to determine PA intensity based on the following thresholds: SED <288 CPM; LPA 288-969 CPM; 970-2,332 CPM and VPA ≥ 2,333 CPM. Time accumulated in each PA category was subsequently calculated. Rowlands et al (2004) validated the RT3 against oxygen uptake relative to body mass in children within a controlled laboratory setting ($r= 0.87$, $P <0.01$) (Rowlands et al., 2004).

CRF

CRF was assessed by an incremental maximal cycle erometer test as outlined in section 3.8. Peak oxygen uptake at maximal exercise was measured using the Cortex MetaMax 3B breath by breath online gas analyser. Of the 105 participants 98 (93.3%) participants completed CRF testing.

Statistical Analysis

Analyses were completed using the Statistical Package for Social Sciences (SPSS Inc., IL.), descriptive statistics are presented as mean and standard deviation (SD). The following variables were non-normally distributed and were subsequently log transformed to improve their distribution (BMI, BF%, WC, energy intake, GL, PRO, fat, CHO, PUFA, MUFA, SFA, sugar, salt, %fat, % PUFA and % SFA). After log transformation the relevant dietary variables were adjusted relative to energy (GL/1000kcal and fibre/1000kcal). Adiposity variables, BMI and BF% were converted to *Z*-scores based on population means for the age group. A raw WC

value has been used as the population mean data does not address the upper age range (17-19 yrs) of the population under investigation. Differences between sexes were assessed by One-way ANOVA. The relationship between dietary variables and adiposity variables were assessed by partial Pearson correlation analysis following adjustment for age, sex, SES and time spent SED. Partial correlations were also used to assess the relationship of PA, CRF and adiposity variables following adjustment for age, Sex, SES, %CHO, %PRO, %FAT and Fibre/1000kcal. MANCOVA was used to assess differences in adiposity (*z*-BMI, *z*-BF% and WC) between upper and lower quantiles of GI and GL these analysis were adjusted for the following covariates: age, sex, SES, %PRO, %FAT, %CHO, FIBRE/1000kcal, % time spent SED. MANCOVA was also employed to explore adiposity across upper and lower quantiles CRF (adjusted for age, sex, ses, %PRO, %FAT, %CHO, FIBRE/1000kcal, % SED) of time spent in PA subcomponents and in those who did and did not achieve >60 mins MVPA (adjusted for age, sex, ses, %PRO, %FAT, %CHO, FIBRE/1000kcal and total PA). As a result of adjusting the MANCOVA analyses for PA and diet a total of 72 participants were assessed due to the combination of participants providing both adequate diet and PA data. No colinearity was observed between covariates of MANCOVA models and the assumption of homogeneity of regression slopes was not violated as no significant interaction effects on adiposity variables were observed between any covariate or independent variables. Significance level was set at P <0.05 for all analysis.

4.2 Results

Table 3. Participant characteristics

	COMBINED (n=98)	**Male**s (n=61)	**Females** (n=37)	***P***
Age (y)	17.36 (1.40)	17.46 (1.22)	17.21 (1.64)	.389
Height (cm)	172.40 (9.85)	177.80 (7.32)	163.64 (6.59)	.000
Weight (kg)	71.25 (16.30)	71.66 (15.45)	70.59 (17.78)	.746
BMI (kg/m^2)	23.89 (5.59)	22.64 (4.65)	25.92 (6.39)	.003
BF (%)	23.30 (10.89)	17.98 (7.49)	31.94 (10.02)	.000
WC (cm)	80.33 (12.20)	79.44 (11.64)	81.76 (13.08)	.346

Table 3 shows anthropometric and adiposity characteristics of the population; *P* significant at <0.05 for comparison of males and females

Males and females had a similar mean age, males were significantly taller, yet females were of a similar weight to males. Females have a significantly greater BMI and BF% compared to males but share a slightly raised WC.

Table 4 displays the mean intakes of dietary variable and the respective recommended intake according to SACN (2008). Mean GI values represented a low-moderate intake in all groups and a similar GI was consumed by males and females. Unadjusted mean GL values represented a high intake with males consuming a significantly greater GL than females (P =0.002), however, when adjusted for energy intake per 1000kcal females consumed a greater GL compared to males but this was not significant. Mean intake values were similar to recommended intakes for the total group as a whole and males and females separately. Minimum fibre intake recommendations were slightly exceeded and total fat intake appears to be within the recommendations. Females consumed significantly greater %CHO (P =0.031) whilst males consumed significantly greater salt (<0.001) and fat intake including: %FAT (P= 0.041), %SFA (P= 0.005), %MUFA (P=0.043) as well as fibre/1000kcal (P= 0.042) than females.

Table 4. Comparison of dietary intake versus current recommendations

	RECOMMENDED INTAKE[a]	TOTAL (n=98)	Males (n=61)	Females (n=37)	*P* Value
GI	<55 Low 55-69 Mod >70 High	58.40	58.22 (4.94)	58.71 (4.23)	.619
GL (g)	<80 Low 81-119 Mod >120 High	166.20 (59.43)	180.44 (56.69)	142.71 (57.00)	.002
Adj GL (g) (GL/1000kcal)		77.37	75.36 (56.69)	80.69 (14.10)	.059
Energy (Kcal)	Males: 2964 [b] Females: 2110	2169.61	2423.60 (811.70)	1750.86 (584.90)	<.001
PROTEIN (g)	Males: 111 [b] Females: 79	87.21 (41.44)	100.13 (45.50)	65.91 (20.72)	<.001
CARBOHYDRATE (g)	Males: 370 [b] Females: 264	283.58 (103.97)	310.80 (101.2)	238.99 (93.74)	<.001
FAT (g)	Males: ≤ 115 [b] Females: ≤ 82	83.47 (36.70)	95.54 (37.15)	63.58 (26.00)	<.001
SATURATED FAT (g)	Males: ≤ 36 [b] Females: ≤ 26	29.99 (14.20)	34.95 (14.12)	21.83 (10.05)	<.001
MONOUNSATURATED FAT (g)	Males: 43 [b] Females: 30	27.08 (12.69)	31.14 (12.84)	20.40 (9.23)	<.001
POLYUNSATURATED FAT (g)	Males: 21 [b] Females: 15	12.48 (6.77)	13.75 (7.11)	10.39 (5.66)	.007
PROTEIN (% of total energy)	**15**	16.12	16.41 (3.78)	15.62 (3.96)	.242
CARBOHYDRATE (% of total energy)	**50**	49.76	48.62 (6.71)	51.63 (6.46)	.031
Sugar %		20.33 (7.77)	19.66 (7.81)	21.45 (7.69)	.221
FAT (% of total energy)	**≤ 35**	34.29	35.38 (7.20)	32.49 (5.76)	.041
SATURATED FAT (% of total energy)	**≤ 11**	12.31	12.99 (3.48)	11.18 (3.16)	.005
MONOUNSATURATED FAT (% of total energy)	**13**	11.07	11.49 (2.59)	10.37 (2.68)	.043
POLYUNSATURATED FAT (% of total energy)	**6.5**	5.17	5.10 (1.96)	5.28 (2.15)	.687
Fibre (g)	**11**	13.82	14.79 (6.77)	12.22 (5.39)	.050
Fibre (g/1000kcal)		6.49 (2.20)	6.14 (2.11)	7.07 (2.43)	.042
Salt (g)	6	7.69 (3.19)	8.80 (3.27)	5.87 (2.02)	<.001

Mean and standard deviation; *P* significant at <0.05 for comparison of males and females; [a], SACN (2008); [b], SACN (2011)

GI was significantly positively associated with BMI (r= .240, P= <0.05), but GL was not correlated with any adiposity variables. GI was significantly inversely associated with fibre (r = -.281, P=<0.05) whilst GL was significantly negatively associated with PRO (r= -.366, P= <0.01) and positively with %CHO (r= .878, P= <0.01). Additionally, %CHO had a significant negative correlation with both %fat (r= -.713, P= <0.01) and PRO (r= -.426, P= <0.01). GL was significantly negatively correlated with % fat (r= -.606, P= <0.01) and with proportions of SFA, MUFA and PUFA (P=<0.01) (table 5).

Table 5. Partial correlation coefficients for diet and adiposity variables.

N=72	GI	GL[a]	% CHO	% FAT	% SFA	% MUFA	% PUFA	% PRO	FIBRE[a]
BMI(kg/m^2)	0.240*	0.038	-0.014	-0.107	-0.137	-.037		0.042	0.181
BF%	0.218	0.128	0.095	-0.107	-0.132	-.042		-0.119	0.301*
WC (cm)	0.191	0.125	0.098	-0.149	-0.123	-.055		-0.116	0.288*
%CHO	0.101	0.878**							
%FAT	-0.037	-0.606**	-0.713**						
%SFA	-0.093	-0.431**	-0.556**	0.825**					
%MUFA	-0.053	-0.642**	-0.712**	0.761**	0.622**				
%PUFA	-0.170	-0.370**	-0.404*	0.434**	0.032	0.489			
%PRO	-0.041	-0.366**	-0.426**	0.045	-0.102	0.037	0.219		
FIBRE[a]	-0.281*	0.015	0.175	-0.219	-0.177	-0.202	-0.094	0.212	

Correlations adjusted for: sex, SES, age, MVPA; [a], Dietary variable per 1000kcal; *, P=<0.05; **, P=0.01

Table 6. Crude mean and standard deviation of adiposity variables across quantiles of GI and GL

N=72	GI				GL			
	1 (GI 54)	2 (GI 62)	F value	P value	1 (GL 66)	2 (GL 88)	F value	P value
BMI	23.16 (5.76)	24.50 (5.68)	7.26	.009	23.14 (5.15)	24.51 (6.24)	0.69	.410
BF%	22.45 (11.06)	23.96 (11.13)	5.88	.018	21.26 (10.01)	25.15 (11.81)	2.42	.125
WC	78.70 (10.67)	81.72 (14.07)	6.07	.017	79.49 (12.69)	80.93 (12.42)	0.985	.325

1 and 2 represent the lower and upper quantile, respectively; analysis adjusted for: sex, age, SES, %PRO, %FAT, %CHO, FIBRE/1000kcal, MVPA

As shown in table 6, the higher quintile of GI (62) vs lower (54) was associated with a significantly greater BMI, BF% and WC (P=0.009, 0.018 and 0.017, respectively). Although adiposity variables were higher in those grouped in the higher quintile of GL, none of these associations were significant.

Table 7. Energy expenditure, physical activity and fitness characteristics

	Total n=98	**Male** n=61	**Female** n=37	***P* value**
TEE (kcal) [a]	2474.48 (523.97)	2636.16 (511.79)	2231.96 (448.70)	<0.001
TotalPA[a]	718.37 (110.10)	712.15 (112.93)	728.82 (106.38)	0.529
SED T (min)[a]	517.82 (107.64)	508.65 (108.48)	533.22 (106.36)	0.342
LPA T[a]	135.77 (57.10)	134.49 (63.08)	137.92 (46.35)	0.804
MPA T[a]	50.90 (23.74)	53.24 (24.41)	46.98 (22.46)	0.273
VPA T[a]	14.67 (12.99)	16.45 (15.01)	11.68 (7.99)	0.212
MVPA T[a]	66.29 (35.87)	71.40 (39.48)	57.71 (27.37)	0.110
SED P[a]	72.16 (10.22)	71.56 (11.33)	73.17 (8.12)	0.513
LPA P[a]	18.63 (7.10)	18.61 (8.00)	18.68 (5.39)	0.966
MPA P[a]	7.12 (3.58)	7.46 (3.38)	6.55 (3.90)	0.363
VPA P[a]	2.11 (2.02)	2.42 (2.36)	1.60 (1.12)	0.149
MVPA P[a] **(>60 mins/day recommended**[b]**)**	9.11 (4.96)	9.69 (5.13)	8.15 (4.57)	0.456
% achieving 60 min MVPA [a]	54.7%	61.7%	42.9%	-
VO2 peak (ml/kg/min)	42.65 (13.0)	49.53 (10.18)	31.18 (8.19)	<0.001

Mean (standard deviations); [a], n= 75, combined; 47, males; 28, females; [b], (DOH, 2011); T, time in minutes; P, percentage; *P,* significant at <0.05 for comparison of males and females.

Table 7 displays PA and CRF characteristics; there were no significant differences between males and females for time spent in any PA category including SED time. Females spent a greater proportion of time SED and engaging in LPA whilst males spent more time in MPA and VPA, but these differences were not statistically significant. On average participants spent > 60 minutes engaging in MVPA (66.29 minutes; 54.7%), however, females only spent 57.71 minutes (42.9%) in MVPA whilst males engaged in 71.40 minutes (61.7%).

The average VO_2 peak for all participants was 42.65 ml/kg/min, in males this value was significantly greater when compared to females (49.53 ml/kg/min vs 31.18 ml/kg/min, respectively; P= <0.001).

Table 8. Partial correlation coefficients for PA and CRF variables with adiposity.

N=72	**SED**	**LPA**	**MPA**	**VPA**	**MVPA**	**CRF**
BMI	-0.209	-0.076	0.059	-0.203	-0.016	**-0.672****
BF%	-0.188	-0.052	0.148	-0.119	0.056	**-0.707****
WC	-0.198	-0.078	0.112	-0.188	-0.013	**-0.668****
LPA	**-0.319***					
MPA	**-0.438****	**0.671****				
VPA	-0.191	**0.295***	**0.453****			
MVPA	**-0.401****	**0.624****	**0.851****	**.683****		
CRF	0.057	0.203	-0.011	.103	-.023	

*, P =<0.05; **, P= <0.01; Correlation adjusted for: Sex, age, SES, %CHO, %PRO, %FAT and Fibre

Table 8, shows that time spent SED is significantly and inversely correlated with engagement in LPA (P= <0.05), MPA and MVPA (P=< 0.001). LPA, MPA, VPA and MVPA were all significantly and positively correlated with each other. Correlations show that no PA variables were significantly correlated with adiposity, however, CRF was significantly and negatively associated with all adiposity variables (P=<0.001).

Table 9. MANCOVA: Mean and standard deviation for adiposity variables across 50th percentiles of time spent in PA categories.

N=72	SED (mins)				LPA (mins)				MPA (mins)			
	1 (43.48)	**2** (598.86)	**F value**	**P value**	**1** (90.74mins)	**2** (178.28)	**f**	**P**	**1** (31.49)	**2** (68.37)	**F value**	**P value**
BMIz	0.84 (1.49)	0.87 (1.67)	0.63	.432	0.78 (1.46)	0.93 (1.69)	0.07	.789	0.83 (1.66)	0.87 (1.51)	1.33	.254
BF%z	1.51 (3.27)	1.95 (3.11)	0.56	.457	1.55 (3.05)	1.92 (3.34)	0.00	.969	1.67 (3.11)	1.79 (3.28)	2.17	.146
WC (cm)	81.75 (14.08)	81.04 (11.74)	0.65	.425	82.36 (13.08)	80.44 (12.78)	0.53	.470	81.19 (12.05)	81.60 (13.77)	0.77	.382

	VPA (mins)				MVPA (mins)				60 mins			
	1 (5.83)	**2** (23.12)	**F value**	**P**	**1** (46.23)	**2** (84.97)	**f**	**P**	**>60**	**<60**	**F value**	**P**
BMIz	1.04 (1.64)	0.67 (1.50)	0.42	.519	0.83 (1.68)	0.87 1.48)	1.64	.205	0.82 (1.52)	0.90 1.65)	1.21	.276
BF%z	1.92 (3.08)	1.54 (3.30)	0.05	.817	1.52 (3.06)	1.95 (3.32)	3.75	.057	1.82 (3.30)	1.64 (3.07)	3.19	.079
WC (cm)	81.80 (12.36)	80.99 (13.53)	0.31	.577	80.83 (11.74)	81.97 14.06)	1.10	.298	81.52 (13.88)	81.25 (11.79)	0.86	.357

1 and 2 represent the lower and upper quantile, respectively; analysis adjusted for: sex, age, ses, PRO, FAT, CHO, FIBRE, total PA and CRF.

MANCOVA revealed that adiposity variables were not significantly different across quintiles of PA (table 9).

Table 10. MANCOVA: Means and standard deviation for adiposity variables across VO_2 peak

N=72	VO2 PEAK				CRF risk			
	1 (31.48 ml/kg/min)	2 (53.00 ml/kg/min)	F value	P value	LOW RISK	HIGH RISK	F value	P value
BMI Zscore	1.95 (0.26)	-0.14 (0.25)	20.74	<.001	0.04 (0.21)	2.05 (0.25)	26.45	<.001
BF% Z score	3.86 (0.51)	-0.19 (0.49)	21.46	<.001	0.82 (0.39)	4.15 (0.48)	31.73	<.001
WC (cm)	1.95 (0.01)	1.85 (0.01)	17.81	<.001	1.87 (0.01)	1.96 (0.01)	26.93	<.001

1 and 2 represent the lower and upper quantile, respectively; analyses adjusted for: Sex, age, SES, PRO, FAT, CHO, FIBRE and MVPA.

Table 10 compares adiposity variables across quantiles of CRF; individuals in the higher compared to lower quintile of VO_2 peak had a significantly lower BMI, BF%

and WC (P= <0.001). When CRF was categorised in terms of health risk; high risk (VO_2 peak < 42 and < 37 ml/kg/min) and low risk (VO_2 peak > 42 and > 37 ml/kg/min) for males and females, respectively, those in the high risk category had a significantly greater BMI, BF% and WC (P= <0.001).

4.3 Discussion

The current study investigated the associations of dietary intake, PA level, and CRF with adiposity (BMI, BF% and WC) in a postpubertal adolescent population from Bedfordshire. The main findings of this investigation were that GI but not GL is significantly associated with adiposity (BMI, BF% and WC). Furthermore CRF but not PA was associated with adiposity when analyses were adjusted for macronutrient intakes. Additionally, this population of postpubertal adolescents from Bedfordshire appear to be consuming a similar proportion of macronutrients (CHO, 49.76; PRO, 16.12; fat, 34.29 % of energy) to the current recommendations (see table 4). MUFA and PUFA are also being consumed in line with government guidelines, % intake of SFA however are being slightly over consumed (by 1.31%) and salt intake appears to be slightly above recommended levels (by 1.69%). Males are consuming significantly greater proportions of energy as fat and less fibre/1000kcal compared to females. Partial correlations demonstrated that PRO and fat were significantly and negatively correlated with CHO. Furthermore males consumed significantly lower proportion of energy as CHO (P=0.031) and a higher proportion of PRO compared to females.

Although the proportions of macronutrients consumed were congruent with government guidelines (table 4), it appears that this sample of adolescents is under consuming energy compared to that of current guidelines (2500 kcal for males and 2000 kcal for females); however, these recommendations are specific to adults and may not be relevant to the younger adolescents in the current sample. Males and females consumed 2423.60 and 1750.86 kcal/day, respectively, which is comparable to findings from a study involving a large sample (n=2869) of UK adolescents (mean age 13.79 years) where males and females consumed 2155 ± 13.4 and 1784.85 ± 10.2, respectively, based on 3 day dietary records (Noel et al., 2010). In contrast, in a sample of Danish 16 year olds from the European Youth Heart Study, mean energy intakes of males and females were 3033.34 and 2149 kcal/day which is considerably higher than that of the current sample; however, this group are younger than those in the current sample and PA engagement was not

reported and thus cannot be compared. Furthermore, data were obtained by a combination of a 24 hr recall and qualitative food records (Nielsen et al., 2005). When considering a more specific UK national average energy intake for adolescents, according to SACN (2011), this discrepancy is more exaggerated; 2964 and 2110 kcal/day, respectively for males and females. It may not, however, be that this group as a whole are consuming less energy than they require as it is possible that some individuals may be underreporting their nutritional intake (Livingstone et al., 2004). Misreporting of energy intake can impact on associations of diet and health markers (Rosell et al., 2003) and therefore should be assessed and potentially controlled for in this population. As energy requirement is determined by PA and body mass, it makes comparison of energy intakes between different studies difficult and thus understanding misreporting, relevant to individual energy requirements, is important when comparing nutritional intakes across studies.

The dietary GI of this group represents a low-moderate value (58.40; moderate GI= 55-69) and this value is similar in both males and females. Unadjusted GL, however, represents a high intake and males consume a significantly greater GL than females (180.44 vs 142.71 g, respectively). After adjustment for total energy, GL/1000kcal becomes borderline-significantly higher in females compared to males (80.69 vs 75.36 g/1000kcal; P=0.059), this is likely due to the higher proportion of energy as CHO consumed by females (51.63%) compared to males (48.62%). These glycaemic CHO data for the group are very similar to that of a sample (n=16) of overweight males and female adolescents from the USA who consumed a mean GI of 58 and GL (g)/1000kcal of 79, based on 7 day food diary data (Ebbeling et al., 2003). In Danish 16 year olds, however, compared to the current sample, GI was 46% and 26% higher and GL was 71.8% and 103% higher, for males and females respectively, assessed by 24 hr recall. Different methods of dietary assessment can influence the assessment of GL (Nielsen et al., 2005), this may therefore explain the differences seen between Danish adolescents and the current sample of UK adolescents. Furthermore, this notion may support the similarities noted between adolescents from the USA (Ebbeling et al., 2003) and the current sample as both studies utilised multiple day food diaries. There appears to be limited investigation into the habitual dietary GI and GL levels of adolescents (Nielsen et al., 2005) and more so for UK adolescent populations. In comparison to the current study, a similar mean dietary GI (59.4 and 58.2, for males and females, respectively) was also identified in a large study (n=1275) utilising UK NDNS data of adults (+ 65 years),

suggesting dietary GI of UK older adults and adolescents may be similar. However, dissimilar GI values compared to the current sample of adolescents have been observed in adults when assessed by FFQ; in males from USA enrolled in the Health Professionals Study (Salmerón et al., 1997) and female adults (Michaud et al., 2002) from the Nurses' Health Study in the UK (GI 72.4 and 73.4), however GL values for male and female adults were similar to adolescents in the current investigation (GL 160 and 126.6, respectively). There appear to be contrasting findings in terms of habitual GI and GL intake amongst adolescents from the UK and Europe, however, apparent differences in dietary assessment method may help explain some of this discrepancy.

In the present study dietary GI was associated with adiposity; partial correlations revealed that GI was significantly and positively correlated with BMI (P=<0.05). The only other dietary variable to be associated with adiposity was fibre, which was significantly positively associated with BF% and WC (P= <0.05). The narrow range of dietary GI consumed by this group makes stratifying the sample into categories of low, moderate and high GI implausible, however, when split into quantiles, mean GI consumption of the two groups represented a low GI (54) and moderate GI (62). MANCOVA demonstrated that individuals in the moderate compared to low GI group have a significantly higher BMI (P=0.009), BF% (P=0.018) and WC (P= 0.017). GL, however, was not associated with adiposity in any analyses. In contrast to these findings, in Italian children (8 years old), BMI was not associated with GL or GI when assessed by FFQ (Scaglioni et al., 2005) and in German youngsters between the ages of 2 and 7 years GI and GL (measured by 3 day records) were not associated with BMI or BF% (Buyken et al., 2008). It is possible that the age difference of the current population is, in part, responsible for these contrasting associations; however, in a longitudinal study of pubertal adolescents from Germany, assessed over 4 years between the ages of 10.3 and 14.3 years old, changes in GI and GL (3 day diet records) were not associated with concurrent alterations in BMI or BF% (Cheng et al., 2009). In line with the current investigation, only a small number of studies have found positive association of GI with adiposity, Nielsen et al (2005) found that fatness assessed by skinfold analysis was significantly and positively associated with both GI and GL in 16 year old males. Association with females, however, were non-significant and it was postulated that the greater degree of reporting bias in females may be responsible for the lack of association (Nielsen et al., 2005), indeed, under reporting seems to increase as

children mature into adolescence (Bandini et al., 2003). This is supported by evidence from Japanese adolescents where higher GL was associated with greater relative risk of overweight (BMI >25 kg/m^2), but only in males (Murakami et al., 2011). It is possible that the apparent reporting bias observed in the current investigation may be responsible for the lack of association seen between GL and adiposity. Under-reporting is likely to result in underestimation of GL, whereas GI may not be affected due to the nature of its calculation (Nielsen et al., 2005). This highlights the importance of considering dietary misreporting when assessing diet and health relationships. It is clear that there is a lack of consistency in terms of dietary assessment method when investigating associations of GI and GL and thus it is difficult to make clear comparisons between these data.

Increased adiposity associated with higher dietary GI have been explained by evidence that low GI foods increase satiety and have been shown to result in less energy intake at subsequent meals (Ludwig., 2000); this has been evidenced in 37 normal and overweight children aged 9-12 yrs who achieved greater satiation and consumed less energy at later meals following a low compared to high GI breakfast (Warren et al., 2003). Indeed, low GI mixed meals have induced greater secretion of satiety hormones (gut peptide cholecystokinin) in male adults (Holt et al., 1992). The slower rates of digestion and thus absorption at the small intestine subsequent to low GI foods are likely to result in extended feedback via gut peptides, including ghrelin, glucagons and glucagons-like peptite-1 to the satiety centre of the brain whilst nutrients receptors are stimulated for a longer period of time compared to more rapidly digested and absorbed high GI foods (Radulian et al., 2009).

Higher fat oxidation has also been associated with low compared to high GI foods; significantly greater fat oxidation over a 3 hour rest period and during a 60 minute bout of moderate intensity exercise, was observed following consumption of a low vs high GI breakfast and lunch in sedentary adult females (Stevenson et al., 2009). Scazzina et al (2011) compared rates of fat oxidation and diet induced energy expenditure elicited from 3 isocaloric breakfasts: 1) high GI (HGI)-high GL (HGL); 2) HGI-LGL (LGI); 3) LGI-LGL in 16 young adult males (23 ± 2 yrs old) , significantly greater fat oxidation was observed following both the HGI-LGL and LGI-LGL compared to the HGI-HGL breakfast; interestingly the authors observed that the LGI-LGL breakfast consistently increased postprandial energy expenditure and fat utilisation compared to the other diets, furthermore this effect was observed even

after the following meal . Therefore, it appears that lowering the GI and GL of the meals induced greater fat utilisation and energy expenditure as compared to lower GL alone. According to Wolfe (1998) postprandial increases in glycaemia and insulinaemia, following high GI meals, results in activation of key rate-limiting enzymes that increase CHO oxidation. Furthermore, malonyl-CoA; an intermediate of CHO oxidation, strongly inhibits fatty acid transport into the mitochondria (Wolfe, 1998). More recently (Roberts et al., 2008) demonstrated that a high CHO diet, comparable to high GI and GL altered fatty acid partitioning towards esterification and away from oxidation. Additionally, the late postprandial hypoglycaemia following high GI foods and subsequent counter regulatory hormone response is associated with an increased propensity for protein metabolism in order to restore euglycaemia (Zurlo et al., 1990; Weyer et al., 2000). Thus, low GI eating has been associated with preserved lean mass compared to high GI (McMillan-Price and Brand-Miller, 2006); a greater BMR would increase energy expenditure and lower energy storing which is likely to have a favourable impact on adiposity (Scazzina et al., 2011). Therefore, it would appear, that in support of current findings, low GI foods increase rates of fat metabolism and postprandial energy expenditure and that reducing the GI of the diet may be more impactful.

Government guidelines for PA state that children and adolescents should achieve ≥60 minutes MVPA in order to maintain or improve health (DOH, 2012). The current investigation identified that 54.7% of the population achieved ≥60 minutes MVPA and, on average, participants achieved 66.29 ± 35.87 minutes of MVPA, 517.82 ± 107.64 minutes of SED and a total PA of 718.37 ±110.10 minutes. Males spent more time in MVPA (71.40 ± 39.48 minutes) than females who as a group did not meet the current recommendations (57.71 ± 27.37 minutes), differences, however, were non-significant. Differences in time spent in any PA category between males and females were non-significant, although females appear to spend more time SED and engaging in LPA where males spent more time in MPA and VPA . A younger sample of children (10-14 years old) also from Bedfordshire, UK (Bailey et al., 2012a), spent less time SED (451.91 ± 79.74) and more time in MVPA (109.24 ± 37.31) as compared to the current sample. The difference in age between Bedfordshire children (Bailey et al., 2012a) and adolescents in the current study is likely to, in part, be responsible for the difference in PA engagement and this is supported by evidence that PA engagement declines as children enter adolescence (Kimm et al., 2002). As Bailey et al (2012a) and the current study used the same

methods for assessing PA (7 day triaxial accelerometry), these comparisons can be made with a relatively good level of confidence. Further comparisons can be drawn from a large sample of pooled data from the International Children's Accelerometry Database (ICAD), where children and adolescents (4-18 years old) engaged, on average, in 835 minutes of total PA, 30 minutes MVPA and 354 minutes SED (Ekelund et al., 2012). In this international study males also engaged in greater MVPA (37 ± 23 vs 24 ± 17 mins) and spent less time SED (345 ± 96 vs 365 ± 96 mins) compared to females, respectively, however, unlike in the current study, in this large sample (n=20,871) differences were significant (P=0.001). It is important to note the difference between the cut-points used to define PA categories between the current study and that of (Ekelund et al., 2012), as this will undoubtedly result in dissimilarities between time spent in different PA categories. Ekelund et al (2012) utilised PA cut points of >3000 counts per minute (CPM) and < 100 CPM to define MVPA and time spent SED, respectively, compared to the current study which utilised cut points of >970 CPM and < 288 CPM, to define MVPA and SED, respectively. Indeed, a study of 104 Bedfordshire children (10-14 yrs old) identified markedly different times accumulated in PA categories when comparing 3 different published cut-points (Bailey et al., 2013). The authors also observed that the use of different cut points resulted in different associations of PA and metabolic health markers including BF%. This highlights the issues related to the lack of continuity between PA cut-points used to calculate PA in the literature.

In the current investigation PA parameters including MVPA and time spent SED were not associated with adiposity. However, in a previous study of 9-10 year old children, BMI, WC and fat mass were associated inversely with MVPA and MPA and VPA individually as assessed by a uniaxial actigraph accelerometer, and adjusted for total energy intake (Steele et al., 2009). In a similar study, which assessed an age range of 4-18 year olds, Eckelund et al (2012) identified that MVPA was inversely associated with WC in a pooled sample of 20,871 4-18 year olds. However these studies (Eckelund et al., 2012 and Steele et al., 2009) did not adjust for macronutrient intake. The present investigation, however, did control for macronutrient intake (fat, PRO, CHO and fibre) and therefore, adjustment for nutritional intake may have attenuated the associations of PA with adiposity.

In the present study CRF standardised for body weight was significantly higher in males than females (P=<0.001), mean CRF values for all participants and males

and females, respectively were very similar to that of 10-14 year old children from Bedfordshire (Bailey et al., 2012); all participants' CRF: 42.65 ± 13.0 vs 41.58 ± 9.38, males: 49.53 ± 10.18 vs 45.96 ± 8.21 and females: 31.18 ± 8.19 vs 38.54 ± 8.98 ml/kg/min, respectively.

In the current sample of postpubertal adolescents, partial correlations revealed that CRF was significantly inversely correlated with BMI, BF% and WC (Table 8). Furthermore, MANCOVA revealed that BMI, BF% and WC were significantly higher (P= <0.001) in individuals in the lower vs higher quintile of VO_2 peak (31.48 vs 53.00 ml/kg/min) and in those grouped into the high risk (< 42 and < 37 ml/kg/min) compared to low risk (> 42 and > 37 ml/kg/min, for males and females, respectively) CRF group. Similarly, Bailey et al (2012) showed that WC was significantly higher when children were stratified as unfit compared to fit (assessed by maximal cycle ergometer test) but no association was observed when grouped in terms of PA engagement (measured by RT3 triaxial accelerometer) in 10-14 year olds from Bedfordshire. In further support of these findings, in a similar age group to the present study, Ortega et al (2007), observed inverse associations for CRF with BMI and WC independent of SED activities and leisure time physical activity (CRF assessed by 20 metre shuttle run) in Spanish adolescents (13-18.5 years old). The current findings and supporting research suggest that CRF is more strongly associated with adiposity than PA.

It is possible that the lack of relationship between PA and adiposity may be explained by limitations surrounding the use of accelerometry; the sporadic nature of PA in youngsters (Baquet et al., 2007) may mean that the 1 minute epochs utilised in the current study may not capture some higher intensity activities, however shorter sampling rates were not possible within the remit of the equipment available. Furthermore, accelerometry is limited in its ability to capture certain activities, for instance, it may not accurately measure upper body movements, cycling or activities on an incline and cannot be used to assess water based activities. Additionally, PA categories were assigned based on the cut-points of Rowlands, which have been validated in children and adults (Rowlands et al., 2004a). However, there are inconsistencies between cut-points used in the literature and thus the most representative PA cut-points for youths are not fully understood (Ridgers and Fairclough, 2011) making comparisons between studies difficult. Another limitation is the study's cross-sectional design, and thus, the direction of

causality cannot be elucidated, however, future work will assess the impact of interventions on health markers in this group.

Strengths of this study include the direct measurement of oxygen uptake during a maximal cycle ergometer test to assess CRF and the use of weighed food diaries to measure nutritional intake. Furthermore, the use of a postpubertal adolescent population means the impact of hormonal changes during puberty are likely to be minimised.

In conclusion, GI but not GL is associated with adiposity in this group of post-pubertal adolescents from Bedfordshire. When adjusting for dietary intake, CRF was also associated with adiposity but PA was not. These data suggest that recommendations for this age group should target improvements in CRF and a lower dietary GI to reduce adiposity. This is of particular importance in light of evidence that the metabolic abnormalities associated with increased adiposity (Despres and Lemieux, 2006) have been shown to track from adolescents into adulthood (Camhi and Katzmarzyk, 2010). It appears that this group are consuming below that of recommended energy intakes. However, it is quite possible that a proportion of this group are misreporting their nutritional intake (de Vries et al., 1994, Posluna et al., 2009) which may distort associations of GI, GL and adiposity. Thus future research should aim to account for dietary reporting bias when assessing the associations of GI and GL with health markers in this population.

Chapter Five: Study Two

The use of objective physical activity monitoring to assess the accuracy of recorded energy intake in adolescents.

5.0 Introduction

Measurement of nutritional intake is an important tool that can be used to guide an individual through a weight loss programme or to assess the relationship between dietary and health parameters during a dietary intervention (Anderssen et al., 2007a) or observational study (Murakami et al., 2013). However, accurately measuring dietary intake is not a straight forward process as currently the only time and cost effective method for assessment is self report. Errors in self reporting of nutritional intakes can lead to inaccurate assessment of dietary intakes, either through failure to recall all foods consumed or by underestimating the amount eaten, known as misreporting. Dietary misreporting is defined as a deviation between reported EI and measured EE when body weight is assumed constant during the assessment period (Poslusna et al., 2009). Population characteristics, such as age, sex and adiposity, have been shown to affect the degree of misreporting (Collins et al., 2010, Murakami et al., 2012) . Ascertaining accurate intakes in overweight and obese can be more challenging than in normal weight individuals, as under reporting generally increases with BMI (Poslusna et al., 2009); according to Subar et al (2003) and Mahir et al (2006) BMI was a significant predictor of underreporting in adults. Additionally, studies have identified that a higher proportion of females and older participants underreport nutritional intakes (Hirvonen et al., 1997; de Vries et al., 1994). Furthermore, there is evidence that under reporting increases with age, from childhood into adolescence (de Vries et al., 1994). This is of particular importance as evidence suggests that misreporting can cause spurious associations between diet and health outcomes; in 301 65 yr old men, Rosell et al (2003), observed that associations of PUFA, Ω 3 fatty acids and fats from milk with fasting insulin concentrations were significantly stronger in under-reporters compared to non under-reporters. Furthermore, although under-reporters had higher food and nutrient densities, suggesting they followed a more healthy diet compared to non under-reporters, the prevalence of the metabolic syndrome was significantly higher in under-reporters (18 vs 9 %; P= 0.029) compared to non

under-reporters. Therefore it appears that misreporting has important implications for studies investigating diet-health relationships (Poslusna et al., 2009).

Traditionally, biomarkers were utilised to assess the validity of self reported nutritional intakes; namely 24 hour urinary nitrogen excretions to assess validity of protein intakes (Isaksson, 1980) and measurement of EE by DLW (Scholler and Van Santen, 1982). Based on the principle that in weight stable individuals EI and EE are equal (Rosenbaum et al., 1996) the comparison of reported EI with EE (DLW) allows for bias in reported intakes to be assessed. This work was furthered by (Goldberg et al., 1991) who assessed reported EI by comparison with estimated ER. The Goldberg et al (1991) equation calculates the confidence limit or cut-off of the sum of EI:BMR ratio below which it is unlikely that mean EI is representative of habitual intake or a random low intake. This technique assumes a PA level (PAL value), relative to BMR, for the population and accounts for the within-subject variation in estimated EI, the variation of the precision of BMR estimates, the total variation in PAL and the sample size being assessed (see literature review, section 2.7, Dietary Misreporting, for more detail). The Goldberg equation has become a useful tool for the assessment of underreporting (Black et al., 1976), and is widely used in more recent research (Vågstrand et al., 2009, Lanctot et al., 2008, Lillegaard et al., 2007). Early studies utilised a PAL value of 1.55 x BMR, based on the value for 'light activity' defined by the WHO (1985) and is a likely minimum ER for sedentary but healthy individuals (Goldberg et al., 1991). If EE is unknown and PAL is therefore assumed, only the lower confidence limit can be calculated and reported EI is classified as 'low' or 'non-low'. If the PA of a population is known an appropriate PAL value can be assigned and the upper confidence limits can be calculated; in these circumstances over-reporters can be identified (Black, 2000). For the purpose of this thesis dietary reporting will be defined as under, valid or over reporting. Studies have utilised the Goldberg equation to assess reporting at the individual level and thus the characteristics of 'under-reporters' has been assessed (Pryer et al., 1997; Rotishauser et al., 1994). However these studies presumed a 1.55 PAL value and would only be able to accurately identify bias in reported food intakes of those leading a low active lifestyle (Black et al., 1997; Black, 2000). Moreover, the degree of misreporting has likely been underestimated in those with a higher PAL value than the traditional 1.55 x BMR. As many dietary surveys include participants with a broad range of EE, a PAL value should be estimated for stratified sub-groups of the population based on physical activity habits, or to be more

specific, an individual PAL value can be applied when calculating a cut-off for EI:BMI. The Goldberg equation was shown to be a good predictor of misreporting compared to directly assessed EE by DLW (r =0.65, P= <0.0001), but only when information regarding PA was known, moreover, when a specific PAL was calculated from directly assessed energy EE (DLW) data the validity of the Goldberg equation improved further (Black et al., 1997). This highlights the importance of PA information and that if an individual PAL can be ascertained then misreporting can be quantified with greater validity. Physical activity data can be gained via questionnaire and can be used to assign a PA sub-group (e.g. sedentary, low active, moderately active or highly active) or individual PAL. Self reported PA, however, can be prone to reporting error (Sirard and Pate, 2001: Trost, 2007) and may lack the accuracy required to gain valid insight into an individual's PA habits. Therefore, more recently, accelerometry has allowed for a more objective assessment of PA that can be applied to the assessment of misreporting by estimating an appropriate PAL based on time spent in different PA subcategories, namely MVPA, however, it seems very few studies have utilised accelerometry in this manner (Samuel-Hodge et al., 2004; Noel et al., 2010). Although, when a more specific sub-grouping of PAL (using accelerometry) is applied to the calculation of the Goldberg cut-offs, it is likely that PAL will be under estimated in some individuals, particularly those who engage in more LPA and less MVPA being that MVPA is used to assign PAL sub-groups (Noel et al., 2010)

If individual EE is known it can be directly compared with EI; and the ratio of EI:EE will therefore represent the degree of reporting bias (Rosenbaum et al., 1996). The expensive and time consuming nature of DLW as well as the requirement for a sophisticated laboratory set up means that it cannot be routinely used to measure EE and validate EI (Poslusna et al., 2009). Although accelerometry has been used to assign a PAL (based on time accumulated in MVPA) when estimating the Goldberg cut-off, very few studies have directly compared accelerometry obtained EE with EI for the assessment of misreporting. Underreporting has been assessed by direct comparison of EI and EE (assessed by 3 x 24 hour recalls and uniaxial accelerometry, respectively) and compared to EI:BMI by calculating a participant specific PAL for use in the Goldberg cut-off (Samuel-Hodge et al., 2004). Participants were classified as under-reporters if the ratio of EI:EE was <0.79 (where EI:EE should equal 1 in weight stable individuals); representing the lower 95% confidence limit of EI:EE, based on studies utilising DLW data (Black and Cole,

2001). According to EI:BMR, 58% of the population underreported compared to 81% as assessed by EI:EE. This suggests that using EE data from accelerometry compared directly to EI yields a greater proportion of underreporters than the Goldberg cut-off utilising an individual PAL derived from the same EE data. However, Samuel-Hodge et al's (2004) study employed uniaxial accelerometry which is likely to underestimate the measurement of activities with limited vertical accelerations (Butte et al., 2012). Moreover, accelerometer data were included if 4 days consisting of >4 hours wear time per day was achieved. A low wear time criteria such as a 4 hour minimum could result in an underestimation of PA, which in turn would result in underestimation of energy underreporting (Samuel-Hodge et al., 2004). Very few studies have used accelerometry to assess misreporting in adolescents, however, Noel et al (2010) used accelerometry to assign individual PAL values and compared three variants of PAL estimations in 13 year olds from the UK: 1) assigned a low-active PAL (1.13 boys 1.16 girls); 2) assigned PAL values from EE(actigraph)/BMR (estimated from Schofield equation); 3) assigned PAL from total minutes of MVPA (based on PAL & PA coefficients (Institute-of-Medicine, 2005)). Both variant 1 and 2 identify similar proportions of misreporters (under reporters: 51.5 vs 51.8%; valid reporters: 40.8 vs 37.9%, respectively; over reporters: 7.7 vs 10.3%), whereas variant 3 performed quite differently, identifying less under reporters and more over reporters (under reporters: 37.1%, valid reporters 42.4% and over reporters: 20.4%). It was proposed that calculating PAL from MVPA will not account for LPA and therefore may assign those who do not engage in MVPA as sedentary and thus under estimate there ER (Noel et al., 2010). Furthermore, a biaxial accelerometer (MTI actigraph) was utilised alongside the Goldberg equation to assess misreporting in African-American female adolescents (Lanctot et al., 2008), where 58.4% were classified as under reporters and 45.3 as valid reporters. In these studies under reporting was significantly and positively associated with BMI, WC and BF% (Noel et al., 2010) as well as unhealthy eating behaviours and older age (P= <0.001) (Lanctote et al., 2008).

To date it appears that only uniaxial and biaxial accelerometry has been used to assign a PAL in the assessment of dietary misreporting in adults (Samuel-Hodge et al., 2004) and adolescents (Noel et al., 2010; Lanctote et al., 2008). The triaxial RT3 accelerometer, which has been validated for use in children and adolescents against oxygen uptake (Chu et al., 2007; Vanhelst et al., 2010; Rowlands et al., 2004), has seemingly not yet been utilised to assess misreporting. Furthermore,

there appears to be a distinct lack of research assessing EI:EE using triaxial accelerometry in any population. The use of accelerometry to derive EE may be more appropriate than using accelerometry to assign a subject specific PAL in conjunction with the Goldberg equation as it does not require calculation of MVPA. Furthermore, accelerometry provides a more cost effective alternative to DLW in the assessment of EE.

Therefore, the purpose of the current investigation is to compare established dietary misreporting techniques: 1) the Goldberg equation incorporating a low activity PAL and 2), the Goldberg equation utilising the application of triaxial accelerometry to assign a specific PAL, to 3), a novel approach utilising EI:EE, based on the direct assessment of EE by triaxial accelerometry. Additionally this investigation will explore the degree of adiposity between misreporters and valid reporters using the different assessment techniques.

5.1 Methodology

Participants

Participants were adolescents recruited as part of the SIRENS and CROSSROADS studies as previously explained in (section 3.0). A total of 72 participants (45 males and 27 females) provided adequate diet and PA data.

Experimental Design

As part of the CROSSROADS and SIRENS studies PA and dietary data were assessed; food diaries and PA monitors were distributed to the participants during data collection (see sections 3.9 and 3.10) in schools and colleges around Bedford and at the COR of the Luton and Dunstable Hospital. The reporting bias of dietary intakes was assessed by three different techniques.

Measurements

Age

Each participant had their age assessed as previously described.

Anthropometry and Body Composition

Stature, body mass, BMI, body fat %, fat free mass and waist circumference were assessed as previously described in section 3.5 and 3.6.

Dietary Intake

Participants were given 3 day weighed food diaries to complete within 1 week of data collectionas outlined in section 3.10. Intakes of energy, nutrients and GI and GL were averaged over the 3 day recording period as described in section 3.10.

Physical Activity

Information on physical activity engagement was required for the calculation of dietary misreporting. The rt3 traxial accelerometer was used to monitor PA and energy expenditure of 7 consecutive days. See section 3.9 for PA monitoring protocol. Time spent in PA subcategories was determined using the thresholds of Rowlands et al., (2004), as previously explained in section 3.9.

Basal Metabolic Rate (BMR)

The schofield equation (Schofield, 1985) for estimating basal energy requirement in 10-19 year olds was used to estimate BMR for each individual base on sex, weight and height and is as follows:

Males: BMR = 0.068 x weight (kg) + 0.574 x height (m) + 2.157

Females: BMR = 0.035 x weight (kg) + 1.948 x height (m) +0.837

Dietary misreporting

The following three methods for assessing dietary misreporting (one, a novel approach) were compared to identify how they performed when classifying reporting bias of adolescents.

The Goldberg et al (1991) equation calculates the confidence limit or cut-off of the EI:BMR ratio below which it is unlikely that mean EI is representative of habitual intake or a random low intake. The ratio of energy expenditure (EE) to BMR (EE:BMR) equals the energy utilised in addition to BMR known as the physical activity level (PAL) and because, in weight stable individuals, EI should equal EE, the ratio of energy intake (EI) to BMR (EI:BMR) should also be equal to PAL (Black, 2000). The equation requires information on physical activity to assign a relevant PAL value or a PAL value can be assumed. The equation accounts for the within-subject variation in estimated EI, the variation of the precision of BMR estimates, the total variation in PAL and the sample size being assessed and is as follows.

To calculate the lower 95% confidence limit (CL) at the individual level below which EI:BMR represents under-reporting:

PAL x exponent [SD_{min} x $\frac{(S/100)}{\sqrt{n}}$]

To calculate the upper 95% CL at the individual level above which EI:BMR represents over-reporting (the upper limit can only be calculated when PAL is estimated rather than assumed for a population or individual):

PAL x exponent [SD_{max} x $\frac{(S/100)}{\sqrt{n}}$] (Black., 2000)

Here, PAL is the mean PAL for the population being examined and SD_{min} is -2 for the 95% lower CL and SD_{max} equals +2 for the upper 95% CL and n (1) is the sample size of the study.

$$S = \sqrt{(CV^2wEI/d) + CV^2wB + CV^2tP}$$

Where, as stated by Black (2002), **CVwEI** is the within subject coefficient of variation (CV) in reported energy intake (23), ***d*** is the number of days of dietary assessment (3) **CVwB** is the CV of BMR estimates (8.5) and **CVtP** is the total variation (within and between subject variation) in PAL.

Method 1) The Goldberg equation - ratio of energy intake to basal metabolic rate **EI:BMR (1.55 PAL)**

This original version of the technique assumes a PAL value, relative to BMR, for the population of 1.55 x BMR, based on the value for 'light activity' defined by the WHO (1985).

Method 2) The Goldberg equation - ratio of energy intake to basal metabolic rate **EI:BMR (MVPA PAL)**

This variant of the Goldberg equation used the information gathered by accelerometry to assigns individual participants a PAL which is determined based on the subcategory of time spent in MVPA that they fall into. This method of assigning PAL values was also used in a large study of UK adolescents (Noel et al., 2010). These categories were based on the dietary reference intakes (DRIs) from

the institute of Medicine description of PAL and PA coefficients which state that engaging in an additional 30 minutes of MPA raises an individual from the 'sedentary' to the 'low-active PAL' where as an additional 60 minutes of MPA would raise them into the 'active PAL category' (Institute-of-Medicine, 2005). Therefore, in the present study, time spent in MVPA was calculated and categorised as follows (table 11):

Table11. PAL categories and respective value based on time spent in MVPA

PAL Category	Minutes in MVA	PAL Value
Sedentary	< 30 mins	1.25
Low active	30 to <60 mins	1.5
Moderately active	60 to120 mins	1.75
Very active	≥60 mins of VPA and/or >120 mins of MPA	2.2

Method 3) RT3 accelerometer assessed energy expenditure and the ratio of energy intake to energy expenditure **(EI:EE)**.

This method used the direct comparison of EI and EE (EI:EE) to assess the bias in reported intakes. Based on this technique the expected ratio of EI:EE should equal 1. The 95% CLs of the ratio of EI:EE when EE was measured by the reference method DLW were reported to be 0.21 above and below 1 (Black and Cole, 2001, Black et al., 1997). Therefore under- and over-reporting were defined as a EI:EE of <0.79 and > 1.21, respectively.

Energy expenditure was estimated using the RT3 accelerometer using a novel approach. The accelerometer was worn based on the requirements of the study protocol as outlined earlier in this section. However, the accelerometry data was analysed differently as compared to the procedures previously outlined for calculating time spent in PA intensities (see section 3.9). Of the days where acceptable accelerometer wear time was achieved (as outlined previously), the energy expenditure for each minute over a 24 hour period (between 00.00 to 00.00 GMT) as calculated by the RT3 software, was summed giving a daily EE. The average of all days assessed was used as the total daily EE. The minute by minute energy expenditure is calculated based on energy expenditure equations utilising information on sex, height and weight and the metabolic equivalents in kilocalories

for CPM registered by the device. Average metabolic rate (AMR) was assigned by the software for each minute when no activity was registered by the device, AMR represents BMR x a factor of 1.1 to account for the thermic effect of food (Erceg., 2012, director of stayhealthy inc. CA, personal communication) which is assumed to be 10%.

Statistical Analysis

Analyses were completed using the Statistical Package for Social Sciences (SPSS Inc., IL.), descriptive statistics are presented as mean and standard deviation (SD). The following variables were non-normally distributed and were therefore log transformed to normalise their distribution (BMI, BF%, WC, energy intake, % of energy from fat, PUFA and SFA). Differences between sexes of participant characteristics were assessed by one-way ANOVA. One way ANOVA was also employed to test the difference between anthropometric, adiposity and nutritional intake variables for each category of misreporting (under, valid and over-report) as assessed by the following misreporting assessment techniques: EI:BMR (1.55 PAL), EI:BMR (MVPA PAL) and EI:EE (RT3). The significance level for this analysis was set at P <0.05 for all analysis.

5.2 Results

Table 12. Anthropometric and dietary reporting characteristics

	Combined n=72	**Male** n=45	**Female** n=27	**P**
Age (y)	17.35 (1.49)	14.48 (1.27)	17.34 (1.50)	.290
Height (cm)	172.05 (9.77)	177.68 (6.79)	162.99 (6.44)	**.000**
Weight (kg)	72.77 (16.97)	71.81 (16.189)	74.31 (18.36)	.544
BMI (kg/m^2)	24.49 (6.03)	22.71 (4.79)	27.34 (6.78)	**.001**
EI (Kcal)	2181.22 (829.74)	2417.91 (854.54)	1800.83 (634.39)	**.002**
EE (Kcal)	2515.45 (519.10)	2652.41 (520.84)	2295.33 (441.55)	**.004**
EI:EE	0.88 (0.31)	0.93 (0.32)	0.80 (0.27)	.087
BMR (Kcal)	1792.57 (285.24)	1925.14 (266.45)	1579.50 (157.80)	**.000**
EI:BMR	1.22 (0.43)	1.27 (0.45)	1.15 (0.40)	.246
EE:BMR (PAL)	1.39 (0.17)	1.37 (0.16)	1.41 (0.18)	.092

Means and standard deviation; P significant at <0.05 for comparison of males and females

Table 12 shows that females had a significantly greater BMI than males (P=0.001). Furthermore, EI (P=0.002), EE (P=0.004) and predicted BMR (P=<0.001) were significantly lower in females than males.

Table 13. Cut-points defining misreporting of energy intakes.

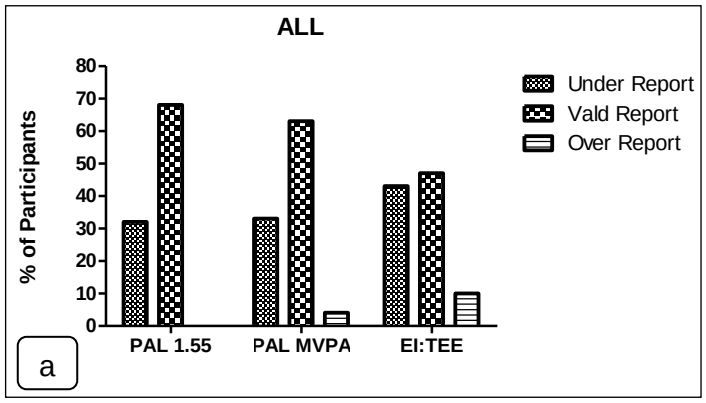
Method	Cut-off value
Goldberg: EI:BMR (1.55 PAL)	1.47 (group level) 1.00 (individual level)
Goldberg: EI:BMR (MVPA PAL) (individual level)	<30 mins MVPA = 0.81 >30 mins MVPA = 0.97 >60 mins MVPA =1.13 >120 mins MVPA or >60 mins VPA = 1.42
EI:EE	0.79

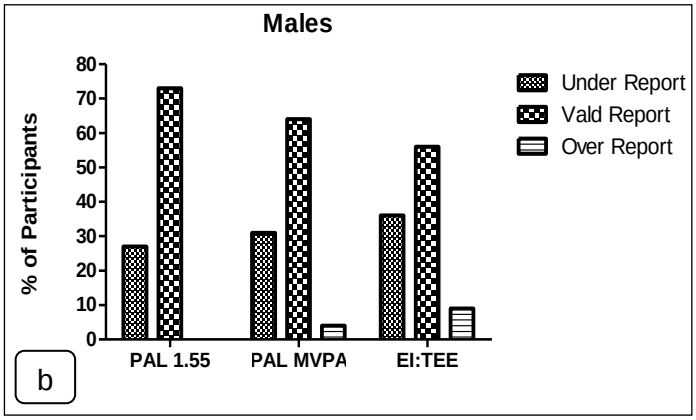
Table 13, Shows the calculated cut-offs for both variants of the Goldberg equation and EI:EE. Cut-offs are for the group (PAL1.55) and individual participants (PAL 1.55, MVPA PAL and EI:EE). MVPA PAL has specific cut-offs for individuals in respective categories of MVPA and VPA.

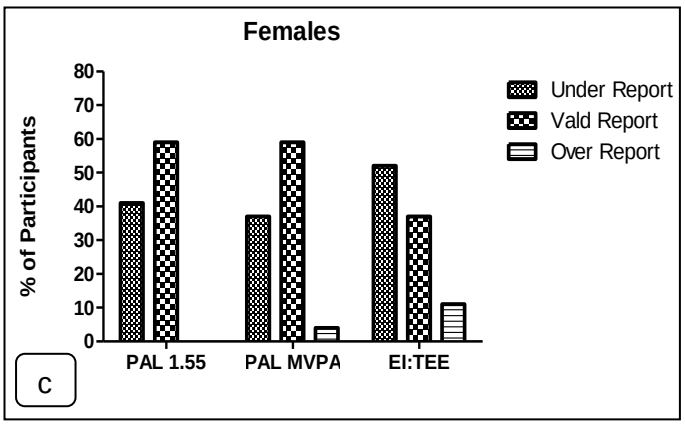
Table 14. Frequency of misreporters across sex and weight status

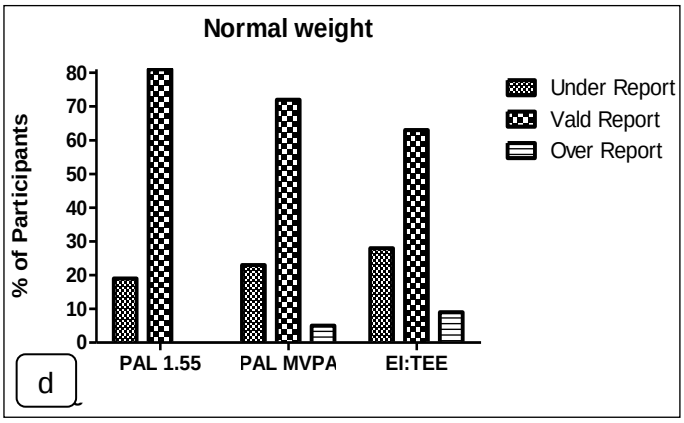
n=72	EI:BMR (PAL 1.55)		EI:BMR (MVPA PAL)			EI:EE		
	UNDER	**VALID**	**UNDER**	**VALID**	**OVER**	**UNDER**	**VALID**	**OVER**
Total (n) **(%)**	23 (31.9)	49 (68.1)	24 (33.3)	45 (62.5)	3 (4.2)	31 (42.5)	35 (47.0)	7 (9.6)
Males (n)	12	33	14	29	2	16	25	4
% of males	26.7 %	73.3 %	31.1 %	64.4 %	4.4 %	35.6 %	55.6 %	8.9 %
Females (n)	11	16	10	16	1	14	10	3
% of females	40.7 %	59.3 %	37 %	59.3 %	3.7 %	51.9 %	37 %	11.1 %
Normal weight	8	35	10	31	2	12	27	4
% of normal weight	18.6 %	81.4 %	23.3 %	72.1 %	4.7 %	27.9 %	62.8 %	9.3 %
Over weight	15	14	14	14	1	18	8	3
% of over weight	51.7 %	48.3%	48.3 %	48.3%	3.4%	62.1	27.6 %	10.3 %

Prevalence of participants within each reporting category for each dietary reporting assessment method









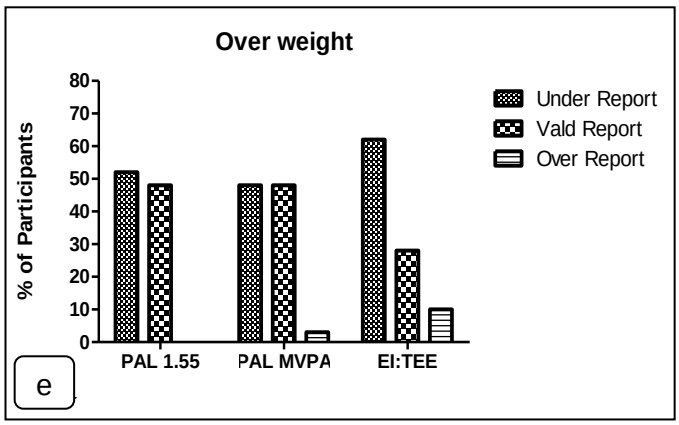


Figure 2. Bar chart showing % of reporters in each reporting category by method of misreport assessment for all (a), male (b), female (c), normal weight (d) and (e), overweight participants.

Figure 2 shows that both of the EI:BMI techniques (PAL 1.55 and PAL MVPA) identified a very similar proportion of under reporters (approximately 30%), whereas EI:EE identified the greatest proportion of the sample as under-reporters (42%) when assessed as an entire group. In males (figure 2a), under reporting was also prevalent in approximately 30 % of the population, however EI:EE identified the greatest proportion of under reporters (35.6%). A greater proportion of females (figure 2b) under reported compared to males, the Goldberg equation identified roughly 40% where EI:EE identified 51.9% of females as under reporters. Normal weight individuals (figure 2c) appeared to under report the least, with only 18.7%, 23.3% and 27.9% underreporting based on PAL 1.55, MVPA PAL and EI:EE respectively. Overweight individuals under reported the most, with the Goldberg equation identifying approximately 50% and EI:EE 62.1% of the group as under reporters (figure 2d). In all cases, EI:EE identified the greatest proportion under and over reporters.

As displayed in table 15, under reporters were significantly heavier, and had a significantly greater WC then valid reporters when assessed by either technique (P=<0.007), when assessed by EI:EE and PAL1.55. Under reporters had a significantly greater BMI (P= 0.014 and 0.004, respectively) and BF% (P=0.009 and 0.007, respectively). Across reporting categories, BMI was not significantly different when assessed by MVPA PAL.

Table 15. Characteristics of misreporters and valid reporters according to EI:BMR (1.55 PAL and MVPA PAL) and EI:EE.

	PAL 1.55			MVPA PAL				EI:TEE			
	UNDER n=23	**VALID n= 49**	***P***	**UNDER n=24**	**VALID n=45**	**OVER n=3**	***P***	**UNDER n=31**	**VALID n=35**	**OVER n=7**	***P***
Age (yrs)	17.01 (1.90)	17.49 (1.28)	0.206	16.91 (1.81)	17.58 (1.28)	17.36 (1.89)	0.206	17.22 (1.80)	17.36 (1.24)	17.69 (1.38)	0.745
Height (cm)	170.75 (10.90)	172.81 (9.29)	0.409	172.88 (11.49)	171.34 (9.39)	178.40 (10.03)	0.443	172.11 (10.58)	172.36 (9.25)	171.29 (9.96)	0.966
Weight (cm)	81.80 (18.86)	68.95 (14.37)	0.003	81.43 (18.37)***†**	68.25 (14.33)	78.17 (17.64)	0.006	80.52 (17.43)***†**	67.21 (14.95)	70.33 (15.55)	0.003
BMI (kg/m^2)	27.60 (7.28)	23.14 (4.82)	0.004	26.80 (7.04)	23.34 (5.06)	24.93 (7.64)	0.081	26.89 (6.65)***†**	22.67 (4.99)	24.07 (5.57)	0.014
BF %	29.92 (12.69)	21.26 (9.93)	0.007	28.30 (11.68)	21.85 (10.66)	22.53 (17.58)	0.081	28.80 (11.52)***†**	20.35 (10.10)	21.96 (11.99)	0.009
FFM (kg)	52.56 (10.77)	52.79 (10.27	0.930	53.93 (11.11)	51.68 (10.35)	58.53 (4.99)	0.428	53.60 (10.18)	51.73 (10.94)	53.89 (9.34)	0.736
WC (cm)	88.01 (15.73)	78.43 (10.63	0.004	86.83 (15.00)***†**	78.62 (11.29)	81.85 (12.97)	0.043	86.81 (14.42) ***†**	77.45 (11.27)	78.49 (11.00)	0.008

Means (± SD); *, P=<0.05; †, significantly different from valid reporters

.

Table 16 shows that energy intake was significantly greater in over and valid reporters compared to under reporters. When looking at macronutrients adjusted for total energy intake there were no significant differences between variables across reporting categories. However, although this was not the case with PAL1.55, when observing nutrients across reporting categories (under through to over-reporting) defined by EI:BMR and EI:TEE there appeared to be a reduced CHO% and a decreasing GI and GL/1000kcal, but none of these differences were significant.

Table 16. Dietary characteristics of misreporters and valid reporters according to EI:BMR and EI:EE

	PAL 1.55			MVPA PAL				EI:TEE			
	UNDER n=23	**VALID n= 49**	***P***	**UNDER n=24**	**VALID n=45**	**OVER n=3**	***P***	**UNDER n=31**	**VALID n=35**	**OVER n=7**	***P***
Energy (kcal)	1523.76 (387.61)	2512 (786. 24)	<0.001	1594.43 (452.91)** †‡	2350.58 (536.04)** ‡	4709.91 (1050.62)**†	<0.001	1664.79 (444.25)**†‡	2328.78 (446.94)**‡	3817.31 (1186.72)**†	<0.001
CHO (% energy)	49.73 (7.59)	50.23 (6.53)	0.774	50.17 (7.59)	50.16 (6.53)	47.97 (3.06)	0.864	50.46 (6.72)	49.96 (6.79)	48.99 (7.78)	0.870
FAT (% energy)	35.21 (9.89)	35.58 (5.88)	0.533	34.48 (9.89)	33.83 (5.88)	35.15 (4.43)	0.927	34.23 (8.76)	34.00 (6.55)	34.08 (3.23)	0.974
PRO (% energy)	16.83 (4.25)	15.90 (3.76)	0.476	16.93 (4.25)	15.79 (3.76)	16.39 (4.02)	0.501	16.57 (4.35)	15.82 (3.11)	16.45 (6.05)	0.853
SFA (% energy)	12.82 (4.96)	11.80 (2.68)	0.475	12.47 (4.96)	11.87 (2.68)	13.17 (1.39)	0.747	11.99 (4.55)	12.25 (2.82)	12.09 (1.97)	0.774
GI	59.04 (4.77)	58.70 (4.27)	0.754	59.08 (4.77)	58.64 (4.27)	55.30 (4.27)	0.342	59.15 (4.79)	59.00 (3.67)	56.41 (3.73)	0.291
GL g/1000kcal	77.65 (13.79)	78.61 (13.21)	0.775	78.60 (13.79)	78.66 (13.21)	70.66 (6.45)	0.590	78.27 (13.20)	79.29 (13.15)	73.51 (11.79)	0.570

Means (± SD). *, P=<0.05; **, P=<0.0001; †, significantly different from valid reporters; ‡, significantly different from over reporters

Scatter plot of EI:BMR (-95% CI) with EI:EE (±95% CI) by sex.

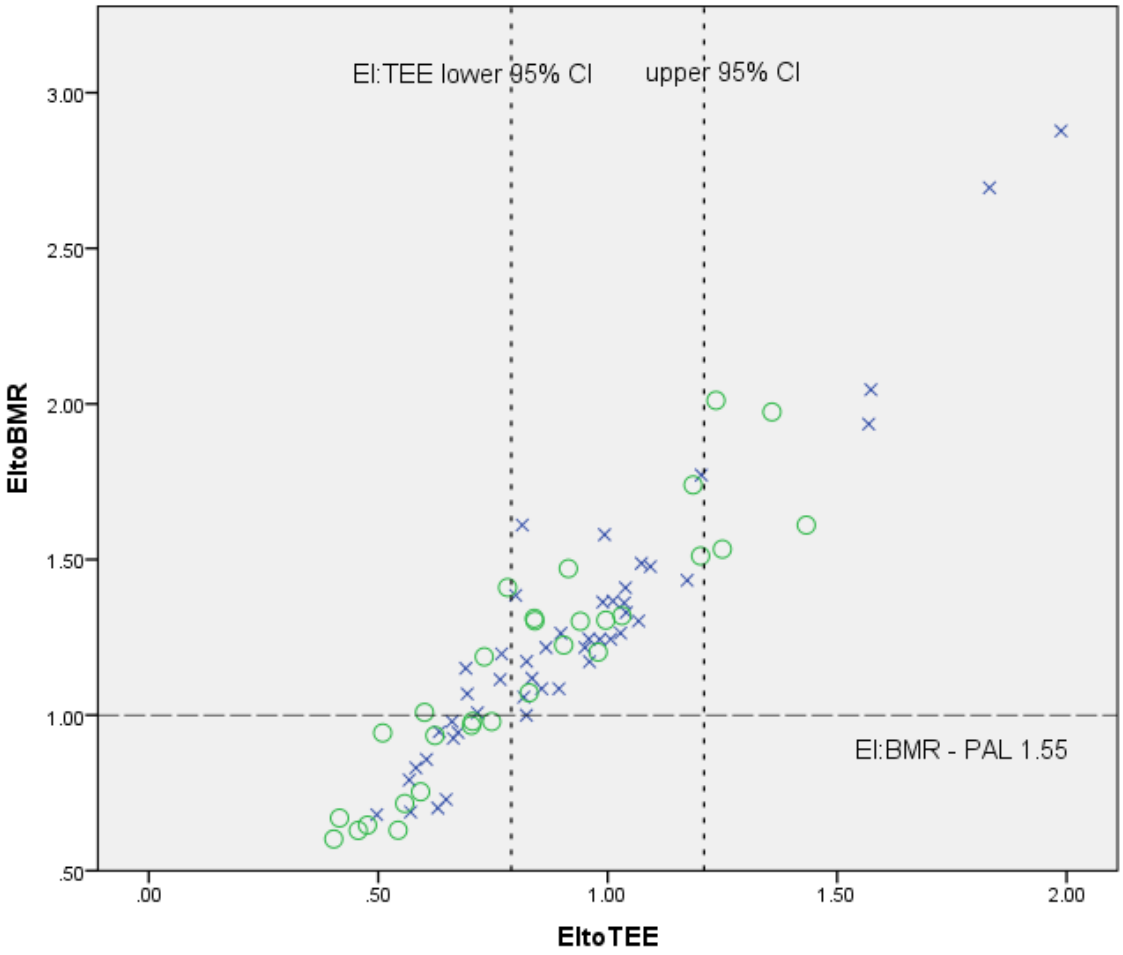


Figure 3. X, Males; O, Females; R^2 =0.887; ------, horizontal dashed line represents the lower 95%CI cut off for EI:BMR (1.55 PAL)

Figure 3, highlights that the ratio of EI:EE identified under reporters that were not detected by the Goldberg equation. Furthermore, females had a lower EI:BMR and EI:EE and are thus are more likely to under report than males, however, males and females both under and over report.

5.3 Discussion

The purpose of the current investigation was to compare a novel approach to assessing dietary misreporting to a method which has been widely used in research (the Goldberg equation), in a group of adolescents from Bedfordshire. Triaxial accelerometry was used to directly estimate EE for the comparison of EI to EE to ascertain the degree of reporting bias at the individual level. This was compared with two variants of the Goldberg equation which defines cut-off values to determine misreporting at the individual level based on the ratio of energy consumed to basal energy requirements (EI:BMR); 1) utilising a minimal physical activity level (1.55 PAL) and 2) applying a sub category of time in MVPA to assign a more specific PAL.

Assessment of reporting characteristics revealed that, as a group, average intakes were under reported (EI:BMR 1.22 ± 0.43) based on the 1.47 cut-off calculated by the 1.55 PAL Goldberg equation. Mean EI:BMR in females was lower compared to males (1.15 ± 0.40 vs 1.27 ± 0.45), but this difference was non-significant. EI:BMR was higher in 9 year olds from Norway compared the current study: 1.55 (boys) and 1.48 (girls) (Lillegaard et al., 2007). PAL was also higher in Norwegian children; 1.86 and 1.78 x BMR, compared to a PAL of 1.37 and 1.41 x BMR (males and females, respectively) in the current study (PAL calculated from EE:BMR assessed by triaxial accelerometry). This difference in PAL between the two studies could be due to a higher habitual PAL in children compared to adolescents as PA has been shown to decline from childhood into adolescence (Riddoch et al., 2004, Kimm et al., 2005). Furthermore, the novel use of the RT3 to estimate EE has not yet been validated and thus EE could have been under estimated. However, previous studies in youngsters comparing EI:EE based on the reference DLW method have found an overall under reporting of 18-23% in 9-10 year olds (Champagne et al., 1998) and 3-16% in 8-10 year olds (Bandini et al., 1997); a range of under reporting which is similar to that of EI:EE in the present study (12%). Moreover, comparison of the present study with a previous study in children (12.6 ± 2 yrs) utilising DLW, show similar EE estimates: 2515.45 vs 2350.24 kcal; 2652.41 vs 2409.96 and 2295.33 vs 2302.47 for all participants, males and females, respectively (Perks et al., 2000).

Prevalence of misreporting was assessed across method of reporting for the group and for males and females separately. Due to the known impact of weight status on misreporting (Poslusna et al., 2009) it was also of interest to assess misreporting of

the group across normal and overweight. Both variants of the Goldberg equation (EI:BMR) identified approximately 30% of participants as under reporters for the group as a whole and in males, however, EI:EE identified a greater proportion of under reporters, 42% and 35.6% (for the group and males, respectively). A greater proportion of females under reported compared to males, 40% according to both Goldberg equations and 51.9% by EI:TEE. Compared with the current sample of female adolescents, similar proportions of females (54.8%) were shown to under report in a study of pre-adolescent Afro-American girls (aged 8-10 yrs), when utilising the Goldberg equation and a very conservative PAL (1.35 x BMR) (Lanctot et al., 2008). Although, there is a difference in age between females of the two studies, yet PAL is similar to the mean PAL of 1.41 x BMR identified in the current sample of female adolescents. In the current study, when assessed across weight status, normal weight participants under reported to a lesser extent than over weight. Underreporting by normal weight was approximately 20% and 27.9% and in overweight participants was 50% and 62.1% according to the Goldberg equations and EI:EE, respectively; highlighting the markedly greater proportion of under reporters in overweight compared to normal weight. It is noteworthy that the females in this group of adolescents have a significantly greater BMI than the males and therefore females and those classed as overweight may consist of similar participants and this should be considered when viewing reporting. The similarities between these groups in terms of reporting prevalence appears to support this notion.

In a study of 13 year olds from the UK, BMI, weight, WC and fatness were significantly higher and FFM significantly lower in under reporters compared to valid and over reporters (P= <0.05) (Noel et al., 2010). The present study also found a positive association between adiposity and under reporting; associations with adiposity were comparable when reporting was assessed by the Goldberg equation and EI:EE method; weight and WC were significantly higher in under reporters compared to valid reporters assessed by either method. BF% and BMI were also significantly higher in under reporters but not when reporting was assessed by Goldberg MVPA PAL (See table 15); a reason for this may be that MVPA PAL identified less overweight under reporters compared to 1.55 PAL and EI:EE. Unlike previous research in youths from the UK (Noel et al., 2010), FFM was not significantly different across reporting categories and adiposity was not significantly different between valid and over reporters in the present study. In contrast, a study

of 9 year olds from Norway (Lillegaard and Andersen, 2005), observed no significant difference between under and valid reporters when reporting was assessed by EI:EE.

In a sample of Norwegian 9 year old children (Lillegaard et al., 2007), under reporting (Goldberg equation) was lower (12.2%) in comparison to the current adolescent sample, this difference may in part be explained by past research that has linked increasing age with higher prevalence of under reporting (de Vries et al., 1994, Posluna et al., 2009). A limitation of Lilllegaard et al's (2007) study is that a 'blanket cut-off' was applied to EI:BMR based on a generalised motion and position sensor-derived PAL (specific to sex) and thus a PAL may have been underestimated. In the current study, however, by utilising EI:EE to assess misreporting, EE was estimated directly for each individual from accelerometry. It is important to note that estimating EE in this manner is also not without limitations and these will be drawn upon later in this section. In a group of 15-18 year olds from Sweden, (Vågstrand et al., 2009) a lower prevalence of under reporters was reported compared to the current study sample; under reporting was 13% in males and 16% in females, however, over reporting was higher in this group compared to the current sample when assessed by the Goldberg equation and grouped into 4 PAL groups: 19% vs 4.4% in males and 17% vs 8.9% in females. These differences, however, can probably be attributed ot the lower BMI of the Swedish adolescents compared to those of the present study, furthermore, diet was assessed by a diet history questionnaire and thus differences could be due to method of dietary assessment (Vågstrand et al., 2009). The authors also reported a significantly increased odds of under reporting females having mothers that under report (P= 0.002) and this was not accounted for in the current study.

A comparable study of 2868 UK adolescents (mean age 13 yrs) (Noel et al., 2010,see section 4.3 for details) reported similar proportions of under reporters to the current study, both studies utilised 3 day diet records. Under reporting was between 37.1 and 51.8% depending on the method used to assign a PAL based on 7 day accelerometry. In the current study approximately 30% under-reported when using an assigned PAL from accelerometry (EI:BMR) whereas EI:EE identified 42.5% of the group as under reporters. It thus appears that EI:EE may identify a reasonable number of misreporters based on previous evidence from a comparable population.

To the authors knowledge only one study has used a device to estimate EE (for comparison with EI) form body movement in youths. Lillegaard and Andersen (2005) used a position and motion sensor (ActiReg ®) worn at the waist to assess nutritional intakes from a pre-coded food diary in 51 Norwegian 9 year olds. EI:EE was similar to that of the current study (see table 12) for all participants 0.83 ± 0.16 and females 0.88 ± 0.17, however boys had a greater discrepancy between EI and EE (0.79 ± 0.13) compared to males in the current study. EI:EE may have been lower in Lillegaard and Anderson's study because the equations used with the ActReg ® to calculate EE have been shown to systematically overestimate EE in children (Müller et al., 2004) thus, potentially resulting in a smaller EI:EE. Furthermore, the position sensor only determines when an individual is standing or sitting and the movement sensor registers movement on an 'all or nothing' principle and thus cannot distinguish activity intensity. Thirty-nine % of the Norwegian children were classed as under reporters and 4% as over reporters, these proportions represent values between those obtained by the MVPA PAL Goldberg equation and EI:EE in the current investigation (See figure 2). EI:EE from a uniaxial accelerometer has been compared to the Goldberg equation utilising a MVPA assigned PAL from accelerometry in 185 African-American, obese women with type II diabetes (Samuel-Hodge et al., 2004). Like the present study, EI:EE identified a greater proportion of under reporters compared to the Goldberg equation 81% vs 58% (Samuel-Hodge et al., 2004). The greater BMI of the African-American women could be responsible for the greater prevalence of under reporting (Forrestal, 2011) compared to females in the present study (35.7 vs 27.3 kg/m^2). This study utilised a uniaxial accelerometer to assess EE of which acceptable daily wear time was 4 days (minimum 4 hours per day) compared to the current study which employed triaxial accelerometry with a minimal wear time of 3 days (minimum 8 hours per day). Uniaxial accelerometry only assesses movement on one plane (vertical) and thus provide a less comprehensive measurement of body movements and has been shown to have weaker correlations than triaxial accelerometry with measured EE (Butte et al., 2012).

In the present study EI:EE consistently identifies a greater proportion of participants as under reporters compared to the Goldberg equation; both variants of the Goldberg equation identified similar proportions of misreporting. Research has shown that PAL (for the Goldberg equation) may be underestimated when assigned

based on MVPA (Noel et al., 2010). This is because individuals that engage in less MVPA are likely to expend more energy in LPA which is not accounted for (Noel et al., 2010), resulting in an under estimation of under reporters. When utilising accelerometry, no assumption or estimation of PAL is required as it is incorporated within each participants total EE and thus EI:EE may identify a more reasonable proportion of participants as under reporters (Forrestal, 2011). It appears this may also be the case with over reporting, a greater proportion of over reporters (at least double) was identified by EI:EE compared to the Goldberg equation (MVPA PAL) in each group of participants. Interestingly, although females and overweight participants under reported to the greatest extent, they also over reported in higher proportion to males and normal weight participants when assessed by EI:EE (figure 2). This was not the case when assessed by EI:BMR, where similar proportions of over reporting were identified by 1.55 PAL and MVPA PAL cut-offs (approximately 4%).

In the present study, total EI and subsequently macronutrient intakes were significantly lower in under reporters compared to valid and over reporters (P= 0.001). Energy adjusted macronutrient intakes, however, were not significantly different between under-, valid- and over-reporters when assessed by either method of misreporting. In Afro-American and European children there was also no significant difference in energy adjusted macronutrient intakes between under and valid reporters (Lillegaard et al., 2007, Lanctot et al., 2008). In adults with Type 2 diabetes, however, although no significant difference was observed in adjusted CHO intakes, energy adjusted protein was significantly higher and fat intakes were significantly lower in under reporters compared to valid reporters (Samuel-Hodge et al., 2004).

A strength of the current study was the identification of over reporting by EI:EE, if over reporting is not accounted for, those identified as ‘valid’ reporters may not all be reporting a plausible dietary intake. A further strength is that obtaining EE data from the RT3 accelerometer is less time consuming than recoding the data to quantify time spent in MVPA and thus may be a more practical approach to assessing misreporting in larger samples with this device. The reference technique for assessing EE in individuals is DLW, however this is expensive and requires the collection of urine samples (Lillegaard and Andersen, 2005). Accelerometers can be

re-issued, making them more cost-effective and potentially more appropriate for younger populations as the technique is less invasive.

Potential limitations of using the RT3 to quantify EE include the fact that sleeping metabolic rate is not accounted for by the system, instead, for each minute of data that a participant is not active, an average metabolic rate (AMR/min) value is quantified which is likely to result in an over estimation of metabolic rate during sleep and ultimately total EE. If EE is over estimated EI:EE will be lower increasing the chances of misclassifying individuals as under reporters. The fact that accelerometry cannot be used to measure certain activities such as swimming or cycling, however, means that aspects of daily EE may also be under estimated, limitations of accelerometry are outlined in section 4.3. Future work may consider the use of waterproof accelerometers such as the Actigraph GT3X+ that may provide more complete data as there is less probability that the device will need to be removed (Rowlands and Stiles, 2012). In the current study EI:EE identified more under-reporters and over-reporters compared to the Goldberg equation. It is thus unlikely that EI:EE was over or under estimating misreporting to a greater extent than EI:BMR using the Goldberg equation. Furthermore, EI:EE values from the current study fell within the range of EI:EE assessed by DLW from a previous study in children (Bandini et al., 1997) suggesting that EE measured by the RT3 may be comparable to the current gold standard. There are also, however, a number of factors that can influence under reporting in youngsters that were not accounted for in the present study, such as BMI and working hours of mothers as well as family income and number of siblings (Vågstrand et al., 2009). Additionally there are factors that affect reporting accuracy that were not accounted for, such as parental assistance with food diaries and weight stability. If individuals are not weight stable they are likely to be in positive or negative energy balance which could be attributed to over- or under-reporting (Forrestal, 2011). Furthermore, future work is required to validate this technique of estimating EE against DLW.

In this sample of adolescents from Bedfordshire between 23% and 31% increasing to 62.1% (in overweight individuals) of participants may under report and up to 11.1% over report. Reporting prevalence varies depending on the method of reporting assessment and the novel application of a triaxial accelerometer to identify EE resulted in more under and over reporters being identified than when compared to the widely used Goldberg equation (EI:BMR). Thus suggesting that the use of

equations that group participants into PAL categories may miss some variance in reporting compared to direct assessment of EE. It appears the EI:EE technique of this study identifies misreporting in reasonable proportions compared to past research. As dietary misreporting can produce distorted associations between diet and health outcomes (Rossell et al., 2003) the high prevalence of under reporting in all groups within this population is likely to have implications for research in this field. To this end, it would be of interest for future work to assess how accounting for underreporting impacts on associations of diet and health when utilising accelerometer derived EE compared to the Goldberg equation and compare this to the reference DLW technique. Although misreporting was more prevalent in females and overweight adolescents, a substantial proportion of normal weight and male participants also misreport, thus, misreporting should be considered in all adolescent populations when assessing dietary intakes.

Chapter Six: Study Three

Associations between GI, GL and other dietary factors with the metabolic syndrome in postpubertal adolescents.

6.0 Introduction

The glycaemic index (GI) and glycaemic load (GL) of the diet have been associated with obesity and associated metabolic risk factors in adult populations (Du et al., 2009, Brand-Miller et al., 2002). Both GI and GL were significantly associated with an adverse lipid profile and CHD in healthy American (Yungsheng et al., 2006; Culberson et al, 2009), Japanese (Murakami et al, 2006) and British (Frost et al 1999) adults. In a large healthy cohort of Dutch adults, greater intakes of GI and GL and lower intakes of fibre were associated with an increased incidence of type II diabetes. In 6,192 adults with type 2 diabetes (57.5 yrs), dietary GL has been positively associated with mortality (Burger et al., 2012) when adjusting analysis for CVD factors, BMI, PA, diet and severity of diabetes, but only in normal weight participants (BMI <25 kg/m^2). This association remained when dietary misreporters (EI:BMR) were excluded from the analysis and thus selective misreporting of overweight individuals was not an attributable factor (Burger et al., 2012). Based on evidence from (Arner et al., 1991) that non-obese individuals with diabetes have less peripheral insulin resistance but a more deficient insulin response compared to overweight, it was thus postulated that a high GL diet may have a more exaggerated BG response in these individuals putting them at greater risk of CVD related mortality.

These associations have been potentially explained by evidence that low glycaemic CHO foods elicit slower rates of digestion and prolonged feedback to the satiety centre of the hypothalamus and thus increasing satiety following their consumption (Radulian et al., 2009, Ludwig., 2000, Holt et al., 1992). Additionally, higher rates of fat oxidation have been observed during exercise, following ingestion of low GI foods (Stevenson et al., 2009) and following consumption of a low GI and low GL meal (Scazzina et al., 2011)

In youths there is less evidence of associations between GI and GL, obesity and the metabolic syndrome. The association of GI, GL and CHO with the metabolic syndrome, as assessed by two metabolic syndrome definitions (the IDF and ATPIII criteria) was assessed in 760 Australian adolescents aged 13-15 yrs old (O'Sullivan

et al., 2010). Associations of insulin resistance (HOMA-IR) and a sample defined metabolic cluster (based on BMI, fasting insulin, glucose, TG and blood pressure values ('high risk' or 'low risk')) were also assessed. The prevalence of the metabolic syndrome was 3.6% (IDF), and 4% (ATPIII); 25.9% of participants were in the high risk cluster. A 20 unit increase in GL and a 30g increase in CHO were associated with significantly increased odds of having metabolic syndrome as defined by the IDF criteria (OR 2.18; 1.26-3.78 and 3.86; 1.80-8.28, respectively). No significant associations were observed between glycaemic CHO and the metabolic syndrome when utilising the ATPIII, cluster-defined or HOMA-IR definitions. The IDF criteria is the only definition to include WC as a mandatory risk factor and, thus this association may be mediated by central adiposity.

An observational study found no significant association between higher glycaemic CHO intake and insulin sensitivity in 120 overweight Latino youths (10-17 yrs old), defined as an 'at risk' population (Davis et al., 2007). Scaglioni et al (2005) also found no association between GI and insulin sensitivity in 95 normal weight 8 year olds. These studies, however, used a 24 hr recall and FFQ, respectively, to identify nutritional intakes, whereas O'Sullivan et al (2010) used 3 day food records; different methods of obtaining food intakes and quantifying GI and GL makes it difficult to make comparisons across studies. Moreover, despite being defined as an 'at risk' group, Davis et al (2007) defined participants as 'at risk' by having a family history of type 2 diabetes and thus not all participants actually expressed an impaired insulin sensitivity. Furthermore, the age group investigated (10-17 yrs) means that participants will be at very different stages of puberty, which is known to impact on insulin sensitivity (Moran et al., 1999). Together, these factors may be attributed to the lack of adverse association between glycaemic CHO and insulin sensitivity.

Although there appears to be a link between an increased glycaemic CHO intake and metabolic risk factors, there is contrasting evidence as to which component, GI or GL, is more strongly linked to health in youths. Adolescents are likely to be making more autonomous lifestyle choices regarding their eating behaviours in comparison to during childhood which are likely to shape their future eating habits (Ebbeling et al., 2005), yet, there is a lack of research investigating these associations in UK adolescents. Understanding such associations in this age group is important as metabolic abnormalities have been shown to track from childhood and adolescents into adulthood (Camhi and Katzmarzyk, 2010). Additionally, the

impact of puberty does not seem to have been accounted for in past research which has assessed the relationships of GI and GL with health markers. This is important since there is a transient increase in insulin resistance which returns to pre-pubertal levels in the last stage of maturational growth (approximately 13 years of age) (Moran et al., 1999). It is understood that this can influence metabolic health parameters (Hannon et al., 2006) which may confound associations between GI and GL with metabolic risk factors. The age range of studies assessing adolescents, typically spanning from 12-18 yrs of age, means that no study has assessed a solely post-pubertal adolescent population.

An important consideration when investigating diet and health relationships is that of dietary misreporting. For example, Rosell et al (2003) found that misreporting of dietary intakes led to spurious relationships between PUFA and fats from milk in under reporters compared to non-under reporters (Rosell et al., 2003). However, Rosell et al (2003) did not identify and account for over reporting in the non-under reporting group, and therefore intakes from this group may not represent valid reporting.

In order to overcome the problems associated with misreporting, some researchers have taken to excluding individual participants who misreport (Sluijs et al., 2010; Lau et al., 2006). This, however, is not recommended, on the basis that it introduces unknown bias into the sample, being that only those participants reporting 'normal' intakes are examined (Gibson, 2005). A different approach, however, is to analyse all participants whilst adjusting statistical analysis for reported EI. If dietary intakes can be observed relative to EI this may attenuate the effect of over and under reporting (Poslusna, et al 2009), this will of course only be of value in those who misreport their entire diet as a whole rather than selectively for different macronutrients.

To this end, the current investigation will assess the associations of GI and GL with risk factors for the metabolic syndrome in a post-pubertal adolescent population from Bedfordshire. As outlined in chapter 2, EI:EE (EE assessed by RT3 accelerometer) may be an appropriate method for assessing misreporting in this population. Due to the potential impact of misreporting, analyses will examine these associations including all participants and also when misreporters are excluded, comparing both the Goldberg equation and EI:EE to assess misreporting.

6.1 Methodology

Participants

Of the 105 14-19 year olds recruited for the SIRENS and CROSSROADS studies 72 adolescents (45 males and 27 females) provided both adequate nutritional intake (3 days weighed food diary including one weekend day) and PA data (at least 3 days of accelerometer wear including one weekend day), see section 3.9 for more details. Participants were recruited as previously outlined in section 3.0.

Experimental Design

Data collection was conducted between 7am and 10 am as participants were required to have fasted from 9pm the previous evening. See section 3.3 for further details.

Measurements

Age, ethnicity and SES

As outlined previously (section 4.1) Age (to two decimal places), ethnicity (white/non-white) and SES (IMD scores) were measured.

Anthropometry and Body Composition

As explained in section 3.5, stature, body mass, BMI, fat mass, fat free mass and WC measurements were taken for all participants.

Dietary Intake

Three day weighed food diaries were completed by each participant as described in section 3.10. For all macronutrient variables the residuals method of energy adjustment was utilised in order to attenuate the impact energy misreporting may have on these variables. The residuals method adjusts for energy intake whilst producing a variable fully independent of energy intake (Mirmiran et al., 2006). This was achieved through linear regression analysis with EI as the independent variable and the intake of the nutrient under adjustment as the dependent variable. An individual's energy adjusted nutrient intake was determined by calculating the residual difference between the observed nutrient value of that participant and the value predicted from the regression equation (Gibson, 2005). To provide a meaningful adjusted value, the residual score was added to the nutrient intake corresponding to the mean EI of the population (Gibson, 2005). When stratifying the

groups by sex or weight status the residual adjustment was made based on the mean energy intake for each respective group separately. GI did not require adjustment for energy since its calculation is independent of energy intake.

Dietary Misreporting

Dietary misreporting was assessed by three separate techniques: comparing EI:BMR using two variants of the Goldberg equation to assign 95% confidence limit (CL) cut-offs beyond which are classified as misreporting (1.55 PAL and MVPA PAL); and comparing EI:EE when EE is estimated directly by triaxial accelerometry (RT3). This method applied the previously defined 95% CL (0.21) for estimating EE from the reference method DLW to define misreporting (Black and Cole, 2001, Black et al., 1997).

Physical Activity

PA engagement was monitored using the RT3 triaxial accelerometer as outlined previously (section 3.9)

Cardiorespiratory fitness

CRF was assessed by incremental cycle ergometer test (see section 3.8) Peak volume of oxygen consumed at maximal exercise was measured by a portable online gas analysis system (Cortex Metamax 3B).

Resting systolic and diastolic blood pressure

Resting SBP and DBP were recorded as per the procedures outlined in section 3.7.

Fasting finger prick blood samples

Fasting blood samples were obtained from each participant; the 40 µL samples were immediately measured for TG, HDL and BG as described in section 3.4.2.

Metabolic syndrome risk factors

For each component of the metabolic syndrome (WC, TG, HDL, SBP, DBP and BG) cut-points were applied to identify the presence of these individual risk factors for metabolic syndrome in children and adolescents as defined by the International Diabetes Federation (IDF) definition (Alberti et al., 2006):

- High WC: WC ≥90th percentile for age and sex according to reference values of UK children and adolescents (McCarthy et al., 2001);
- Hypertriglyceridemia: TG ≥1.7 mmol.L;
- Low HDL-C <1.03 mmol.L;
- high blood pressure: SBP ≥130 mmHg or DBP ≥85 mmHg;
- high fasting BG: BG ≥5.6 mmol.L.

According to the IDF criteria, adolescents who have central adiposity (high WC) plus at least two additional risk factors are classed as having the metabolic syndrome (Alberti et al., 2006).

Clustered metabolic risk

Clustered metabolic risk score was constructed by standardising to the mean (by sex) and summing the standardised scores of the following variables: DBP, TG, inverse HDL (HDL x -1) to confer higher risk with increasing values, BG and WC. Prior to standardising to the mean, the following non-normally distributed variables were log transformed: WC, HDL (prior to inverting the score), TG and BG. Having a high clustered metabolic risk (Crisk) score was defined as ≥ 1 SD above the mean Crisk score for the population (Andersen et al., 2006).

Statistical Analysis

Statistical analyses were conducted using SPSS, descriptive data are presented as mean and standard deviation. The following variables were non-normally distributed and were subsequently log transformed to improve their distribution (BMI, BF%, WC, EI, GL, PRO, fat, CHO, PUFA, MUFA, SFA, sugar, salt, %fat, % PUFA and % SFA). BMI z-score was calculated using UK 1990 reference values (Culberson et al., 2009), as were BF% z-scores, however a raw WC was utilised as the population reference data does not address the upper age range (17-19 yrs) of the population investigated. Differences between sex and weight status were assessed using One-way ANOVA. When assessing the relationship between dietary and metabolic risk variables time spent SED was adjusted for based on the evidence that being SED mediates GI- and GL-health relationships (Hare-Bruun et al., 2006). Nutrient intakes (including GL) were adjusted for energy using the residuals method. The relationship between nutritional variables and metabolic risk factors was assessed using partial correlation analysis adjusted for age, sex, SES, zBMI and the

percentage of time spent SED. Binary logistic regression analysis was employed to assess the relationship between GI and GL as continuous variables with metabolic risk factors as defined by the IDF criteria of the metabolic syndrome for adolescents (Alberti et al., 2006) and high clustered metabolic risk score as dichotomous variables. The following covariates were included in the logistic regression analysis: sex, age, ethnicity, SES, % MVPA and residual intakes of fat, PRO, fibre and respectively for GI or GL. Associations of Glycaemic CHO variables (GI and GL) with metabolic risk factors were further assessed by MANCOVA with metabolic risk factors as continuous variables. The difference in risk variables was assessed across upper and lower quantiles of GI and GL intake adjusted for the following covariates: sex, age, ethnicity, SES, % MVPA and residual intakes of fat, PRO, fibre. Logistic regression and MANCOVA were conducted in all participants and separately for males and females. Associations of diet with metabolic health risk factors were explored when including all participants and when excluding those identified as misreporters as assessed by three techniques for identifying dietary misreporting: The Goldberg equation 1.55 PAL and MVPA PAL and EI:EE (RT3 triaxial accelerometry). For MANCOVA the assumption of homogeneity of regression slopes was met as no significant ($P > 0.05$) interaction effects were observed for metabolic risk factors between any independent or covariate variables. Furthermore there was no collinearity between covariates entered into the MANVOA models. In order to avoid bias in the logistic regression model residual adjusted CHO was removed as a covariate because multiple linear regression analysis revealed that the variances of the regression coefficients for residual adjusted CHO and glycaemic CHO variables (GI and GL) were dependent and thus the assumption of collinearity was violated. In order to make additional models comparable residual adjusted CHO was also excluded from adjustment of MANCOVA. For all analyses the significance level was set at $P > 0.05$.

6.2 Results

Table 17, shows anthropometric and adiposity characteristics of Bedfordshire adolescents (this table is the same as table 3 but with the addition of SES and FFM). Males and females have a similar mean age, on average males are significantly taller, yet females are of a similar mean weight. Females have a significantly greater mean BMI (P=0.003) and BF% (P= <0.001) compared to males but on average exhibit as lightly raised WC compared to males.

Table 17. Participant characteristics

	COMBINED (n=98)	**Male**s (n=61)	**Females** (n=37)	***P***
Age (yrs)	17.36 (1.40)	17.46 (1.22)	17.21 (1.64)	0.389
Height (cm)	172.40 (9.85)	177.80 (7.32)	163.64 (6.59)	0.000
Weight (kg)	71.25 (16.30)	71.66 (15.45)	70.59 (17.78)	0.746
BMI (kg/m^2)	23.89 (5.59)	22.64 (4.65)	25.92 (6.39)	0.003
BF (%)	23.30 (10.89)	17.98 (7.49)	31.94 (10.02)	0.000
FFM (kg)	51.78 (11.60)	56.83 (8.72)	43.45 (11.01)	0.000
WC (cm)	80.33 (12.20)	79.44 (11.64)	81.76 (13.08)	0.346
SES (IMD)	14.54 (11.00)	13.86 (11.43)	15.66 (10.30)	0.435

Means (± SD); *P* significant at <0.05 for comparison of males and females

According to the IDF criteria for the metabolic syndrome in children and adolescents (Alberti et al., 2006) the prevalence of participants with low HDL levels and thus an adverse value was 34.7% and for high TG the prevalence was 12%. 38% of participants had a high WC, although the prevalence of participants with a raised fasting BG was just 6.7%. DBP and SBP were classed as high in 13.2% and 10.5% of participants, respectively. With regard to clustered risk score 17.3 % of participants had a high score and 13.2% were classed as having the metabolic syndrome (table 18).

Table 18. Metabolic syndrome risk factors and prevalence in Bedfordshire adolescents

	All participants (N=75)		**Males (N=46)**		**Females (N=29)**	
	Mean (SD)	Participants with risk factor (%)	Mean (SD)	Participants with risk factor (%)	Mean (SD)	Participants with risk factor (%)
HDL (mmol/L)	1.13 (0.32)	26 (34.7%)	1.12 (0.31)	15 (32.6%)	1.15 (0.35)	11 (37.9%)
TG (mmol/L)	0.91 (0.70)	9 (12%)	0.82 (0.43)	4 (9.7%)	1.04 (0.98)	5 (17.2%)
WC (cm)	81.39 (12.88)	29 (38.2%)	79.71(12.18)	8 (17%)	84.12 (13.71)	21 (72.4%)
BG (mmol/L)	4.81 (0.51)	5 (6.7%)	4.89 (0.48)	2 (4.3%)	4.69 (0.53)	3 (10.3%)
DBP (mmHg)	72.06 (8.17)	10 (13.2%)	70.67 (8.27)	5 (10.6%)	74.32 (7.60)	5 (17.2%)
SBP (mmHg)	115.64 (11.83)	8 (10.5%)	118.58 (11.78)	6 (12.8%)	110.87 (10.45)	2 (6.9%)
Clustered risk score	0.21 (3.19)	13 (17.3%)	-0.28 (3.04)	6 (12.8%)	0.99 (3.31)	7 (25%)
Prevalence of Met S	-	10 (13.2 %)	-	5 (10.6%)	-	5 (17.2%)

Metabolic risk factors: HDL, <1.03mmol.L; TG, ≥1.7 mmol.L; WC, ≥90th percentile for age and sex; BG, ≥5.6 mmol.L; DBP, ≥ 85 mmHg; SBP, ≥ 130 mmHg; high clustered risk, ≥1 SD above the mean.

Table 19. Partial correlations of dietary variables and metabolic risk factors

		GI	GL	Fibre	PRO	Fat	CHO	Sugar
WC †	Correlation	.183	.120	.290	-.109	-.145	.095	-.064
	P Value	0.133	0.324	**0.016**	0.372	0.235	0.435	0.602
HDL	Correlation	.138	.192	-.036	.099	-.096	.161	-.127
	P Value	0.262	0.116	0.768	0.422	0.434	0.189	0.303
TG	Correlation	-.008	-.008	.025	-.225	.127	-.017	.042
	P Value	0.947	0.945	0.840	0.065	0.304	0.889	0.732
BG	Correlation	-.094	-.106	-.139	-.092	.234	-.075	.151
	P Value	0.446	0.388	0.257	0.455	0.054	0.545	0.219
SBP	Correlation	.007	-.006	-.175	.217	-.011	-.074	.033
	P Value	0.953	0.960	0.153	0.075	0.930	0.548	0.787
DBP	Correlation	.007	-.046	-.057	.052	.128	-.129	-.157
	P Value	0.955	0.712	0.645	0.673	0.298	0.293	0.200
Clustered Risk score	Correlation	-.078	-.068	.020	-.243	.225	-.068	.076
	P Value	0.525	0.579	0.874	**0.046**	0.065	0.581	0.535

Correlations controlled for age, sex, SES, BMIzscore and MVPA; **†,** ZBMI not controlled for in analysis.

Metabolic risk factors were assessed in terms of their clinical significance based on cut points that define a non-risk or high risk value for each parameter (Alberti et al., 2006). Subsequently, binary logistic regression analyses were conducted to assess the odds of being in a high risk category according to dietary GI or GL. Due to the nature of group analysis, some models were not viable due to the low participant numbers in some risk categories e.g. TG risk. Models which were not viable (BG, DBP, SBP) have not been included.

Table 20. Multivariate adjusted OR (and 95% CIs) of having a cardiometabolic risk factor with increasing dietary GI and GL

	ALL PARTICIPANTS (n = 72)							
	TG	***P***	**HDL**	***P***	**WC**	***P***	**High clustered Risk score**	***P***
GI	1.05 (0.71-1.55)	0.822	1.15 (0.92-1.45)	0.215	-	-	1.04 (0.63-1.73)	0.879
GL	0.00 (0.00-0.00)	0.087	0.00 (0.00-1.80)	0.057	-	-	0.00 (0.00-1.30)	0.051
GI†	1.25 (0.92-1.70)	0.148	1.21 (0.97-1.50)	0.092	1.70 (1.16-2.50)	0.007*	1.25 (0.96-1.63)	0.101
GL†	0.00 (0.00-0.26)	0.030*	0.00 (0.00-0.17)	0.034*	0.00 (0.00-2.55)	0.055	0.00 (0.00-0.93)	0.049*

Models adjusted for sex, age, SES, MVPA, BMI, fat, PRO and fibre; †, model is not adjusted for BMI; GI (1 unit increase), GL (1g increase). - , model not viable.

In model 1 of the logistic regression models of all participants, a 1 unit increase in GI was associated with increased odds of having hypertriglyceridemia, low levels of HDL, and high clustered risk score. A 1g increase in GL was associated with reduced odds of having hypertriglyceridemia, low levels of HDL and high clustered metabolic risk. However, as these are non significant associations (confidence intervals range from <1 to >1) it makes it difficult to suggest if the odds ratio would actually fall above or below 1 and thus the direction of association cannot be ascertained.

A second set of models were conducted without adjustment for BMI (†) this was done because WC is associated with BMI and so that the impact of weight status on the logistic regression models could be assessed. Excluding BMI strengthened associations to the extent that some became significant. According to these data a 1g increase in GL is associated with significantly reduced odds of having hypertriglyceridemia (OR 0.00, P =0.030), low HDL (0.00, P =0.034, high clustered risk (0.00, P =0.049). Although GI models came closer to indicating significant increased odds ratios (reduced P value) for HDL, TG and Crisk, associations remained non-significant. A 1 unit increase in GI, however, was associated with significantly increased odds of having a high WC (P =0.007), where as GL was associated with a non-significant reduced odds of having a high WC.

Table 21. Odds ratio (and 95% CIs) for cardiometabolic risk factors with dietary GI and GL in males

	Males (n = 45)			
	HDL	***P***	**Clustered risk**	***P***
GI	2.04 (1.07-3.90)	0.031*	-	
GL	0.00 (0.00-0.00)	0.014*	-	
GI†	2.09 (1.11-3.93)	0.022*	2.57 (1.02-6.46)	0.045*
GL†	0.00 (0.00-0.00)	0.011*	0.00 (0.00-16.15)	0.059

Models adjusted for sex, age, SES, MVPA, BMI, fat, PRO and fibre. †, model is not adjusted for BMI; GI (1 unit increase), GL (1g increase). - , model not viable.

When associations were assessed in males, there was a significantly increased odds of having low HDL per unit increase in GI (2.04,1.07-3.90; P =0.031). When BMI was excluded from the model the odds ratio is slightly increased (2.09, 1.11-3.93; P = 0.022). Per unit increase in GI there was a significantly increased odds of having a high clustered risk score (2.57, 1.02-6.46; P= 0.045). Per unit increase in GL there was significantly lower odds of having low HDL (0.00, 0.00-0.00; P = 0.014) and this association is strengthened following removal of BMI from the logistic regression model.

Due to the issues of running binary logistic regression analysis with limited group sizes, ANCOVA was employed to assess any associations between dietary GI and GL with metabolic risk factors. For this technique the dependent variable is continuous and thus issues of small group numbers when split into a risk or non-risk category are eliminated.

Table 22. Mean (±SD) cardiometabolic risk factor values across quantiles of GI and GL for all participants

N=72	GI				GL RES			
	1 (54.96)	2 (62.04)	F	P	1 (75.50)	2 (94.74)	F	P
HDL (mmol.L)	1.17 (0.34)	1.12 (0.31)	0.78	0.381	1.06 (0.30)	1.21 (0.34)	3.62	0.062
†			1.75	0.190			3.69	0.059
TG (mmol.L)	0.91 (0.81)	0.92 (0.65)	0.03	0.865	0.82 (0.65)	1.00 (0.81)	0.84	0.364
†			0.58	0.449			0.60	0.443
WC† (cm)	79.14 (10.67)	83.56 (10.41)	4.84	0.031	79.49 (12.69)	80.93 (12.42)	0.02	0.880
BG (mmol.L)	4.79 (0.60)	4.74 (0.41)	2.50	0.119	4.80 (0.54)	4.73 (0.49)	0.06	0.813
†			1.50	0.226			0.05	0.831
SBP(mmHg)	115.06 (11.20)	115.34 (11.17)	1.39	0.242	116.53 (10.01)	114.31 (12.27)	0.04	0.851
†			0.04	0.968			0.01	0.935
DBP(mmHg)	72.22 (8.73)	71.33 (7.69)	1.47	0.229	71.16 (8.14)	72.28 (8.47)	0.94	0.336
†			0.04	0.840			0.69	0.408
Crisk score	0.11 (3.17)	0.35 (3.23)	0.78	0.380	0.04 (3.32)	0.41 (3.07)	0.65	0.424
†			0.98	0.327			0.21	0.647

1 and 2 represent the lower and upper quantile, respectively; analysis adjusted for covariates: sex, age, ethnicity, SES, MVPA and residual intakes of fat, PRO, fibre; †, model is not adjusted for BMI.

WC is significantly higher in participants within the highest quintile group of GI (P = 0.031) no other associations are significant. However, there is a non-significant trend for a higher mean clustered risk score in those from the highest quantiles of GI and GL. HDL is lower in the highest quantile of GI group and higher in the highest quartile of GL group, and this association approaches statistical significance after removal of BMI from the model (P = 0.059). There also appears to be a trend for TG to increase with increasing GI and GL but this association is non-significant. Both SBP and DBP only slightly vary according to quintile of GI and GL intake. Furthermore, BG appears to decrease slightly (P= 0.119 and 0.813, respectively) as GI and GL consumption increases.

When ANCOVA was run according to sex and weight status, no associations between GI, GL and metabolic risk factors were significant (results not presented). However, associations between diet and risk factors were similar for males and normal weight participants, this was also the case when comparing female and

overweight participants. It is noteworthy that the trends in these non significant data show a negative association of GI and GL with fasting BG in all groups. Furthermore WC appears to reduce with increasing GL in male and normal weight participants and TG appear to reduce with increasing GI in females (see appendix 7).

Table 23. Mean (±SD) metabolic risk factor values across quantiles of GI and GL with dietary misreporters excluded from analysis (EI:BMR PAL 1.55).

	GI				GL RES			
N=72	1 (55.37)	2 (61.90)	F	P	1 (74.60)	2 (87.90)	F	P
HDL (mmol.L)	1.15 (0.25)	1.17 (0.44)	0.13	0.722	1.11 (0.39)	1.21 (0.32)	1.13	0.294
†			0.37	0.548			0.92	0.343
TG (mmol.L)	0.91 (1.08)	0.89 (0.49)	0.14	0.712	0.79 (0.54)	1.00 (1.03)	0.01	0.933
†			0.79	0.380			0.01	0.909
WC† (cm)	76.82 (8.86)	79.96 (12.08)	0.72	0.402	76.58 (8.81)	80.20 (12.04)	0.43	0.517
BG (mmol.L)	4.88 (0.61)	4.67 (0.42)	1.03	0.316	4.75 (0.55)	4.80 (0.52)	0.26	0.615
†			1.22	0.276			0.23	0.637
SBP(mmHg)	115.19 (11.56)	111.60 (11.03)	3.72	0.061	115.56 (11.24)	111.24 (11.21)	0.08	0.773
†			1.11	0.298			0.00	0.959
DBP(mmHg)	71.35 (8.87)	69.24 (7.27)	.922	0.343	70.43 (7.79)	70.12 (8.51)	0.08	0.786
†			0.36	0.551			0.02	0.877
Crisk	-0.53 (2.81)	-0.55 (3.09)	0.43	0.516	-0.85 (2.88)	-0.24 (3.00)	0.20	0.659
			0.07	0.794			0.01	0.935

1 and 2 represent the lower and upper quantile, respectively; analysis adjusted for covariates: sex, age, ethnicity, SES, MVPA and residual intakes of fat, PRO, fibre; †, model is not adjusted for BMI.

Table 24. Mean (±SD) metabolic risk factor values across quantiles of GI and GL with dietary misreporters excluded from analysis (EI:BMR PAL MVPA).

N=72	GI				GL RES			
	1 (55.61)	2 (62.05)	F	P	1 (74.67)	2 (88.49)	F	P
HDL (mmol.L)	1.22 (0.36)	1.13 (0.36)	1.51	0.227	1.16 (0.38)	1.19 (0.35)	0.24	0.628
†			2.42	0.128			0.16	0.689
TG (mmol.L)	0.92 (1.13)	0.91 (0.85)	0.02	0.894	0.72 (0.36)	1.10 (1.13)	0.30	0.585
†			0.43	0.518			0.39	0.535
WC† (cm)	76.23 (9.23)	80.90 (12.98)			75.73 (8.45)	81.37 (13.28)		
			1.30	0.261			0.14	0.707
BG (mmol.L)	4.86 (0.61)	4.71 (0.44)	0.26	0.614	4.86 (0.61)	4.71 (0.44)	0.17	0.684
†			0.48	0.494			0.20	0.654
SBP(mmHg)	113.09 (11.11)	112.28 (11.28)	0.21	0.646	114.68 (11.32)	110.76 (10.73)	0.14	0.706
†			0.03	0.870			0.05	0.819
DBP(mmHg)	71.42 (8.87)	66.61 (7.35)	0.62	0.438	70.92 (8.16)	70.09 (8.18)	0.06	0.802
†			0.17	0.681			0.03	0.858
Crisk	-0.65 (3.07)	-0.21 (3.18)	0.07	0.792	-0.78 (2.88)	-0.09 (3.33)	0.16	0.689
			0.38	0.543			0.03	0.873

1 and 2 represent the lower and upper quantile, respectively; analysis adjusted for covariates: sex, age, ethnicity, SES, MVPA and residual intakes of fat, PRO, fibre; †, model is not adjusted for BMI.

Table 25. Mean (±SD) metabolic risk factor values across quantiles of GI and GL with dietary misreporters excluded from analysis (EI:EE)

N=72	GI				GL RES			
	1 (55.88)	2 (61.95)	F	P	1 (71.40)	2 (90.39)	F	P
HDL	1.31 (0.35)	1.17 (0.38)	4.85	0.037	1.21 (0.39)	1.26 (0.35)	0.02	0.894
			6.09	0.020			0.00	0.993
TG	0.63 (0.17)	0.94 (0.53)	2.78	0.108	0.77 (0.55)	0.80 (0.27)	0.00	0.968
			3.94	0.058			0.01	0.906
WC	74.28 (0.66)	80.46 (12.85)	0.68	0.416	75.44 (9.85)	79.36 (12.45)	0.01	0.934
BG	4.87 (0.66)	4.70 (0.44)	0.02	0.893	4.72 (0.64)	4.84 (0.48)	3.63	0.068
			0.01	0.943			4.01	0.056
SBP	114.62 (11.63)	112.36 (9.35)	0.04	0.845	115.41 (10.86)	111.61 (9.93)	0.24	0.629
			0.00	0.955			0.35	0.558
DBP	72.16 (9.10)	70.39 (6.59)	0.27	0.606	72.14 (8.22)	70.42 (7.60)	0.84	0.368
			0.14	0.709			0.97	0.333
Crisk	-1.46 (2.04)	-0.28 (2.90)	0.96	0.336	-1.08 (2.75)	-0.63 (2.40)	0.09	0.772
			2.07	0.162			0.00	0.992

1 and 2 represent the lower and upper quantile, respectively; analysis adjusted for covariates: sex, age, ethnicity, SES, MVPA and residual intakes of fat, PRO, fibre.

Table 26. Summary of the impact of excluding dietary misreporters on associations between metabolic risk factor variables and GI or GL.

Risk factor	Misreporters included	Misreporters excluded EI:EE (RT3)
WC	Higher in high GI quintile (P = 0.031)	Association attenuated
HDL	Not associated	Lower in high GI quintile (P = 0.020)
TG	Not associated	Higher in high GI quintile (P = 0.058)
BG	Not associated	Higher in high GL quintile (P = 0.056)

Only when model was not adjusted for BMI.

ANCOVA was run with misreporters excluded, comparing the association of GI and GL with metabolic risk factors with misreporting assessed by three different techniques. When misreporters were identified by EI:BMR PAL1.55 and excluded,

there was a trend for HDL to increase with GI but this was non-significant. The removal of misreporters based on the EI:BMR MVPA PAL technique showed a trend for HDL to decreases with dietary GI, indeed, this association is non-significant. The EI:EE model, however, was the only of the three to show significant associations between glycaemic CHO and risk factors (summarised in table 26); HDL was significantly lower in the higher quintile of GI (P =0.037) and TG is borderline significantly higher in those with in the higher GI quintile (P =0.058). Furthermore, following removal of under reporters assessed by EI:EE, there is a non-significant trend for BG to increase in those in the higher quintile for GL, and after exclusion of BMI from the model BG is borderline significantly higher in those consuming a higher GL diet (P =0.056)

6.3 Discussion

The observational associations of dietary GI and GL with risk factors for the metabolic syndrome were assessed in postpubertal adolescents from Bedfordshire. Additionally, these associations were examined following removal of misreporters. Misreporting was assessed by 3 separate techniques; 2 variants of the widely used Goldberg equation (EI:BMR) and a novel approach to identifying EE (RT3 accelerometer) for the application of EI:EE; the impact of removing misreporters was compared between the 3 techniques.

When analysing the group as a whole, binary logistic regression revealed that a unit increase in GI significantly increased the likelihood of having a high WC (OR 1.70, CI 1.16-2.50; P= 0.007), however, this was only significant when BMI was excluded from the model. GL was not associated with a high WC. Similarly, in adult females, Hare-Bruun et al (2006) identified that increasing dietary GI was associated with gains in body fat and WC; GL shared no significant associations with adiposity variables. These data suggest that reducing the GI of the diet rather than GL could be more impactful on lowering adiposity.

In the present study, in contrast to a positive association between risk and GI, a unit increase in GL was associated with a reduced likelihood of having the following metabolic risk factors: TG (OR 0.00, CI 0.00-0.26; P= 0.030); HDL (OR 0.00, CI 0.00-0.17; P= 0.034) and cumulative risk as assessed by clustered risk scores: Crisk (OR 0.00, CI 0.00-0.93; P= 0.049). On the contrary to these findings, in

Australian adolescents identified that GL was significantly associated with incidence of the metabolic syndrome (O'Sullivan et al., 2010) as defined by the IDF criteria (Alberti et al., 2006).

Furthermore, a reduced odds of having low HDL per unit increase of GL is not consistent with many past research studies (Murakami et al., 2006; Yungsheng et al., 2006; Culberson et al., 2009; Denova-Gutierrez et al., 2009) in which GL was associated with an adverse lipid profile (low HDL and high TG), but these were conducted in adults. Moreover, although these studies controlled for intake of other macronutrients and in some cases PA, diet was assessed by FFQ or self report diet history and PA was self reported via questionnaire. The current investigation, however, used a more objective assessment of PA (traixial accelerometry), which is less prone to reporting errors than PA questionnaires (Sirard and Pate, 2001, Trost, 2007). Furthermore, more precise information regarding the type and quantity of foods consumed may have been acquired by employing a weighed food diary, compared to a FFQ; improving calculations of GI and subsequently GL (Slyper et al., 2005).

The finding of the present study that GI is associated with a high WC is in agreement with past research in adults and previous work in chapter 4 of this thesis. Few published studies of youngsters, however, appear to have demonstrated positive associations between GI and adiposity variables. GI and GL were positively associated with adiposity in 16 year old adolescents (Nielsen et al., 2005), however, this was only observed in males. Furthermore, like the present population under investigation, the adolescents assessed by Neilsen et al (2005) were of an age that could be entirely postpubertal (16 yrs), but, unfortunately, they did not assess pubertal status.

Limitations associated with running logistic regression with small sample sizes meant that stratifying the sample into sub groups produced little valid data, however, it was possible to assess logistic regression in males due to the greater group size compared to females. When males were analysed separately compared to the group as a whole, a unit increase in GI was associated with an increased odds of having a high Crisk score (OR 2.57, CI 1.02-6.46; P= 0.045) (following exclusion of BMI from the model) and low HDL (OR 2.04, CI 1.07-3.90; P= 0.031), and this association was strengthened when BMI was removed from the model (P= 0.022).

For GL the negative association with HDL risk remained but associations were stronger (P= 0.014 and 0.011 including and excluding BMI, respectively), the association of GL with Crisk was not present in males alone.

As logistic regression was not always a viable statistical method, MANCOVA was employed so that associations could be assessed with risk factors as continuous variables. The narrow range of dietary GI consumed by this group made stratifying the sample into categories of low, moderate and high GI implausible, however, when stratified into 2 quantiles of GI intake for MANCOVA, the lower GI quintile (54.96) and upper GI quintile (62.04) represented a low GI (<55) and moderate GI (55-69) group, respectively.

In the present study, when assessed as a whole group stratified by low and high quantiles of GI (54.96 vs 62.04) and GL (75.5 vs 94.74); WC was significantly greater in the higher compared to lower quintile of GI (83.56 vs 79.14 cm; P= 0.031), consistent with earlier analysis by logistic regression and with previous studies of European adults (Hare-Bruun et al., 2006) and adolescents (Neilsen et al., 2005). Contrasting findings, however, have been observed in British youths; a study of 818 children (4-10 yr olds) and 818 adolescents (11-18 yr olds) based on data from the NDNS showed that GL was independently associated with higher risk of overweight (BMI) in children (P= 0.04) and central obesity (waist to height ratio) in adolescents (P= 0.02), whereas GI was not associated with adiposity (Murakami et al., 2013). Although the dietary assessment techniques of both studies are comparable (weighed food diaries) and an almost identical range of confounding variables was adjusted for, Murakami et al (2013) employed a much larger sample size, providing greater statistical power than the current study (Lenth, 2001). However, they also utilised an age range of adolescents (11-18 yrs) that is likely to encompass individuals of varying pubertal status (Moran et al., 1999). Puberty has been shown to impact on metabolic risk factors (Hannon et al., 2006), via a transient increase in insulin resistance (Moran et al., 1999), which may confound associations of diet and health markers. Importantly, the present investigation analysed a solely postpubertal population to avoid the potential effects of puberty.

In the present study GL was not associated with any risk factors for the metabolic syndrome when expressed as a continuous variable. HDL was, however, greater in the higher compared to lower quintile of GL (1.06 vs 1.21 mmol/L; P= 0.062) and this association approached significance (P=0.059) when BMI was not included in

the model. Chapter 1 showed that adjusted GL is negatively correlated with %fat (r -0.61; P= <0.01), this could be a reason for the lack of association with GL and health markers, being that fat intake was controlled for in these analyses. This may also explain the borderline significant positive association between GL and HDL cholesterol, as it may be that those consuming a higher GL diet concomitantly consumed less dietary fat. However, although in the past, lower fat intakes have been associated with a more beneficial lipid profile and cardiometabolic health (Hu et al., 2001), more recently, it appears that high fat-low CHO, or specifically, low GI and GL diets can have a favourable impact on lipid profile and CVD risk (Mozaffarian et al., 2011, Chess and Stanley, 2008). It does appear, however, that the type of fat rather than the total amount consumed, such as replacing SFA with PUFA and MUFA (Sirtori et al., 2009; Cicero et al., 2010) can be beneficial for cardiometabolic health (Abete et al., 2011) and in chapter 4 it was observed that adjusted GL was also significantly negatively correlated with %SFA but also %MUFA and %PUFA (P=<0.01). Therefore, a lower GL was associated with a lower fat intake in general, making it difficult to suggest why there was a lack of association between increased GL and poor metabolic health.

MANCOVA was run with the population stratified separately for sex and also weight status, since associations between glycaemic CHO and health markers may be mediated by these factors in adults and youths (Hare-Bruun et al., 2006, Nielsen et al., 2005). This analysis revealed that no associations were significant between metabolic syndrome risk factors and glycaemic CHO. A reason for this lack of association could be due to the reduced statistical power as a result of stratifying an already small sample population into smaller groups (Lenth, 2001). Interestingly, however, these analyses revealed that in the female group and overweight group the associations between glycaemic CHO and metabolic risk factors were similar, this was also the case for male participants and those classed as overweight. Table 17 shows that the males indeed have an average BMI of 22.64 kg/m^2, classing them as a normal weight group, where as the mean BMI of the females was 25.92 kg/m^2 classing them as overweight. It thus appears that caution may need to be taken when comparing these groups to populations from previous research.

Misreporting has been found to impact on associations between diet and health (Rosell et al., 2003) and a small number of studies have assessed associations between glycaemic CHO and health markers with and without misreporters included in analyses. In adults previous research has found that dietary misreporting had

minimal impact on associations between glycaemic CHO (assessed by FFQ) and BMI (Lau et al., 2006), whereas, Sluijs et al (2010) observed that removal of misreporters strengthened associations of GI and GL with type 2 diabetes risk, but the effect was minimal. A study of British children and adolescents (Murakami et al 2013), reported that adjusting for and excluding misreporters did not alter any associations between GI and GL with adiposity. This suggests that associations of glycaemic foods with health may not be hindered by inaccurate food intakes. However, this may only be the case in circumstances where food intakes are merely underestimated, rather than entirely omitted since the latter is more likely to produce false values for GI and subsequently GL. However, Sluijs et al (2010) assessed misreporting by means of the Goldberg equation and did not account for the PAL of the population. Moreover, Murakami et al (2013) assessed misreporting by comparison of EI to estimated energy requirement, however, this was calculated by equations published from the US Dietary Reference Intakes (Institute-of-Medicine, 2005); encompassing estimated PAL categories based on PA diary data and thus errors in PAL estimation may under estimate EE and subsequently undereporting. Burger et al (2010), however, found that a 22g GL increase was positively and significantly associated with an increased total mortality risk (HR 1.42; 1.07-1.88) in normal weight subjects with type 2 diabetes and that this association was augmented by the exclusion of energy misreporters. Individuals with type 2 diabetes have been shown to under report to a greater extent than overweight individuals (Sallé et al., 2006) and thus the impact of excluding misreporters may have been greater in this group compared to previous studies (Sluijs et al., 2010; Murakami et al., 2013). Furthermore, these associations were still observed even though misreporting was assessed using the Goldberg equation and the low activity PAL of 1.55 x BMR which is known to under estimate misreporting (Black, 2000).

In the current study, removing misreporters resulted in altered associations between glycaemic CHO and metabolic risk factors. Using either variant of the Goldberg equation (1.55 PAL and MVPA PAL) to identify and exclude misreporters resulted in attenuation of the positive association observed between GI and WC prior to excluding those who inaccurately report. Interestingly, however, when misreporters were excluded subsequent to being identified by EI:EE and stratified by low and high quantiles of GI (55.88 vs 61.95) and GL (71.40 vs 90.37); HDL was significantly reduced in those individuals who consumed a higher compared to lower GI diet (HDL 1.17 vs 1.31 mmol/L; P= 0.037) and this association was stronger when BMI

was excluded in the model; this is an association that was not seen in earlier analyses. Furthermore, following removal of BMI from the model, TG were borderline significantly raised in those consuming a higher compared to lower glycaemic index diet (TG 0.94 vs 0.63 mmol/L); P= 0.058). This suggests that increased dietary GI is associated with an adverse lipid profile and that these associations may only be observed after removal of misreporters. It is important to consider the characteristics of non-misreporters who were included in this analysis. It was reported in chapter 5 (Table 15) that the valid reporters had a significantly lower BMI and WC than underreporters, and furthermore, when identified by EI:EE, BMI was lower in valid reporters than when identified by EI:BMR (1.55 PAL and MVPA PAL, respectively) (22.67 vs 23.14 and 23.34 kg/m^2) as was WC (77.45 vs 78.43 and 78.62 cm). Thus, the observed associations of GI with HDL and TG were present in valid reporters who were also a more normal weight group. It is not possible to suggest that these associations were only present due to the fact that valid reporters are of a normal weight as it was observed that misreporting and valid reporting occurred in normal and overweight individuals (chapter 5).

Although the associations of glycaemic CHO with lipid profile appear to be consistent with past research, there are inconsistencies as to whether GI or GL is most important. Slyper et al (2005), demonstrated that although GI and GL (assessed by 3 day food diary) were shown to significantly negatively correlate with HDL; GL, and not GI, was shown to be an independent negative predictor of HDL (P= 0.009) as assessed by multiple regression analysis in 32 children and young adults aged 11-25 yrs from the USA (Slyper et al., 2005). Slyper et al (2005) also observed a negative correlation between GL and %fat, like in the present study, however, unlike the present investigation did not control for (other than CHO) macronutrient intake including fat and fibre in their regression analysis. Furthermore, they did not assess and account for pubertal status, nor was misreporting considered. Together, these factors may explain the discrepancy between findings of the two studies. Slyper et al (2005) did not report mean dietary GI or GL intakes making it difficult to draw comparisons between these studies in terms of glycaemic CHO consumption.

Another interesting effect of excluding misreporters in the current study was in relation to associations between BG and GL. There appeared to be a non-significant trend for BG to be lower in those individuals consuming a higher GI and GL diet prior to removal of misreporters, a finding that is comparable to a study investigating

glycaemic CHO and glucose control in adults (Du et al., 2008). However, following removal of misreporters, assessed by EI:EE, the direction of the association between glycaemic CHO and BG appeared to reverse, and a borderline significant increase was observed in those in the higher compared to lower quintile of dietary GL (4.84 vs 4.72 mmol/L; P= 0.056), after removal of BMI from the model.

According to (Livingstone and Black, 2003) under reporters tend to specifically misreport foods that contribute to GI and GL; fruits and vegetables appear to be over reported whilst biscuits, milk products and sugars tend to be under reported. The fact that excluding misreporters resulted in altered associations of health markers with GI suggests that some misreporters are very likely to be entirely excluding certain CHO foods rather than just underestimating their reported intakes. This is because the nature of GI calculation, i.e. not effected by quantity, means that underestimating the amount consumed will have little effect on the diet's overall GI.

It appears that assessing and excluding misreporters based on the novel application of the RT3 accelerometer to estimate EE and comparing the ratio of EI:EE altered associations between glycaemic CHO and metabolic risk factors in a manner that was not observed when misreporting was assessed by either variant of the Goldberg equation (EI:BMR). The fact that EI:EE identified a greater proportion of misreporters compared to the Goldberg equation, and provided a potentially more accurate assessment of misreporting as outlined in chapter 4, could be a reason for the difference seen following removal of misreporters between the techniques. It is important, however, to highlight that EI:EE(RT3) has not been validated against a reference method such as DLW, however, this technique does identify a comparable proportion of misreporters compared to past research, as outlined in chapter 4.

One of the main findings from this investigation is that HDL levels appear to be lower in those individuals consuming a higher GI diet. Reduced levels of HDL can have implications for heart disease CVD and stroke; HDL facilitates the catabolism of surplus cholesterol from peripheral tissues (including arterial walls) (McArdle et al., 2007). This cholesterol is transported as LDL which can contribute to an atherogenic state (Despres and Lemieux, 2006). In these circumstances, fibrous plaques formed with in the lining of arterial cell walls can obstruct blood vessels potentially leading to myocardial infarction and ischemic stroke (Daniels et al., 2008).

The following mechanisms have been proposed, linking increase GI to an adverse lipid profile: The rise in circulating insulin, following consumption of a high GI meal, subsequently results in the uptake of surplus energy (glucose) from the blood stream for use or storage in insulin sensitive tissues such as the liver, adipose tissue and skeletal muscle as well as the inhibition of lipolysis and glycogenolysis (Radulian et al., 2009). This leads to a faster down regulation of BG; often to below fasting level. This hypoglycaemia stimulates the release of hormones such as adrenaline and glucagon to restore euglycaemia and activate fat oxidation to meet the energy demands of the body (Weiss & Gills 2008). Subsequent increased levels of circulating FFAs at rest, however, has been associated with an adverse lipid profile and CVD as well as peripheral insulin resistance in insulin sensitive (non-adipose) tissue (Weiss & Gills 2008). Whereas, following a low GI meal, postprandial rises in gut hormones and insulin are reduced and the prolonged absorption of CHO suppresses the counteregulatory response and FFA release (Jenkins et al., 2002). Moreover, a low GI meal is likely to suppress lipoprotein production, as a lower insulin response has been shown to reduce activity of insulin–stimulated 5-hydroxi-3-methylglutoryl-CoA reductase which is the rate-limiting enzyme in cholesterol synthesis (Rodwell et al., 1976). The link between these factors and the development of the metabolic syndrome has been detailed more extensively in section 2.1.

Further important implications of this study surround the impact of misreporting on relationships between glycaemic CHO and health parameters in this adolescent population. Not only will this have implications for observational research but also intervention studies assessing the impact of altered dietary glycaemic CHO on health outcomes. Some scientists have argued that excluding misreporters will introduce unknown bias into the sample, being that only those participants reporting valid intakes are examined (Gibson, 2005). Alternatively energy intakes can be controlled in an attempt to attenuate the effect of misreporting energy (Posluna et al., 2009). The current study, however, identified that it may be important to exclude misreporters from examination since even after controlling for energy intake via the residuals method, different associations with health parameters following exclusion of misreporters emerged.

This investigation has a number of strengths being that dietary intakes were assessed by weighed food diaries and PA was assessed via the objective method of triaxial accelerometry. Furthermore, although residual confounding cannot be fully

accounted for, analyses were adjusted for a number of known confounding factors and thus may be less likely. Furthermore associations were observed both in the presence and absence of misreporters. Certain limitations should also be noted: As this study is observational the direction and causality of the observed associations cannot be established and thus further work is required in this postpubertal adolescent population to identify the impact of altering dietary glycaemic CHO on metabolic risk factors. Furthermore, the novel technique of identifying EE from the RT3 accelerometer for the assessment of misreporting by EI:EE has not been validated and the limitations of this method have been outlined in chapter 5, however, the fact that this technique resulted in different associations between GI and health markers compared to a widely used yet potentially less accurate method (the Goldberg equation) is of great importance and this requires further work.

In conclusion, this study supports the evidence in adults and a limited number of studies in youths that increased dietary GI is associated with increased odds of having a high WC. Associations between GL and risk factors are less clear, logistic regression analysis revealed that GL appears to be associated with lower odds of having low HDL, hypertriglyceridemia and high clustered metabolic risk. Excluding misreporters from analysis had important implications for these associations. After removal of misreporters by EI:EE(RT3), MANCOVA revealed that although GL was not significantly associated with any risk factors, a borderline significant positive association with BG emerged that was not present in prior analyses. Increased GI (moderate vs low GI) was significantly associated with reduced HDL and increased TG (borderline significant) after removal of misreporters. These findings have implications for future dietary recommendations for adolescents who are an age group that are making more autonomous lifestyle decisions regarding their eating behaviours (Ebbeling et al., 2003) and a group in which metabolic abnormalities have been shown to track into adulthood (Camhi and Katzmarzyk, 2010). Further intervention analysis is required to assess causality and direction of these associations.

Chapter Seven: Study Four

Associations between physical activity and fitness with the metabolic syndrome in postpubertal UK adolescents: the impact of different PA thresholds.

7.0 Introduction

PA (Wareham et al., 2005) and CRF (Blair et al., 2001b) are well regarded as important determinants of metabolic health. This has been evidenced in adults and youths, however, there appear to be inconsistencies as to whether PA or CRF is more strongly associated with health outcomes. Lee et al (2010) assessed metabolic risk factors and the prevalence of the metabolic syndrome across levels of CRF (treadmill VO_2max test) in 909 young (24yrs) Korean males. The authors identified that having a low and moderate, versus high CRF was independently associated with an increased likelihood of having the metabolic syndrome following adjustment for age, smoking status and body composition. Karelis et al (2008) identified in a group of overweight and obese sedentary women (57.7 ± 4.8 yrs) that those classed as having the metabolic syndrome had significantly lower CRF and PA energy expenditure compared to those without the metabolic syndrome; however, logistic regression revealed that PA energy expenditure was independently associated with increased odds of having the metabolic syndrome. Although Karelis et al (2008) estimated PA energy expenditure from the reference technique of DLW, other research has observed that the intensity of PA undertaken may be more influential on health outcomes than total PA. Studies of adults provide evidence that being more SED may have a stronger negative impact on health markers than MVPA (Healy et al., 2008b, Healy et al., 2011), whereas evidence in youths suggest that MVPA is a more important predictor of metabolic risk factors (Ekelund et al., 2012). A widely utilised PA assessment method is accelerometry which measures acceleration of the body in one (uniaxial), two (biaxial) or three (triaxial) planes (Rowlands, 2007). Arbitrary 'activity counts' derived from accelerometry data require recoding in order to provide biological meaning. This is achieved through the use of PA cut-points for different PA intensities that have been derived according to different EE validation studies (Rowlands et al., 2004b, Vanhelst et al., 2010a, Chu et al., 2007b). Employing such cut-points allows for the

exploration of associations between health markers and total PA or accumulated minutes of PA at various intensities: time spent SED; in LPA; in MPA; in VPA and in MVPA to be assessed (Ekelund et al., 2007c, Bailey et al., 2012b).

Accelerometry has been used to explore associations between PA and health markers in youths; Ekelund et al (2007) investigated the independent associations of PA (measured via 3-4 day accelerometry) and CRF (via a maximal cycle ergometer test) with metabolic risk factors in 1709 9-10 and 15-16 yr olds. Both PA and CRF were separately and independently associated with individual and clustered metabolic risk factors, a potential confounding limitation of this study is that CRF was estimated from HR and oxygen consumption was not measured (Rowlands et al., 1993). Moreover, in a large sample (20,871) of pooled data in 4-18 year old male and females; MVPA was independently negatively associated with WC and SBP and positively with HDL, when controlled for SED time (Ekelund et al., 2012). However, SED time was not associated with cardiometabolic risk factors when accounting for time spent in MVPA. Subsequent to the evidence that MVPA is an important determinant of health outcomes, the UK government recommend that at least 30 and 60 minutes of MVPA per day should be engaged in by adults and youths, on 5 and 7 days per week, respectively (DOH, 2011). A previous government report (DOH, 2008) demonstrated that only 24% of girls and 32% of boys were meeting the minimum 60 minute MVPA per day recommendations (DOH, 2008). In contrast to evidence that MVPA is most beneficial to health, in adults, SED time has been observed as an independent positive predictor of WC, TG insulin resistance, CRP, BP, clustered metabolic risk score and negatively of HDL when analyses were adjusted for MVPA (Healy et al., 2008b, Healy et al., 2011). Thus, there appear to be contrasting findings regarding which components of PA might be most beneficial to health. This evidence would suggest that recommendations based on associations of MVPA with health parameters may be flawed and thus better understanding of these associations is very important.

A potential confounding factor concerning these studies is the lack of standardised procedures for the reduction of raw accelerometry data. Previous research has defined non-wear time as 10 consecutive minutes of zero counts (Bailey et al., 2013 and Husset et al., 2007) whereas others have removed non wear time defined as 60 minutes of consecutive zero counts (Ekelund et al., 2012). This will impact upon the number of participants classed as providing acceptable PA data and therefore the

number of participants assessed. Furthermore, this is likely to bias the sample towards individuals engaging in physical activity and exclude those who spend less time physically active (Denton et al., 2013). Additionally there are various thresholds which are inconsistently utilised in the literature for calculating PA intensities (Ekelund et al, 2011). A review of trends in PA levels as assessed by accelerometry demonstrated that the prevalence of youths (9-19 year old males and females) achieving the recommended 60 minutes per day of MVPA ranged between 1% and 100%. There are vast inconsistencies amongst studies, which have been predominantly attributed to differences in intensity thresholds (Ekelund et al., 2011).

There are a range of accelerometers available to objectively assess PA levels that feature in the literature. The most widely used monitors include the omnidirectional Actical and Actiwatch (Rowlands, 2007) as well as the ActiGraph range, which features the triaxial and water-proof GT3x (Robusto and Trost, 2012) and the triaxial Stayhealthy RT3 (Rowlands, 2007). Triaxial accelerometry has been utilised in past studies to assess free-living PA and its relationship with health in children and adolescents using the RT3 (Stayhealthy, Inc.) accelerometer. For instance, (Krekoukia et al., 2007) assessed associations of PA (measured via RT3 over 4 consecutive days) and CRF (estimated from heart rate using the physical working capacity test at 170 bpm (PWC170) with insulin resistance, lipid profile and inflammation in 110 lean and obese 9-11 year old children. CRF was negatively associated with insulin resistance but this disappeared following adjustment for age, sex and fat mass (FM), however total daily PA (negatively) and WC (positively) explained 49% ($P< 0.01$) of the variance in insulin resistance (HOMA-IR). The use of PWC170 to estimate CRF is a flaw of this study; PWC170 has been shown to have a wide variability with a 10-15% error in children (Rowland et al., 1993) and thus the CRF of the these youths may not have been accurately represented (Krekoukia et al., 2007). In contrast to this, in a sample of 100 10-14 year old children, Bailey et al (2012) identified that clustered metabolic risk was significantly lower in those identified as having a high CRF as opposed to low CRF, following a maximal cycle ergometer test that predicts oxygen uptake from a formula based on work rate. High and low fitness were defined as >37.0 and >42.1 mL/kg/min for girls and boys, respectively, based on evidence that these values are associated with a reduced metabolic risk in children examined in the EYHS (Ruiz et al., 2007). PA subcomponent thresholds were derived for 7 day RT3 accelerometry from the cut-points of Rowlands et al (2004) (see table 27); clustered risk was not associated

with any PA subcomponents. Unlike previous studies utilising RT3 data in youths (Hussey et al., 2007, Krekoukia et al., 2007), Bailey et al., (2012) collected accelerometry data over 7 consecutive days; participants were excluded from analysis if they failed to wear the accelerometer for 3 or more days. The authors explain that a minimum daily wear time of 9 hours for weekdays (Mattocks et al., 2008) and 8 hours for weekend days was required (Rowlands et al., 2008). This was based on evidence that this wear time criteria is sufficient for assessing habitual PA in youngsters. Many authors neglect to note this information, making comparison across studies difficult.

Table 27. PA category thresholds of Rowlands and Chu

Variable	Rowlands	Chu
SED (cpm)	<288	<420
LPA (cpm)	288-969	420-1859
MPA (cpm)	970-2,332	1860-4109
VPA (cpm)	≥2,333	≥4110

CPM, counts per minute.

Rowlands et al (2004) produced cut-points for setting thresholds of activity intensities (SED, LPA, MPA, VPA) whilst validating the RT3 in 19 boys (mean age 9 ±1 years), which have since been used to assess PA levels in a number of studies of young people (Bailey et al., 2013, Rowlands, 2007, Bailey et al., 2012a). More recently, however, different cut-points have been developed using the RT3 accelerometer which have also been validated against oxygen consumption in 35 8-12 year old Chinese children (Chu et al., 2007), See table 27. Chu et al (2007) derived cut-points from receiver operator curves (ROC) analysis which gave sensitivity and specificity values of 72-98% indicating that the intensity thresholds gave an accurate distinction between intensity categories. The difference between these validated cut-points (table 27) could affect the proportion of individuals classed within PA categories and subsequently any associations with health markers. For instance, the Chu activity count cut-point for MPA is 890 counts higher than that of Rowlands, and thus time spent in MVPA would be lower when utilising this cut-point. Only one study has compared the use of different PA cut-points and this was conducted in a group children; this study employed a sample of 104 10-14 year olds from the UK and collected PA data by means of 7 day accelerometry (RT3) (Bailey et al., 2013). The authors observed a greater mean time spent SED (+

40.2 and 67.7 mins) and a lower mean time in MVPA (- 65.1 and 50.6 mins) as assessed by the Chu compared to Rowlands cut-points in boys and girls, respectively. Associations of PA with CRF and cardiometabolic risk factors were different depending on the cut-points utilised; BF% was significantly positively associated with MPA, but only in girls when using the Chu thresholds. SED time was significantly and positively associated with TG using the Rowlands cut-points whereas DBP was negatively associated with MPA but only when utilising the Chu cut-points.

As outlined above, there is a lack of uniformity in terms of methodological protocol used across studies utilising accelerometry and CRF data. This may be attributable to the differences observed between these studies with regards to associations of PA and CRF with metabolic health in youths. There is a need for consensus when utilising accelerometer thresholds for determining PA levels at different intensities to provide more accurate data regarding physical activity engagement of youths in relation to recommended guidelines. This is of particular importance as current recommendations are defined in terms of MVPA levels and are based on the association with health outcomes (DOH, 2011). Different cut-points for determining PA have not been compared in adolescents, furthermore, few studies control for nutritional intake when analysing associations of PA, CRF and health markers; this may have implications for associations of PA and health as outlined in chapter 1.

Therefore the current investigation aims to assess the associations of CRF and PA with metabolic risk factors in a sample of UK adolescents whilst controlling of nutritional intake. Additionally the study will compare the associations of metabolic health parameters with PA as determined by the thresholds of Rowlands et al (2004) versus Chu et al (2007).

7.1 Methodology

Participants

Of the 105 participants recruited as part of the SIRENS and CROSSROADS studies as outline previously (in chapter 4), 76 participants met the wear time criteria for sufficient physical activity data and of those participants, a total of 72 participants also completed 3 day weighed food diaries.

Experimental Design

Data collection was conducted between 7 and 10 am as participants were required to have fasted from 9am the previous evening. See section 4.1 for further details. Associations of PA with metabolic health risk factors were assessed. Two thresholds for determining time spent in different PA subcategories (SED, LPA, MPA, VPA and MVPA) were compared and the impact of using the different thresholds on PA-health relationships explored.

Measurements

Age, ethnicity and SES

Age (to two decimal places), ethnicity (white/non-white) and SES (IMD scores) were measured as explained previously (Section 4.1)

Physical Activity

Participants were issued with a RT3 triaxial accelerometer which was worn at the hip for 7 consecutive days; see accelerometer protocols as outlined previously (section 3.9). Time spent in PA intensity categories was calculated by applying two sets of thresholds that have been validated for use in youths, and for RT3 accelerometer: the (Rowlands., 2004) thresholds, and the thresholds of (Chu et al., 2007). The following different intensity cut-points were utilised for calculating engagement in individual PA categories according to CPM registered by the RT3 as displayed previously in the table 27.

Cardiorespiratory fitness

Peak volume of oxygen consumed at maximal exercise was measured using a portable online gas analysis system (Cortex Metamax 3B) during an incremental cycle ergometer test as outlined previously (section 3.8).

Resting systolic and diastolic blood pressure

Resting SBP and DBP were recorded as described in section 3.8

Fasting finger prick blood samples

Fasting blood samples were obtained from each participant and then immediately analysed for TG, HDL and BG as described in section 3.5.2.

Metabolic syndrome risk factors

Metabolic risk was quantified by calculating the prevalence of individual IDF metabolic syndrome risk factors and determining a clustered metabolic risk score as outlined in chapter 6 section 6.1.

Statistical Analysis

Statistical analyses were conducted using SPSS, descriptive data are presented as mean and standard deviation. The following variables were non-normally distributed and were subsequently log transformed to improve their distribution (VPA, BMI, BF%, WC, PRO, fat, CHO). Adiposity variables, BMI and BF% were converted to *Z*-scores based on population means for the age group (Culberson et al., 2009), however a raw WC was utilised as the population reference data does not address the upper age range (17-19 yrs) of the population investigated. Differences between sex and weight status were assessed by One-way ANOVA. Partial Pearson's correlation analysis was used to assess the relationship between time spent in different PA categories for both thresholds and were adjusted for sex, age and total PA. Partial correlations were also utilised to compare the relationship between PA and metabolic risk variables adjusted for sex, age, SES, total PA and *z*BMI. MANCOVA were conducted to assess the differences in metabolic risk variables between upper and lower quantiles of time spent in each PA category adjusted for the following covariates: sex, age, SES ethnicity, SES, zBMI, total PA and dietary variables (residual intakes of fat, PRO and CHO). There were no covariates sharing colinearity assessed by multiple linear regression analysis. The assumption of homogeneity of regression slopes, however, was violated by the covariate variables

sex and SES when their interaction with the dependant variables was assessed using a customised MANCOVA, therefore the MANCOVA model was rerun with sex and SES excluded as covariates to ensure assumptions were not violated. Binary logistic regression analysis was employed to assess the associations between PA variables as continuous variables with metabolic risk factors as defined by the IDF criteria of the metabolic syndrome for adolescents (Alberti et al., 2006) and high clustered metabolic risk score as dichotomous variables. Logistic regression was also adjusted for age, ethnicity, SES, zBMI, total PA and dietary variables (residual intakes of fat, PRO and CHO), however, SES and sex were excluded as covariates so that logistic regression and MANCOVA models were comparable. MANCOVA and logistic regression analysis were utilised using the thresholds of Rowlands and Chu separately so that the impact of using the different thresholds could be compared. For all analyses the significance level was set at P <0.05.

7.2 Results

Table 28 displays participant characteristics for the group (total) and males and females, separately. As a group, mean age was 17.35 yrs and according to mean BMI the group are classed as being of normal weight status (24.49 kg/m^2). Males were significantly taller and had a greater FFM than females. Females had a significantly greater BF% and BMI than males.

Table 28. Participant characteristics, mean and standard deviation

	Total n=72	**Male** n=45	**Female** n=27	**P**
Age (y)	17.35 (1.49)	14.48 (1.27)	17.34 (1.50)	0.290
Height (cm)	172.05 (9.77)	177.68 (6.79)	162.99 (6.44)	**0.000**
Weight (kg)	72.77 (16.97)	71.81 (16.189)	74.31 (18.36)	0.544
BMI kg/m^2	24.49 (6.03)	22.71 (4.79)	27.34 (6.78)	**0.001**
BF (%)	23.30 (10.89)	17.98 (7.49)	31.94 (10.02)	**0.000**
FFM (kg)	51.78 (11.60)	56.83 (8.72)	43.45 (11.01)	**0.000**
WC (cm)	80.33 (12.20)	79.44 (11.64)	81.76 (13.08)	0.346
SES (IMD)	14.54 (11.00)	13.86 (11.43)	15.66 (10.30)	0.435

P significant at <0.05 for comparison of males and females

As shown in table 29, below, time spent in MPA, VPA and MVPA were significantly higher in males compared to females, when utilising either threshold, additionally SED time was significantly greater in females versus males, but only when assessed by the Chu thresholds. When assessed using Rowlands thresholds, the average total accumulated time of MVPA was above the 60 minute guideline (65.60 mins); 53% of participants achieved >60 mins MVPA. Males achieved a mean time of 73.14 mins in MVPA (63.8% >60 mins MVPA) whilst females undertook 53.38 mins per day (37.9% >60 mins MVPA). When assessed by Chu, however, the total time accumulated of MVPA was considerably lower than 60 minutes (27.20 mins); only 6.6% of all participants achieve >60 mins MVPA, Males achieve 32.45 mins (10.6% >60 mins MVPA) whilst females accumulate just 18.55 mins of MVPA per day (0% of females achieve >60 mins MVPA).

Table 29. Time accumulated in each PA category using the PA thresholds of Rowlands and Chu.

	Rowlands			Chu		
	Total n= 72	**Males n= 45**	**Females n= 27**	**Total n= 72**	**Males n= 45**	**Females n= 27**
SED (mins)	517.67(106.93)	504.79 (111.73)	538.54 (96.88)	560.17 (102.93)	540.00 (102.13)	**592.85 (97.22)***
LPA (mins)	134.51 (57.77)	128.43 (58.47)	144.37 (56.22)	129.25 (55.06)	130.98 (57.87)	126.44 (51.04)
MPA (mins)	50.42 (23.96)	54.93 (25.75)	**43.11 (18.95)***	24.05 (15.51)	27.80 (16.23)	**17.97 (12.25)***
VPA (mins)	14.48 (13.01)	17.02 (14.84)	**10.35 (7.94)***	3.46 (14.15)	5.06 (17.86)	**0.85 (1.02)***
MVPA (mins)	65.60 (36.13)	73.14 (40.34)	**53.38 (23.95)***	27.20 (23.84)	32.54 (27.45)	**18.55 (12.59)***
% >60 mins MVPA	**53%**	**63.8%**	**37.9%**	**6.6%**	**10.6%**	**0%**

Means (±SD); *, significantly different from males (P=<0.05).

Table 30, displays partial correlations between PA components. Regardless of threshold used SED time was negatively correlated with LPA, MPA, VPA and MVPA. Other than Chu-LPA, which was not significantly correlated with Chu-VPA, all other PA components were positively, significantly correlated with each other

Table 30. Partial correlations of PA components respectively assessed by the Rowlands et al (2004) 2004 and Chu et al (2007) thresholds.

	SED		LPA		MPA		VPA		MVPA	
N=72	r	P	r	P	r	P	r	P	r	P
Rowlands										
SED		.	**-0.936**	**0.000**	**-0.823**	**0.000**	**-0.470**	**0.000**	**-0.786**	**0.000**
LPA	**-0.936**	**0.000**		.	**0.625**	**0.000**	**0.245**	**0.039**	**0.549**	**0.000**
MPA	**-0.823**	**0.000**	**0.625**	**0.000**		.	**0.476**	**0.000**	**0.842**	**0.000**
VPA	**-0.470**	**0.000**	**0.245**	**0.039**	**0.476**	**0.000**		.	**0.696**	**0.000**
MVPA	**-0.786**	**0.000**	**0.549**	**0.000**	**0.842**	**0.000**	**0.696**	**0.000**		.
Chu										
SED		.	**-0.929**	**0.000**	**-0.575**	**0.000**	**-0.275**	**0.020**	**-0.502**	**0.000**
LPA	**-0.929**	**0.000**		.	**0.337**	**0.004**	0.015	0.902	**0.277**	**0.019**
MPA	**-0.575**	**0.000**	**0.337**	**0.004**		.	**0.463**	**0.000**	**0.850**	**0.000**
VPA	**-0.275**	**0.020**	0.015	0.902	**0.463**	**0.000**		.	**0.536**	**0.000**
MVPA	**-0.502**	**0.000**	**0.277**	**0.019**	**0.850**	**0.000**	**0.536**	**0.000**		.

Correlations adjusted for: age, sex and total PA.

Table 31 shows that partial correlations revealed that time spent in LPA was significantly and positively associated with CRF but only when PA was assessed by the Rowlands thresholds. SBP and DBP were significantly and positively associated with time spent SED as assessed by both thresholds, associations with SBP were stronger when PA was assessed by the Chu thresholds. SBP and DBP were negatively associated with LPA and MVPA and clustered risk scores were also significantly and negatively associated with LPA when utilising the Rowlands thresholds. When assessed by the Chu thresholds, LPA was negatively associated with SBP and DBP; DBP was also significantly, negatively associated with VPA and MVPA

Table 31. Partial correlations of PA, as assessed by the Rowlands et al (2004) and Chu et al (2007) thresholds, with CRF and metabolic risk factors

	CRF (VO_2 peak)		WC		HDL		TG		BG		SBP		DBP		Crisk	
N=72	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Rowlands																
SED	-.020	0.865	-0.086	0.479	-0.036	0.772	-0.114	0.352	0.122	0.316	**0.300**	**0.012***	**0.328**	**0.006****	0.133	0.276
LPA	**.234**	**0.050***	0.032	0.794	0.086	0.480	0.015	0.902	-0.192	0.115	**-0.283**	**0.019***	**-0.348**	**0.003****	**-0.261**	**0.030****
MPA	-.005	0.967	0.167	0.166	0.015	0.901	0.216	0.074	0.027	0.826	-0.227	0.061	-0.177	0.146	0.077	0.530
VPA	-.087	0.472	-0.09	0.458	-0.107	0.382	0.08	0.515	-0.017	0.890	-0.109	0.373	-0.169	0.164	0.021	0.867
MVPA	-.116	0.334	-0.009	0.944	-0.081	0.510	-0.113	0.356	0.106	0.385	**-0.331**	**0.005****	**-0.354**	**0.003****	0.139	0.256
Chu																
SED	.031	0.799	-0.117	0.326	-0.081	0.510	-0.113	0.356	0.106	0.385	**0.331**	**0.005****	**0.354**	**0.003****	0.139	0.256
LPA	.156	0.195	0.093	0.437	0.125	0.305	0.125	0.308	-0.171	0.161	**-0.326**	**0.006****	**-0.356**	**0.003****	-0.188	0.121
MPA	-.153	0.204	0.044	0.716	-0.038	0.755	0.062	0.614	-0.015	0.903	-0.214	0.078	-0.164	0.179	0.015	0.906
VPA	-.089	0.461	-0.039	0.747	-0.038	0.754	-0.03	0.804	-0.079	0.521	-0.036	0.766	**-0.261**	**0.030***	-0.157	0.198
MVPA	-.054	0.655	-0.002	0.986	0.002	0.987	0.031	0.800	-0.017	0.890	-0.175	0.150	**-0.243**	**0.045***	-0.063	0.605

Correlations adjusted for: Sex, SES, age and *z*BMI; *, P value <0.05; ** P <0.01.

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Table 32. All participants: MANCOVA-Metabolic risk factors (mean and standard deviation) across 2 quantiles of time in respective PA categories assessed by the Rowlands et al (2004) and Chu et al (2007) thresholds.

N=72	Rowlands SED T				Chu SED T			
	1 (436.48[a])	2 (598.85[a])	F	P	1 (482.72[a])	2 (637.61[a])	F	P
HDL (mmol.L)	1.15 (0.37)	1.13 (0.27)	0.12	0.728	1.15 (0.37)	1.12 (0.27)	0.00	0.975
TG (mmol.L)	1.02 (0.92)	0.80 (0.33)	1.52	0.222	1.02 (0.92)	0.80 (0.33)	1.40	0.241
WC (cm)†	81.75 (14.07)	81.04 (11.74)	1.05	0.310	82.17 (13.80)	80.63 (12.02)	0.28	0.599
BG (mmol.L)	4.77 (0.48)	4.87 (0.55)	1.19	0.280	4.78 (0.47)	4.85 (0.56)	0.52	0.473
SBP (mmHg)	114.87 (13.92)	116.42 (9.42)	1.51	0.224	115.61 (13.41)	115.68 (10.20)	0.26	0.610
DBP (mmHg)	**70.24 (8.94)**	**73.88 (6.97)**	**6.50**	**0.013**	**70.46 (8.84)**	**73.67 (7.20)**	**5.38**	**0.024**
†			**7.89**	**0.007**			**5.90**	**0.018**
Crisk	-0.05 (3.83)	0.47 (2.43)	1.16	0.286	0.03 (3.74)	0.38 (2.58)	0.77	0.382

	Rowlands LPA T				Chu LPA T			
	1 (90.74[a])	2 (178.28[a])	F	P	1 (85.45[a])	2 (173.04[a])	F	P
HDL (mmol.L)	1.08 (0.28)	1.19 (0.35)	2.24	0.139	**1.08 (0.24)**	**1.19 (0.38)**	**5.26**	**0.025**
TG (mmol.L)	0.79 (0.26)	1.03 (0.94)	1.81	0.183	0.76 (0.26)	1.06 (0.93)	2.63	0.110
WC (cm)†	82.36 (13.08)	80.44 (12.73)	0.63	0.430	80.16 (10.06)	82.64 (15.23)	0.26	0.615
BG (mmol.L)	**4.94 (0.54)**	**4.69 (0.46)**	**4.62**	**0.035**	4.84 (0.55)	4.80 (0.48)	0.54	0.466
SBP (mmHg)	**119.29 (12.16)**	**112.00 (10.42)**	**7.76**	**0.007**	**117.64 (11.91)**	**113.64 (11.57)**	**4.03**	**0.049**
DBP (mmHg)	73.26 (7.21)	70.86 (8.96)	2.17	0.146	73.32 (7.05)	70.80 (9.07)	3.50	0.066
Crisk	0.58 (2.95)	-0.15 (3.41)	3.64	0.061	0.15 (2.71)	0.27 (3.65)	0.92	0.340

	Rowlands MVPA T				Chu MVPA T			
	1 (39.95[a])	2 (91.24[a])	F	P	1 (13.00[a])	2 (41.40[a])	F	P
HDL (mmol.L)	1.13 (0.25)	1.15 (0.38)	0.42	0.518	1.12 (0.25)	1.15 (0.38)	0.03	0.854
TG (mmol.L)	0.91 (0.85)	0.91 (0.51)	0.25	0.618	0.98 (0.87)	0.84 (0.47)	0.21	0.648
WC (cm)†	80.83 (11.74)	81.97 (14.06)	0.46	0.501	82.54 (12.82)	80.26 (13.01)	0.13	0.724
BG (mmol.L)	4.73 (0.46)	4.90 (0.56)	1.16	0.285	4.80 (0.48)	4.84 (0.55)	0.00	0.991
SBP (mmHg)	115.05 (11.75)	116.24 (12.05)	0.00	0.969	114.08 (11.68)	117.21 (11.93)	1.69	0.199
DBP (mmHg)	**73.76 (8.25)**	**70.37 (7.83)**	**4.20**	**0.044**	73.32 (8.56)	70.80 (7.67)	0.52	0.474
Crisk	0.27 (2.91)	0.14 (3.49)	0.08	0.779	0.65 (3.32)	-0.25 (3.02)	0.01	0.907

1 and 2 represent the lower and upper quantile, respectively; analysis adjusted for: sex, age, SES, ethnicity, SES, zBMI, total PA and residual intakes of fat, PRO and CHO; †, model is not adjusted for BMI; [a], minutes

Table 33. Male participants: MANCOVA-Metabolic risk factors (mean and standard deviation) across 2 quantiles of time in respective PA categories assessed by the Rowlands and Chu thresholds.

N=72	Rowlands SED T				Chu SED T			
	1 (417.44 [a])	2 (588.50 [a])	F	P	1 (464.26 [a])	2 (619.03 [a])	F	P
HDL	1.10 (0.35)	1.15 (0.27)	0.53	0.470	1.10 (0.34)	1.15 (1.15)	0.71	0.406
TG	0.93 (0.55)	0.72 (0.26)	3.00	0.091	**0.93 (0.53)**	**0.72 (0.26)**	**4.23**	**0.047**
WC†	4.85 (0.45)	4.94 (0.53)	0.03	0.857	83.16 (14.73)	76.12 (7.56)	0.10	0.749
BG	83.14 (15.06)	76.43 (7.54)	0.02	0.900	4.84 (0.45)	4.95 (0.54)	0.05	0.832
SBP	117.28 (13.22)	119.83 (10.35)	3.29	0.078	118.60 (14.56)	118.57 (8.46)	0.52	0.474
DBP	**69.07 (8.75)**	**72.21 (7.65)**	**5.85**	**0.020**	69.75 (9.19)	71.63 (7.27)	2.37	0.132
Crisk	-0.16 (3.84)	-0.39 (2.14)	0.22	0.644	-0.06 (3.79)	-0.50 (2.12)	0.02	0.903

	Rowlands LPA T				Chu LPA T			
	1 (84.82 [a])	2 (170.22 [a])	F	P	1 (85.14 [a])	2 (174.91 [a])	F	P
HDL	1.08 (0.19)	1.17 (0.39)	1.19	0.281	**1.07 (0.24)**	**1.18 (0.36)**	**5.99**	**0.019**
TG	0.76 (0.25)	0.89 (0.56)	1.62	0.211	0.74 (0.24)	0.91 (0.55)	1.67	0.204
WC†	4.84 (0.34)	4.95 (0.65)	1.51	0.227	79.30 (7.58)	80.11 (15.54)	0.00	0.971
BG	82.81 (13.87)	76.75 (9.70)	0.06	0.813	4.89 (0.54)	4.90 (0.45)	0.02	0.887
SBP	**122.20 (11.41)**	**115.13 (11.30)**	**8.50**	**0.006**	**122.43 (11.81)**	**114.90 (10.73)**	**7.17**	**0.011**
†			**9.36**	**0.004**			**5.40**	**0.025**
DBP	72.41 (7.42)	69.00 (8.84)	1.59	0.215	72.24 (6.86)	69.17 (9.33)	1.89	0.177
Crisk	0.20 (2.72)	-0.76 (3.33)	0.26	0.613	-0.14 (2.00)	-0.43 (3.86)	0.64	0.430

	Rowlands MPA T				Chu MPA T			
	1 (34.79 [a])	2 (74.23 [a])	F	P	1 (15.91 [a])	2 (39.20 [a])	F	P
HDL	1.13 (0.27)	1.12 (0.35)	0.69	0.413	1.14 (0.27)	1.11 (0.34)	0.32	0.576
TG	0.72 (0.22)	0.92 (0.56)	1.51	0.226	0.86 (0.37)	0.79 (0.49)	3.11	0.086
WC†	4.90 (7.80)	4.89 (0.43)	0.22	0.639	77.36 (8.51)	81.98 (14.72)	2.32	0.136
BG	78.18 (0.55)	81.18 (15.30)	0.02	0.883	4.87 (0.36)	4.92 (0.60)	0.00	0.953
SBP	**121.98 (12.23)**	**115.33 (10.58)**	**4.87**	**0.033**	119.48 (11.73)	117.73 (12.02)	1.69	0.201
DBP	72.48 (7.83)	68.94 (8.47)	2.67	0.111	71.35 (8.42)	70.02 (8.25)	0.80	0.378
Crisk	-0.28 (1.91)	-0.28 (3.91)	0.28	0.600	-0.23 (2.65)	-0.33 (3.45)	1.26	0.269

1 and 2 represent the lower and upper quantile, respectively; analysis adjusted for: sex, age, SES, ethnicity, SES, zBMI, total PA and residual intakes of fat, PRO and CHO; †, model is not adjusted for BMI; [a], minutes

Table 34. Female participants: MANCOVA-Metabolic risk factors (mean and standard deviation) across 2 quantiles of time in respective PA categories assessed by the Rowlands and Chu thresholds.

N=72	Rowlands				Chu			
	MVPA T				MVPA T			
	1 (33.26[a])	2 (72.17[a])	F	P	1 (7.85 [a])	2 (28.53 [a])	F	P
HDL (mmol.L)	1.08 (0.24)	1.22 (0.42)	3.66	0.071	1.12 (0.27)	1.13 (0.35)	0.75	0.393
TG (mmol.L)	0.91 (0.31)	1.18 (1.34)	0.01	0.933	0.86 (0.38)	0.80 (0.49)	1.93	0.173
WC (cm)†	87.73 (14.28)	80.76 (12.70)	1.10	0.308	77.84 (8.50)	81.51 (14.86)	0.94	0.337
BG (mmol.L)	4.69 (0.53)	4.69 (0.55)	0.00	0.974	4.86 (0.36)	4.93 (0.60)	0.04	0.842
SBP (mmHg)	113.25 (7.42)	108.67 (12.50)	0.65	0.432	119.61 (11.72)	117.60 (12.00)	0.75	0.391
DBP (mmHg)	**78.11 (6.17)**	**70.79 (7.25)**	**5.44**	**0.031**	72.04 (8.15)	69.35 (8.35)	1.60	0.214
Crisk	1.88 (3.14)	0.17 (3.35)	2.72	0.116	-0.07 (2.76)	-0.50 (3.35)	1.33	0.256

1 and 2 represent the lower and upper quantile, respectively; analysis adjusted for: sex, age, SES, ethnicity, SES, zBMI, total PA and residual intakes of fat, PRO and CHO; †, model is not adjusted for BMI; [a], minutes

When participants were assessed as a whole group (table 32), using both thresholds resulted in a significantly higher mean DBP (P= 0.024 and 0.013, Rowlands and Chu respectively) in the higher SED time group. When assessed by the Chu threshold, HDL (P= 0.025) was significantly increased and SBP reduced (p= 0.049) in the high LPA group compared the lower LPA group. MPA, however was not associated with any cardio-metabolic risk factors. When assessed by the Chu threshold the higher VPA group had a significantly lower mean DBP (P= 0.031), no associations with VPA were significant when assessed by Rowlands (not presented). Accumulating <60 mins compared to >60mins MVPA was not associated with any risk factors, however when stratified into a lower vs higher group of MVPA and assessed by Rowlands DBP was significantly lower (P=0.044) in the higher MVPA group.

In males when assessed by the Rowlands thresholds (table 33) DBP was significantly greater (P= 0.02) in the higher SED time group, however, when assessed by the Chu threshold mean TG levels were significantly lower (P= 0.047) in the higher SED time group. In the higher LPA time group SBP was significantly lower (P= 0.004 and 0.025) when PA was assessed by both Rowlands and Chu thresholds, respectively; associations were strengthened when BMI was excluded from the model. Additionally, HDL was significantly higher (p= 0.019) in the high LPA group when assessed by the Chu thresholds. SBP was significantly lower in the higher MPA group when employing the Rowlands thresholds (P= 0.033).

In females only (table 34) MVPA was associated with any cardio-metabolic risk factors; DBP was significantly lower (P= 0.031) in the higher MVPA group when PA was assessed using the Rowlands cut-points. Results for other levels of PA were non-significant in the female group and thus not presented. Achieving greater than 60 minutes of MVPA was not associated with any metabolic risk factors in any group.

Table 35. All participants: Associations between CRF and metabolic risk factors

	VO2 Peak				CRF risk			
N=72	1	2	F	P	Low risk[a]	High risk[a]	F	P
HDL	1.09 (0.36)	1.18 (0.29)	0.31	0.904	1.19 (0.36)	1.05 (0.37)	0.59	0.455
TG	1.11 (0.94)	0.74 (0.27)	1.53	0.221	**0.75 (0.30)**	**1.17 (1.01)**	**4.83**	**0.032**
†			**8.68**	**0.004**			**15.17**	**0.000**
WC†	**88.19 (14.49)**	**75.25 (7.23)**	**12.93**	**0.001**	**75.34 (7.89)**	**90.65 (13.82)**	**23.63**	**0.000**
BG	4.81 (0.54)	4.85 (0.50)	0.01	0.904	4.81 (0.52)	4.86 (0.51)	0.89	0.348
SBP	**114.24 (12.10)**	**116.95 (11.88)**	**8.48**	**0.005**	115.42 (12.06)	115.93 (12.07)	2.40	0.127
DBP	73.67 (7.02)	70.13 (8.90)	0.07	0.785	**70.12 (8.43)**	**74.40 (7.20)**	0.00	0.962
†			3.04	0.086			**4.44**	**0.037**
Crisk	1.63 (3.52)	-1.05 (2.31)	0.04	0.840	**-1.12 (2.25)**	**2.29 (3.43)**	**4.07**	**0.048**
†			**13.90**	**0.000**			**30.91**	**0.000**

1 and 2 represent the lower and upper quantile, respectively; [a], low risk defined as CRF ≥37.0 and ≥42.1 mL/kg/min; high risk defined as <37.0 and <42.1 mL/kg/min, for females and males, respectively; Adjusted for: sex, age, SES, ethnicity, SES, zBMI, total PA and residual intakes of fat, PRO and CHO; †, model is not adjusted for BMI

Increasing CRF was associated with a significantly increased SBP but reduced WC, TG and both clustered risk scores were also reduced but only when BMI was excluded from model. When CRF was expressed as high or low risk, those in the CRF risk group had significantly higher TG, WC DBP and clustered risk scores (Table 35).

Table 36. Male participants: Associations between CRF and metabolic risk factors

	VO2 Peak				CRF risk			
N=72	1	2	F	P	Low risk[a]	High risk[a]	F	P
HDL	1.04 (0.27)	1.21 (0.32)	0.01	0.945	1.18 (0.29)	0.93 (0.30)	0.06	0.813
TG	**0.98 (0.54)**	**0.66 (0.18)**	0.93	0.341	**0.74 (0.28)**	**1.11 (0.70)**	1.45	0.236
†			**5.56**	**0.023**			**8.28**	**0.006**
WC†	**85.13 (13.91)**	**74.06 (6.52)**	**7.22**	**0.011**	**75.98 (6.68)**	**91.93 (17.68)**	**14.56**	**0.000**
BG	4.85 (0.39)	4.94 (0.58)	1.45	0.236	4.87 (0.50)	4.99 (0.46)	2.19	0.148
SBP	120.12 (10.70)	116.98 (12.85)	0.01	0.909	117.72 (11.31)	121.41 (13.40)	0.15	0.701
DBP	72.08 (8.12)	69.20 (8.34)	0.00	0.952	70.19 (8.41)	72.23 (7.98)	0.37	0.545
Crisk	0.83 (3.41)	-1.39 (2.17)	0.15	0.697	**-0.95 (2.30)**	**2.11 (4.19)**	0.73	0.398
†			4.01	0.052			**12.72**	**0.001**

1 and 2 represent the lower and upper quantile, respectively; [a], low risk defined as CRF ≥37.0 and ≥42.1 mL/kg/min; high risk defined as <37.0 and <42.1 mL/kg/min, for females and males, respectively; Adjusted for: sex, age, SES, ethnicity, SES, zBMI, total PA and residual intakes of fat, PRO and CHO; †, model is not adjusted for BMI

In males increasing CRF was associated with a significantly reduced WC and TG (Table 36). When CRF was expressed as high or low risk, those in the CRF risk group had a significantly higher TG and WC (BMI excluded).

Table 37. Female participants: Associations between CRF and metabolic risk factors

	VO2 Peak				CRF risk			
N=72	1	2	F	P	Low risk[a]	High risk[a]	F	P
HDL	**1.02 (0.27)**	**1.28 (0.40)**	**7.86**	**0.011**	1.23 (0.23)	1.11 (0.40)	1.14	0.300
†			**10.03**	**0.005**			2.25	0.149
TG	0.98 (0.41)	1.19 (1.41)	1.20	0.287	0.79 (0.39)	1.20 (1.16)	2.38	0.140
WC†	**90.70 (11.10)**	**78.29 (14.37)**	**7.88**	**0.011**	**72.43 (12.04)**	**89.91 (11.50)**	**7.55**	**0.012**
BG	**4.91 (0.56)**	**4.51 (0.46)**	**5.65**	**0.028**	4.57 (0.56)	4.78 (0.54)	0.72	0.406
†			**6.71**	**0.017**			1.36	0.258
SBP	115.64 (9.68)	104.92 (9.03)	0.95	0.342	105.06 (10.19)	112.76 (10.31)	0.05	0.834
DBP	76.14 (6.58)	71.52 (8.42)	0.03	0.862	69.79 (9.07)	75.66 (6.61)	0.39	0.538
Crisk	**2.66 (3.00)**	**-0.54 (3.08)**	**10.86**	**0.004**	**-1.88 (2.16)**	**2.39 (3.08)**	**6.76**	**0.018**
†			**19.99**	**0.000**			**14.94**	**0.001**

1 and 2 represent the lower and upper quantile, respectively; [a], low risk defined as CRF ≥37.0 and ≥42.1 mL/kg/min; high risk defined as <37.0 and <42.1 mL/kg/min, for females and males, respectively; Adjusted for: sex, age, SES, ethnicity, SES, zBMI, total PA and residual intakes of fat, PRO and CHO; †, model is not adjusted for BMI

In females, a higher VO_2 peak was associated with an increased HDL (P=0.011) and a significantly reduced WC, BG and clustered risk score. Adjustment for BMI attenuated associations of CRF with HDL, clustered risk and BG; P values were lower when BMI was excluded from the model. When CRF was classified as low or high risk to health, those in the high risk group (CRF <37.0 and < 42.1 mL/kg/min, for females and males, respectively) had significantly higher mean WC and clustered risk scores; again, adjusting for BMI attenuated the associations observed between clustered risk scores and CRF risk (Table 37).

Table 38. Odds ratio and 95% CI for expressing a metabolic risk factor per unit increase of time (mins) spent in respective PA categories

	ALL PARTICIPANTS (n = 72)					
ROWLANDS	VPA	*P*	MVPA	*P*	VO_2 peak	*P*
HDL†	2.35 (0.23-24.29)	0.472	0.99 (0.95-1.04)	0.738	**0.90 (0.83-0.97)**	**0.008**
TG†	**0.00 (0.00-0.31)**	**0.013**	1.00 (0.91-1.05)	0.556	**0.83 (0.72-0.96)**	**0.014**
WC†	0.09 (0.01-1.63)	0.104	0.98 (0.92-1.04)	0.526	**0.77 (0.67-0.89)**	**0.000**
BG†	0.08 (0.00-27.28)	0.389	0.95 (0.60-1.50)	0.810	0.93 (0.80-1.07)	0.284
Crisk†	**0.02 (0.00-0.69)**	**0.030**	1.00 (0.94-1.07)	0.940	**0.58 (0.40-0.83)**	**0.003**
CHU	VPA	*P*	MVPA	*P*		
HDL†	3.55 (0.34-36.64)	0.287	0.48 (0.02-10.88)	0.646		
TG†	0.15 (0.00-6.93)	0.330	[a]**0.01 (0.00-0.74)**	**0.036**		
WC†	1.32	0.852	0.12	0.330		
BG†	0.18 (0.00-92.16)	0.590	0.00 (0.00-1.05)	0.052		
Crisk†	**0.02 (0.00-0.86)**	**0.041**	0.13 (0.00-7.80)	0.326		

Analysis adjusted for: sex, age, SES, ethnicity, SES, zBMI, total PA and residual intakes of fat, PRO and CHO; †, model is not adjusted for BMI; [a], model is adjusted for BMI.

Logistic regression analysis was run in order to assess associations of PA and CRF with metabolic risk factors expressed as a clinical health marker (Table 38). Per unit increase in VO_2 peak there was a significantly reduced odds of being classed as having a risk factor for HDL (P= 0.008), TG (P= 0.014), WC (P= <0.001) and having

a high clustered risk score (P= 0.003), these associations were attenuated and were non-significant when BMI was included in the model apart for HDL which remained significant (P= 0.025). As outlined below, significant associations were only observed in PA models assessing associations of metabolic risk factors with VPA and MVPA. When assessed by the Rowlands thresholds, a 1 minute increase in time spent in VPA was associated with significantly reduced odds of having hypertriglyceridemia (P= 0.013) and having a high clustered risk score (P= 0.030), these associations were non-significant when BMI was adjusted for. When PA was assessed by the Chu thresholds, VPA was associated with a significantly reduced likelihood of having a high clustered risk score (P= 0.041) whilst increasing MVPA was associated with reduced odds of hypertriglyceridemia (P =0.036), but only when BMI was not adjusted for in the model. BP was not associated with PA or CRF.

7.3 Discussion

To the author's knowledge, this is the first study to assess the associations of CRF and PA with risk factors of the metabolic syndrome in a group of postpubertal adolescents. In addition, this is the first study to compare two published thresholds for determining PA intensity (Rowlands et al., 2004, Chu et al., 2007) and the respective associations of PA with metabolic health in adolescents whilst controlling for dietary intake. The main findings of this study were that CRF and PA are both associated with individual risk factors for the metabolic syndrome but that associations vary according to sex. However, there is a discrepancy in the mean time accumulated in different PA intensities depending on the threshold used (Chu et al., 2007 or Rowlands et al., 2004). Furthermore, it appears that this discrepancy subsequently results in different associations between PA and metabolic risk factors, depending on the threshold utilised.

The mean daily time accumulated in different PA categories varied according to the threshold used to assess PA. Time spent SED and in LPA appeared to be least affected by the use of different thresholds. Time in MPA (50.42 vs 24.05 mins) and VPA (14.48 vs 3.46 mins) was much greater when assessed by the Rowlands compared to the Chu thresholds, respectively. Therefore time spent in MVPA was considerably higher when assessed by Rowlands compared to when employing the

Chu thresholds. On average the Rowlands thresholds classify the whole group (65.60 mins MVPA) and males (73.14 mins MVPA) as sufficiently active (based on the government recommendations of >60 mins MVPA per day) whereas according to the Chu thresholds neither group is sufficiently active (males: 32.54; females: 18.55 mins MVPA). Females were significantly more SED than males when using the Chu thresholds and both thresholds identified that females engaged in significantly less MPA, VPA and MVPA. There are clearly implications of using different thresholds to calculate time accumulated in PA in this population, especially when such large discrepancies are evident when estimating time in MVPA.

Partial correlations revealed that LPA was the only PA variable to be correlated with CRF when controlling for sex, SES, age and *z*BMI but this was only observed when PA was assessed by the Rowlands thresholds (r = .234; P= 0.05). In contrast to this, Bailey et al (2013) observed that CRF was significantly negatively associated with LPA using the Rowlands and Chu thresholds in children. The authors also observed a significant positive association of CRF with VPA (Rowlands) and MPA (Chu). As both studies have utilised the same thresholds and data reduction protocols these differences in PA and CRF associations may be attributable to the difference in age of the two populations. As children progress through their adolescent years PA engagement declines (Kimm et al., 2002), indeed Bailey et al (2013) observed that the boys in their study engaged in 55.00 and 22.70 minutes more MPA than the adolescent males of the current study, when using the Rowlands and Chu thresholds, respectively. This was also observed in girls, who engaged in 44.09 and 35.00 minutes more MPA than females in the current study when using the Rowlands and Chu thresholds respectively; this difference may explain the contrasting associations between the two studies. Eklund et al (2007) also observed that CRF was negatively associated with SED time and positively with LPA, MPA and VPA in 9-10 year old children; however, this association was also present in adolescent 15-16 year olds. In addition, this study (Eklund et al., 2007) used thresholds (>3000 CPM and < 100 CPM to define MVPA and time spent SED, respectively) which are different to those used in the current study and that of Bailey et al (2013), and may therefore be an additional factor associated with these contrasting relationships.

Having higher CRF levels has been consistently related to having a cardio-protective effect and has been associated with a lower incidence of CVD and mortality rates in adults and youths (Blair et al., 2001a, Ekelund et al., 2007a). In the present study, MANCOVA revealed that WC and clustered risk score were significantly lower in those individuals with greater CRF; this association was present in all participants and in males and females. In all participants as a group and in males TG levels were significantly reduced in those with a higher CRF. Additionally, in females, HDL was significantly increased and BG reduced in the higher CRF group. Therefore in this population it appears that having a higher CRF is beneficial for lipid profile and central adiposity and BG in females, furthermore clustered risk score was consistently reduced in those who had a greater CRF. Clustered metabolic risk represents a constellation of metabolic risk markers that may detect an array of cardiometabolic disturbances; health status is worse in individuals with multiple risk factors than those with a single risk factor (Gami et al., 2007). Assessing clustered risk may be more informative as it can ameliorate the daily fluctuations in individual markers (Anderssen et al., 2007b).

The government recommendation that adolescents should engage in at least 60 minutes of MVPA (DOH, 2011) is based on evidence in youths and adults that higher intensity activities such as MPA and VPA are more beneficial to health (Ekelund et al., 2007, Husseyet al., 2009). In the present study however, MPA, VPA and MVPA were only favourably associated with blood pressure. MPA was inversely associated with SBP (p=0.033) in males when using the thresholds of Rowlands. Bailey et al (2013), who compared the same thresholds but in 104 children (10-14yrs) found that MPA was not associated with any metabolic risk factors apart from sharing an inverse relationship with DBP (p=0.028) as well as BF% in girls when utilising the Chu thresholds. Differences may be attributed to the fact that children engaged in more MPA than adolescents in the current study, as outlined earlier in this section. In the present study VPA was only associated with DBP when participants were assessed using the Chu thresholds; DBP was significantly lower in those engaging in more VPA (P= 0.031); the same association was observed using partial correlation analysis (r -.261; P= 0.030). Similarly, Bailey et al (2013) also observed that VPA was not associated with metabolic risk factors in boys nor in girls. This similarity between boys (Bailey et al., 2013) and these male adolescents could be explained by the fact that time in VPA accumulated by the two populations

was very similar (only 11.56 minutes more, according to Rowlands and 1.36 minutes less based on the Chu thresholds). Furthermore, (Ekelund et al., 2007a) also demonstrated that MPA and VPA were inversely associated with DBP and SBP (as did (Hussey et al., 2007) and were not associated with TG or HDL in 1709 children (9-10yrs old) and adolescents (15-16yrs old), conversely however, BG was also negatively associated with MPA and VPA. The present study has observed, in all participants (males and females), that those in the higher quintile of MVPA had a significantly lower DBP (P= 0.044) but only when assessed by the Rowlands thresholds, this was also observed in partial correlation analysis using both thresholds. In a large (n= 20871) multi-site study of children and adolescents (4-18 yrs old) (Ekelund et al., 2012) it was observed that increasing MVPA (assessed by the Actigraph accelerometer) was associated with a reduced SBP; however, unlike the present investigation, MVPA was also associated with reduced WC, TG, insulin and increased HDL and the authors reported that SED time did not share any significant associations with cardiometabolic risk factors. The PA thresholds utilised by Ekelund et al (2012), compared to the current study, are markedly higher for assessing MVPA and lower for SED PA; >3000 CPM for MVPA and <100 CPM for SED whereas the Rowlands and Chu cut-points are >970 and >1860 CPM for MVPA and <288 and <420 CPM respectively. Therefore, those engaging in the Ekelund et al (2012) study had to engage in considerably higher intensity activities compared to the adolescents of the current study to accumulate MVPA, and this higher intensity of activity might be more favourably associated metabolic risk factors. However, Ekelund et al (2012) ran accompanying analyses using MVPA cut-points of >2000 CPM (similar to those of the Chu thresholds) and found their associations with metabolic risk were unchanged.

This highlights the inconsistencies in terms of PA data analysis used between different studies and, although its impact is not clear, this difference might impact on the associations between PA and metabolic health. A further difference between these studies is the protocol for obtaining and reducing data from the accelerometer. The present investigation used at least 2 acceptable week days (540 mins) and 1 weekend day (480 mins) of accelerometer wear time, yet, contrastingly, Ekelund et al (2012) used data from children who provided only 1 day of wear time ≥500 mins, which may provide a less accurate representation of habitual PA than the current study. Furthermore, the present study classed non-wear time (which was discarded) as any period of zero counts lasting ≥10 consecutive mins as opposed to Ekelund et al (2012) who defined non-wear time as

≥60 mins of consecutive zero counts, allowing non zero interruptions lasting 2 minutes. Therefore the current study may have discarded more data as non wear time compared to Ekelund et al (2012) potentially resulting in more participants meeting the required daily wear time and being subsequently included in the statistical analysis. Therefore, Ekelund et al (2012) may have examined a less biased sample, than the present study. In addition, the greater sample size employed by Ekelund et al (2012) in comparison to the current study, means that their study will have a greater statistical power.

When exploring associations with lower intensity components of PA such as time spent SED and in LPA, more associations with metabolic risk factors emerged compared to associations with MVPA. When utilising either the Rowlands and Chu thresholds, in all participants, an increased SED time was associated with a higher mean DBP (P= 0.024 and 0.013, respectively). This was also the case in males assessed by the Rowlands thresholds. When assessed by the Chu thresholds, TG levels were significantly lower in males engaging in more SED time, although such a finding is not in agreement with previous research in youths (Ekelund et al., 2007). Conversely, although Bailey et al (2013) observed that TG was also the only risk factor associated with SED time in boys, they identified a positive association suggesting that being SED may be detrimental to lipid profile as supported by previous research in children, adolescent and adults (Ekelund et al., 2007a, Hussey et al., 2007). This lack of agreement between the two studies may be related to the differences in accumulated SED time; the adolescents of the current study spent approximately 75 minutes more time SED than the children of Bailey et al's (2013) investigation.

Furthermore, Bailey et al (2013) reported that LPA was not associated with any metabolic risk factors in children, although they did observe positive associations between LPA and BF%. In the present investigation however, engaging in more LPA was associated with a reduced mean BG (P= 0.035), SBP (P= 0.007) and clustered risk (VO_2) score (P=0.055) in all participants using the Rowlands thresholds; partial correlations also revealed that LPA was associated with reduced clustered risk (r -.261; P= 0.030). Additionally, in males, SBP was significantly lower in those completing more LPA when assessed by both thresholds. In males and the group as a whole, increased LPA as assessed by Chu was also associated with significantly higher HDL levels (P= 0.019 and P=0.025, respectively). Similarly, in

the EYHS (Ekelund et al., 2007a) LPA was inversely associated with clustered risk, DBP and SBP, in contrast however; LPA was the only PA component not to be associated with BG. In this group of adolescents being less SED and engaging in more LPA appears to be associated with improved BG and BP values and may also be a benefit to lipid profile, however, there appear to be conflicting associations of HDL with LPA and TG with SED activities in this group.

In order to further assess the associations of CRF and PA with cardio-metabolic risk factors, binary logistic regression analysis conducted in all participants allowed for risk factors to be assessed in terms of clinical cut points for health risk. Per unit increase in CRF there were reduced odds of having hypertriglyceridemia (HTG) (P= 0.014), low HDL (P= 0.008), high WC (P=<0.001) and high clustered metabolic risk score (P=0.003). Apart from HDL, these associations were not significant when analyses were adjusted for BMI, therefore it seems that these associations are mediated by weight status, yet the association of HDL with CRF appears to be independent of BMI.

When risk factors were examined using logistic regression, the only PA components to share a significant association with risk were VPA and MVPA. When assessed by the Rowlands thresholds an increase in VPA was associated with a reduced likelihood of having HTG (P= 0.041) and high clustered risk score (P= 0.030) but the association with clustered risk was mediated by BMI. VPA was also associated with a reduced clustered risk score (P= 0.041) when utilising the thresholds of Chu, which when used to assess MVPA revealed a significantly reduced odds of having HTG (P= 0.036), moreover, this association with clustered risk was attenuated by the inclusion of BMI in the model. These associations appear to be in contrast to the findings of Bailey et al (2013) who did not observe any associations between VPA, MPA and risk and to the findings from MANCOVA analysis in the current study of which VPA and MVPA were only associated with BP (confusing). The different associations observed from logistic regression analysis compared to MANCOVA in the present study may have been observed due to the nature of the different analysis. Logistic regression split the sample into a non-risk and risk group for each metabolic risk factor (respectively); therefore the statistical power of this analysis depends on the number of participants exhibiting risk factors. Of the 75 participants examined, the prevalence of these associated risk factors was: 12% TG, 17.3% Clustered risk and 34.7% HDL. Although the group size for TG and clustered risk

appear small the relatively high prevalence of low HDL in this group compared to other risk factors might suggest that this finding is more statistically robust.

In the current study WC was not associated with any PA components as assessed by either PA thresholds, however, it was associated with CRF in all groups. In a study of 152 children (7-10 yrs old), SED time (positively) and VPA (negatively) were significantly correlated with WC in boys only, whereas CRF was significantly negatively correlated with BMI and WC in boys and girls (Hussey et al., 2007). Similarly to the current study, Hussey et al (2007) also used RT3 accelerometry and the thresholds of Rowlands to assess PA, as did Bailey et al (2013); both studies found that PA (MPA and VPA) was associated with WC and BF% in children. The greater time accumulated in MVPA by children (Bailey et al., 2013, Hussey et al., 2007) as compared to the adolescents of the current study (Bailey et al., 2013) may be responsible for the contrasting findings between the adolescents of this investigation and past research in children.

The proportion of participants classified as sufficiently active (>60 mins of MVPA) by the two thresholds were markedly different, (Table 29) the Rowlands thresholds classified 53% of the group, 63.8% of males and 37.9% of females as sufficiently active. Contrastingly, the Chu thresholds classified only 6.6% of the group and 10.6 % of males as sufficiently active; no females achieved >60 mins MVPA when assessed by the Chu thresholds. These variations can be attributed to the difference in MVPA cut-points between the Rowlands and Chu thresholds. Bailey et al (2013), observed a very similar difference in terms of time accumulated in MVPA when using the Chu et al (2007) and Rowlands et al (2004) thresholds.

The associations observed between CFR and metabolic risk factors were stronger than those observed between PA and health parameters. It is important to highlight that when assessing the associations of PA and health risk a secondary analysis was conducted that controlled for CRF. Following adjustment for CRF, PA was no longer associated with any metabolic risk factors and thus it seems that CRF mediates associations of PA and health markers in this population. Metabolic health parameters may be mediated by CRF through increased mitochondrial volume and density, increased electron transport chain enzyme activity (Hawley, 2002) and increased capillary and limb blood flow (Holten et al., 2004). The mediating effect of CRF over PA could be explained by the fact that increased PA may have a

beneficial effect on CVD and type 2 diabetes risk but through increases in CRF subsequent to these physical activities (Rennie et al., 2003). The current study revealed that although MPA and VPA were associated with reduced BP, LPA appeared to be associated with improved BP, BG and lipid profile. Evidence in adults has revealed that low to moderate and moderate to high PA modulates insulin secretions, increases fatty acid oxidation and promotes glucose uptake from the blood through increased AMP-activated protein kinase (AMPK) activity (Chen et al., 2003). However, a 4-fold increase in AMPK activity has been observed immediately following high intensity exercise (~75% VO_2max) but not after lower intensity exercise (~50% VO_2max) (Wojtaszewski et al., 2000). The current findings then may be in line with evidence that breaking SED activities and engaging in more LPA is associated with health improvements (Healy et al., 2008b, Healy et al., 2011).

There are some limitations of the present study that should be noted; the relatively small sample size of this study means that the strength of the associations may be weaker than compared to some previous studies in this age group. It is possible that the data acquisition period of this study (minimum of 3 days of 900 and 800 minutes for week days and weekend days) and the one minute epoch may not have fully represented the true PA habits of this population. However, it was ?larger than that of previous research (Ekelund et al., 2012), and a shorter epoch was not possible with the technology available. Participants were recruited from schools and colleges from the Bedfordshire area and therefore the findings from this group of post-pubertal adolescents cannot be generalised to other populations. Furthermore, as this research is observational, the direction of causality of the associations cannot be determined.

This study demonstrates that that using different PA thresholds to assess PA intensity impacts on the relationships between PA components and metabolic risk factors, however a consistent relationship emerged between MVPA (negative) and a SED (positive with BP) suggesting that limiting SED behaviour and engaging in higher intensity activity is beneficial for BP in this adolescent population. There was also evidence that increasing LPA is related to an improved fasting BG and lipid profile but this was not consistent between males and females. Furthermore, regardless of threshold used, increasing VPA was associated with reduced odds of having a high clustered risk score. The associations observed between CRF and

risk factors were stronger than those observed with PA; CRF was inversely associated with WC, clustered risk and factors associated with an adverse lipid profile.

The effect of using different thresholds will clearly have implications for identifying sufficiently active youths, as these recommendations are endorsed by the government and based on the health benefits associated with MVPA, it is important that consensus is arrived at regarding the thresholds used to accurately assess MVPA engagement.

Chapter Eight: Study Five

The impact of a low glycaemic index diet on the metabolic health of postpubertal adolescents with features of the metabolic syndrome.

8.0 Introduction

In adults and youths, consumption of a diet high in GI and GL has been associated with central obesity and individual risk factors for the metabolic syndrome, as well as increased clustering of cardiometabolic risk (Culberson et al., 2009, O'Sullivan et al., 2010). GI and GL have been independently associated with adverse lipid profile and CVD in adults (Yungsheng et al, 2006; Culberson et al, 2009). Furthermore, increased GI and GL intakes have been associated with incidence of type 2 diabetes in healthy adults (Sluijs et al, 2010) and in subjects with type 2 diabetes GL has been positively associated with CVD related mortality risk (Burger et al., 2012); these associations are thus evident in adults classed as healthy and 'at risk'. In 769 Australian adolescents (13-15 yrs), O'Sullivan et al (2010) assessed the associations of glycaemic CHO with the prevalence of the metabolic syndrome as defined by the ATPIII and IDF (Alberti et al., 2006) criteria, separately. A 20 unit increase in GL was associated with significantly increased odds of having the metabolic syndrome as defined by the IDF criteria (O'Sullivan et al., 2010), but not when the metabolic syndrome was defined by the ATPIII criteria. The IDF criteria is the only definition to include WC as a mandatory factor for having the syndrome (see section 6.1); the authors postulated that WC may partly explain the relationship between GL and metabolic risk factors. In contrast, however, Davis et al (2007) observed that GI and GL were not associated with adiposity or insulin dynamics in a group of overweight Latino youths (10-17 yrs) with a family history of type 2 diabetes. Davis et al (2007), however, utilised two weekday 24 hour recalls, one of which was via telephone whereas O'Sullivan et al (2010) employed a 3 day food record (2 week 1 weekend day) to assess diet which may have contributed to the equivocal findings of these studies. By not including a weekend day, Davis et al (2007) may not have captured a true representation of habitual intake and the fact that food items were recalled may compromise the accuracy of type and quantity of foods recorded (Rockett and Colditz, 1997, Kipnis et al., 2001). An issue with a number of investigations that assess the association of glycaemic CHO and health

parameters is that the different dietary reporting methods they employ makes comparisons between studies difficult. The difference between the types of information obtained from different reporting methods may pose particular problems when assessing the GI and GL of the diet, due to the detail required on the type and quantity of food consumed (Collins et al., 2010). 24 hour recalls and FFQs require participants to remember and recall their food intakes (Hu, 2008) whereas food diaries are designed to be completed immediately after eating and are thus less prone to reporting errors (Kipnis et al 2001).

There is also observational evidence that these associations extend beyond the specific risk factors for the metabolic syndrome; glycaemic CHO consumption has been linked to increased markers of poor liver function and markers of systemic inflammation (CRP) (Valtueña et al., 2006), as well as a reduction in the anti-inflammatory cytokine adiponectin (Qi et al., 2005) and some researchers have suggested these factors may mediate poor metabolic health (Eckel et al., 2005, Fernández-Real and Ricart, 2003, Sutherland et al., 2004). Dietary GI has been associated with liver steatosis (as measured by liver echography) in 247 apparently healthy adults (Valtuena et al, 2006); being insulin resistant strengthened associations suggesting that high GI foods are a greater burden to those who have difficulty controlling BG levels. In a sample of 902, type 2 diabetic women from the Nurses' Health study, higher GL was associated with significantly lower adiponectin levels, this was observed across normal weight, overweight and obese participants, according to BMI (Qi et al., 2005). This research group also presented the same findings in a sample of diabetic male adults (Qi et al., 2005).

Although observational evidence suggests that glycaemic CHO is associated with metabolic risk factors, these studies cannot determine the direction and causality of such associations and thus, it is important that intervention studies are conducted to further inform this interaction. Intervention studies in both adults and youths have revealed that reducing dietary GI and GL can reduce adiposity (Spieth et al., 2000, Pereira, 2004) and have a beneficial effect on metabolic health (Rizkalla et al., 2004, Jebb et al., 2010). Some intervention studies in overweight and obese adults have reported that greater weight loss is observed following a low compared to high GI diet, however, this is only observed in those with reduced insulin sensitivity (Pittas et al., 2005, Ebbeling et al., 2007). In 39 overweight and obese young adults (18-40yrs) a diet designed to elicit a 10% weight loss (60 % of required energy) low

in GL compared to low fat (high in CHO and GL) elicited greater improvements in insulin resistance, TG, CRP and blood pressure (SBP and DBP), despite very similar reductions in weight and FM (Pereira, 2004). Those on the low GI diet had a smaller reduction in resting metabolic rate (RMR) and reported feelings of less hunger compared to the low fat diet. A limitation of this work is the lack of matched macronutrient intake between the two diets and thus health improvements may be a result of additional dietary factors other than GL. Furthermore, Pereira et al (2004) used a heavily controlled dietary intervention, supported by daily checkups and strict menus; this may not be a practical approach for the general population. It may be that an adlibitum diet is a more 'adherable' approach to health improvement through lowering dietary GI and GL (Ebbeling et al., 2005). In adult males, just 4 weeks on a low GI diet compared to high (using a crossover design) resulted in significant improvements in glucose control ($HbA1_c$) and lipid profile (Rizkalla et al., 2004), this was also observed in 210 type II diabetic men and women (Jenkins et al., 2008). There is little evidence for the effects of low GI interventions on health in UK populations, however, one study conducted within the UK, compared 4 diets over a 4 week period: a low GI compared to high GI diet of either low or high SFA content in 548 adults with existing metabolic risk factors (Jebb et al., 2010). Improved lipid profile; reduced TC, LDL and apoB concentrations resulted from reducing dietary SFA, however reducing dietary GI further enhanced TC and LDL improvements. All groups failed to significantly improve insulin sensitivity, although, the low fat/LGI group improved insulin sensitivity to the greatest extent. Despite the fact that these diets were designed for weight maintenance, body weight was slightly reduced as a result of lowering dietary fat intake; there was no evidence of an effect of GI on weight management (Jebb et al., 2010). A limitation of the above studies (Jebb et al., 2010, Pereira et al., 2004, Ebbeling et al., 2007) is that CRF or PA were not adjusted for in their analysis. In a prospective cohort of 13,621 men and women, (Héroux et al., 2010) observed that accounting for fitness (maximal exercise test) substantially attenuated the previously significant relationship between an unhealthy eating index and all cause mortality. Interestingly initial adjustment for self-reported PA had very little impact on the original association; the mortality risk estimates were reduced by 13.5 and 55.0 % after controlling for PA (self-report) and fitness, respectively. According to (Pischon, 2010) objectively assessed fitness and additionally PA should be accounted for due to the apparent intermediary effects of these variables on diet-health relationships. Although it is not possible to say if this would affect the associations of GI and GL on health

outcomes it is important to account for these factors based on this evidence. Despite these limitations it appears that reducing dietary GI and GL, particularly in those exhibiting obesity and metabolic risk factors, may promote weight loss and be beneficial in the amelioration of metabolic risk.

There is limited and equivocal evidence in youngsters of the impact of altering glycaemic CHO consumption on health outcomes. Unfortunately, the low GI/GL dietary interventions have tended to employ small sample sizes and are not well controlled. In a study of 16 obese but otherwise healthy adolescents (13-21 years old) from the USA, Ebbeling et al (2003) employed an *ad libitum* low GL diet and observed greater fat loss in comparison to a reduced fat intervention over 6 months. Although the two diets were described as not significantly different in energy, there was a mean difference of 692 kcals between baseline and the low GL diet compared to only 148 kcals in the low fat diet and thus it is unclear as to whether greater fat loss was observed due to reduced GL or energy (Ebbeling et al., 2003). Furthermore, although the diet was described as *ad libitum*, in order to reduce the GL it was necessary to reduce CHO intake and thus the intervention was not truly *ad libitum*. Although Ebbeling et al (2003) observed a beneficial effect of low GL compared to low fat on adiposity, they found that insulin resistance increased in both groups but the change was non-significant for the low GI group. It was suggested that the increased insulin resistance was a result of hormonal changes during puberty (Ebbeling et al., 2003). The ages of the adolescents studied by Ebbeling et al (2003), like the majority of observational studies, span across a range encompassing individuals at different stages of maturational growth. Individuals still in puberty are likely to have a greater central adiposity and insulin resistance compared to those who have finished puberty (Staiano and Katzmarzyk, 2012, Moran et al., 1999); these factors have been evidenced to influence metabolic risk factors (Hannon et al. 2006). Therefore puberty may confound the influence of lowering glycaemic CHO on adiposity and health outcomes. Moreover, there appears to be a distinct lack of research investigating the effects of lowering dietary GI in postpubertal adolescents. However, in otherwise healthy but overweight and obese prepubertal children (11 yrs old), from Hungary, Fajcsak et al (2008), observed the impact of an *ad libitum* low GL diet on adiposity and metabolic risk markers. The intervention centred on exchanging 50% of the high GI foods consumed with low GI alternatives and there was no significant difference between calories consumed at baseline and during the intervention. Significant reductions in

fat mass and the prevalence of metabolic risk factors were observed after the six week intervention. Unfortunately this study did not report the GI and GL of the diet during the intervention period nor did the authors employ a control group, furthermore, in lowering the GL of the diet, portion sizes were indirectly restricted and the thus the diet was not truly ad *ad libitum*. Speith et al (2000) examined the effects of an unrestricted low GI diet compared to a standard reduced fat for the management of obesity in 190 children (10 yrs old) from the USA. The low GI diet centred on food selection rather than restriction; children were advised to consume low GI CHO, PRO and fat at every meal and snack. The low fat diet emphasised consumption of low fat, low sugar and foods of a low energy density and was thus energy restricted (approximate energy restriction of 250-500 kcals per day). The authors observed a significant decrease in BMI of 1.15 kg/m^2, by contrast the low fat diet group showed no change in BMI. This low GI intervention had no restriction of energy or specific macronutrients, children were encourage to eat to satiety and snack when hungry, however, the low fat diet was heavily restricted on energy intake. These effects may be attributable to the lack of adherence to a heavily restricted compared to a more flexible diet, but as dietary change was not monitored throughout the intervention the impact of either dietary prescription on nutritional intakes cannot be observed. Furthermore, because the macronutrient intake of these diets was very different it is difficult to attribute the effect solely to GI. However, this evidence does provide support for an *ad libitum* diet in the reduction of obesity in children yet there are no studies exploring its effect on metabolic risk factors. Like the majority of GI interventions in adults, none of the above studies in youths adjusted their analysis for physical activity of fitness and thus the true extent of the impact of these dietary changes on health may not have been demonstrated (Pischon, 2010).

In adults and youths, the majority of interventions examining the impact of lower glycaemic CHO on health outcomes alter the GL of the diet (Ebbeling et al., 2007, Ebbeling et al., 2003, Pereira, 2004, Fajcsak et al., 2008) and in order to manipulate dietary GL, CHO intake must be limited, thus restricting the flexibility. In youths an *ad libitum* approach may be more beneficial than restricted diets since adolescents have been shown to value the opportunity to make autonomous choices regarding their food choices and are less likely to adhere to energy restricted diets (Ebbeling et al., 2005). Therefore, if a truly *ad libitum* low GI diet can have a positive impact on health outcomes and or weight loss in adolescents, it may be an approach that can

be generalised to the wider population; however, there is currently no research that has explored this in an adolescent population. Additionally much of the evidence in youth is based within the USA and thus, the application of an *ad libitum* low GI diet has not been assessed in a UK population. This is important because many of the GI tables used to define the GI of individual foods are based on research from the USA and Australia (Aston et al., 2008) and therefore little is known of its application within a UK based intervention in an adolescent population. Identifying a dietary intervention that can be well adhered to by adolescents is of particular importance because metabolic complications observed in this age group have been shown to track into adulthood (Camhi and Katzmarzyk, 2010). Furthermore, although studies of low GI interventions in youths have assessed overweight participants, none appear to be exhibiting existing metabolic complications; despite evidence in adults suggesting that reduced GI may be more beneficial for those 'at risk' (Pittas et al., 2005, Ebbeling et al., 2007, Valtueña et al., 2006). It is therefore important to determine the impact of an *ad libitum* low GI diet on metabolic risk factors in adolescents exhibiting poor metabolic health. There also appears to be no research in adolescents examining the effect of a low GI intervention on additional important markers of cardiometabolic health such as liver function and inflammation.

To this end, the current investigation will explore the impact of a truly *ad libitum* low GI dietary intervention on metabolic risk factors compared to a control group, in postpubertal adolescents from the UK. These associations will be assessed in a 'metabolically unhealthy' population as defined by the IDF criteria for the metabolic syndrome, based on the evidence that reducing dietary glycaemic CHO may be more beneficial for 'at risk' groups and may be mediated by a high WC in adolescents (Pittas et al., 2005, O'Sullivan et al., 2010). When analysing the effect of this intervention, the impact of adjusting for objectively measured PA and CRF will also be considered. Furthermore, due to the extent and impact of dietary misreporting on glycaemic CHO and metabolic health relationships, as previously observed in UK adolescents within chapter 3, dietary misreporting will also be assessed in this group.

8.1 Methodology

Participants

Participants from Bedfordshire were recruited for the SIRENS study as outlined in section 3.2. In order to take part participants had to be overweight (high WC) and exhibit 1 additional metabolic syndrome risk factors for the metabolic syndrome as defined by the IDF child and adolescent criteria. Participants were screened as part of initial data collection procedures at the Centre for Obesity Research (COR) within the Luton and Dunstable Hospital to identify if they were eligible to participate.

Experimental design

The SIRENS study was a randomised controlled trial assessing the impact of a 12 week, *ad libitum*, low GI dietary intervention compared to control group on metabolic risk factors. Prior to enrolment into either intervention or control group, participants provided baseline data; the measures are outlined below. At week 6 and after the participants had completed week 12 of the intervention these measures were repeated. Participants attended the COR for all measures.

Measures

Age, ethnicity, SES, pubertal status

As previously outlined in section 4.1, age (years), ethnicity (white or non-white) and SES (IMD scores) were measured at baseline. Age was calculated for follow up data collection sessions based on the date of each participants data collection, respectively. Pubertal status was self assessed by each participant when they consented to take part. The study consent and information form included a sex specific line drawing diagram of the Tanner scale of sexual maturation (stages 2-5), as shown in Appendix 2; individuals who were in Tanner stage 5 were classed as having finished puberty (Marshall and Tanner, 1970a, Marshall and Tanner, 1969). Participants were thus informed (on the information sheet) that they could only participate if they deemed themselves to be at Tanner stage 5.

Anthropometry and body composition

Stature, WC, body mass, fat mass and BMI were measured as outlined in section 3.5.

Diet

Three day weighed food diaries (included 2 week day and 1 weekend day) were used to assess habitual dietary intake, GI and GL as outlined in section 3.10. Participants were required to complete follow up food diaries in weeks 6 and 12; participants were required to record their food intakes on the same three consecutive days at each dietary follow up.

Physical activity

PA level was assessed at baseline via 7 day accelerometry (RT3) as outlined in section 3.9. The thresholds of Chu (Chu et al., 2007a) were used to determine time spent in difference categories of PA intensity as outlined in section 6.2.

CRF

Peak oxygen uptake was measured during a maximal incremental cycle ergometer test at baseline, this was outlined in detail in section 3.9.

Venous blood sampling

Venous blood samples were collected at baseline, week 6 and after week 12 of the intervention. These procedures have been described in section 3.4.1. Once samples were collected, they were immediately delivered to the L&DH biochemistry laboratories where the samples were assayed by L&DH biochemistry staff and stored in aliquots at -80˚C for further assessment at a later stage.

Metabolic risk factors and liver function markers were assayed at the L&DH biochemistry laboratories, as per their laboratory procedures. Markers of inflammation and fasting insulin were assayed by the biochemistry laboratory of the Addenbrookes, Cambridge University Hospital, see Appendix 4, for assay details.

- Lipid profile: Fasting HDL and TG
- Inflammation: Adiponectin; Tumor Necrosis Factor alpha (TNF-α); Interleukin-6 (IL-6); and high sensitivity C-reactive protein (hs-CRP).
- Liver function: gamma-glutamyl transpeptidase (GGT); alkaline phosphatise (ALP); alanine transaminase (ALT); albumin; and bilirubin.
- Glucose control: Fasting plasma glucose, Fasting insulin; HbA1c.
- Insulin resistance was calculated using the homeostatic model of insulin resistance (HOMA-IR) and was calculated using the following formula:

HOMA-IR = fasting insulin (mU/L) x fasting glucose (mmol/L) (Wallace et al., 2004).

Metabolic risk factors and clustered metabolic risk score was calculated as outlined in section 6.2

Intervention groups

All participants who consented to taking part and met the inclusion criteria (see section 3.2) provided baseline data by attending the COR and were subsequently randomised to either the control or low GI dietary intervention group using stratified minimisation via the MINIM computer software package for windows. The groups were matched based on sex, age, ethnicity and WC into either the low GI dietary group or the control group. Prior to enrolment, baseline metabolic health risk factors were checked to ensure participants were classed as 'at risk'. The experimental design of the SIRENS study is summarised below in figure 4. Prior to starting the intervention, both the control and low GI groups were made aware that they were required to maintain their usual exercise and PA habits during the entire intervention period.

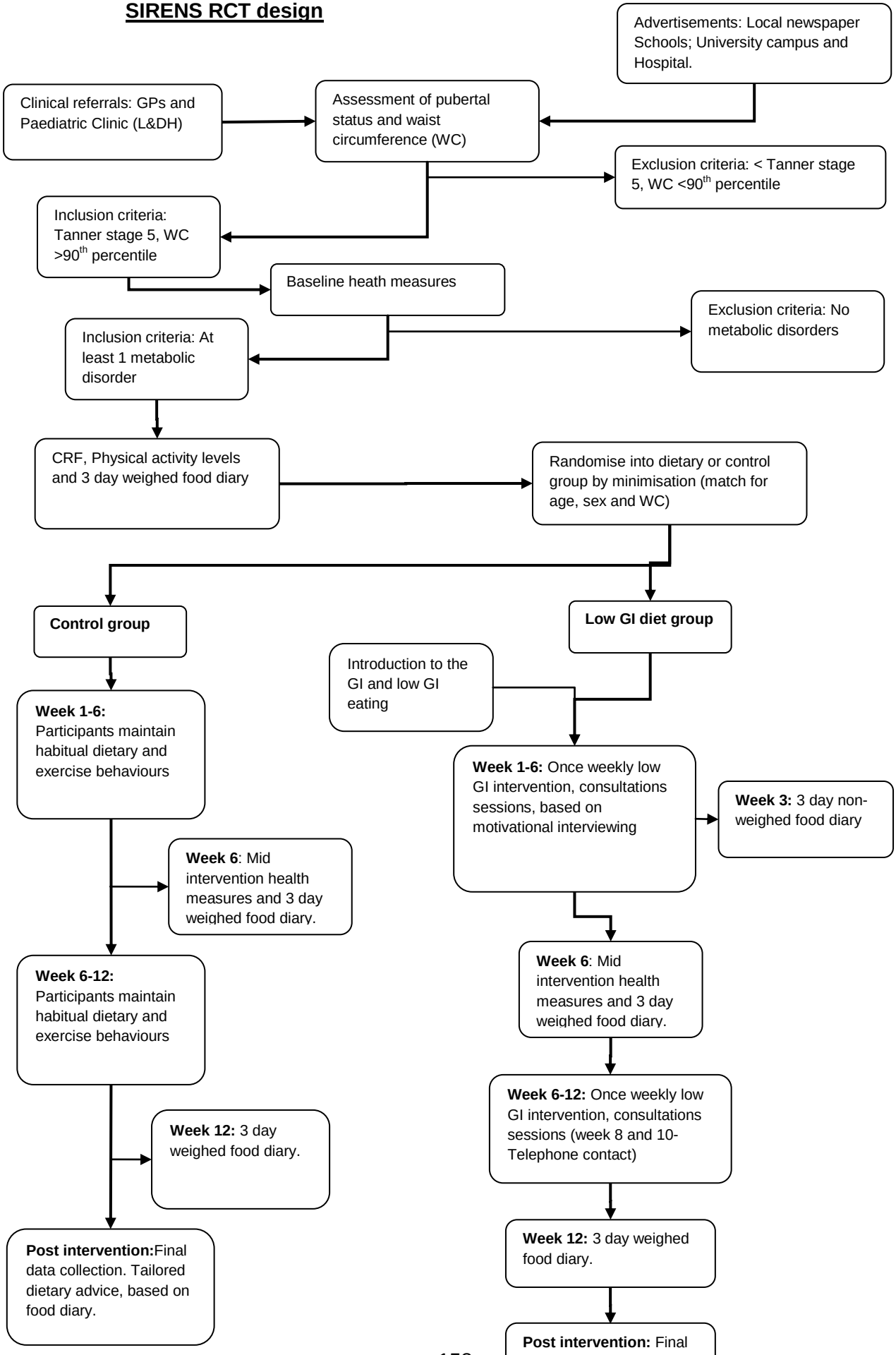


Figure 4. SIRENS trial design

Low Glycaemic index dietary intervention

The low GI diet regime was developed in consultation with dieticians at the COR, L&DH. Participants in the low GI intervention group attended weekly (apart from weeks 8 and 10) consultations sessions over the 12 week period (see intervention schedule, figure 6). Each session was guided by the PhD candidate who had been trained in the use of motivational interviewing for behaviour change and this approach formed the basis for all consultation sessions (see figure5) for consultation design). Participants were given pre arranged time slots to attend their consultation, which lasted 20-30 minutes. However, any consultation session that followed a dietary assessment required up to 40 minutes allowing for extra time to discuss the food diary with the participant and correct any unclear entries or anomalies.

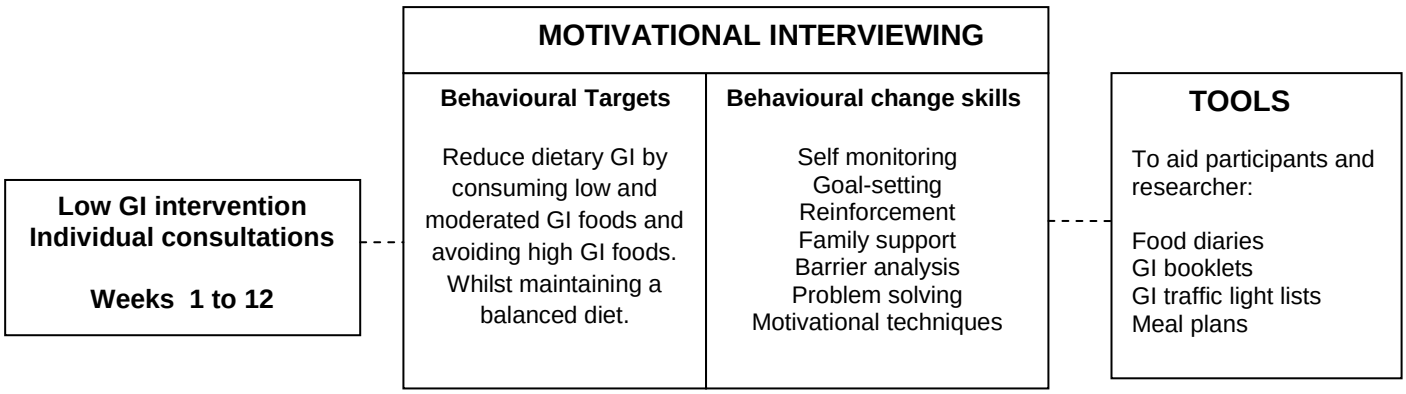


Figure 5. Low GI consultation design.

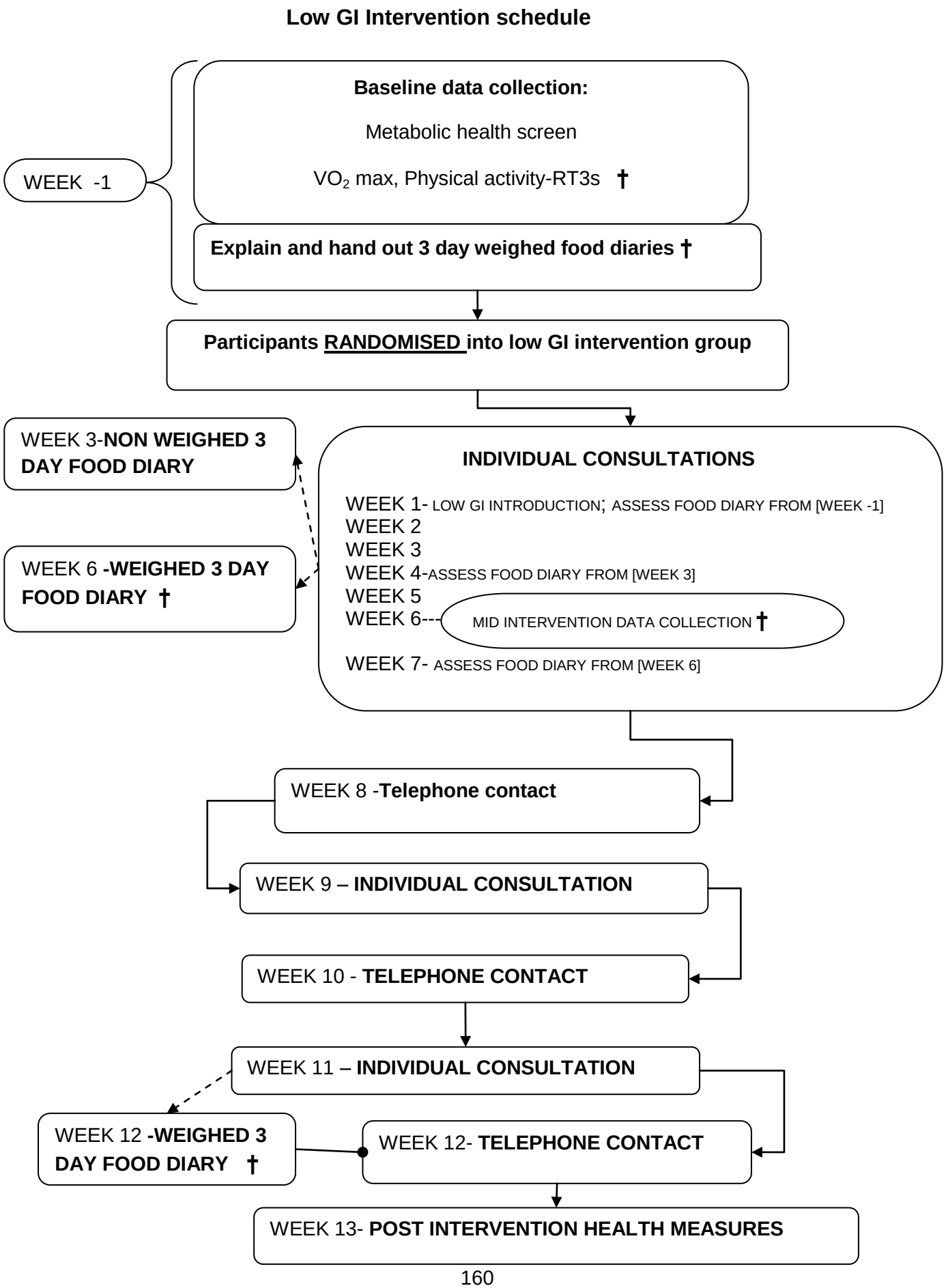


Figure 6. Low GI intervention flow chart; †, also required by control group.

Low GI regime

Because a number of low GI foods are high in fat it was important to ensure that participants were not over consuming these foods. Thus the low GI intervention was centred on regular balanced meals based on food groups from the Eatwell plate, and this was encouraged for all meals and snacks. Participants were guided on how to make simple replacements for high GI foods in their diet with lower GI foods. The regime was fully *ad libitum* and thus not restricted by energy or controlled for macronutrient intake. Therefore the participants were not required to make substantial changes to their normal diet. Evidence suggests that the flexibility of an *ad libitum* diet suits the desire for autonomy that many youngsters and in particular, adolescents, seek (Ebbeling et al, 2003), and thus is likely to improve adherence to the diet.

The initial consultation session in week 1 lasted approximately 1 hour and consisted of a 10 minute introductory power point presentation that explained the purpose of the intervention and the concept and benefit of a low GI diet. Participants were also required to bring their completed 3 day weighed food diary that was given to them during the previous week. Weighed food diaries were a primary tool in the reduction of dietary GI; in the first consultation session (See intervention flow chart, figure 6) they were used to gain a rapid impression of the habitual dietary intake of each participant and explore with the participant which foods they consume that could be swapped for lower GI options. This was done as a collaborative process allowing the participants to decide on their own food choices. In this session participants were also given a GI traffic light list of staple foods that included high GI foods to avoid (in red), moderate GI foods to consume in moderation (in orange) and low GI foods that should be consumed in all meals and snacks (in green) (see appendix 5). Because many of the foods listed on the international table of GI are from Australia and the USA, the GI traffic light list was developed specifically for this intervention and included foods within the international tables which most represented those consumed within the UK. This list was used in all consultation sessions and was also given to the participants to guide their food choices during the 12 week intervention. The international tables of GI were also utilised in these sessions as tools for identifying the GI of foods consumed and potential low GI swaps. As the intervention progressed participants were required to complete a non-weighed food diary at week 3 which was used as a guidance tool for the participants to identify if they were making the right low GI food choices. At this stage participants were also

given a low GI recipe booklet which they could use to help make meal choices; recipes were based on those published in the New Glucose Revolution for Diabetes (Brand-Miller et al., 2007) . The intervention flow chart highlights the time points throughout the intervention at which follow up data collections and consultations took place (see figure 6).

Control group

The control group were required to attend 3 consultation sessions throughout the 12 week period. At week 1, participants handed in their completed 3 day weighed food diary. Like the low GI group, at this point the control group were given the same guidance on eating healthy balanced meals using the Eatwell plate (FSA., 2012). This was done in order to match the underlying guidance for balanced meals issued to the low GI group in order to avoid over consumption of high fat low GI foods. Participants were required to attend follow up data collection and nutritional assessment at week 6 and 12. The control group were offered an additional consultation at the end of the intervention that matched the guidance given to the intervention group at week 1. See study design flow diagram above (figure 6).

Statistical analysis

Analyses were completed using the Statistical Package for Social Sciences (SPSS 19, IL.); descriptive statistics are presented as mean and standard deviation (SD), dietary intakes for the intervention period are presented as mean and standard error (SE). The following variables were non-normally distributed and were subsequently log transformed to improve their distribution to acceptable levels: FM, WC, HDL, TG, fat, PUFA, SED, LPA, MPA, and MVPA. All nutrient intakes (g) were adjusted for energy intake using the residuals method as outlined previously in section 6.1. BMI and BF% were converted to *Z*-scores based on population means for the age group. Differences between group baseline characteristics were assessed by One-way ANOVA. MANCOVA was used to assess between group (LGI and control) differences by time period (baseline, week 6 and week 12) and the interaction of group by time period of dietary variables (CHO, PRO, fat, fibre SFA, MUFA, PUFA), hunger and satiety as well as all health parameter variables. MANCOVA were adjusted for the following covariates in a sensitivity analysis approach: model 1(age, sex, SES, ethnicity, zBMI); model 2 additionally included (energy, PRO, fat, CHO, fibre); model 3 additionally included (CRF and % MVPA) and model 4 additionally included (EI:EE). For the same variables as the MANCOVA (apart for dietary

variables) analyses were also run as linear mixed models ANCOVA in order to account for within subject correlations of repeated observations and these analysis were adjusted for using the covariates of model 4. No colinearity was observed between covariates of MANCOVA models apart for EI:BMR and thus EI:EE (RT3) was included as a covariate in model 4. The assumption of homogeneity of regression slopes, however, was violated; there were significant interaction effects observed for group by: age and DBP, ALT, CRP, bilirubin; zBMI and CRP, bilirubin; energy and CRP, ALT, bilirubin; SES and bilirubin, ALT, CRP; and fibre and ALP; therefore results of MANCOVA and mixed models for these independent variables should be interpreted with caution. The assumption of homogeneity of variance between groups was not violated as assessed by critical F values.

8.4 Results

Recruitment and enrolment schematic

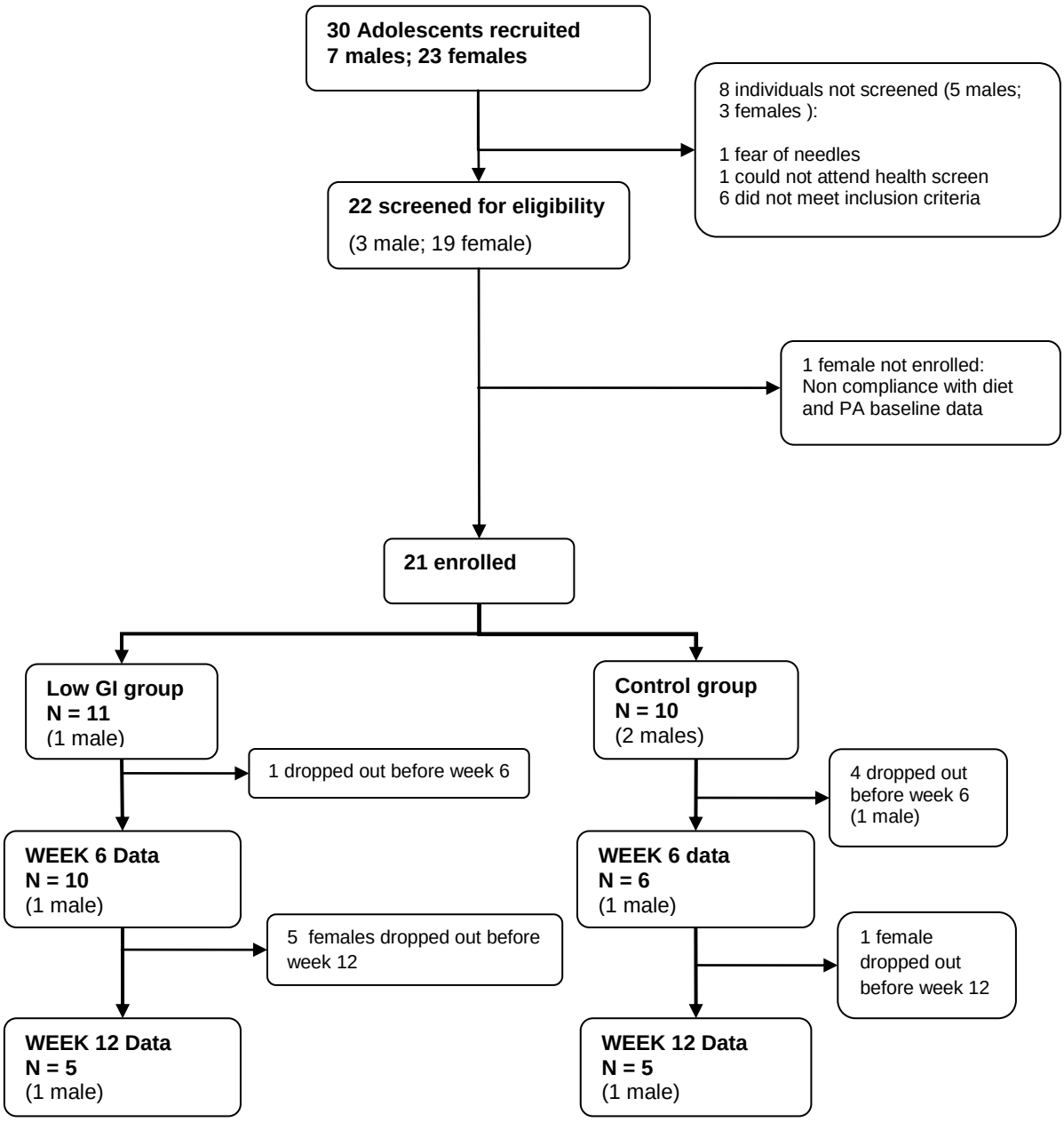


Figure 7. Flow of participants through trial.

Figure 7 shows that 11 and 10 participants were enrolled into the LGI and control group, respectively. However, due to unequal dropout rates between enrolment and week 6 data collection, the control group was comprised of 6 and the LGI group 10

participants. By week 12 data collection group sizes had become even due to further unequal dropout rates.

Table 39. Baseline participant characteristics, physical activity and CRF levels for intervention and control group.

	Low GI group n= 10		**Control group n= 6**		**P**
Males (n)	1		1		
Age (yrs)	16.74	(2.27)	15.95	(0.95)	0.44
Ethnicity non-white [n (%)]	7	(70%)	4	(66.7%)	[a]N/S
SES (IMD)	21.29	(14.65)	20.24	(9.75)	0.88
Height (cm)	160.10	(4.99)	168.38	(6.90)	0.01
Weight (kg)	81.07	(13.90)	92.87	(10.26)	0.09
BMI (kg/m^2)	31.63	(5.31)	32.82	(3.60)	0.57
zBMI	2.56	(0.78)	2.74	(0.51)	0.62
BF(%)	41.12	(4.65)	41.43	(3.91)	0.89
FM (kg)	33.89	(9.61)	38.62	(6.63)	0.25
FFM (kg)	47.18	(4.63)	54.25	(5.78)	0.02
WC (cm)	93.80	(9.82)	95.73	(6.46)	0.63
PA and CRF					
Total PA mins	688.07	(104.18)	842.25	(48.44)	0.01
SED mins	558.77	(110.88)	633.37	(98.36)	0.24
LPA mins	98.45	(45.34)	143.92	(38.42)	0.08
MPA mins	15.15	(13.81)	27.83	(23.08)	0.17
VPA mins	0.27	(0.38)	4.65	(10.23)	0.16
MVPA mins	15.43	(14.13)	32.48	(32.63)	0.15
>60 mins MVPA [n (%)]	0		1	(16.6%)	-
VO_2 peak ($ml/kg/min^{-1}$)	24.40	(5.70)	26.00	(3.69)	0.55

P, significant at <0.05 for between groups comparison; [a]N/S, Chi-squared test no significant difference between groups.

Baseline characteristics of those participants providing at least 6 week data (Table 39) show that there were more participants in the low GI group (LGI) compared to control (n= 10 and 6, respectively) and that there was only 1 male in each group. The LGI group was older and had a slightly greater proportion of non-white participants; SES scores were very similar between the two groups. The control group was significantly taller, heavier and had a greater FFM than the LGI group, WC was approximately 2 cm greater in the control group but this was non-significant; %BF and zBMI, however, was not different between the two groups. The control group engaged in significantly greater total PA and LPA than the LGI group.

Although non-significant, SED, MPA, VPA and MVPA were greater in the control group. On average neither group achieved > 60 minutes of MVPA and only one male participant in the control group achieved >60 minutes of MVPA. CRF as measured by VO_2 peak was similar between the two groups.

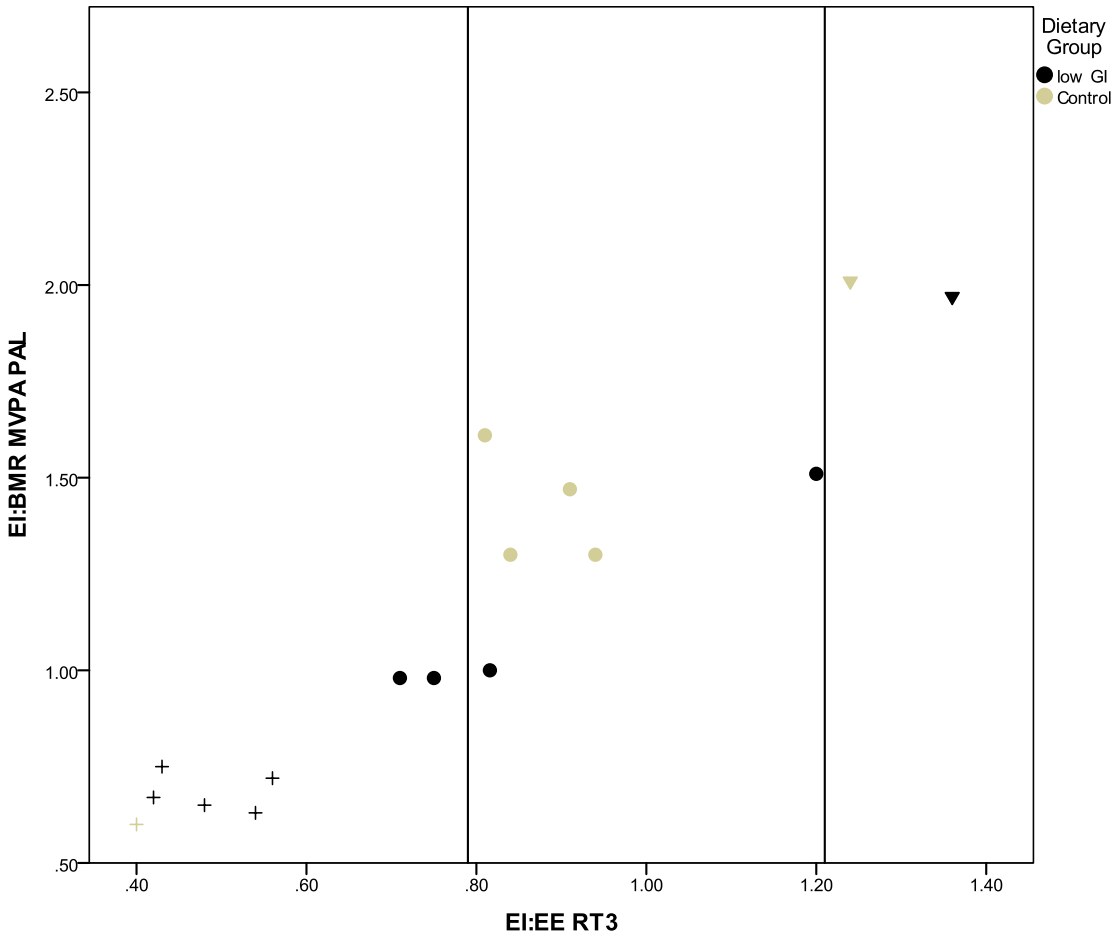
Table 40. Baseline dietary intakes of the intervention and control groups

	Low GI group n = 10		**Control group n = 6**		**P value**
KCAL	1624.72	(584.49)	2600.34	(1050.63)	0.03
GI	57.80	(3.91)	60.47	(4.27)	0.22
GL[a] (g)	163.11	(18.95)	162.65	(13.05)	0.96
CHO[a] (g)	281.75	(28.88)	276.35	(27.32)	0.72
PRO[a] (g)	64.21	(16.88)	75.01	(9.39)	0.18
Fat[a] (g)	75.5	(5.04)	70.97	(13.76)	0.35
SFA[a] (g)	25.84	(4.17)	22.13	(8.23)	0.25
MUFA[a] (g)	23.9	(3.55)	24.05	(6.04)	0.95
PUFA[a] (g)	12.91	(0.2)	12.94	(0.17)	0.74
Fibre[a] (g)	15.21	(2.55)	14.73	(5.80)	0.82
%CHO	52.03	(6.56)	52.83	(3.62)	0.78
%PRO	14.82	(4.66)	15.27	(3.22)	0.83
%FAT	32.89	(3.00)	31.74	(6.56)	0.63
%SFA	11.60	(2.50)	9.81	(3.29)	0.23
%MUFA	9.90	(2.22)	10.50	(3.08)	0.65
%PUFA	5.03	(2.24)	6.30	(2.42)	0.30

Mean (± SD); *P* significant at <0.05 for between groups comparison.

As shown in table 40, baseline the control group consumed a significantly greater energy intake compare the LGI group, this may be attributed of the greater height and mass of the control group. No other dietary variables as adjusted means or % of energy were significantly different. However the LGI group appear to be consuming less PRO and more SFA than the control group. GL was similar between the two groups, GI intake was slightly higher in the control group; for both groups, mean GI intake was classified as moderate.

Scatter plot comparing baseline EI:BMR (MVPA PAL) and EI:EE (RT3)



For EI:BMR (MVPA PAL): +, under-reporter; ●, valid-reporter; ▼, over-reporter. Horizontal lines represent cut-points for EI:EE (RT3)

Figure 8, Misreporting of energy as assessed by EI:BMR (MVPAL) and EI:EE (RT3) between groups.

According to EI:BMR (MVPA PAL), of the 16 overweight participants, 8 were valid-reporters, 6 were under-reporters and 2 over-reported their energy intakes. Whereas, EI:EE (RT3) classified 6 as valid-reporters, 8 as under-reporters and 2 as over-reporters. Of the 6 control group participants only 1(16.6%) individual under-reported and 1 over-reported, as determined by both EI:BMR and EI:EE. Of the 10 low GI group participants, 5 (50%) under-reported according to EI:BMR and this increased to 7 (70%) when assessed by EI:EE (figure 8).

Table 41. Comparison of mean (SD) baseline metabolic syndrome (IDF criteria) risk factors (prevalence of risk) and prevalence of metabolic syndrome between groups.

	low GI n = 10		**Control N = 6**		**P value**
HDL mmol.L	1.22	(0.24)	1.25	(0.17)	0.70
<1.03mmol.L [n (%)]	3	(30%)	1	(16.7)	[a]NS
TG mmol.L	1.34	(1.10)	1.05	(0.93)	0.45
≥1.7 mmol.L [n (%)]	1	(10%)	1	(16.7)	[a]NS
SBP mmHg	113.10	(9.08%)	116.58	(12.68)	0.53
≥ 130 mmHg [n (%)]	0		1	(16.7)	[a]NS
DBP mmHg	78.30	(7.00)	75.08	(5.22)	0.35
≥ 85 mmHg [n (%)]	2	(20%)	0		[a]NS
BG mmol.L	5.02	(0.45)	4.96	(0.55)	0.81
≥5.6 mmol.L [n (%)]	1	(10%)	1	(10%)	[a]NS
Crisk Score	0.34	(2.90)	-0.75	(2.51)	0.46
>1 SD [n (%)]	2	(20%)	0		[a]NS
Metabolic Syndrome IDF [n (%)]	2	(20%)	0		[a]NS

P significant at <0.05 for between groups comparison ; [a]NS, Chi-squared test no significant difference between groups.

Table 41, Mean fasting TG levels and clustered risk score were higher and more individuals were classed as having a low HDL and high clustered risk score in the LGI group compared to the control group. Two participants were classified as having the metabolic syndrome; both were in the LGI group. However, none of the risk factor mean values or frequency of individuals at risk were significantly different between the two groups.

Table 42. Dietary intake during the intervention period for intervention and control group.

	low GI						Control group					
	Baseline (n=11)		WK 6 (n=10)		wk 12 (n=5)		Baseline (n=10)		WK 6 (n= 6)		WK 12 (n= 5)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Energy (kcal)	1624.7	211.2	1329.3	222.6	1454.8	298.7	2600.3	272.7	2254.9	272.7	2295.2	298.7
GI (%)	57.80	1.55	**49.55** ◊◊	1.64	52.73	2.20	60.47	2.00	59.74	2.00	58.51	2.20
GL (g)	163.11	6.47	**117.32**◊◊	6.82	**138.90** ◊	9.15	162.65	8.35	139.32	8.35	**137.10** ◊	9.15
PRO (g)	64.21	4.35	70.81	4.58	**85.43** ◊◊	6.15	75.01	5.61	68.66	5.61	84.31	6.15
Fat (g)	75.50	4.28	**57.99** ◊◊	4.51	66.83	6.05	70.97	5.52	**55.10** ◊	5.52	70.91	6.05
SFA (g)	25.84	2.18	**18.75** ◊	2.30	**17.69** ◊	3.08	22.13	2.81	17.21	2.81	24.75	3.08
PUFA (g)	11.66	2.07	10.96	2.18	16.92	2.93	12.39	2.68	10.59	2.68	13.84	2.93
MUFA (g)	23.90	2.09	18.57	2.21	18.87	2.96	24.05	2.70	18.70	2.70	23.57	2.96
CHO (g)	281.75	10.37	**221.59** ◊◊	10.94	**246.94** ◊	14.67	276.35	13.39	**231.77** ◊	13.39	242.80	14.67
Fibre (g)	15.21	2.55	14.36	2.18	15.79	4.03	14.73	5.80	12.19	7.02	12.45	6.62
Sugar (g)	129.12	54.40	**91.13** ◊	23.66	**78.96** ◊	26.61	103.98	46.55	93.68	34.17	96.68	42.43
% PRO	14.82	1.13	17.69	1.13	15.62	1.60	15.27	1.46	16.45	1.46	18.88	1.60
% Fat	32.89	2.25	29.37	2.25	31.94	3.18	31.74	2.91	31.31	2.91	34.23	3.18
% SFA	11.60	1.12	9.58	1.12	9.11	1.58	9.81	1.45	9.52	1.45	11.60	1.58
% PUFA	5.03	1.00	6.33	1.00	7.97	1.41	6.30	1.29	6.14	1.29	6.01	1.41
% MUFA	9.90	0.97	8.57	0.97	8.13	1.37	10.50	1.25	10.46	1.25	12.00	1.37
% CHO	52.03	2.28	52.68	2.28	51.96	3.23	52.83	2.95	52.11	2.95	47.84	3.23
% Sugar	22.87	2.52	23.49	2.52	17.36	3.56	19.23	3.25	19.60	3.25	19.01	3.56
Hunger score[a]	39.57	7.06	36.92	9.34	25.11	10.79	32.20	8.36	41.17	13.21	42.00	13.21
Satiety score[a]	50.62	6.12	52.25	8.10	53.11	9.35	50.03	7.24	40.33	11.45	48.00	11.45

Mean and standard error; [a],low GI group baseline n= 7; week 6 n= 4; week 12 n= 3. Control group baseline n= 5; week 6 n= 2; week 12 n= 2. †, significant between group*time-period interaction P= <0.05 ◊◊, significantly different from baseline P <0.01 (◊, P=<0.05).

As displayed in Table 42, Energy intake declined at week 6 and increased slightly at week 12 in both groups, however, there was no significant interaction between group*time period and there was no significant within-subjects change in energy intake in either group.

There was a significant reduction in GI in the LGI group between baseline and week 6 (P=0.001), at week 12, however, GI had increased and was no longer significantly different from, yet remained lower than, baseline. In the control group there was a slight but non-significant reduction in GI at week 6 and 12 compared to baseline. MANCOVA revealed a significant reduction in GI of 7.52 in the LGI group relative to the control group at week 6 compared to baseline (β = -7.52 (SE 3.62); P= 0.045)); at week 12 compared to baseline this interaction was no longer significant. GL was also significantly reduced at week 6 (P= <0.001) as well as week 12 (P= 0.038) compared to baseline in the LGI group. In the control group, however, there was also a significant reduction in GL at week 12 compared to baseline (P=0.047) similar to that of the LGI group.

Total PRO intake increased in both groups by week 12 compared to baseline and this increase was significant in the LGI group. Fat intake significantly declined at week 6 (P= 0.008 and 0.050) for the LGI and control groups. Similar declines in SFA were observed in both groups at week 6, however, at week 12 SFA continued to decline at week 12 whereas it increased in the control group; the reduction in SFA for the LGI group was significant at week 6 (P=0.032) and 12 (P= 0.038). Changes in MUFA and PUFA were non significant for both intervention groups. CHO intake significantly declined at week 6 in both groups, however, the reduction was greater in the LGI group (P= <0.001) compared to the control (P= 0.024). CHO intakes increased from week 6 at week 12 in both groups, however the difference from baseline remained significant for the LGI group (P= 0.061).

Sugar intake significantly declined in the LGI group from baseline at week 6 (P= 0.050) and decreased further at week 12 (P= 0.031). Fibre intakes appeared relatively unchanged in both groups. There were no significant changes in macronutrients relative to energy and thus, other than for total PRO which increased in both groups, the decline in total macronutrients observed may be attributed to the reduction in energy consumed at week 6 and 12 relative to baseline in both groups.

Overall daily hunger and satiety score were not significantly altered during the intervention, however, hunger scores decreased from baseline at week 6 and 12 in the LGI group, but increased in the control group. There was also a slight increase in satiety scores in the LGI group, where as it marginally decreased in the control group. See figure 9, for a graphical representation of changes in energy, glycaemic CHO and hunger scores.

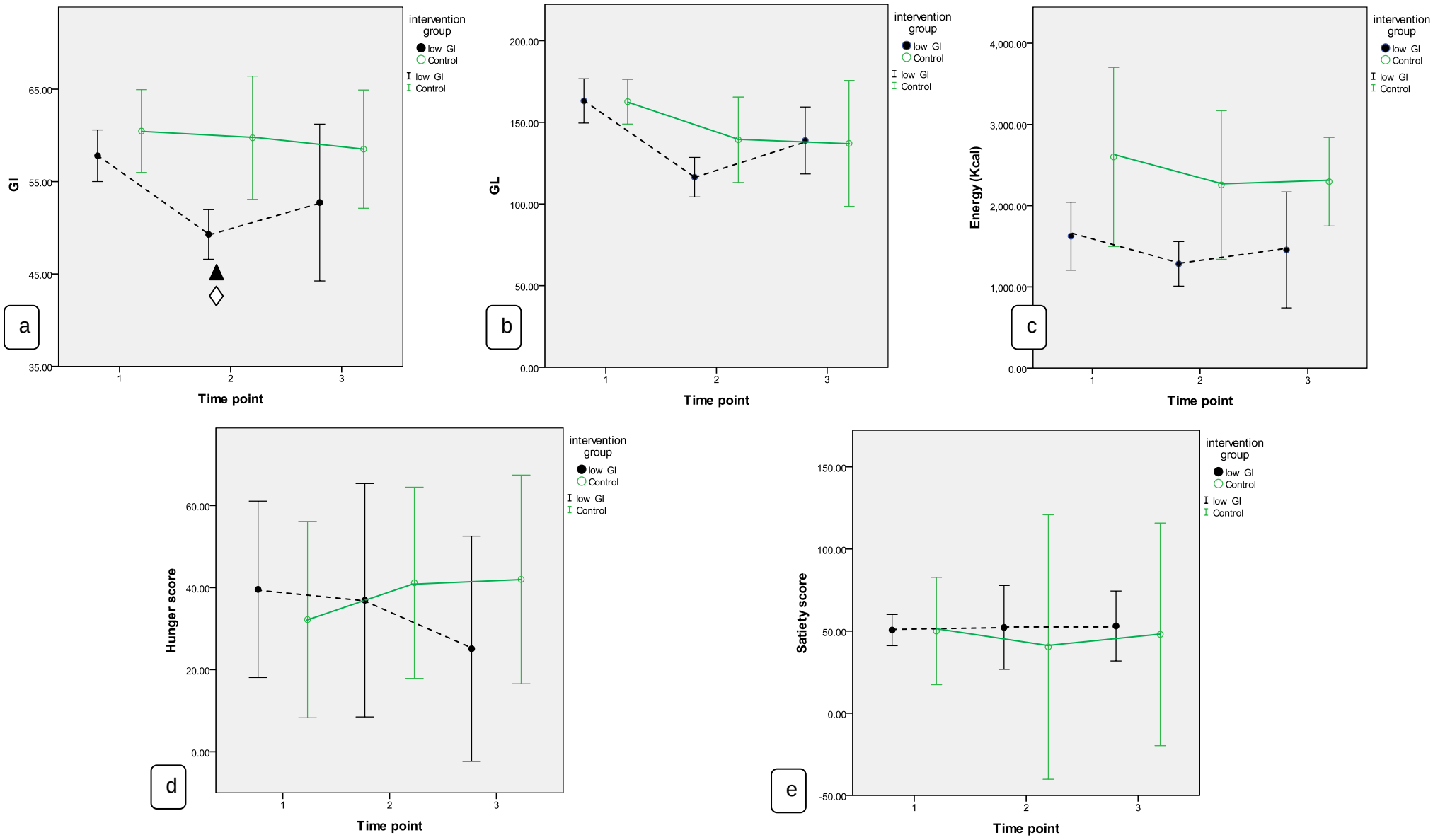


Figure 9. Mean dietary GI (a), GL (b), energy (c), Hunger (d) and satiety (e) scores for intervention and control group across study time points (1 = Baseline; 2 = week 6; 3= week 12). ▲, P= < 0.01 for within group change compared to baseline; ◊, P= < 0.05 for interaction of group*time period compared to baseline.

MANCOVA and linear mixed models were adjusted using the sensitivity analysis approach, 4 separate models were analysed but adjusted for the following covariates: Model 1) age, sex, ethnicity, SES, *z*BMI; Model 2 built on model 1, additionally adjusting for energy, and residual adjusted dietary variables (fibre, fat, PRO, CHO); Model 3 also included CRF and %SED and model 4 additionally included EI:EE and thus finally controlled for dietary misreporting.

Weight and Adiposity

Figure 10 displays adjusted (model 4) mean weight and adiposity variable values for each group during the three intervention time-points: 1 (baseline); 2 (week 6); 3 (week 12).

Mancova and linear mixed model analysis revealed that there was no significant change in weight or adiposity in either group. FFM changes were similar for both groups during the intervention. For body weight, FM and zBMI there was little change in the LGI group, however, there does appear to be a non significant trend for an increase from baseline in these variables for the control group.

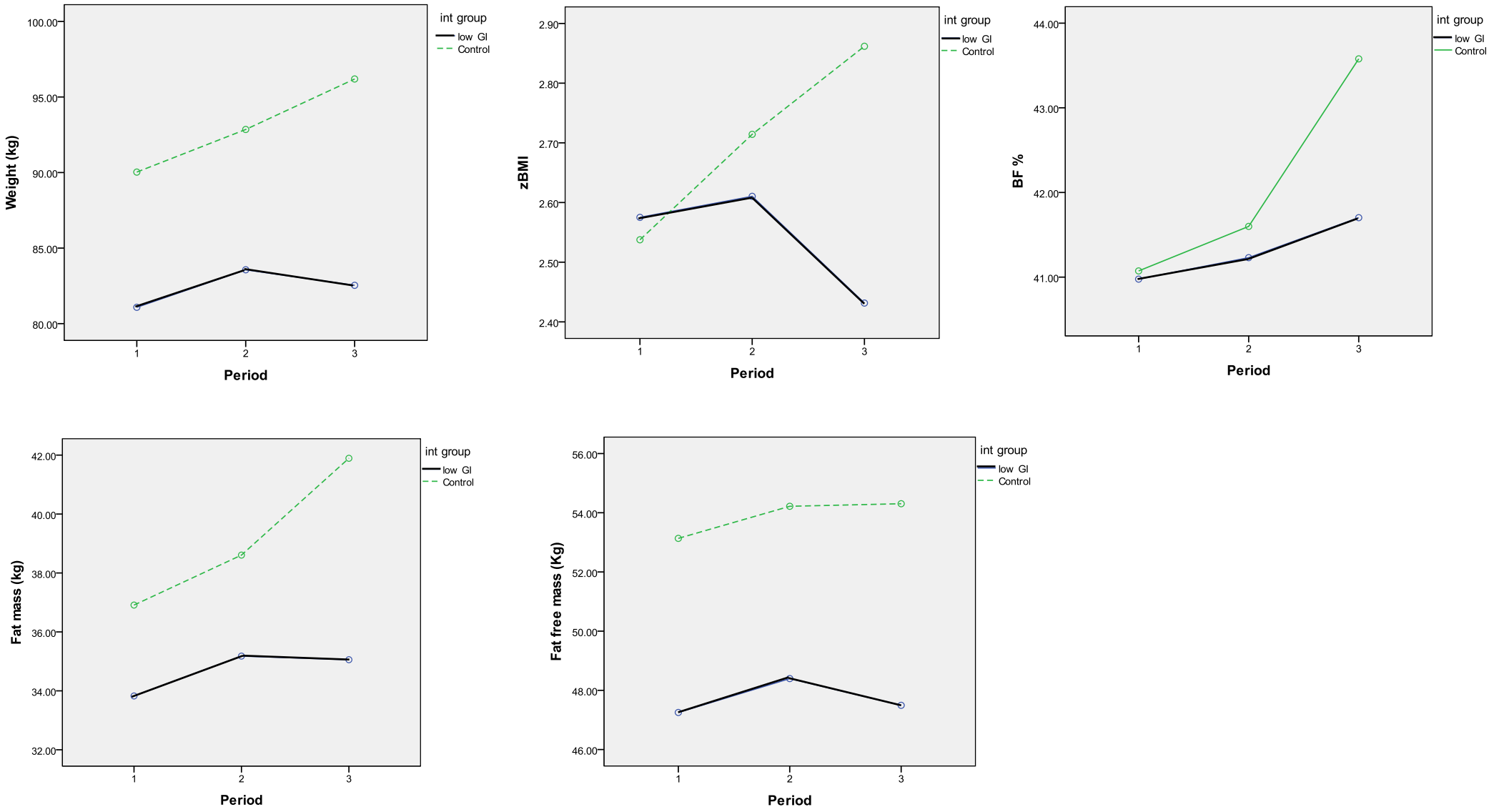


Figure 10. Graphs showing weight and adiposity changes for intervention and control groups during the intervention: a (weight); b (zBMI); c (BF %); d (fat mass); e (fat free mass). Analysis adjusted for age, sex, ethnicity, SES, kcal, and residual adjusted dietary variables (fibre, fat, PRO, CHO), CRF, MVPA and EI:EE.

Metabolic syndrome risk factors

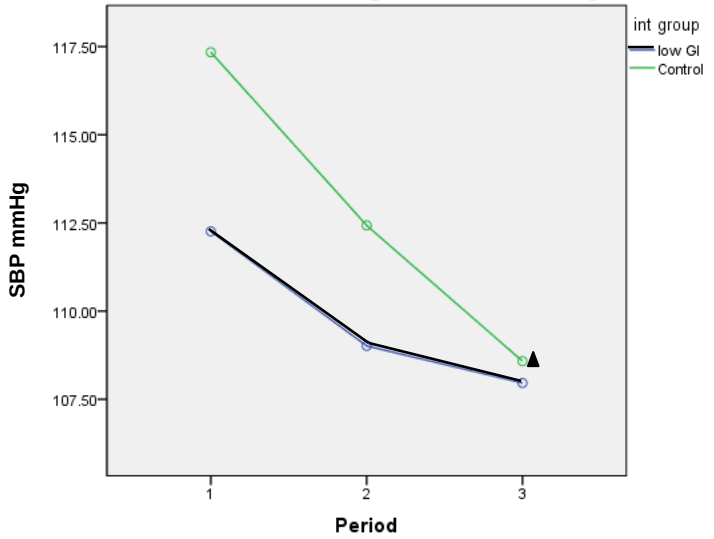
Table 43, figure 11 and figure 12, show that none of the adjusted MANCOVA or Linear mixed models revealed a significant group*time-period interaction for any metabolic risk factor variables; according to MANCOVA there were also no significant within group changes observed. However, pairwise comparisons of adjusted means from linear mixed model analysis revealed a significant within group reduction in clustered risk score at week 12 compared to baseline in the LGI group (P= <0.01). In the control group there was a significant reduction in SBP at week 12 compared to baseline (P= <0.05). Mixed models also revealed that BG and DBP were marginally significantly reduced at week 6 compared to baseline in both groups (P= <0.1), however, by week 12 BG had continued to decline in the LGI group where as it had slightly increased in the control group. SBP was also marginally significantly reduced at week 12 compared to baseline in the LGI group.

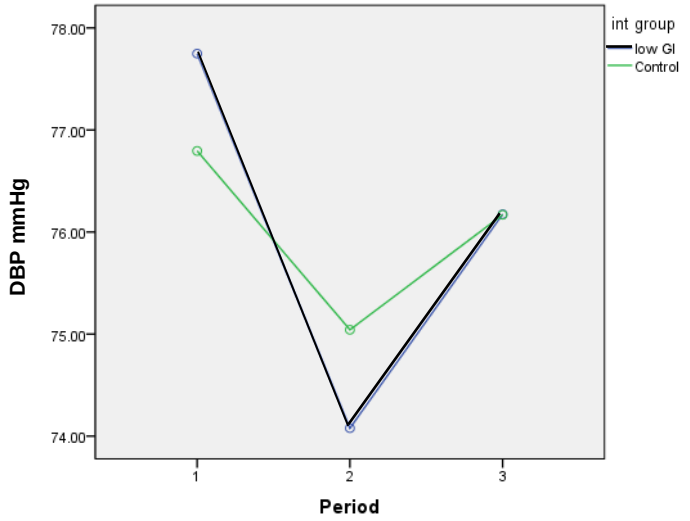
Trends in outcome variables over time for intervention and control groups using line graphs. For SBP, DBP, TG, Clustered risk and BG changes appear to be similar in both the LGI and control groups. However, WC can be seen to decrease considerably at week 6 and remain stable between weeks 6 and 12 in the control group compared to the LGI group which appears to increase slightly between week 6 and 12. Additionally HDL cholesterol reduced in both groups between baseline and week 6 but increased above baseline in the control group between week 6 and 12 and continued to decline at week 12 in the LGI group

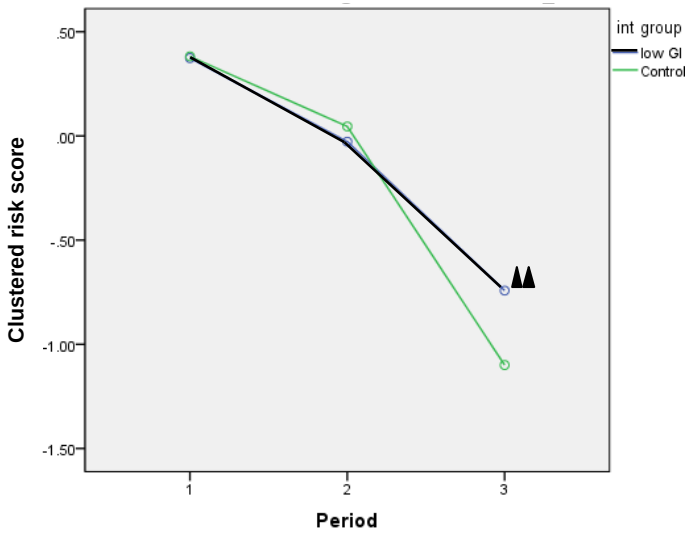
Table 43. Linear mixed effects for metabolic syndrome risk factors between intervention groups

Model 4	[a]Linear Mixed Effects Intervention group*time period			
	β Estimate Period 2 vs 1		**β Estimate Period 3 vs 1**	
	β	P Value	β	P Value
SBP	0.23 (4.96)	0.964	3.53 (3.90)	0.380
DBP	-4.05 (3.32)	0.238	-3.88 (1.79)	0.052
BG	0.04 (0.16)	0.811	0.02 (0.19)	0.920
TG	-0.10 (0.10)	0.305	0.09 (0.21)	0.679
WC	-0.17 (1.47)	0.906	-0.14 (1.50)	0.926
HDL	0.04	0.587	-0.04	0.644
Crisk	-1.01 (0.78)	0.209	-0.62 (0.40)	0.146

[a] LGI group as reference group; analysis adjusted for age, sex, ethnicity, SES, *z*BMI, kcal, and residual adjusted dietary variables (fibre, fat, PRO, CHO), CRF, MVPA and EI:EE







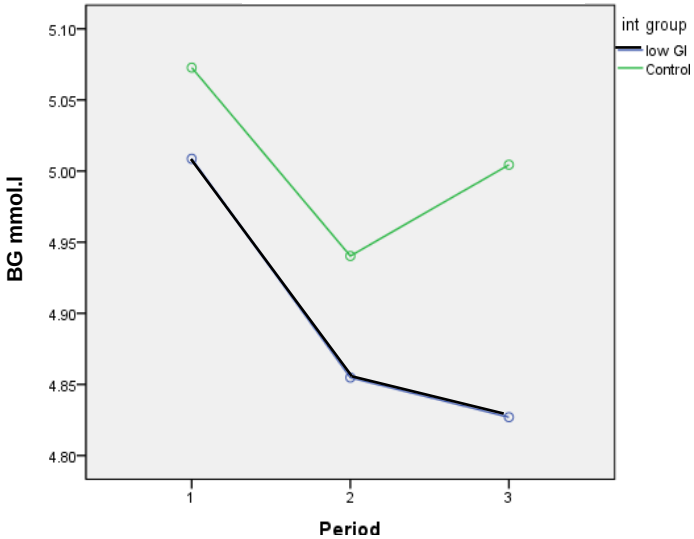


Figure 11. Graphs showing change in mean values of metabolic risk factors for the control and intervention group: SBP, DBP, clustered risk score, BG. Model 4: analysis adjusted for age, sex, ethnicity, social economic status, *z*BMI, kcal, and residual adjusted dietary variables (fibre, fat, PRO, CHO), CRF, MVPA and EI:EE; for mixed models: ▲, significantly different from baseline (P= <0.05).

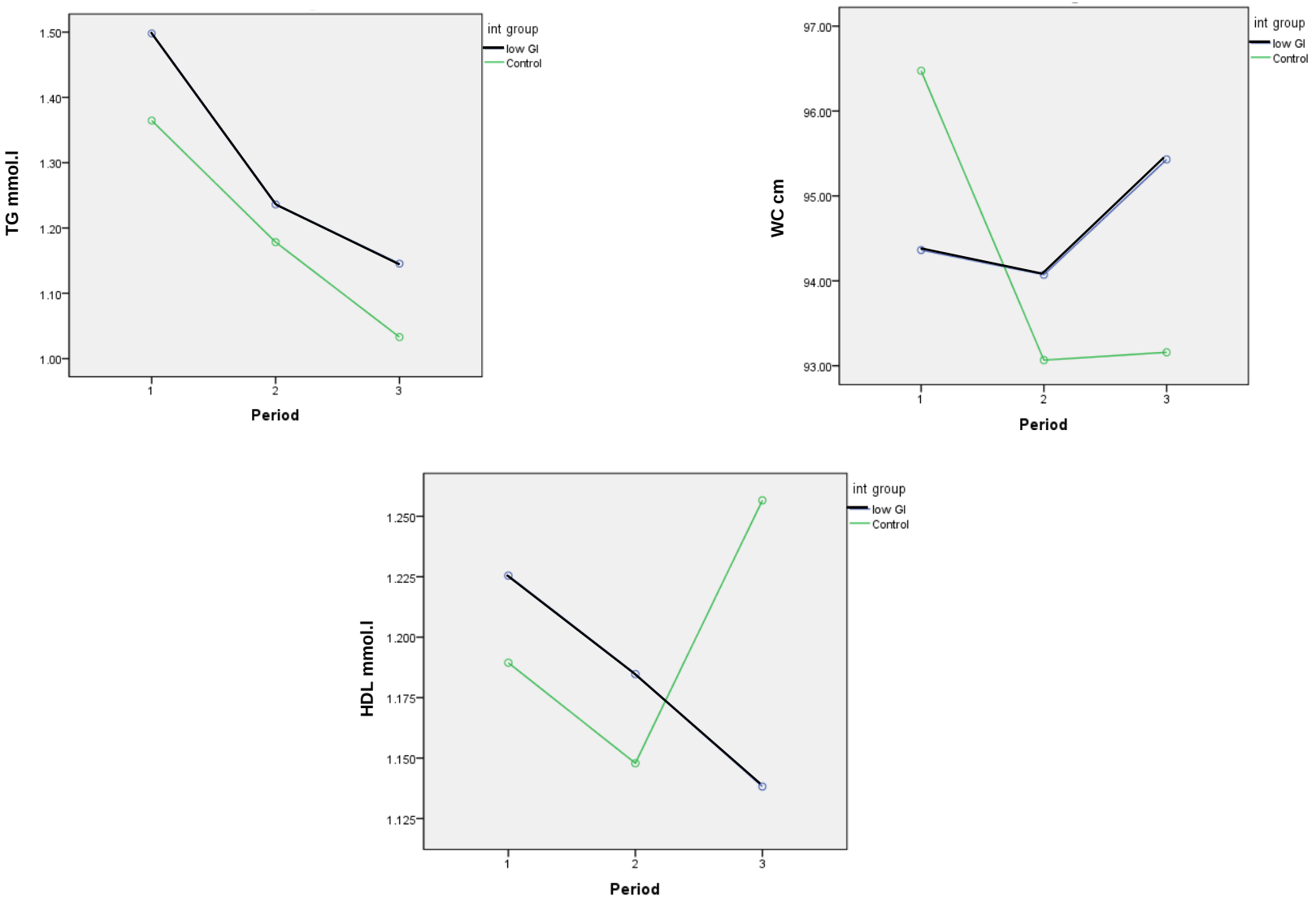


Figure 12. Graphs showing change in mean values of metabolic risk factors for the control and intervention group: TG, WC,HDL. Analysis adjusted for age, sex, ethnicity, social economic status, *z*BMI, kcal, and residual adjusted dietary variables (fibre, fat, PRO, CHO), CRF, MVPA and EI:EE; for mixed models: ▲▲, significantly different from baseline (P= <0.01).

Inflammatory markers and cytokines

For inflammatory markers and cytokines there were no significant interaction effects for group* time-period, however as the models progress to include dietary and fitness variables as covariates the P value can be seen to decline suggesting the strength of associations is increasing as diet and CRF and PA are adjusted for (see appendix 6).

Figure 13 Displays adjusted means for inflammatory markers and cytokines. Using MANCOVA did not identify any significant within-subjects differences over the intervention time-period. However, linear mixed models (which adjust for the correlation of repeated observations) did identify a marginally significant reduction in adiponectin and IL-6 from baseline to week 12 in the LGI group (P= <0.1) (Table 44). In the control group there was a marginally significant increase in TNF from baseline to week 12 (P= <0.1).

Although adiponectin is marginally reduced at week 12 compared to baseline it can be observed in figure 13 that adiponectin begins to increase at week 6 and Hs-CRP starts to decrease at week 6 suggesting a favourable effect in the LGI group at week 6 which appears to reverse between weeks 6 and 12. It can be observed that changes in TNF in each group relative to baseline are very similar

Table 44. Linear mixed effects for inflammatory markers and cytokines between intervention groups

Model 4	**[a]Linear Mixed Effects Intervention group*time period**			
	β Estimate Period 2 vs 1		**β Estimate Period 3 vs 1**	
	β	P Value	β	P Value
Adiponectin	0.34 (0.72)	0.641	-0.48 (0.48)	0.354
CRP	-0.23 (0.86)	0.793	0.10 (1.10)	0.927
TNF	-1.07 (1.25)	0.403	-1.19 (1.07)	0.290
IL-6	-0.33 (0.28)	0.270	-1.38 (0.87)	0.142

[a] LGI group as reference group; analysis adjusted for age, sex, ethnicity, SES, *z*BMI, kcal, and residual adjusted dietary variables (fibre, fat, PRO, CHO), CRF, MVPA and EI:EE

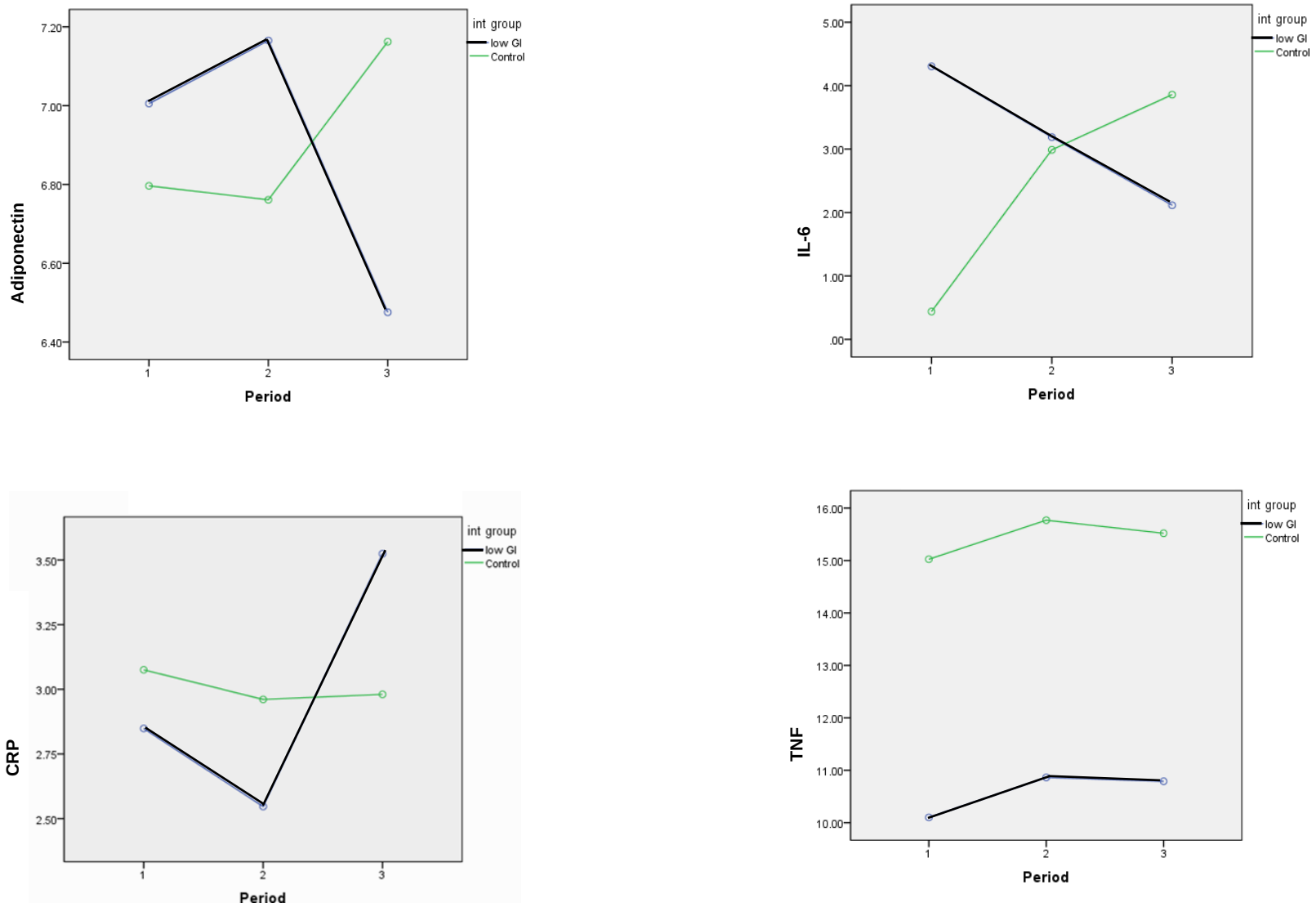


Figure 13. Graphs showing change in adjusted mean values of inflammatory markers for the control and intervention group: Adiponectin, IL-6, CRP, TNF. Analysis adjusted for age, sex, ethnicity, social economic status, *z*BMI, kcal, and residual adjusted dietary variables (fibre, fat, PRO, CHO), CRF, MVPA and EI:EE.

Glucose control and liver function

In table 45, linear mixed models showed that insulin was significantly reduced at week 6 compared to baseline and week 6 and 12 in the control group (P= <0.05). As displayed in figure 14, the LGI and control group ALP was significantly reduced at week 12 versus baseline (P =<0.01) and between week 6 and week 12 (P= <0.05), furthermore in the LGI group ALP was significantly reduced at week 6 compared to baseline (P= <0.05). In the control group ALT significantly increased at week 6 (P= <0.05), in the LGI group, ALT decreased (non-significantly) at weeks 6 and 12. However, at week 12 in the control group ALT reduced significantly (P= <0.01) at week 12 compared to week 6 and to below baseline values. In figure 15, MANCOVA revealed a significant increase in insulin at week 12 compared to baseline and week 6 (P=<0.05) in the LGI group. In the control group HOMA-IR was significantly reduced at week 6 (P= <0.01) and week 12 (P= <0.05) compared to baseline. There were no other significant within group changes in $HbA1_c$, GGT, albumin and bilirubin in either group.

Adjusted beta estimates (β) for linear mixed models showed, however, that there were marginally significant reductions in $HbA1_c$ at week 6 (β -0.20; P= 0.078) and week 12 (β -0.43; P= 0.062) versus baseline in the LGI group relative to changes in the control group (Table 45). Insulin at week 6 and 12 significantly increased from baseline in the LGI group relative to the control group by 48.74 (P= 0.017) units and 30.71 (P= 0.045), respectively. The same interaction was observed for HOMA-IR with an increase of 1.43 at week 6 versus baseline in the low GI group relative to the control group (β = 1.43, P= 0.024). Interactions were significant following adjustment for CRF and PA in model 3; additional adjustment for EI:EE in model 4 did not alter these associations (see appendix 6). This was observed when the interaction was assessed by MANCOVA and when the additional adjustment for the correlation of repeated observations was made using linear mixed model analysis.

Linear mixed models also revealed a significant group*time-period interaction for the liver function marker ALT with a significant reduction of 8.57 at week 6 compared to baseline in the LGI relative to the control group (β= -8.57, P= 0.012).

Trends in the data show that despite a significant and marked increase in insulin and HOMA-IR at week 12 compared to baseline for the LGI group relative to the control group (assessed by ANCOVA and mixed effects), at week 6, the within groups comparison for these variables remains relatively unchanged. Furthermore, for those variables where there was no significant effect of the intervention such as GGT and bilirubin there appears to be a reduction in these values at week 6 versus baseline in the LGI group relative to the control group. This was also the case for some metabolic risk factor and inflammatory variables; thus it seems the LGI intervention may be associated with a favourable effect on some outcome variables at week 6 that may be lost between week 6 and 12. Conversely, $HbA1_c$, appears to remain stable at week 6 compared to baseline and begins to decline between weeks 6 and 12 as opposed to the control group where this value increases throughout the intervention. Moreover for ALT and ALP values decline throughout the intervention compared to baseline in the LGI group.

Table 45. Linear mixed effects for glucose control and liver function markers between intervention groups

Model 4	[a]Linear Mixed Effects Intervention group*time period			
	β Estimate Period 2 vs 1		**β Estimate Period 3 vs 1**	
	β	P Value	β	P Value
HbA1c	-0.20 (0.11)	0.078	-0.43 (0.19)	0.062
Insulin	48.74 (18.31)	0.017	30.71 (13.45)	0.045
HOMAIR	1.43 (0.56)	0.024	0.91 (0.51)	0.113
ALP	-12.73 (9.66)	0.242	-10.49 (6.21)	0.125
ALT	-8.57 (3.13)	0.012	-3.94 (4.22)	0.365
GGT	0.17 (0.98)	0.863	0.21 (0.84)	0.806
Albumin	-3.67 (3.13)	0.257	-1.41 (1.63)	0.398
Bilirubin	3.55 (4.92)	0.481	2.41 (5.04)	0.638

[a] LGI group as reference group; analysis adjusted for age, sex, ethnicity, SES, *z*BMI, kcal, and residual adjusted dietary variables (fibre, fat, PRO, CHO), CRF, MVPA and EI:EE

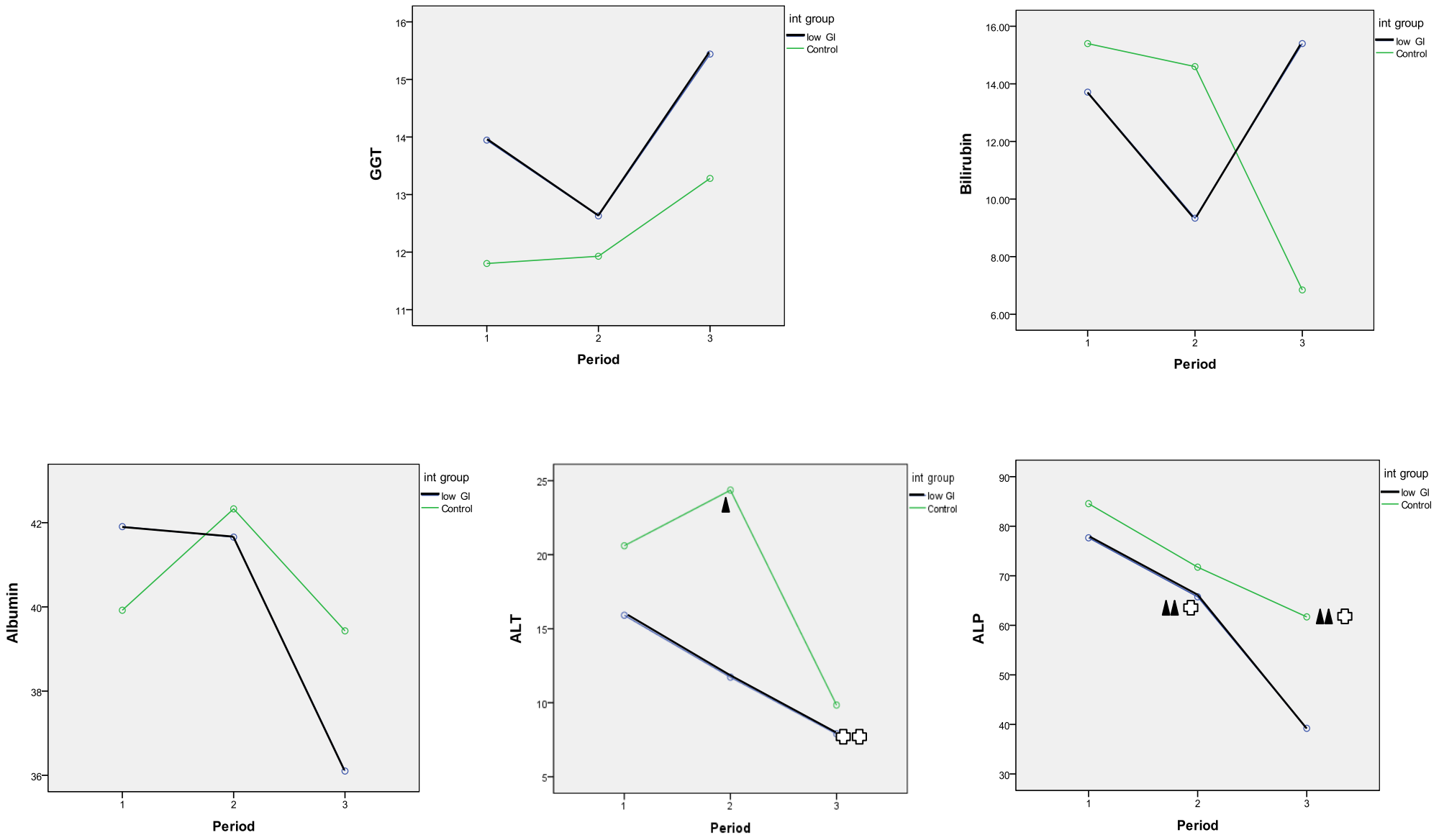


Figure 14, Graphs showing change in adjusted mean values of liver function markers for the control and intervention group: GGT, Bilirubin, Albumin, ALT, ALP. Analysis adjusted for age, sex, ethnicity, social economic status, *z*BMI, kcal, and residual adjusted dietary variables (fibre, fat, PRO, CHO), CRF, MVPA and EI:EE; for mixed models: ▲, significantly different from baseline (P= <0.05); ▲▲, (P= <0.01); within groups: ✠, significantly different from baseline (P= <0.05); ✠✠, (P= <0.01).

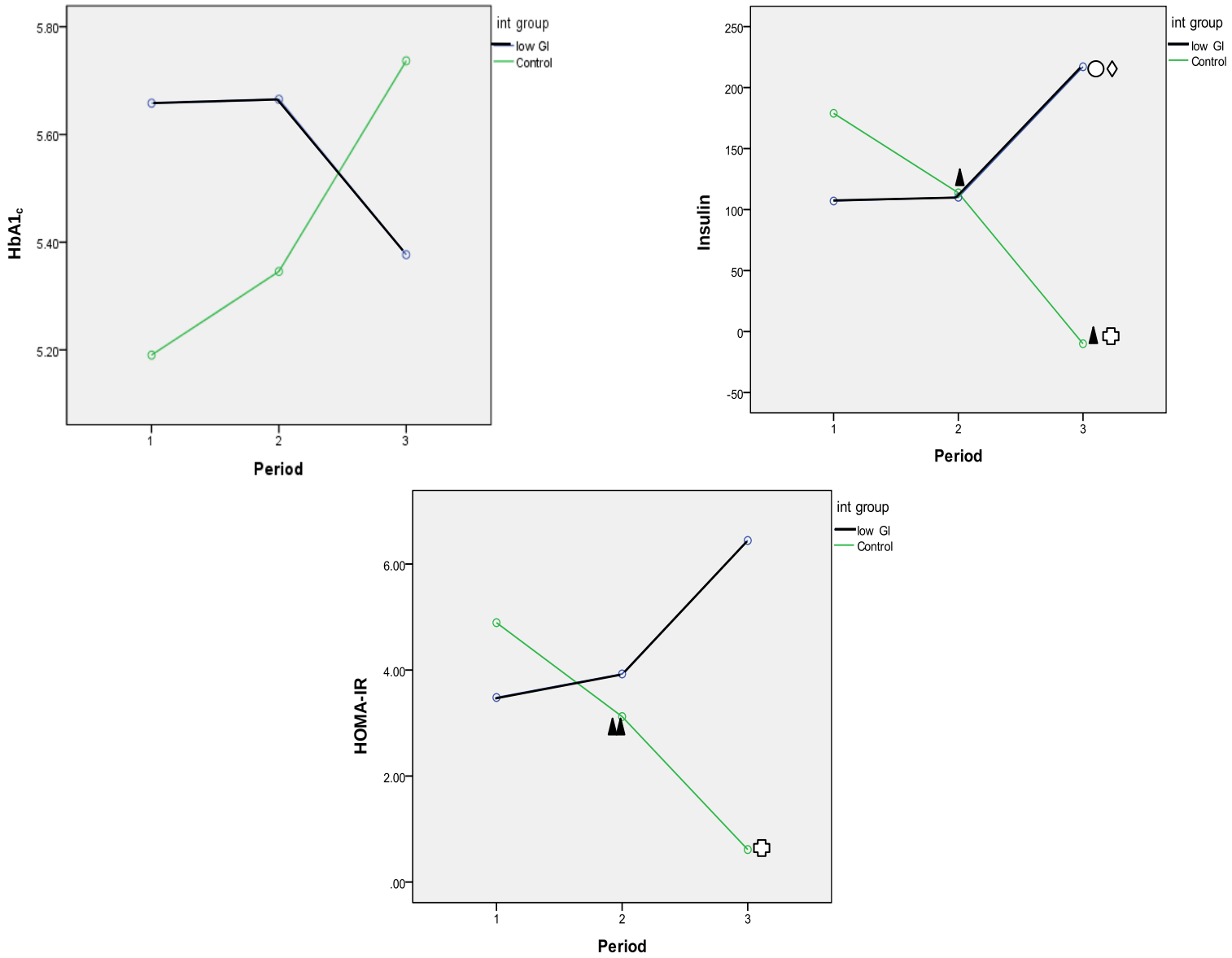


Figure 15. Graphs showing change in adjusted mean values of glucose control markers: for the control and intervention group: HbA1c, insulin, HOMA-IR. Analysis adjusted for age, sex, ethnicity, social economic status, *z*BMI, kcal, and residual adjusted dietary variables (fibre, fat, PRO, CHO), CRF, MVPA and EI:EE. MANCOVA: ○, significantly different from baseline (P= <0.05). ◊, significantly different from baseline (P= <0.05). Mixed model: ▲, significantly different from baseline (P= <0.05); ▲▲, (P= <0.01); for within groups:✣, significantly different from baseline (P= <0.05).

8.3 Discussion

The present study investigated the impact of an *ad libitum* 12 week low GI dietary intervention on risk factors of the metabolic syndrome, markers of glucose control, liver function and inflammation in a sample of overweight and obese postpubertal adolescents, with a high WC and one additional metabolic risk factor, from Bedfordshire.

This work provides novel insight into the feasibility of prescribing an *ad libitum* low GI dietary intervention on the dietary intakes of a UK adolescent population. Three day weighed food diary data recorded at baseline showed that mean dietary GI and GL was moderate both for the LGI and control group (GI 57.8 and 60.47, respectively). GI was significantly reduced (-8.25, P= 0.001) in the LGI group at week 6 compared to baseline. Between week 6 and 12, however, GI had increased in the LGI group by 3.18 units and was no longer significantly different from, yet remained below, baseline. In the control group GI remained relatively stable, slightly reducing by <1 unit at week 6 and <2 units by week 12 compared to baseline.

The reduction in GI seen in the LGI group is comparable, despite a considerable age difference, to a 6 month low GI dietary intervention prescribed to 106 overweight adults with type 2 diabetes (mean age 60 yrs) from Canada who reduced their GI from baseline (81.5) by over 10 units to 69.6 and their GL by 37.8 (g) (Jenkins et al., 2008). This diet aimed to evoke a 10 – 20 % reduction in GI and food options and quantities were controlled based on the individual energy requirements of each participant. In 110 UK adults with high central adiposity, and thus deemed at risk of developing the metabolic syndrome, a 6 month low GI intervention also elicited a similar change in GI of -7.2 units, however this study also heavily restricted fat and energy intakes to maintain weight (Jebb et al., 2010). An *ad libitum* approach was used by Ebbeling et al (2008) to lower GL in 11 obese young adults (18-35 yrs) form the USA over 6 months. Although, CHO intakes were indirectly controlled in order to reduce GL there was a similar reduction in GI to that of the present study of approximately 10 units.

Furthermore, in the present study, when assessing the interaction of time-period and intervention group a significant reduction in GI of 7.52 in the LGI group relative to the control group at week 6 compared to baseline was evident (P= 0.045). In

contrast to GI, GL decreased in both groups from baseline at week 6 and 12. This change in GL appeared to be in line with decreases in total CHO seen in both groups. It is likely that the very similar reduction in energy in both groups at week 6 and 12 in relation to baseline is attributable to the reduced CHO intake and subsequently GL; by week 12 GL values in both groups had significantly reduced (P=<0.05) to 138.90 and 137.10, respectively. However, the reduction in GL in the LGI group (P= <0.001) was greater than that of the control group (-45.79 and -23.33, respectively) at week 6 and it is likely that this is related to the considerable GI reduction in the LGI group at this time point. It seems that a significantly reduced GL in the control group was achieved due to a non-significant but 5% decrease in %CHO and a concomitant slight increase in %Fat and %PRO at week 12 compared to baseline. Because dietary fat and protein slow rates of gastric emptying and digestion they are associated with a number of low GI foods. Some dieticians have expressed concern in the past that an *ad libitum* low GI diet is thus likely to have an unfavourable effect on fat consumption (Franz, 2003). However, in the present study this was not observed, in fact, data trends show that there was a slight reduction in %fat in the LGI group at week 6 and %SFA reduced at both week 6 and 12 approximately 2.5%. Comparatively, in the control group, %fat and %SFA had both increased by approximately 2% at week 12 of the intervention compared to baseline. However, none of these changes were found to be significant. It thus appears that a flexible diet centred on the substitution of high for low GI foods consumed within the context of a balanced diet, (Eatwell plate, FSA, 2013) elicited a potentially beneficial change in BF% and SFA intake, that was not observed in the control group who consumed habitual diet in the context of the eatwell plate. It also appears that this approach resulted in similar glycaemic CHO changes to a relatively restricted low GI approach (Jenkins et al., 2008) and *ad libitum* low GL diet (Ebbeling et al., 2008) in adults.

Unfortunately, few studies that have prescribed low glycaemic CHO diets in adolescents, particularly those described as *ad libitum*, report the change in GI and GL during the intervention. Ebbeling et al (2003) prescribed a 6 month *ad libitum* low GL diet compared to a conventional low fat diet in obese 13-20 yr olds from the USA. At 6 months in the LGL group, GL (g/1000 kcal) had significantly reduced from baseline (86) to 68 g/1000 kcal and this was the product of a significantly reduced % CHO intake and a non significant reduction in GI from baseline (58) to 53. In the conventional low fat group % fat intake had significantly declined but GL, GI and

CHO were unchanged. The difference between this study and the present investigation is that Ebbeling et al (2003) required of their participants to reduce their CHO intake in order to elicit a reduction in GL and was thus not truly *ad libitum*. The present study only focused on swapping high GI foods with low GI alternatives to maintain flexibility of the diet and thus macronutrients as a % of energy were relatively unchanged in both groups. Contrastingly, the adolescents from the US maintained a reduced GL and GI for a markedly greater time period compared to those in the current study, who could not maintain the reduction in GI observed at 6 weeks for a further 6 week period. Furthermore, a 12 month follow up conducted by Ebbeling et al (2003) identified that GI and GL reductions were maintained for a further 6 months. In their study (Ebbeling et al., 2003), participants attended 2 nutritional education and behavioural therapy session each month for 6 months, although this is less intensive than the current study which employed a weekly consultation session, perhaps the reduced frequency presented less burden to the participants and thus eliciting a greater adherence to the intervention.

Although, in these Bedfordshire adolescents, no significant changes in hunger and satiety scores were observed in either group, it can be seen in figure 10, that hunger scores decreased from baseline in the LGI group, but increased slightly in the control group. Moreover, there was a non significant trend of increased satiety scores for the LGI group, whereas there was a decline in the control group. This is supported by evidence that satiety was significantly more prolonged (delayed subsequent *ad libitum* meal intake) following consumption of a low compared to high GI meal in 16 overweight 12-18 year olds (Ball et al., 2003). It is important, however, to highlight the lack of compliance with completing the hunger and satiety questionnaires of which at baseline n = 7 and 5; at week 6 n= 4 and 2 and at week 12 n = 3 and 2 for the LGI and control group, respectively. Furthermore, to avoid hindering food record compliance, the hunger and satiety questionnaire was completed once for each of the 3 dietary recording days to obtain an ‘overall’ score rather than repeating the questionnaire after each meal.

A number of past intervention studies have shown that a reduced glycaemic CHO diet can have a beneficial effect on metabolic health. There is however limited evidence in both adults and youngsters and there does not appear to be any evidence of a beneficial impact on clustered metabolic risk score in youths. In the present study, however, clustered risk score was shown to be significantly reduced

(P= <0.01) at week 12 from baseline in the LGI group and although it was reduced in the control group the difference was not significant when within-subject correlations were accounted for by mixed model analysis. Previous research (Pereira, 2004) involving 39 young adults comparing a low GL and low fat diet showed a trend (P=0.07) for greater improvements in DBP and SBP in the low GL group after approximately 9 weeks. This was observed despite similar relative energy intakes and adiposity changes in both groups. In the present study, marginally significant improvements in SBP and DBP occurred after 6 weeks in the LGI group, however, a similar trend was observed for DBP at 6 weeks in the control group and these trends were observed despite no reductions in adiposity. The present investigation did not identify significant improvements in lipid profile (HDL or TG) in either group, which could relate to a lack of adiposity change during the 12 week intervention. In light of this, however, Pereira et al (2004) also observed significantly greater improvements in TG in the low GL group, suggesting that the benefit on lipid profile as a result of reduced GL outweighed that of weight loss. The authors observed a significantly smaller decline in resting energy expenditure in the low GL diet group vs low fat group, but not to the extent that a significant change in adiposity could occur over a short period. Because of the evidence that hyperinsulinemia may play a critical role in the development of dyslipidemia and other metabolic risk factors, via enhanced hepatic VLDL rich TG secretion (Reaven, 1995, Fried and Rao, 2003) it was postulated that the greater improvement in lipid profile in the low GL group may have been caused by the larger reduction in insulin concentrations seen in this group (Pereira et al., 2004). Improvements in metabolic health risk factors were also observed in a group of 8 overweight and obese Hungarian children (11 year olds) prescribed a 6 week *ad libitum* low GL dietary intervention. Slight improvements in lipid profile were observed; with a small increase in HDL and a decrease in TG. Moreover, reductions in BG were similar to those of the present study at week 6: 5.31 vs 4.96 mmol.L and 5.01 vs 4.85 mmol.L, respectively. Furthermore, Fajcsak et al (2008) observed a significant reduction in the number of metabolic risk factors present at baseline following the intervention 28 vs 15, however they did not calculate a clustered risk score. These changes occurred in the presence of a significant reduction in BF% and although described as *ad libitum*, this intervention restricted portion sizes in an attempt to control dietary GL and thus it is quite possible that energy intake was reduced as a consequence of this intervention. Yet, because dietary changes were not assessed and there was no control group in Fajcsak et al's (2008) study it is very difficult to distinguish which

dietary factors were likely to have attributed to the improved metabolic health or if they were linked to reduced adiposity.

The significant improvement in clustered metabolic risk score in the LGI group occurred despite no change observed in lipid profile or WC in this group. It is noteworthy, however, that assessing clustered risk may provide more insight into metabolic changes than individual risk factors alone, as a clustered score can compensate for daily fluctuations that exist amongst individual risk markers (Anderssen et al., 2007b). Furthermore, the reductions in BG, DBP and SBP, may have contributed to the overall reduction in clustered risk. A reduced BP following the low GI diet could be explained by the mechanism of reduced nitric oxide (NO) oxidation (Blaak et al., 2012). NO, a potent vasodilator, is oxidised at an increased rate in the presence of hyperglycaemia induced oxidative stress, leading to reduced NO concentrations and subsequent impairments in vasodilatation (Williams et al., 1998). This may be supported by the current work, which also observed marginal reductions in some inflammatory markers in the LGI group, as outlined below.

A number of intervention studies in adults have shown that reducing dietary GI and GL has a beneficial effect on inflammatory markers. In the present study there was no significant interaction of group and time-period. Within group comparisons, however, demonstrated that there was a marginally significant reduction in IL-6 between baseline and week 12 in the LGI group, furthermore, despite similar trends in both groups, there was a marginally significant increase in TNF in the control group at the same time-point. Thus it appears that the low GI diet may have had a beneficial effect on inflammation that was not observed in the control group. In contrast to improved inflammation the LGI group there was a marginally significant decrease in adiponectin (a potent anti-inflammatory cytokine) observed at week 12 compared to baseline. It is noteworthy, however, that despite a potentially beneficial effect of the LGI diet on inflammation, at week 12, CRP levels had increased above baseline. Thus there seem to be inconsistent findings in this study for inflammation changes as a result of lowering dietary GI but the lack of reduction in adiposity as a result of the intervention may have hindered improvements in inflammation. (Pittas et al., 2005) observed that a 24 week low GI diet was not associated with a significantly reduction in plasma CRP concentrations once body weight change was controlled for. Similarly, it was identified that a 10 week low vs high GI diet resulted in a reduced concentration of CRP (16%), however, this change was not significant

(Sørensen et al., 2005). Moreover, in the study of Pereira et al (2004) in adults (outlined earlier in this section), in addition to improved BP and TG, a significantly greater reduction in CRP was observed in the low GL compared to the reduced fat control group. This occured despite equal reductions in body fat between the two groups, and this was attributed to reduced insulin concentrations in those following the low GI diet. It has also been observed that both a traditionally 'healthy American diet' and an energy matched legume rich low GI diet had a beneficial effect on inflammation in 64 healthy 35-75 yr old males; (Hartman et al., 2010), observed a significant reduction in CRP and TNF during a 4 week intervention period separated by a 2 week washout diet period. Unfortunately, the lack of evidence for younger populations makes comparison to other adolescent populations difficult.

Previous research has highlighted that a high GI diet was associated with increased liver steatosis in 247 Italian adults who were otherwise healthy but had a high WC (Valtueña et al., 2006) they reported significantly higher ALT values (a marker of liver damage) in those consuming a high GI diet. The authors proposed that a high GI diet could exacerbate hepatic fat deposition via increased lipogenisis and reduced FFA oxidation at the liver and that the effects would be heightened in those with insulin resistance (Valtueńa et al., 2006). In the present study it was observed that there may have been favourable changes in liver function markers in the LGI group compared with detrimental changes in the control group. The liver function marker ALP significantly reduced (P= <0.01) at week 12 compared to baseline during the LGI intervention and in the control group ALT significantly increased at week 6 (P= <0.05), whereas it was non-significantly reduced in the LGI group. However, in a group of elderly, non diabetic, normal and over-weight individuals, over a 5 year longitudinal study, glycaemic CHO was not associated with markers of liver function GGT or ALT, but a reduced dietary fibre intake was associated with increased GGT (Arner et al., 1991). It is possible that glycaemic CHO may only be associated unfavourably with liver function in those with insulin resistance, or in the case of the current study, those who are metabolically at risk.

There were no significant changes in glucose control marker, $HbA1_{c,}$ within either group. In the LGI group, however, values decline between weeks 6 and 12 by 0.31, whereas this value increases throughout the intervention in the control group. The decline in $HbA1_c$ at week 12 in the LGI group may be attributable to greater reduction in GI observed at week 6; this is because $HbA1_c$ is a long term marker

indicating glucose control over the preceding 120 days (approximately) therefore, during the 42 days between baseline and week 6 measures, the LGI diet may not have had a sufficient effect on glucose control to show an improvement**.** A longer intervention period may have produced a significant reduction in $HbA1_c$ for the LGI group. Subsequent to the contrasting $HbA1_c$ values between groups, mixed model interactions demonstrated that the LGI intervention produced a marginally significant reduction in $HbA1_c$ relative to the control group at weeks 6 of - 0.20 (0.11); P= 0.078 and a greater reduction at week 12 of -0.43 (0.19); P= 0.062. Thus it appears that the LGI intervention may have elicited lower long term BG levels. Similarly in type II diabetic adults a reduction in $HbA1_c$ of -0.50 was observed after a 6 month low GI diet and a subsequent reduction in GI of 10 units (P= <0.001). Furthermore in adult males $HbA1_c$ levels were significantly reduced after a low GI intervention of just 4 weeks compared to a high GI diet separated by a 4 week crossover (Jenkins et al., 2004).

The evidence that long term glucose control was improved in the LGI group of this investigation is not supported by any improvements in insulin or HOMA-IR values. According to adjusted mean values, insulin and HOMA-IR were significantly reduced at week 6 and 12 compared to baseline in the control group, whereas insulin had in fact doubled at week 12 in the LGI group. Subsequently, there was a significant group by time period interaction for insulin, showing an increase in insulin at week 6 of 48.74 (P= 0.017) and week 12 of 30.71 (P= 0.045) compared to baseline in the LGI group relative to the control group. This interaction was also observed at week 6 for insulin resistance with an increase in HOMA-IR of 1.43 (P=0.024) in the LGI group relative to changes in the control group. In light of a significant reduction in $HbA1_c$ in the LGI group (interaction of group with time-period), these findings for insulin could be regarded as contradictory. However, figure 15, shows that this increase in insulin and HOMA-IR in the LGI group does not appear to occur until after week 6. Therefore, the marginally significant reduction in BG at week 6 may be attributable to the improved $HbA1_c$ level observed at week 12 which was potentially induced by the significant reduction in GI observed in this group at week 6. These data introduce the question of why would improvements in $HbA1_c$ in LGI but not control group be observed when there was little variation in fasting BG scores between the two groups and a reduction in insulin and HOMA-IR values in the control group. This may be attributable to the fact that BG is a fasting value; it does not highlight the specific postprandial impact on BG and insulin

following the control diet relative to that of the LGI diet. In support of this notion, it has been identified that postprandial hyperglycaemia contributes up to 70% of daily hyperglycaemia and postprandial glucose concentrations were positively associated with $HbA1_c$ (Bonora et al., 2001), however, as this was observed in adults with existing type 2 diabetes, it may not apply to the metabolically 'at risk' adolescents of the current study. The dietary intake data suggests that there would be a reduced postprandial BG response in the individuals consuming the lower GI and GL diet shown at the week 6 time point and thus this may be reflected in the reduce $HbA1_c$ value observed at week 12 in the LGI group. The potential for this intervention to improve long term glucose control is an important finding, as evidence supports that a reduction in long term plasma glucose levels, measured by $HbA1_c$ is inversely associated with CVD risk (Blaak et al., 2012).

In agreement with the current study, Ebbeling et al (2003) also observed that insulin resistance (HOMA-IR) increased in overweight adolescents. This occurred following prescription of both an *ad libitum* low GL diet or a reduced fat control diet, however, HOMA-IR increased significantly less in the low GL group (P= 0.02). This alteration occurred despite a significant reduction in BMI and BF% observed in the low GL group and this effect remained after adjustment for BMI change. It was postulated that the increased insulin resistance was a result of hormonal changes during puberty (Ebbeling et al., 2003) and that the low GL diet had slowed the increase in insulin resistance initiated by puberty. Perhaps the reduced GI and GL observed in the LGI group of the SIRENS study at week 6 may have slowed a puberty related increase in insulin resistance (between baseline and week 6). However, that does not explain why the control group saw significant reductions in insulin resistance during the intervention. Perhaps one explanation could be that the LGI group consisted of more participants still under the influence of puberty and thus experiencing increased HOMA-IR levels, however, this study attempted to control for pubertal development by only including individuals who self reported being at ≥Tanner stage 5 based on comparison with reference images of pubic hair growth (Marshall and Tanner, 1970a, Marshall and Tanner, 1969). It could be possible that self reporting of Tanner stage lead to inaccurate classification of pubertal status, and thus a confounding of results by pubertal hormones, however, in the context of this study, self report was deemed the most appropriate technique. Thus, in adolescents, pubertal changes may be hindering low GI and GL induced improvements in insulin resistance that have not been seen in studies of adults; in

39 overweight adults, Pereira et al (2004) found that insulin resistance improved significantly in those prescribed a low GL (experimental) and low fat (control) 10% weight loss diet, yet improvements were significantly greater in the low GL group. Nonetheless, a GI reduction of 10 units, similar to that of the present study, has been shown to improve insulin sensitivity in overweight men, however, the GI intake of these adult males was considerably higher at baseline than the adolescents of the present study (80 vs 57.8) (Radulian et al., 2009). Moreover, the authors did not measure fibre intakes. According to (Hu et al., 2001) there is a lack of support for decreasing GI and GL intakes improving insulin sensitivity, and that dietary fibre may be more strongly responsible for its attenuation. Studies have shown that increased fibre intake, particularly from wholegrain foods, was associated with improved insulin sensitivity in healthy and insulin resistant adults; whereas, GI and GL were not associated with any improvements (Mozaffarian et al., 2011, Chess and Stanley, 2008). In the present study, however, fibre intakes were similar between both groups and were not significantly altered as a result of the intervention. Moreover, fibre intakes actually slightly declined (non-significantly) in both groups between baseline and week 6 (to a greater extent in the control group) and in the LGI group, fibre intakes returned to baseline levels by week 12. In the control group, however, fibre intakes had remained reduced at the end of the intervention, and thus, although modest, the reduction in fibre in the control group does not support the previous evidence in adults (Mozaffarian et al., 2011, Chess and Stanley, 2008) that suggest increased dietary fibre is associated with improved insulin sensitivity.

This improvement in insulin and insulin resistance in the control group could also be explained by the Hawthorne effect; there may have been an increase in healthy behaviours by the control group despite being specifically asked not to alter their normal eating or physical activity habits, other than to consume all meals and snack in the context of the eatwell plate (FSA, 2012). However, the reported dietary changes do not support a notion that the control group changed their diet during the intervention to the extent at which insulin levels might be improved. Nor were beneficial changes in adiposity observed for this group. A limitation of this study however is the lack of follow up CRF and PA assessment after baseline data collection. It is possible that PA and CRF changes may have occurred during the intervention in either group that were not accounted for. It was decided that PA and CRF assessment during the intervention may have added too great a burden to the

participants and therefore was only conducted at baseline. Additionally it is possible that the nutritional intakes reported in food diaries was not a true reflection of the foods and drinks that were consumed during the 12 week period. However, the use of weighed food diaries to assess nutritional intake at baseline and throughout the intervention was considered a strength of this study, yet this method is not without limitations. It is known that in order to make recording intakes easier, individuals may reduce or alter the foods they eat to decrease the number of foods that require recording (Livingston et al., 2004). Additionally, because of the nature of the intervention it is quite plausible that individuals may under record or entirely exclude foods they regard as less healthy. Excluding foods would affect the calculation of dietary GI and subsequently GL. However, misreporting just the quantity of foods may have little effect on GI calculations but would more likely impact upon the calculation of GL. Indeed in the present study dietary misreporting was prevalent in over 50% of adolescents and only 1 member of the control group was classed as an under-reporter. This is a common issue relevant to studies involving dietary assessment, yet the current study attempted to control for misreporting by adjusting the analysis (model 4) for baseline EI:BMR, this is something that does not appear to have been done in previous low GI or GL intervention studies of youths.

The change observed in metabolic risk factors during this 12 week intervention appears to have occurred with no significant changes in weight or adiposity in either group. However, BF%, fat mass and body weight do appear to be increasing at week 6 and further at week 12 from baseline in the control group, where as they remain relatively stable in the LGI group. Perhaps if the intervention period extended past 12 weeks a significant change in adiposity may have been observed between the intervention groups. This was the case in a 6 month intervention of 16 overweight adolescents; Ebbeling et al (2003) employed an *ad libitum* low GL diet and observed greater fat loss in comparison to a reduced dietary fat intervention. However, their low GL group consumed considerably less calories (-692 kcals) during the intervention compared to the control group (-148 kcals) and thus fat reductions may be the result of a reduced energy intake. Unfortunately, satiety was not measured and thus differences in energy intake cannot be attributed to an altered appetite (Ebbeling et al, 2003). Furthermore, dietary misreporting was not accounted for which may impact associations of glycaemic CHO with health markers, as highlighted in chapter 3 of this thesis. In a more recent intervention study comparing 4 diets over a 4 week period: a low GI compared to high GI diet of

either low or high SFA content in 548 adults; lowering dietary fat was associated with a slight reduction in body weight, however, reducing dietary GI appeared to have no impact on weight loss (Jebb et al., 2010). However, this diet was heavily restricted in terms of proportions of macronutrients and energy, and thus adherence to this intervention may have been affected by its restrictive nature. This notion is supported by evidence from Speith et al (2000) who examined the effects of an unrestricted low GI diet compared to a standard reduced fat diet. The low GI diet centred on food options rather than restriction (like in the present study), where as the low fat diet aimed to restricted energy by 250-500 kcals, despite this there was a significant reduction in BMI in the LGI group; there was no change in BMI in the low fat diet group (Spieth et al., 2000).

Trends in the data highlight that there may have been an unfavourable effect of the LGI diet between weeks 6 and 12 of the intervention (apart from HDL). Whereas, between baseline and week 6 trends suggest that outcome variables were either improved or at least maintained at baseline levels. This consistent trend may be related to the greater reduction in GI and GL relative to controls at week 6. Furthermore, any favourable alterations in outcome variables appear to be lost at week 12 when GI has subsequently also increase in the LGI group.

The fact that GI began to increase between week 6 and 12 highlights that adherence to the LGI diet after 6 weeks may have decreased. There are a number of factors that could contribute to reduced adherence; it is possible that participants began to lose interest in the study after 6 weeks, however, in comparison to a number of studies that have maintained dietary adherence, 12 weeks is a markedly reduced time period. Because participants were required to travel to the Luton and Dunstable Hospital for data collection and or the University of Bedfordshire campus (depending on their location) for consultations, this often meant depending on parents for transportation or taking public transport. Unfortunately it was not possible to provide any form of financial inducements to cover transport or parking expenses. Additionally, a number of participants commented on the fact that they were not noticing weight loss, even though it was made very clear that the SIRENS study was focused on health improvement rather than weight loss. This appeared to reduce enthusiasm and motivation for the study in some individuals. Those intervention participants that were particularly enthusiastic tended to be those that felt they were losing weight. Unfortunately, no structured interviews were conducted

to provide robust qualitative information regarding the feasibility of the study. However, those participants that remained on the intervention at 12 weeks did report that the intervention was easy to understand and felt it was something they found straight forward to follow.

A limitation of the existing low glycaemic CHO intervention studies in adults and youngsters (Jebb et al., 2010, Pereira et al., 2004, Ebbeling et al., 2007, Ebbeling et al., 2003, Fajcsak et al., 2008) is that CRF and PA were not adjusted for in their analyses. In a prospective cohort of 13,621 men and women, (Héroux et al., 2010) identified that accounting for CRF substantially attenuated the associations of a healthy eating index and reduced all cause mortality rates. Therefore the current investigation accounted for baseline CRF and PA (%MVPA) in its statistical analysis. This was done in a sensitivity analysis approach building more covariates into each new MANCOVA model to determine the effect of accounting for diet, CRF and PA (see methodology section 8.1). In most cases the inclusion of CRF and PA (model 3, see appendix 6) increased the strength of interaction effects observed between the two intervention groups. Furthermore, the current study attempted to account for dietary misreporting at baseline by including EI:EE as a covariate (model 4); this appeared to slightly strengthen the interactions observed between groups but had little impact on P values to the extent that significance was greatly altered. However, for the liver function variables ALP and ALT, there appeared to be an attenuation of interaction effects as the covariate models included CRF, PA (model 3) and EI:EE (model 4).

The use of accelerometry to objectively assess PA and CRF is a strength of this investigation, however this technique is not without limitations, and these have been previously outlined in 4.3. There are a number of additional limitations of this study that should be considered, firstly the small sample size means that the statistical power of the investigation is limited. However, an a-priory sample size calculation was conducted before conducting this study which identified that to detect a significant change in HOMA-IR between two groups, with a power of >0.85, a sample size of n= 3 per group would be required. Furthermore, there was only one male participant in each group, thus making it difficult to generalise the findings to a wider mixed sex population.

In summary, this study provides new insight into the effectiveness of an *ad libitum* low GI diet centred on low GI food exchanges on the nutritional intakes of overweight and obese adolescents form the UK. The evidence suggests that this approach was an effective avenue for reducing dietary GI and GL in comparison to previous studies of which the diets tend to be more controlled. Furthermore this change was achieved despite no significant change in proportions of macronutrients; thus providing a potential platform for future studies to explore the effects of changing dietary GI without confounding from other dietary changes. Despite a lack of significant improvement in individual metabolic risk factors, there was a significant reduction in clustered risk score for the LGI group at week 12. There was also a borderline significant improvement in a long term marker of glucose control as a result of the LGI intervention compared to those in the control group, which could have important clinical significance and may warrant further investigation over a longer time period than the 12 weeks. This, however, was contradicted by an increase in insulin resistance in the LGI group compared to the control group and thus there appears to be equivocal evidence for a beneficial effect of this LGI dietary intervention, although pubertal status may have been an influencing factor. Interestingly, the greatest reduction in GI and GL occurred between baseline and week 6, however, at week 12 there was an increase in GI and GL in the LGI group; to this end, it appears that there may have been a decline in adherence to the intervention after 6 weeks and this may be attributable to the trends for improvement in BG, liver function markers and TG between baseline and week 6. Unfortunately the small sample size makes it difficult to draw definitive conclusions on the impact of this intervention on health parameters; future work is required to further understanding of how this flexible dietary intervention may impact on metabolic health in a larger sample of adolescents.

Chapter Nine: General discussion

9.0 Metabolic health in Youths

Childhood and adolescent obesity has risen in recent decades and by the year 2015 it is estimated that 1 in 3 of the world's adult population will be obese. In England, between 1995 and 2007, childhood and adolescent obesity rates increased from 3.1% to 6.9% and 5.2% to 7.4% in boys and girls, respectively and the prevalence of overweight and obese in the England is 17.9% and 21.8% for boys and girls, respectively (Stamatakis et al., 2010). The metabolic syndrome, represents a clustering of obesity related risk factors (dyslipidemia, hypertension, impaired fasting glucose) for CVD, insulin resistance and type 2 diabetes (Despres and Lemieux, 2006). The prevalence of this syndrome is rising in youngsters (Eckel et al., 2005) and has been observed in 4.5% of US (Ford et al., 2008) and 4.1 % of European (Vissers et al., 2007) adolescents. Young people with the metabolic syndrome are more likely to express these risk factors in adulthood (Camhi and Katzmarzyk., 2010), and thus, appropriate strategies for improving metabolic health in youths is of great importance.

Observational studies have provided evidence in healthy and 'metabolically at risk' adults and youths, that modifying the quantity and or quality of dietary CHO intake (Rizkalla et al., 2004, Jebb et al., 2010) can beneficially alter a number of risk factors linked to the development of type 2 diabetes and CVD. However, little is known of the current dietary glycaemic CHO intakes of UK adolescents and thus it was important to gain understanding of the current general macronutrient as well as glycaemic CHO intakes of this under researched group.

9.1 Dietary intakes of Bedfordshire adolescents

In the adolescents assessed by this work, as shown in chapter 4, the dietary GI represented that of a low-moderate intake 58.40 and energy adjusted GL (g/1000kcal) was 77.37. This was very similar to a previous study of overweight adolescents from the USA (Ebbeling et al., 2003) adolescents where intakes of GI and GL (g/1000kcal) were 58 and 79, respectively. This group appeared to be consuming macronutrients in line with current government recommendations (SACN, 2008. Although GI intakes appear to be moderate (GI 55-60), evidence has shown that small changes in dietary GI, within the range observed by this work are associated with improved adiposity and metabolic health (Hare-Bruun et al., 2006, Du et al., 2009). This evidence, despite in a relatively small sample, provides an up

to date insight into the current glycaemic CHO intakes which may help inform future dietary health improvement strategies for adolescents.

9.2 Glycaemic CHO and adiposity

Despite evidence that reducing both GI and GL can promote reduced adiposity, in adults, it seems that lowering GI might be more beneficial than GL (Du et al., 2009, Hare-Bruun et al., 2006). In youths, however, the evidence is less clear (Nielsen et al., 2005, Murakami et al., 2013). A stronger relationship between GI and adiposity compared to GL may be explained by evidence that rates of fat oxidation and diet induced energy expenditure were consistently higher following a low GI-low GL breakfast when compared to an iso-caloric high GI-low GL breakfast (Scazzina et al., 2011). Therefore reducing the GI of the diet may be more beneficial for adiposity than reducing the GL alone; this effect was also observed after the following meal (Scazzina et al., 2011). This is supported by the current work; chapter 4 revealed that in postpubertal adolescents, consuming a moderate dietary GI of 62 compared to a low GI of 54 was associated with a significantly greater BMI (P=0.009), BF% (P=0.018) and WC (P= 0.017). GL, however, was not associated with adiposity in any analyses. It appears that dietary GI and not GL is an important determinant of obesity and therefore potential poor metabolic health, although such results should be viewed with caution due to observed dietary reporting issues.

9.3 Dietary misreporting in Bedfordshire adolescents

Due to the impact that misreporting of dietary intakes can have on associations of diet and health, (Rosell et al., 2003) it was important to quantify the prevalence of dietary misreporting in this group and to attempt to control for its impact. Chapter 4, identified that that underreporting may be an issue, as this group consumed a markedly lower energy compared to that of the reported UK national average for adolescents (SACN, 2011) 2423.60 vs 2964 and 1750.86 vs 2110 kcals for males and females, respectively. A limitation of much past research in youths is the lack of consideration for dietary misreporting when assessing diet and health relationships.

To quantify the extent of misreporting in this group, an established technique (the Goldberg equation EI:BMR) was compared to that of a novel approach for assessing dietary misreporting which utilised EE obtained directly from accelerometry data; EI:EE (RT3), as shown in chapter 5. This data highlighted the

relatively high prevalence of dietary misreporting, particularly underreporting in this group. The EI:EE (RT3) technique identified approximately 10% more participants as underreporters compared to the Goldberg equation; 42.5 vs 30.3 %, respectively. A comparable study of 2868 UK adolescents (13 years old), which utilised accelerometry to calculate misreporting, also identified similar proportions of under reporters (between 37.1 and 51.8%) (Noel et al., 2010), compared to the current study. Furthermore, in comparison to DLW studies in children it appeared that the EI:EE (RT3) technique identified a plausible proportion of misreporters (Champagne et al., 1998) and RT3 estimates of EE were comparable to those measured by DLW in 12 year olds (Perks et al., 2000). Furthermore, this technique is likely to more accurately account for PA energy expenditure than the Goldberg equation which may underestimate dietary misreporting (Noel et al., 2010). Thus the novel application of EI:EE, using the RT3 accelerometer, may be suitable as a future assessment method of dietary misreporting, is a less complex and potentially more practical application of accelerometry than previously used. However, future validation studies of this technique compared to DLW are required.

9.4 Glycaemic CHO and metabolic health

In adults, there is a strong base of evidence in support of a positive association between metabolic risk factors and the risk of developing the metabolic syndrome, yet evidence is lacking in youths. A number of studies in youths have also evidenced that glycaemic CHO is adversely associated with lipid profile, BG and blood pressure (O'Sullivan et al., 2010, Marukami et al., 2013). However, these studies incorporate an age group of participants of varying pubertal status and thus findings may be confounded by puberty. In this postpubertal group, however, it was identified in chapter 6, that no significant associations were observed between dietary GI, GL and metabolic risk factors when using either variant of the Goldberg equation (1.55 PAL and MVPA PAL) to identify and exclude misreporters. Interestingly the significant positive association observed between GI and WC was attenuated following the exclusion of misreporters. This may be attributable to the high proportion of overweight underreporters; after their exclusion from analysis this association may be lost. However, after excluding misreporters (as identified by EI:EE (RT3)) and stratifying participants by low and high quantiles of GI (55.88 vs 61.95); HDL was significantly lower (1.17 vs 1.31 mmol/L; P= 0.037) and TG was

borderline significantly raised (TG 0.94 vs 0.63 mmol/L); P= 0.058) in those individuals who consumed a higher compared to lower GI diet. Furthermore when stratified by low and high GL (71.40 vs 90.37), the direction of the non-significant association between BG and GL appeared to reverse, and a borderline significant increase was observed in those in the higher compared to lower quintile of dietary GL (4.84 vs 4.72 mmol/L; P= 0.056) after removal of BMI from the model. These are associations that were not observed in earlier analyses and thus highlights the importance of accounting for misreporting, being that an apparent positive association between GI and an adverse lipid profile, as well as GL and BG, emerged only after excluding dietary misreporters from statistical analysis, when identified by a novel approach (EI:EE RT3).

Contrastingly, in overweight adults (Sluijs et al., 2010) and youths (Marukami et al., 2013) it has been identified that accounting for and adjusting analysis for misreporting resulted in a minimal effect on associations between glycaemic CHO and metabolic health. However, these studies did not account for directly assessed PA when calculating misreporting and thus may have underestimated underreporting. Similarly to the current findings, however, Burger et al (2010) reported that GL was positively and significantly associated with mortality but only after excluding under reporters, in a type II diabetic population. Individuals with type 2 diabetes under report to a greater extent than overweight individuals (Sallé et al., 2006) and thus the impact of excluding misreporters may have been greater in this group compared to previous studies (Sluijs et al., 2010; Murakami et al., 2013). This suggests that accounting for dietary misreporting in 'at risk groups' such as those in the current work may be of particular importance when assessing diet and health relationships. Excluding misreporters presents a limitation in that only 'valid' dietary reporters were assessed, yet, it appears that the inclusion of misreporters and the type of assessment method of misreporting resulted in quite different findings and therefore is something that should be considered in future studies of similar populations. Because dietary GI is not affected by the quantity but rather glycaemic quality of the diet, and the fact that exclusion of misreporters altered associations between GI and health, it is likely that underreporters were entirely omitting certain CHO containing foods rather than just misreporting the quantity consumed. However, it is important to consider the fact that misreporters were more overweight than valid reporters in the current work and thus these altered associations could be mediated by adiposity, yet, it is difficult to confirm this notion

being that misreporting occurred across a broad range of weight statuses. An important consideration in this work was to account for PA and CRF when assessing the associations of diet and health. It does not appear that previous studies have controlled for these factors when assessing the associations of glycaemic CHO and health parameters, and this is strength of the current work.

9.5 Physical activity, cardiorespiratory fitness and metabolic health

CRF (Blair et al., 2001b) and PA (Wareham et al., 2005) as well as the type of PA that individuals engage (Healy et al., 2011, Ekelund et al., 2012) in are important determinants of adiposity and associated metabolic health complications, yet there is little evidence of these associations in postpubertal adolescents. Additionally, there are inconsistencies in the way in which PA behaviours are measured and quantified in youths which may be a contributing factor explaining equivocal findings in the literature (Ekelund et al., 2011a). Therefore the current thesis explored the associations of PA (comparing two different PA thresholds for youths; (Rowlands et al (2004) and Chu et al (2007)) and CRF with metabolic health, but importantly accounted for the potential confounding effects of dietary intake.

Chapter 7, demonstrated that in this group, the use of different PA thresholds resulted in disparate proportions of time spent in different PA categories. Importantly, the Rowlands thresholds defined the group as sufficiently active according to time spent in MVPA (65.60 mins) whereas, according to the Chu thresholds, the group were not sufficiently active (<60 mins MVPA) as they only engaged in 27.20 mins of MVPA. Furthermore, it was identified, when utilising either threshold that the adolescents (aged 14-19 yrs) of the current investigation engaged in less PA than a comparable sample of children age 10-14 yrs; for example, when applying the Rowlands thresholds, adolescent males engaged in 55 minutes less MPA than boys from the same region of the UK and female adolescents engaged in 44.09 mins less MPA than girls. This comparison is consistent with past research that PA declines as children enter their adolescent years (Kimm et al., 2002) and highlights the importance of maintaining PA levels as children progress into adulthood.

In this group and consistent with previous literature (Blair et al., 2001a, Ekelund et al., 2007a), higher CRF was associated with a significantly reduced WC, TG and

clustered risk score in all participants. In females, CRF was associated with more favourable fasting BG and HDL levels. However, in contrast, PA engagement appeared to be less associated with metabolic health, as supported by previous research in children (Bailey et al., 2012a). Despite government recommendations stating that young people should engage each day in at least 60 mins MVPA (DOH, 2011), in the present work, the only risk factors to be associated MPA and VPA were DBP and SBP and this was observed using either sets of intensity thresholds. In contrast, in a large study of children and adolescents, increased time spent in MVPA was associated with more favourable SBP, WC, TG and HDL levels (Ekelund et al., 2012), the disparity between these studies may be explained by the different thresholds used by Ekelund et al (2012); as they utilised much higher cut-offs to determine MPA and VPA than this in this work. Furthermore, Ekelund et al (2012) assessed PA using an Actigraph accelerometer and utilised a 60 second as opposed to 10 second zero count protocol for classifying and removing non-wear time from raw accelerometer data.

In this work, being in the highest compared to lowest quintile of LPA was associated with a reduced BG, SBP and clustered risk score in all participants when utilising the Rowlands thresholds. But when using the Chu thresholds higher LPA was, instead, associated with an improved HDL level. Additionally, being more SED was associated with a higher DBP using either set of thresholds. However, TG levels were significantly lower in males engaging in more SED time when assessed by the Chu thresholds; a finding which is contradictory to previous evidence in youths (Ekelund et al., 2007). On the contrary to the current work, Bailey et al (2013) observed that being more SED was associated unfavourably with TG in a comparable group of children. However, these children (Bailey et al., 2013) accumulated more time SED than the adolescents in the current study, which may be attributable to these confounding associations.

Although applying either sets of intensity thresholds identified significant associations between PA and health parameters, there are discrepancies as to the relationship between metabolic risk factors and time spent in different PA categories. Therefore, clear consensus on which thresholds are most appropriate for this age group is required to allow accurate comparisons to be made between studies.

Directly assessing CRF via a maximal exercise test is strength of this study, however, there were limitations of this procedure that should be considered. Firstly, cycle ergometer tests have been shown to elicit a 7-12% lower oxygen consumption compared to treadmill running when assessing VO_2 max in adults and adolescents (Hermansen and Saltin, 1969, Loftin et al., 2004) and thus CRF levels may have been undersetimated. However, a cycle ergometer test was regarded as more appropriate given that some participants may not have had experienced treadmill running before and may be more comfortable on a cycle ergometer. Furthermore, being seated on a stationary bike posed less risk if a participant was to become faint and fall which is a rare yet potential consequence of intense exercise (Pina et al., 1995). Furthermore, there was limited time to familiarise participants with the cycle ergometer test, since much of the testing was conducted within schools and colleges during lesson time.

The objective assessment of PA using accelerometry is usually regarded as a superior method compared to pedometry and the completion of self-report PA questionnaires which are prone to reporting errors (Sirard and Pate, 2001, Trost, 2007). Accelerometry is not without its limitations, however, as addressed in section 4.1 of this thesis. For example, in the current study, the 1 minute epoch used to capture activity data meant that the sporadic nature of some higher intensity exercises may not have been captured (Rowlands., 2007). More modern devices compared to those available for this research, such as the Actigraph GT3x can measure in a 10 second epoch over an extended period, due to its superior battery life and memory capacity and thus may have more validly in detected high intensity PA. It is possible that a shorter epoch and a detection of higher intensity activity might have resulted in a significant association between MVPA and metabolic health risk factors in the adolescent population. The RT3, however, has been shown to be a valid measure of PA in youth compared to DLW (Krekoukia et al., 2007, Rowlands et al., 2004a).

In addition, employing a shorter epoch (such as 10 seconds) would have more accurately estimated EE and thus predictions of energy requirements, improving the assessment of dietary misreporting further. It does not appear that assessment of EE for the calculation of misreporting has been compared whilst using different epochs in previous studies. Future work should investigate the potential of varying

epoch length on the effectiveness of methods designed to account for misreporting and the subsequent impact on associations between diet and health.

9.6 Impact of a flexible low GI diet on metabolic health in postpubertal adolescents

In order to identify the impact of altering dietary GI on health outcomes in an adolescent population, a 12 week, *ad libitum* low GI intervention was conducted in a subsample of overweight and obese postpubertal adolescents who expressed risk factors for the metabolic syndrome as presented in chapter 8. Currently there is a lack of research into the effects of reducing dietary GI and GL in adolescent populations, however, in children and adolescents from the USA and children from Hungary, it has been observed that a low GL diet reduced BMI, adiposity and the prevalence of metabolic risk factors (Speith et al., 2000, Ebbeling et al., 2003, Fajcsak et al., 2008). Although these studies describe their intervention as *ad libitum*, they remain restrictive in that proportions of macronutrients may be set to manipulate dietary GL and often indirectly restrict energy intake. Such parameters may mean that the diet is more complex for the general population to follow. However, the intervention of the current work only focused on manipulating dietary GI and is a truly flexible, *ad libitum* diet, and may promote better compliance and adherence than previous studies.

Data gathered from 3 day weighed food diaries provided novel insight into the impact of this 12 week low GI intervention in a group of postpubertal adolescents. Despite identifying no significant changes in proportions of macronutrients, dietary GI and GL significantly reduced between baseline and week 6 by 8.25 units in the LGI group. In the LGI group, GI and GL intake increased at week 12 compared to week 6 yet values remained below baseline. In the control group GI was relatively unchanged, however GL did decline throughout the intervention and by week 12 was similar to that of the LGI group and significantly different from baseline.

The increase in GI between week 6 and 12 suggests that there may have been issues with adherence to the intervention diet following 6 weeks. It was postulated that because this was not an intervention targeting weight loss, but rather metabolic health, that individuals may have lost motivation if they did not feel they were losing weight, despite being made aware of the focus of the intervention before taking part, although such evidence was anecdotal. Furthermore, there was no significant change in mean BMI, BF% or fat mass in either group. However, the dietary

changes observed at week 6 suggest this *ad libitum* low GI diet was successful in reducing GI and GL whilst maintaining original proportions of macronutrients. This allows for the effect of a reduced GI diet on metabolic health to be examined in relative isolation to other macronutrients. However, although non- significant, there was a slight reduction in the proportions of fat and SFA consumed following the LGI diet that were not observed in the control group. This is encouraging as it suggests that a flexible diet focusing on substitution of high GI foods with low GI foods in the context of habitual diet and the Eatwell plate (FSA, 2012) may promote a more healthful dietary profile than just consuming habitual diet in the context of the Eatwell plate, however, more studies utilising this approach would be required to confirm this. The current intervention elicited a similar reduction in GI in adolescents to previous research in adults which employed more heavily restricted diets (Jebbet al., 2010, Ebbeling et al., 2008, Jenkins et al., 2008), where GI was reduced by approximately 10 units. Unfortunately, of the evidence in youths, dietary changes were not monitored during the intervention and thus comparison cannot be made between these studies. Some authors have stated it is too complex to be applied to a general population (Franz, 2003) but the current study contradicts this notion, as it appears that the application of low GI eating in a health context may be a beneficial approach to improving the diet quality of UK adolescents.

Furthermore, there was a significant reduction in clustered metabolic risk score in the LGI group (P= <0.01) that was not observed in controls when within-subject correlations were accounted for. In addition, mixed models showed that BG was marginally significantly reduced at week 6 compared to baseline in both groups (P= <0.1), however, by week 12 BG had continued to decline in the LGI group whereas, in the control group it had slightly increased. There were also marginally significant improvements in DBP and SBP in the LGI group, however, this was also observed for DBP in the control group at week 6. In overweight adults, a low GL diet was shown to improve BP in comparison to a reduced fat diet, however this study also observed significantly greater improvements in lipid profile, which was not observed in the current study (Pereira, 2004), although these diets were designed to reduce body weight by 10%. Contrastingly, in the current study, weight and adiposity were relatively unchanged. In three previous studies, two of children and one of adolescents, fat mass and BMI have been reduced following low GL diets, however in two instances dietary change was not monitored during the intervention (Speith et al., Fajcsak et al., 2008) and in another, it was not clear if adiposity changes were a

result of altered GL or a reduced energy intake (Ebbeling et al., 2003) and thus the evidence in youths is not clear. Despite there being no significant improvements in lipid profile in the current intervention, the significant improvement in clustered metabolic risk score in the LGI group suggests that there may have been a beneficial effect in this group of overall metabolic health. To the author's knowledge this is the first low GI intervention in youths to show an improved clustered metabolic risk score.

Despite being inversely associated with GI in the current observational evidence, HDL was not increased subsequent to reducing dietary GI. Evidence suggests that the assessment of HDL particle size and functionality may be more important markers of atherogenic risk than total HDL levels (Kypreos et al., 2013, Kontush and Chapman, 2012). There appears to be a lack of research assessing the relationship between glycaemic CHO and HDL functionality. Impaired HDL functionality can effect anti-inflammatory activity and accelerate atherosclerosis (Kontush and Chapman, 2012) and may play a potential role in glucose homeostasis and the development of diabetes (Soran et al., 2012). It is possible that the impact of the low GI diet may have altered HDL functionality instead of total HDL levels, the failure to assessment this marker is a limitation of the current work.

The LGI group had a marginally significant improvement in long term glucose control compared to those in the control group; relative to the control group, $HbA1_c$ was reduced at weeks 6 by 0.20 (0.11); P= 0.078 and at week 12 by 0.43 (0.19); P= 0.062. In contrast to evidence showing an improved glucose control, insulin resistance significantly increased in the LGI group relative to the control group at week 6 compared to baseline by 1.43 (P= 0.024). The fact that insulin declined in the control group relative to the LGI group is an unexpected finding, and the dietary changes observed in the control group do not represent those associated with improved insulin resistance. Furthermore, it is important to note that the above metabolic health changes occurred in the absence of a significant reduction in adiposity. Therefore, it is likely that additional factors are influencing the variation in insulin resistance between the two groups. It was postulated that the impact of puberty may still be impacting on insulin levels, despite attempting to control for the effects of puberty in the experimental design. As pubertal status was self assessed, it is possible that certain individuals may not have accurately assessed their own stage of maturational growth. Additionally, within the remit of this PhD, it was only

possible to assess insulin resistance using an indirect marker, for the current investigation the HOMA-IR method, which utilises fasting BG and insulin levels to estimate the degree of insulin resistance for each individual was utilised. Unfortunately this method does not provide as accurate results as direct methods such as the clamp technique or an insulin suppression test (Muniyappa et al., 2008) and thus some caution should be used when interpreting these data. However, direct methods are expensive, time intensive and require expertise to be conducted. Despite this the HOMA-IR method has been shown to have reasonable linear correlations with clamp studies (Wallace et al., 2004, Radziuk, 2000).

9.7 Application of the GI in health promotion

Evidence provided from this current work and past research (Scazzina et al., 2011) appears to demonstrate that reducing the GI of the diet may be more beneficial than reducing dietary GL in improving metabolic health. Furthermore, this work appears to be the first to show that a flexible, *ad libitum*, low GI diet in the context of a balanced diet, was an appropriate approach to lowering dietary GI whilst eliciting a healthful diet in UK adolescents. This is promising for future health improving strategies, as despite criticism that the GI may be a difficult concept to grasp by the general public (Franz., 2003, Pi-Syunyer., 2002), in the context of low GI food lists and high for low GI food swaps, it appeared that adolescents found the diet straight forward to understand and follow. This is supported by the apparent compliance in lowering dietary GI, as observed in the food diaries, but also anecdotally, in conversation during low GI consultations. However, the poor retention rate of intervention participants should not be overlooked. It became apparent during dietary consultations, however, that individuals were not aware of the concept of the GI before their participation in this intervention and they had never been introduced to low GI food lists. If future health promotion or improvement programmes in the UK were to truly incorporate low GI eating as a strategy, much action is required to raise awareness of it as a concept. Indeed, in the current intervention, difficulties arose when trying to find appropriate low GI substitutes for certain food items, despite the fact a UK low GI list was created for this intervention. This was because the most comprehensive lists are based on analysis of foods from the USA and Australia (Aston et al., 2008), so even when referring to the extensive international GI tables (Chess and Stanley, 2008) it was often difficult to identify appropriate

substitutes. A meal time that seemed consistently difficult to identify appropriate low for high GI substitutes was breakfast, as many of the breakfast cereals consumed by youngsters tend to consist of refined grains and or be high in added sugars. The potential low GI breakfast cereal substitutes, such as muesli, porridge or bran based cereals, where often regarded as unappealing by the participants.

A feasible way of increasing a youngster's ability to make healthy GI choices could be to include a GI value on packaging of CHO containing foods and beverages; currently, only a very limited number of products on the UK market have this value displayed, yet it would be a very simple way of allowing individuals, especially adolescents who are making more autonomous decisions regarding their nutrition (Ebbeling et., 2003), to make more healthy food choices. A recent international study comparing diets of varying protein and GI content targeting weight control identified that individuals reported better scores of acceptance and positive experiences for a diet high in protein rather than low in GI (McConnon et al., 2013). This could be related to a better understanding of the benefits of a high protein diet due to the increased media attention they have received. In Australia, however, there has been a large campaign raising awareness of dietary GI and its importance on health, many foods on the Australian market have been tested and display a GI value on their packaging. It is also important to consider that certain foods are manufactured differently in different countries, regardless of whether or not the food item has the same branding and name, therefore the GI of foods tested in the USA and Australia, which make up the majority of foods on the current international GI tables, can be different to those on the market in the UK and the rest of Europe (Chess and Stanley, 2008). Thus, GI tables should be used with some caution when utilising data that was obtained from different countries and to this end, more studies assessing the GI of foods consumed within the UK are required. Recently a culmination of international nutrition and health experts met in June 2013, to form an International Scientific Consensus Summit, entitled, 'Glycemic Index, Glycemic Load and Glycemic Response'. In the preliminary draft consensus statement it was agreed that given the rise in obesity and diabetes and with the evidenced potential for low GI to be beneficial for these factors, there is an increasing requirement to disseminate information regarding the GI and GL to the general public and health professionals. Also, it was stated that low GI should be considered in the context of future healthy diets. (www.gisymbol.com.au/cmsAdmin/uploads/GI-Summit-Consensus-Statement.pdf, accessed June, 2013).

9.8 Issues with food diary compliance

In order to estimate dietary GI and GL it was important to gain detailed information regarding the type, brand and quantity (weight) of a food or drink as well as considering food that was weighed but not consumed (leftovers). This can pose a burden to the participant in that weighing and recording what they eat can be an inconvenient, laborious and time consuming task (Livingstone et al., 2004) and thus requires a great deal of self motivation to complete. Respondents must be literate and numerate to be able to complete their food diary independently; in the case of the SIRENS study, one participant required support from a parent to complete their food diary. Evidence has shown that some individuals are likely to alter their eating habits to simplify the process of recording or due to a need to consume a diet they consider 'socially desirable' (Livingston et al., 2004), such as eating less during recording days or consciously neglecting to record certain foods. Although biased EI is a common issue when assessing diet generally, unfortunately, in adolescents, nutrient intake data is particularly prone to bias in the form of underreporting in comparison to children and adults (Bandini et al., 1990, Livingstone et al., 2004). However, as the current work accounted for misreporting the impact of this should have been minimised.

Alternative techniques to dietary assessment such as dietary recalls and FFQs can be less labour intensive for respondents; however, these techniques require food items to be recalled from memory which can hinder the accuracy of these methods. More recently technological advances have introduced the prospect of capturing dietary intake using a mobile smart phone device, such as the DietCam (Kong and Tan, 2012) which aims to use algorithm within its software to estimate food type and volumes from 3 separate images of a plate of food. This may be a good prospect for assessing diet in youngsters who are likely to be more familiar with new technologies than their older counter parts. A study assessing the preferences for dietary assessment methods in adolescents identified a strong preference for methods incorporating technology such as those that take images of foods (Alberti et al., 2006). The authors postulated that this may improve compliance and accuracy when measuring dietary intakes in this group. However, analyses of these images are far from implantation in research. Other similar systems that use camera

technology require intensive user interaction and images to be sent to multiple paid individuals who manually analyse the images which can make these processes labour intensive and expensive (Kong and Tan, 2012).

9.8 Issues with accelerometer compliance

With measurement of PA, compliance issues can result in long periods of non-wear time and this was seemingly observed in the current work by the 28.6% of participants that did not wear their accelerometer for a sufficient time period. This impacted on the statistical power of the study, in that assessment of associations of PA and diet (controlled for PA) with health markers could only be conducted on those who wore their accelerometer for a sufficient time period. An issue which became apparent during this work was that some of the adolescents expressed reluctance to wear the RT3 accelerometer; as the device must be attached to the waistline of trousers or a belt (much like a traditional pager), it is usually constantly visible to others. Female adolescents, in particular, expressed that the device was not aesthetically pleasing and "did not go with their clothing", nor could it be worn with a dress. Additionally, participants may have been weary of their peers being aware that they were having their activity monitored, especially in the context of the SIRENS study which was centred on individuals with poor health. Future work would benefit from using a device that can be worn underneath clothing out of view from others. An alternative could be to use the Actigraph GT3x triaxial accelerometer, which can be worn underneath clothing as it is attached to the participant via its own elastic waist belt. Furthermore, as previously outlined, this device is also water proof, which allows for the device to be worn during some water based activities. Together these factors could improve compliance and widen opportunities to wear the monitors and thus providing richer PA data.

9.9 Issues with intervention studies in youths

The high dropout rates in this intervention study and subsequent low sample size means that its findings must be interpreted with caution. Retention of intervention participants was an issue; from those who were fully enrolled at baseline (n=21), dropout rates at week 6 were 24% and at week 12 dropout had increased to 52%. Additionally, recruiting the target sample size of 25 per group was highly challenging and took much longer than initially anticipated.

Recruitment comprised of firstly forming links with and disseminating the study details to schools, colleges and GP surgeries. This often required formal meetings with senior members of staff before being allowed to advertise the study at these sites, which proved time intensive. Many schools and even some GP practices didn't seem comfortable with being part of study that broached the issue of obesity in youngsters and declined participation. Recruitment strategies evolved over time to achieve recruitment targets and included creating information for the Hospital website, additional newspaper advertisements and a press release that lead to an interview on a local regional radio station to promote awareness of the study in the Bedfordshire area. It appears that participants were less motivated to take part and adherence to the intervention reduced as the trial progressed; dropout rates and dietary GI and GL increased in the LGI group at week 12 compared to week 6. Unfortunately a limitation of this study is a lack of qualitative data gaining insight into reasons for dropping out or not taking part initially. Such information would be helpful when designing future studies in this area.

Although no official qualitative data was collected to inform the research team of possible reasons for recruitment and retention challenges, anecdotal evidence from this group would suggest that some parents were more concerned about their child's weight and health than the participants themselves and that parental encouragement was key for some individuals in determining their participation. However, some individuals clearly wanted to take part for their own reasons; for instance, a number of participants had older family members with obesity related health issues and expressed concern for their own health. In the case of many of the females, participants wanted to change their appearance and be slimmer. Where participants had actively enrolled for their own reasons, they appeared to be more motivated to take part in all aspects of the study. These individuals may have felt the study had relevance to them making participation more interesting, subsequently increasing compliance (Ross et al., 1999). Unfortunately, no record of motives to take part in the SIRENS study was taken. This is a limitation of the work, and future studies should seek to explore motives and barriers to participation and adherence to this flexible low GI diet in adolescents.

A fear of needles was a significant hindrance to recruitment, often, when a non-returned consent form was followed up, the reason given for not taking part was a fear of needles. In some instances parents would cancel screening appointments at

the last minute due to the fact that they could not encourage their child to have a venous blood sample taken. This challenge also prolonged data collection sessions when participants were reluctant to have blood samples taken which, at times, disrupted testing schedules. In addition, it was, occasionally, difficult to locate an appropriate vein, which may have been influenced by the young age of the participants and high adiposity. However, this was certainly not the case in all participants, in fact, in those individuals who actively enrolled onto the study for their own reasons, blood sampling was much more straight forward regardless of whether or not they were averse to having their blood sampled.

Another issue with conducting this intervention was the lack of independence in this sample regarding transportation. A large number of participants had to depend on their parents for transportation or use public transport to attend data collections and weekly consultations, as the majority of participants were not within walking distance of the Hospital or University campus. This meant that in many instances scheduling of data collection and consultations had to account for the participant and their parent's free time, limiting availability. In addition, there was no provision for this study to provide any financial support for transportation or to cover the expense of parking at the Hospital. The relatively low availability of funding for this RCT and thus the lack of financial inducement or 'thank you' gift is a possible hindrance to recruitment and retention rates. Previous studies have shown that children and adolescents are more likely to overlook risks to participating in research or withdraw from participation when monetary gain is part of participation(Wendler et al., 2002, Field et al., 2004). This may have improved the statistical power of the study and subsequently strengthened associations between reduced dietary glycaemic CHO and metabolic risk factors.

9.10 Representativeness of findings

The findings of this thesis provide novel insight into the associations of glycaemic CHO and metabolic health and the impact of different PA thresholds when assessing PA and health relationships in a postpubertal adolescent population that will be important for future health improvement strategies and research. However, limitations lie in the generalisability of these findings. Although the observational work was on a general adolescent population, participants were only recruited from within Bedfordshire; mainly from the towns of Bedford and Luton, thus these

findings may not be generalisable to a greater UK population. Yet it is important to note that this sample is likely to be representative of other towns in the South East of England, outside of the greater London area. Moreover, according to the sample size calculation conducted a-priory, as described in section 3.11, this observational work is statistically under-powered, a sufficient sample size was estimated as n=137, despite not achieving this with the total number of participants recruited and assessed (n=105), the lack of compliance for sufficient accelerometer wear time meant that the sample size was reduced further when adjusting analysis for PA (n=75). Furthermore, with regard to those enrolled in the intervention, the findings are limited to overweight and obese individuals with a risk factor for the metabolic syndrome, however, this could be regarded as a strength of this research being that there appears to be a lack of interventions in youths targeting an 'at risk population'. Moreover, the intervention sample was small and heterogeneous; the majority of participants were females; as only 2 male participants were enrolled in the trial, it is not possible to generalise the effects of this intervention to male adolescents and the small sample size limits the statistical power of the findings. However as described in section 3.11 the a-priory sample size calculation estimated that only 3 participants per group would be required to detect an effect of the intervention on insulin resistance (HOMA-IR). However, to detect a significant difference between the control and intervention groups a required sample size of 15 in each group was estimated, and thus, as only 11 intervention and 10 control participants were assessed at baseline, the intervention was also statistically underpowered and the results should be interpreted with caution.

9.11 Conclusion

Together this evidence highlights the potential of low GI for the improvement of cardiometablic health. The observational and intervention study evidence, supports the future promotion of low GI in the context of a healthy diet in youngsters, and specifically in adolescents from the UK, for whom there is limited information regarding their current glycaemic CHO intake and association with metabolic health, or the impact of a low GI intervention. This work also highlights the importance of assessing and accounting for dietary misreporting when investigating diet and health relationships in this population. There was promising evidence that this highly flexible low GI diet was an appropriate avenue for reducing dietary GI whilst

maintaining a healthful macronutrient composition and that this approach may have a beneficial effect on long term glucose control, blood pressure and clustered metabolic risk score. Interestingly, however, insulin resistance was not improved by the intervention. Unfortunately, the small sample size utilised for the intervention means that its findings should be interpreted with some caution. Future studies should endeavour to overcome challenges of recruitment and retention in such populations to more clearly identify any associations between reducing GI and metabolic health improvement.

Chapter Ten: Reference List

10.0 References

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Chapter Eleven Appendices

Appendix 1

CROSSROADS Physical Activity, Nutrition and Health.

University of Bedfordshire

Dear Potential participant

Re: Cross sectional Study: Risk of Adolescent Disease (CROSSROADS)

We would like to invite you to take part in a school-based nutrition, physical activity and health research study. The study aims to assess how diet, physical activity engagement and fitness levels relate to the health of 15-19 year olds from schools, sixth-forms and colleges in the Bedford area.

If you would like to take part you must sign the consent form (**a**) attached to this document. If you are under 16 your parent or guardian will also need to read this information sheet and sign the consent form (**b**). If you have any questions about this information sheet, or would like further details, please ask a member of the research team - you can find their contact details at the bottom of this sheet.

The study will be conducted at your school, sixth-form or college. You will only be required for testing on 1 day for a relatively short time period, and will be asked to wear a physical activity monitor and keep a weighed food diary and return these to us at your place of education a few days later.

What happens in the data collection?

On the night prior to the data collection day, we will remind you to fast over night (10-12 hours). Therefore you must not consume any food or drink for 10 hours prior to the school start time (you will be allowed to sip water). Please come to your school with a snack so that you can eat something immediately after you have seen the researchers for the initial data collection.

When you arrive at the school, you will immediately see the researchers (who will be in a room on the school or college grounds) to have your initial data collection (about 20 minutes) which includes a measure of your height, weight and muscle and fat levels (by standing on a scale with two metal plates) and a measure of your BG and cholesterol levels (by taking a fingerprick blood sample from your finger). This is a very simple and easy procedure and requires the drops of blood collected to be put into a machine to measure cholesterol and glucose. Once these measurements are completed you will be able to eat your snack and continue your lessons. You will then visit the research team again later during the day to complete the remaining measurements.

For the remaining data collection (about 30 minutes) you will have your waist circumference, blood pressure and heart rate measured. You will also be required to undertake some exercise on a stationary bike and to wear a plastic mask over your mouth and nose to enable us to measure gases in your expired air. It is expected that towards the end of the session you will find it hard work but you will be advised that

you can stop at any point should you wish to. The exercise session will last between 8-12 minutes. You will also be asked to complete some questionnaires relating to physical activity levels.

After the testing in school / college, we will also ask you to keep a 7-day physical activity diary and to wear a small box on your waist for 7-days. This is an accelerometer which monitors physical activity levels. This is very expensive and must be removed when swimming or bathing/showering. Please make sure you take good care of this and please bring it back when asked so that we may use it straight away for the next person. During the same week that we ask you to wear the physical activity monitor you will also be asked to complete a 3 day weighed food diary. You will be given a food diary and weighing scales, so that you can provide us with an accurate measure of the food and drink you regularly consume. We will explain how to do this in more detail when we meet you. Once this has been completed and returned to us at your school or college the testing will be complete.

There is a risk that we might find some abnormal results such as high blood pressure, high blood cholesterol or glucose readings. If so, we will inform you and as a precaution will suggest you visit your general practitioner (GP) or we may be able to offer you a place on one of our physical activity / nutrition projects.

It is your responsibility to provide information regarding your health status or previous experiences of unusual feelings with exercise. You cannot take part in the study if you have a chronic medical condition such as heart disease, asthma (not controllable with medication) or any other condition that may put you at risk when performing having the fitness test.

Your participation in this study is entirely voluntary and the results will be strictly confidential (only the researchers and you will see the individual results). All data will be stored in a secure locked filing cabinet or on a password protected computer file that can only be accessed by members of the research team.

Before giving your full consent to the study, we ask that you discuss this with your parent or guardian to make sure you understand what the study involves. If you have any questions about the study, please contact a member of the research team (contact details below). Remember, this study is voluntary and you can leave the study at any time by telling a member of the research team that you no longer wish to take part.

It is hoped that the work from this study will be published in a scientific journal but your data will be kept confidential at all times.

CROSSROADS research team contact details:

Ben Davies, E-mail: Ben.Davies@beds.ac.uk, Telephone: [redacted]

Dr. Catherine Kerr, E-mail:Catherine.Kerr@beds.ac.uk, Telephone: [redacted]

Informed Consent

CROSSROADS (Cross Sectional Study: Risk of Adolescent Disease)

If the participant is under 16 years of age, please sign the below informed consent (a) to assent to taking part. However, your parent or guardian must also consent to your participation by signing the below parental/guardian informed consent (b)

Informed Consent Form

Please complete all the details below. All information obtained will be treated as confidential. Completed forms must be returned to _____or the **PE office**, before any testing commences. Please return as soon as possible.

Please read the following statements carefully. Please sign only when you have agreed with the statements.

I have been asked to participate in a school-based nutrition, physical activity and health research study in 15-19 year olds. I give my free consent by signing this form.

I understand that I will be involved in the following:

• The research will be carried out as described on the information sheet

• If I decide to withdraw from participation, my decision will be accepted.

• I understand that I am responsible for providing information regarding my health status or previous experiences of unusual feelings with physical effort.

• I have no injury or illness that will affect my ability to successfully complete the tests.

• I have read and understood the information above, and my questions have been answered to my satisfaction.

Informed consent (a) **(If participant is over 16)**

Name: .....……

Date of Birth: ……..

Permanent Address:
……....
.....
.....

Contact Telephone No:…….

Signature of participant…… Date……

Signature of researcher .....

Parental/Guardian Informed consent form (B) **(If participant is under 16)**

I have been asked to allow my child to participate in a school-based nutrition, physical activity and health research study in 15-19 year olds. I give my free consent by signing this form.

- By signing this form I give my free consent to allow my child to participate in the research project, should my child wish to take part.
- I have read and understood the information above, and my questions have been answered to my satisfaction.

Name of Child: ……

Date of Birth: ……..

Permanent Address:

……

……

……

Parent/Guardian Contact Telephone No:……

Signature of parent/guardian…… Date……

Signature of Researcher ……

Along with this form, please also return the following completed form: Physical activity readiness questionnaire – this checks that you are well enough to complete the exercise test.

Adapted Physical Activity Readiness Questionnaire for Youth

This questionnaire offers a safe, preliminary health-screening prior to participation in exercise...

- Has your doctor ever said that you have a heart condition?

  Yes ☐ **No** ☐

- Do you have chest pain brought on by exercise?

  Yes ☐ **No** ☐

- In the past month, have you experienced chest pain when you were NOT doing exercise?

  Yes ☐ **No** ☐

- Do you lose consciousness or lose balance as a result of dizziness?

  Yes ☐ **No** ☐

- Do you have a bone or joint problem that could be aggravated by exercise?

  ☐ ☐

  Yes **No**

- Does your doctor currently prescribe medication for blood pressure or a heart condition (e.g., diuretics or water pills)?

  Yes ☐ **No** ☐

- Do you know of <u>any other reason</u> why you should not participate in exercise?

  Yes ☐ **No** ☐

If 'yes', please give the reason;

.....

Blood sampling question

- Can you answer yes to any of the following? Have you ever had any form of Hepatitis? Have you any reason to think you might be HIV positive? Could taking blood be hazardous to your own or the researchers health?

Yes ☐ **No** ☐

<u>Participant</u> name (Please PRINT NAME)……..

Signed……. Date…….

Appendix 2

Non sex specific SIRENS information and consent form

Centre for Obesity Research

Luton and Dunstable Hospital NHS
NHS Trust

Direct Telephone: 01582 497172/497421 Direct Fax: +44 01582 564543

Participant Information Sheet – Part 1

The SIRENS study (Study of Insulin Resistance factors using Exercise and Nutritional Strategies)

Dear Participant or Parent/Carer

Re: Study

We would like to invite you to take part in a research project to find the answer to the following question: Can we improve the health of 14-19 year-olds with above average waist circumference using aerobic exercise (e.g. cycling, jogging), resistance exercise (e.g. lifting weights), or a special diet?

Before you decide if you want to take part, it's important to understand why the research is being done and what we might be asking you to do. So please consider this letter carefully and talk to your family, friends, doctor or nurse if you want to. If you would like to take part you must sign the consent form attached to this document. If you have any questions about this information sheet, or would like further details, please ask a member of the research team - you can find their contact details at the bottom of this sheet.

Please be aware that if you have any of the following medical conditions: Type 1 or Type 2 diabetes mellitus, heart dysfunction, thyroid problems, or are receiving long-term treatment for a health condition, or if you: think you may be pregnant, have an eating disorder, have experienced any recent dramatic weight

changes, use medication that contains steroids, are an alcohol or drug abuser, have a known family history of hypercholesterolaemia (high levels of cholesterol in the blood), haemoglobinopathy (abnormal structure of haemoglobin in the blood, e.g. sickle-cell anemia), cardiac disease or renal disease, then unfortunately you cannot take part in this study. This is because the above conditions can affect your metabolic health and could influence the findings of our research project.

Purpose of the Study

More and more young people are becoming overweight and showing signs of being at risk of disease later in life. Being overweight (having a higher than average waist circumference) can lead to poor metabolic health (e.g. high blood pressure and high levels of fat and sugar in the blood), and possibly disease (e.g. heart disease or stroke or Type 2 diabetes) later on in life. It is important that we try to find the best way to help overweight people lose body fat and improve their health, particularly when there may already be signs of increased risk of developing type 2 diabetes, for example.

Exercise and diet can be used to help lose body fat and improve the chances of not developing disease, but we are not sure what type of exercise or diet is best. We would like to compare 2 different exercise programmes (aerobic and resistance), and a special diet programme against a control group (who receive a lifestyle consultation at the end of the study) to see which is best for improving the health of young people who are overweight and are at more risk of poorer metabolic health (as shown by having high blood pressure or raised cholesterol etc).

Why have you been invited?

You have been invited to join our study because you are 14-19 years-old; we are aiming to recruit 100 people like you to take part. We are recruiting 14-19 year olds as little is known about the best way to treat people of this age who are overweight and have poorer metabolic health status. We also only want to include people who have gone through puberty as we know that hormone levels will be more stable (which is important for this study). We do not know yet if you are one of these people, so we need to test this first.

Do you have to take part?

No, it is up to you. We will speak to you individually to make sure you clearly understand the nature of the study and will give you a copy of an information sheet which provides details of the study. If after a minimum of 24 hours you decide that you would like to take part in the study we will ask for your consent

by signing a form to indicate that you understand what is being asked of you and that you are happy to take part (we will provide you with a copy of this to keep). You are free to stop taking part at any time during the research without giving a reason. If you decide to stop, this will not affect any care you receive.

What will happen to you if you take part?

1. We first need to check that you have finished or are at the end of puberty. Females who are classified as Tanner Stage 5 for pubic hair (see Tanner Pubic Hair Ratings Scale on Page 8) can take part in this study. Males who are classified as Tanner Stage 5 for pubic hair (see Tanner Pubic Hair Ratings Scale on Page 8) can take part in the study. Anyone in Tanner Stage 1 to 4 cannot take part in the study.

2. If you are at the end of puberty (Tanner Stage 5) and would like to take part in this study, we will first need to measure your waist circumference (using a tape measure) at a suitable location (either at your school, GP surgery, college, the University of Bedfordshire, or at the Luton & Dunstable Hospital [L&DH]). If you have been referred to us, your GP or health practitioner at the L&DH may have taken this measurement for us.

3. If your waist circumference is high for someone your age and sex, we will then ask you to visit the L&DH on a separate morning so that we can assess your metabolic health e.g. blood pressure, fats and sugar in the blood (if this is during an academic term, we should be able to offer testing times before the start of the school or working / college day). If we are measuring your waist circumference at the L&DH, we may then take measurements of metabolic health immediately afterwards. When you arrive at the hospital, you will be asked to come along to where we are taking these measurements (we will give you directions to our location).

   A blood sample (to measure insulin levels, liver function and other indicators of metabolic health) will first be taken from your lower arm. This will involve a needle being inserted into a vein on the lower part of your arm so that blood can be drawn out into a container. This procedure is similar to the one you would normally expect at a hospital or your GP's surgery and we would not take more than 12 ml in any one sample. We will then take a finger prick blood sample. This is a very simple procedure and simply requires drops of blood to be squeezed from the finger and collected into a tube. The blood will immediately be put into a machine to measure cholesterol and sugar levels. You will then have your muscle and fat levels measured (by asking you to stand on a platform which looks like weighing scales with two metal plates) along with your blood pressure and height. On the night before we take these measurements, we will ask you to fast overnight (10-12 hours). It is very important that you do not

consume any food or drink in the 10 hours before your measurement time (although you can sip water). Please bring a snack which you can eat after your measurements have been taken.

4. If we find that you have above normal or slightly undesirable values for one or more of the following: blood pressure, blood cholesterol measures (high-density lipoprotein, triglycerides), blood sugar (glucose), insulin resistance (which means that your body doesn't respond well to insulin which can lead to T2DM), then you will be eligible to take part in the study.

5. If eligible, you will be randomly (by chance) assigned to either an exercise programme, a diet programme, or to act as a control (which means you will not take part in the diet or exercise programmes but will have your metabolic health and fitness assessed and be offered a diet and exercise counseling session with a trained advisor at the end of the study). If you have normal values for each of these measures you will not be invited to take part in any of the interventions but your data will be used so we can report on how common it is for young people to have poor metabolic health. Half of the people eligible to take part in the study will be assigned to an exercise group, a quarter to the special diet group, and a quarter to the control group.

What the interventions involve

Each programme will last 12 weeks. Before you take part in the programme you will be asked to undertake a fitness test on a stationary bike. It is expected that towards the end of the exercise you will find it hard work but you can stop at any point if you wish to. The fitness test will last approximately 8-12 minutes. We will also ask you to keep a 7-day physical activity diary and to wear a small box (accelerometer) on your waist for 7-days to monitor physical activity levels. This must be removed before swimming or washing and we ask you to take great care of this equipment.

If you are entered into the aerobic exercise programme, you be asked to visit the L&DH or the University of Bedfordshire 3 times per week (for 12 weeks) and to undertake approximately 45 minutes of moderate-intensity exercise on a stationary bike or treadmill (or cross-trainer). If you are randomized into the resistance exercise programme you will need to visit the L&DH or the University of Bedfordshire twice per week to complete a circuit of various weight exercises to improve strength. This will last approximately 40 minutes. All of these sessions will be supervised by a qualified fitness trainer.

If you are entered into the diet programme you will be given advice on eating foods that have a low glycaemic index (or GI) whilst maintaining a healthy balanced diet. Low GI foods are foods that cause smaller increases in your blood sugar levels compared to high GI foods, which cause bigger increases in blood sugar after you eat them. The diet does not restrict the number of calories that you can eat.

You will be given a lot of help and guidance as to what foods to choose whilst on this dietary programme. You will be asked to attend 2 separate group meetings at the L&DH or the University of Bedfordshire before the diet starts. On the 1st meeting you will receive information about completing weighed and non-weighed food diaries, which you will need to complete a total of 4 times during the study (for 3 days at a time). During the 2nd group meeting (about a week later) you will be provided with information about the importance of eating low GI foods and the diet itself. You will then be required to attend one-to-one discussions lasting approximately 15-30 minutes, once per week for the first 6 weeks of the diet and then at weeks 9 and 11. During other times during your 12 week dietary programme the research team may wish to contact you by telephone, text and/or e-mail (subject to your consent) to provide you with further support.

If you are entered into the control group, you will be asked to continue your normal lifestyle so that we may compare the results of our intervention groups to a group that does not change their lifestyle. You will still have your health and diet assessed and will be given feedback on your current health status as well as dietary and physical activity advice once the study has finished, if you wish.

All exercise and diet sessions will take place late afternoon/evening on each weekday (i.e. Monday to Friday). A variety of times will be offered from which you can pick those that are best for you.

6 weeks into the programme you will have your height, waist circumference, muscle and fat levels, blood pressure and a venepuncture blood sample taken. At the end of the programme, you will take part in all measurements again. During the study please do not take part in any other research weight management or health research projects.

If at the end of the study we feel that you may benefit from further health improvements, we will advise your GP and may put you in contact with a healthcare professional at the L&DH.

Expenses and Payments

As you will be required to visit the L&DH or the University of Bedfordshire a number of times, you may be required to pay for car parking. However, you may wish to walk, cycle, or use public transport to travel to the hospital, and you may also want to ask your parent or another member of your family to drive you by car and pick you up once your session has finished. Alternatively it may be possible to park a short distance away from the hospital or university and walk in from there with or without a companion depending on your circumstances.

What are the possible risks of taking part?

There are risks associated with exercise such as feeling light headed and fainting but every effort will be made to minimise these, such as the completion of a physical activity readiness questionnaire (attached). There are also risks when we are taking blood samples from you, such as infection and pain/discomfort, but anyone taking blood samples from you will have been trained to do so and will be following the hospital's guidelines for carrying out such procedures.

What are the possible benefits of taking part?

All participants will receive a thorough assessment of fitness and metabolic health (includes assessment of cardiovascular and metabolic health measures). If you take part in one of the exercise or diet programmes, it may be that your body fat levels will be reduced and that your metabolic health (blood pressure, cholesterol, blood sugar, insulin resistance) might be improved. Your fitness levels and ability to take part in exercise may also be improved by taking part in the exercise programmes. You may also find it easier to live a healthy lifestyle in the future once the study has finished. Participants acting as controls will receive individualised one-to-one guidance on lifestyle changes that could be made to improve health and reduce waist circumference.

Thank you for reading so far. If you are still interested please go to Part 2.

<u>Participant Information Sheet – Part 2</u>

What happens when the study ends?

When the study ends, you will no longer take part in the programme you took part in during the study. It will be suggested that you continue to be physically active and to eat a healthy diet.

If you are in the control group, you will be offered guidance on how to improve your health during a 30 minute one-to-one diet and physical activity discussion at the end of the study and may be put in contact with a health practitioner at the L&DH if appropriate. In this session your current food intake and exercise habits can be discussed and advice will be given based on this information.

What will happen if I don't want to carry on with the study or I can no longer consent to participate during the study?

If you withdraw from the study, or can no longer make the decision to continue with the study, the information and samples collected from you so far may still be used for research purposes.

What if there is a problem?

If you have a concern about any part of the study, you or your parent/guardian should ask to speak to the researchers who will do their best to answer your questions (Dr Catherine Kerr, Tel: 01234-793268 / e-mail: catherine.kerr@beds.ac.uk for general queries or those to do with the exercise and diet interventions; Ben Davies, Tel: [redacted] / e-mail: ben.davies@beds.ac.uk). If you are unhappy with answers to your queries and should wish to complain formally, you can contact the University of Bedfordshire Research Institute (Professor Mark Lewis: Telephone (Department secretary): 01234-793268).

In the event that something does go wrong and you are harmed during the research and this was because of someone being careless then you may be able to take legal action for compensation against the University of Bedfordshire, but you may have to pay your legal costs. The above formal complaints procedures will still be available to you. You will not receive compensation for any injury caused by a procedure outside of the study procedures or if you do not carry out the study procedures in the way you have been asked**.**

Will anyone else know I am taking part in this study?

We will keep your information and any data collected during the study in confidence. This means we will only tell those who have a need or right to know (appropriate research team members). Your data will be stored securely in a locked filing cabinet or on a password protected file on a computer and will only be used for the current study. Wherever possible, we will only send out information that has your name and address removed. Your GP will be notified that you are taking part in this study.

What will happen to any samples I give?

As we said earlier, we will be collecting blood samples from you to measure your metabolic health. These samples will be collected by Hospital staff or trained members of the research team who will transport the samples to the L&DH phlebotomy laboratory for immediate analyses. Some samples may be frozen and stored securely in a laboratory at the L&DH or the Sport & Exercise Sciences laboratory at the University of Bedfordshire for analysis at a later date, but will only be used for the current study. Only the Hospital/laboratory staff and the research team will have access to your samples. Blood samples and their results will be kept in confidence and will only be handled and seen by those who have a need or right to know. Any remaining samples that are no longer required will be destroyed following routine Hospital procedures.

What will happen to the results of the research study?

The findings of this study identified as scientifically important will be provided to you in a summary document once the data has been analysed. It is intended that this information is published in a scientific research journal. The published data will be fully anonymous and therefore no-one will be able to identify you in any published material.

Who is organising and funding the research?

The organisers and funders of this project are the L&DH Centre for Obesity Research and the University of Bedfordshire.

Who has reviewed the study?

Before any research goes ahead it has to be checked by a Research Ethics Committee. They make sure that the research is fair. Your project has been checked and approved by the Essex 1 Research Ethics Committee.

Further information and contact details.

For further information on the study, please follow the guide below:

1) For general information about the research, visit the National Research Ethics Service website at: www.nres.npsa.nhs.uk, where the study is outlined. 2) For specific information about this research project or advice as to whether you should participate, contact Ben Davies, a member of the research team (E-mail: ben.davies@beds.ac.uk; Telephone: [redacted]). 3) If you are unhappy or have concerns during the study, please contact Catherine Kerr, E-mail: catherine.kerr@beds.ac.uk; Telephone (Department secretary): 01234-793268.

If you would like to take part in this study, please return the following completed forms to the below address or by email to ben.davies@beds.ac.uk:

1. **Physical activity readiness questionnaire** – this checks that you or your child is well enough to complete the exercise test.
2. **Consent form** - this is to confirm you understand what is expected if you participate and you are happy to do so.

Please also check the Tanner Pubic Hair ratings scale below. If you are in Tanner stage 5 you **can** take part in this study. If you are not in Tanner stage 5, you **cannot** take part in this study.

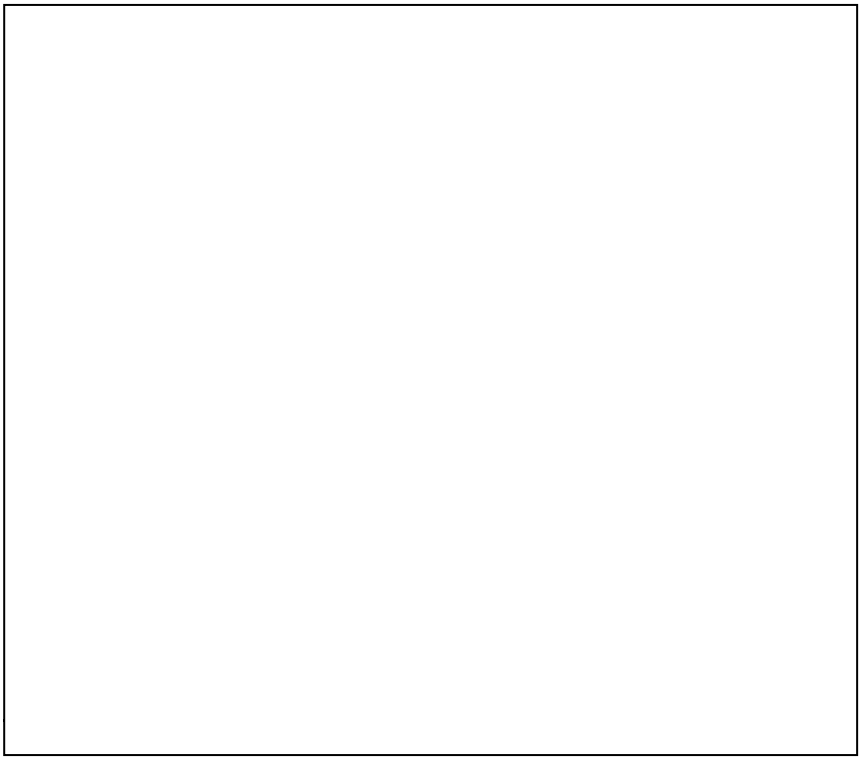
Please remember, as mentioned earlier, that if you have any the following medical conditions: Type 1 or Type 2 diabetes mellitus, heart dysfunction, thyroid problems, or are receiving long-term treatment for a health condition, or if you: think you may be pregnant, have an eating disorder, have experienced any recent dramatic weight changes, use medication that contains steroids, are an alcohol or drug abuser, have a known family history of hypercholesterolaemia (high levels of cholesterol in the blood), haemoglobinopathy (abnormal structure of haemoglobin in the blood, e.g. sickle-cell anemia), cardiac disease or renal disease, then unfortunately you cannot take part in this study.

Please send completed forms to the following address:

Ben Davies
Department of Sport & Exercise Sciences
University of Bedfordshire
Polhill Avenue
Bedford
Bedfordshire
MK41 9EA

Tanner Pubic Hair Ratings Scale for Boys

Tanner Pubic Hair Ratings Scale for Girls



Adapted Physical Activity Readiness Questionnaire for Youth

This questionnaire offers a safe, preliminary health-screening you prior to participation in exercise.

- Has your doctor ever said that you have a heart condition?

  Yes ☐ **No** ☐

- Do you have chest pain brought on by exercise?

  Yes ☐ **No** ☐

- In the past month, have you experienced chest pain when you were NOT doing exercise?

  Yes ☐ **No** ☐

- Do you lose consciousness or lose balance as a result of dizziness?

  Yes ☐ **No** ☐

- Do you have a bone or joint problem that could be aggravated by exercise?

  Yes ☐ **No** ☐

- Does your doctor currently prescribe medication for blood pressure or a heart condition (e.g., diuretics or water pills)?

  Yes ☐ **No** ☐

- Do you know of <u>any other reason</u> why you should not participate in exercise?

  Yes ☐ **No** ☐

If ‘yes’, please give the reason on the next page;

……

……

……

Participant name (Please PRINT NAME)……..

Signed……. Date…….

Consent Form

The SIRENS study (Study of Insulin Resistance factors using Exercise and Nutritional Strategies)

Please tick box

1. I confirm that I have read, or have had explained to me, the information sheet for the above study and fully understand what is involved. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily by a member of the research team, or my GP. ☐

2. I understand that my participation is voluntary and that I can stop taking part at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of data collected during the study may be looked at by members of the research team from the University of Bedfordshire and Luton & Dunstable Hospital, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data. ☐

4. I agree to take part in the above study. ☐

Name of Participant:……..

Date of Birth:…….

Permanent Address:……

.....

Contact No:……. Telephone

Contact address:……..... Email

Name of participant's GP:……

GP Address:……

Signature:…….. Date:…….

Signature of researcher: .....

Please send completed forms to the following address:

Ben Davies
Department of Sport & Exercise Sciences, University of Bedfordshire, Polhill Avenue, Bedford, Beds, MK41 9EA

Appendix 3

3 day weighed food diary instruction sheet

Introduction

We would like you to fill out a weighed food diary of all the food and drink you consume over 3 days in a row, one of these days must be a Saturday or Sunday (therefore you could choose, Thursday, Friday and Saturday or Sunday, Monday and Tuesday). You will need to take the food diary with you so you can record what you eat and drink whilst you are not at home.

Each diary entry should include (See example sheet):

- Everything you eat and drink, it could be a main meal or small snack (including sweets and even water).

- Please include extras like sauces, gravies and dressings that you put on you your food.

- Please give as much information as possible about the amount and type of food or drink you are consuming (weight of the food or drink, any brand names e.g. Heinz baked beans).

- **If possible, please put any packaging in clear bags provided. Especially if you cannot weigh the items (i.e. crisp packet eaten out and about)**

- Include the time of day, try to say if the meal or snack is for breakfast, lunch or dinner and where the food was eaten (e.g. home or elswhere).

- We have provided a food description prompt card to help you list the type of foods you eat **(PLEASE SEE PROMPT SHEET).**

- Please list all of the ingredients in whatever item you eat or drink. e.g. If at lunchtime you have a ham sandwich you should list the amounts and type of foods (e.g. 25 grams of Sainsbury's honey roast ham (sliced); 15 grams of iceberg lettuce; two slices of kingsmill wholemeal bread [85 grams], SEE EXAMPLE DIARY.
- There are sections on the diary to enter the different meals you eat (breakfast, lunch, dinner etc), separated by a gray bar. If you need more space to write in each section, just go over the gray shaded bar into the next section and start the next meal after the next gray shaded area (see example).

When recording

- Record what you consume at the same time as eating or drinking it, this way you won't forget what you have eaten.
- Any meals or snacks you don't prepare, try to get help from the person making the meal to weigh the amounts and list the type of foods in the meal.

Weighing your food and drink

Because this is a weighed food diary you have been provided with weighing scales so that you can weigh the foods and drinks you consume. This is very important so we can accurately work out how much of each type of food you are eating or drinking. If you cannot weigh your food or drink, please provide a description of the amount of food or place any packaging in the plastic wallet provided and explain the quantity of the packaged food you have eaten.

Here is an example of how to weigh a ham sandwich with lettuce (**make sure the scales are set to weigh in <u>grams</u>**):

- Turn on the scales by pressing the ON button

- **Weigh the empty container.** Place the container (e.g. plate) you will be eating from, on the scales. You should use this plate to weigh the ingredients on. **Make sure you write the weight of the empty plate.** You should repeat this process if you eat out of or off an additional container (e.g. if you have a plate of toast and a bowl of cereal)

- Before putting any food on the plate press the **ZERO** button, the scales should now have the plate on but display no weight (**0.00 grams**)

- Add to the plate both pieces of bread (remember to list the type (e.g. wholemeal) and if possible the brand of bread (e.g. Hovis) in the **'Food & Drink'** column. Record the weight (in grams) of the bread in the **'Weight served'** column.

- Press zero to reset the scales to **0.00 grams** and add any butter or spread to the bread. List the type of and brand of spread and record the weight of the butter in the appropriate sections.

- Press zero to reset the scales and repeat the process with the lettuce.

- Whenever a new ingredient is added, the scales should be zeroed so that the weight of each ingredient can be recorded separately as it is added to the plate.

- Use the weight on packaging to help diary entries if you consume the total contents of the packet.
- Any drinks you have should be weighed in the same way

- Any **leftovers**, not eaten, should be re-weighed and noted in the diary. If there is food leftover, re-weigh the container or containers that you have eaten or drunk from (with any leftovers on/in) and

write this in the **‘weight leftover’** column on the same line as where you have written the weight of the empty container. Put a tick next to the foods or drinks that are left on/in the container.

Make sure you eat or drink from the same container or containers you weighed at the beginning.

Example food diary

	Today is_Thursday___ Today's date is_07/03/11__ Recording day (1) 2 3 (please circle) Name: Participant number:				
	Food & **Drink** (Please describe in detail)	Weight of empty container (g)	Weight served (g)	Weight leftover(g) (tick items left)	**Leave blank** (office use only)
			- remember to weigh your empty plate or container-		
	Sainsbury's own- Cornflakes.	Bowl	**40 g**	**411 g**	
remember to include	**Sainsbury's Semi-skimme, pasturised milk**		**110 g**	✓	
	Toasted wholemeal pitta bread, Sainsbury's	Plate	**55 g**	----------	
	Flora Pro active, polyunsaturated, spread.		**34 g**		
Drinks	**Bovril beef extract**		**5 g**		
Snacks	**Sainsburys - Muliti-vitimin tablet with iron-**		**1 g**		
	Tap water		**240g**		
Wrappers	**Tetley tea bag and hot water**	cup	**202 g**	**364 g**	
	Sainsbury - Semi-skimmed milk, pasteurised		**40 g**	✓	
Time eaten:					
7.45am					
	Hardboiled egg	Plate	61 g	640 g	
remember to include	**Sainsbury Clementine**		**65 g**		
	sainsburys value - Strawberry yoghurt		**125g**		
Drinks	**Granny smith apple**		**100 g**		
Snacks	**sainsburys - Wholemeal pitta bread**		**109 g**		
	lettuce		23g	✓	
	sainsburys - Wafer thin honey cured ham		28g	✓	
Wrappers	Sliced tomato		31g	✓	
	Tap water		**440 g**	✓	
	Tea bag and hot water	Cup	200g	355 g ✓	
Time eaten:	**Londis - Skimmed milk, pasturised**		**45g**		
1pm				✓	

Appendix 4

Venous blood assay methodologies

a) Conducted by the L&DH biochemistry Laboratories

(Synchron ® Clinical Systems, Beckman Coulter®, Chemistry Information manual, 2005)

ALP

ALP reagent is used to measure alkaline phosphatase activity by a kinetic rate method using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction, alkaline phosphatase catalyzes the hydrolysis of the colorless organic phosphate ester substrate, p-nitrophenylphosphate, to the yellow colored product, p-nitrophenol, and phosphate. This reaction occurs at an alkaline pH of 10.3.

ALT

ALT reagent is used to measure analyte activity by a kinetic rate method. In the reaction, alanine aminotransferase catalyzes the reversible transamination of L-alanine and alpha-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of reduced
β-nicotinamide adenine dinucleotide (NADH) to β-nicotinamide adenine dinucleotide (NAD).

Albumin

Albumin reagent is used to measure albumin concentration by a timed endpoint method. In the reaction, albumin combines with bromcresol purple (BCP) to form a colored product.

CRP

CRP reagent is used to measure the C-Reactive Protein concentration by a turbidimetric method. In the reaction, C-Reactive Protein combines with specific antibody to form insoluble antigen-antibody complexes.

GGT

GGT reagent is used to measure the γ-glutamyl transferase activity by an enzymatic rate method.1 In the reaction, the γ-glutamyl transferase catalyzes the transfer of a gamma-glutamyl group from the colorless substrate, γ-glutamyl-p-nitroaniline, to the acceptor, glycylglycine with production of the colored product, p-nitroaniline.

Glucose

GLU reagent is used to measure the glucose concentration by a timed endpoint method.1 In the reaction, hexokinase (HK) catalyses the transfer of a phosphate group from adenosine triphosphate (ATP) to glucose to form adenosine

diphosphate (ADP) and glucose-6-phosphate. The glucose-6-phosphate is then oxidized to 6-phosphogluconate with the concomitant reduction of β-nicotinamide adenine dinucleotide (NAD) to reduced β-nicotinamide adenine dinucleotide (NADH) by the catalytic action of glucose-6-phosphate dehydrogenase (G6PDPH).

HDL

LDL and VLDL in human serum or plasma are precipitated by dextran sulfate (50 000 Mw) and magnesium in the separating reagent. The LDL and VLDL portions are then removed by centrifugation. The cholesterol in the HDL fraction which remains in the supernatant is assayed with an enzymatic cholesterol reagent HDLC reagent is used to measure the cholesterol concentration by a timed-endpoint method. In the reaction, the cholesterol esterase (CE) hydrolyzes cholesterol esters to free cholesterol and fatty acids. The free cholesterol is oxidized to cholestene-3-one and hydrogen peroxide by cholesterol oxidase (CO). Peroxidase (HPO) catalyzes the reaction of hydrogen peroxide with 4-aminoantipyrine (4-AAP) and phenol to produce a colored quinoneimine product.

$HbA1_c$

The SYNCHRON® System(s) utilizes two unique cartridges, Hb and A1c, to determine hemoglobin A1c concentration as a percentage of total hemoglobin.

Hemoglobin reagent is used to measure total hemoglobin concentration by a colorimetric method. The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio usedis one part sample to 8.6 parts reagent. The System monitors the change in absorbance at 410 nanometers. This change in absorbance is directly proportional to the concentration of total hemoglobin in the sample and is used by
the System to calculate and express total hemoglobin concentration.

Triglycerides

GPO reagent is used to measure the triglycerides concentration by a timed endpoint method.Triglycerides in the sample are hydrolyzed to glycerol and free fatty acids by the action of lipase. A sequence of three coupled enzymatic steps using glycerol kinase (GK), glycerophosphate oxidase (GPO), and horseradish peroxidase
(HPO) causes the oxidative coupling of 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) with 4-aminoantipyrine to form a red quinoneimine dye.

b) Conducted by the Addenbrookes, Cambridge University Hospital, Biochemistry Laboratories

IL-6 and TNFα

The cytokines (IL-6 and TNFα) were measured using an ultrasensitive 7-plex electrochemical luminesecence immunoassay from MesoScale Discovery, MD, USA. The assay was run according to

the manufacturer's instructions (MesoScale Discovery 2013, Human Inflammatory 7-plex ultra-sensative kit package insert, (online), Available, http://www.mesoscale.com/CatalogSystemWeb/Documents/Human_ProInflam_7-plex_US.pdf, (July 2013).

Adiponectin

Adiponectin was measured using a two-site microtitre plate-based DELFIA assay. The microtitre plate is coated with a monoclonal anti-adiponectin capture antibody. Diluted sample is added to the plate. After incubation and washing a biotinylated polyclonal anti-adiponectin detection antibody is added to the plate. After incubation and washing Europium labelled Streptavidin is added to the plate. After another wash fluorescence is generated in the wells by the addition of Enhancement Solution. The assay uses 20µl of diluted sample per well (1 in 50 dilution).

Insulin

For insulin measures, samples were assayed in singleton on a 1235 AutoDELFIA automatic immunoassay analyser using a two-step time resolved fluorometric assay (Kit No. B080-101). All reagents, standards and consumables are those recommended and supplied by the manufacturer.

Appendix 5

Low GI traffic light list example (breads)

Healthy Low GI List

High = more than 70

Medium = 56-69

Low = 55 or less

Try to eat LOW GI foods more often and avoid HIGH GI foods!

BREAD	
FOOD	GI value
Garlic bread	**High**
Fruit loaf (Tesco Value)	**90**
Multigrain bread (Sainsbury's)	**80**
Hovis, wholemeal	**68**
Hovis, white	**73**
Baguette/ French stick	**77**
Bagel, white	**72**
White bread, sliced	**71**
Bread roll, white	**71**
Wholemeal bread (Sainsbury's)	**71**
Fruit and cinnamon bread (Tesco Finest)	**71**
Crusty white bread	**70**
Bread roll, whole wheat	**70**
Pita bread, white	**68-Meduim**
Wholemeal, stoneground (whole wheat) bread	**66**
chapatti	**63**
Malt loaf, organic (Tesco)	**62**
Oatmeal batch bread (Tesco)	**62**
Multi-grain batch bread (Tesco)	**62**
Fruit loaf, sliced (Tesco)	**57**
Pita bread, wholemeal	**56**
Multi-seed bread	**56**
Crusty malted wheat bred (Tesco Finest)	**52-Low**
Corn Tortilla wrap	**52**
100 % whole grain bread (must be 100%!)	**51**
Pumpernickel bread	**50**
Sourdough rye bread	**48**
Burgen® Mixed grain bread	**44**
Burgen® Soy and linseed bread	**36**

Appendix 6

Unpresented data from chapter 8: Study 5

MANCOVA: Test of between subject effects met risk

source		Type III Sum of Squares	df	Mean Square	F	Sig.
Period2 * intervention	MeanSys	19.592	2	9.796	.144	.867
model 1	MeanDias	54.697	2	27.348	.661	.523
	BG	.022	2	.011	.063	.939
	Crisk_score	1.883	2	.941	.221	.803
	TG	.211	2	.106	.138	.871
	WC	.017	2	.008	.000	1.000
	HDL	.021	2	.011	.345	.711
Period2 * intervention	MeanSys	35.763	2	17.881	.307	.738
model 2	MeanDias	23.994	2	11.997	.347	.710
	BG	.097	2	.048	.265	.769
	Crisk_score	.109	2	.055	.020	.980
	TG	.089	2	.044	.062	.940
	WC	9.912	2	4.956	.269	.766
	HDL	.011	2	.006	.192	.826
Period2 * intervention	MeanSys	44.902	2	22.451	.372	.693
	MeanDias	7.940	2	3.970	.113	.894
model 3	BG	.000	2	.000	.001	.999
	Crisk_score	.040	2	.020	.007	.993
	TG	.167	2	.083	.151	.861
	WC	17.720	2	8.860	.542	.588
	HDL	.023	2	.012	.411	.668
Period2 * intervention	MeanSys	21.569	2	10.784	.174	.841
	MeanDias	6.007	2	3.003	.083	.921
Model 4	BG	.015	2	.008	.051	.950
	Crisk_score	.248	2	.124	.046	.955
	TG	.010	2	.005	.010	.990
	WC	24.315	2	12.157	.736	.490
	HDL	.035	2	.018	.608	.553

Parameter estimates mets risk factors

B	Std. Error	t	Sig.	95% Confidence Interval			95% Confidence Interval	
				Lower Bound	Upper Bound	Sig.	Lower Bound	Upper Bound
WC	model 1	2v1	-0.07	3.51	-0.02	0.985	-7.21	7.08
		3v1	0.03	3.85	0.01	0.993	-7.80	7.87
	model 2	2v1	2.03	3.31	0.61	0.546	-4.78	8.83
		3v1	2.45	3.75	0.65	0.519	-5.25	10.15
	model 3	2v1	2.92	3.18	0.92	0.368	-3.64	9.47
		3v1	3.30	3.68	0.90	0.379	-4.30	10.91
	model 4	2v1	3.117	3.203	.973	.341	-3.509	9.742
		3v1	4.383	3.911	1.121	.274	-3.707	12.473
TG	model 1	2v1	-0.09	0.64	-0.15	0.885	-1.39	1.21
		3v1	0.27	0.70	0.38	0.704	-1.16	1.69
	model 2	2v1	0.20	0.65	0.31	0.757	-1.14	1.54
		3v1	0.22	0.74	0.30	0.769	-1.30	1.73
	model 3	2v1	-0.14	0.58	-0.24	0.812	-1.35	1.06
		3v1	-0.37	0.68	-0.55	0.589	-1.77	1.03
	model 4	2v1	-.076	.568	-.133	.895	-1.251	1.100
		3v1	-.021	.694	-.030	.976	-1.456	1.414
HDL	model 1	2v1	0.04	0.13	0.34	0.734	-0.22	0.31
		3v1	-0.07	0.14	-0.52	0.608	-0.36	0.21
	model 2	2v1	0.05	0.13	0.36	0.722	-0.22	0.32
		3v1	-0.04	0.15	-0.26	0.796	-0.35	0.27
	model 3	2v1	0.01	0.13	0.06	0.950	-0.27	0.28
		3v1	-0.11	0.15	-0.74	0.466	-0.43	0.20
	model 4	2v1	.001	.134	.006	.995	-.276	.278
		3v1	-.154	.164	-.944	.355	-.493	.184
SBP	model 1	2v1	0.28	6.03	0.05	0.963	-12.00	12.56
		3v1	3.28	6.60	0.50	0.623	-10.17	16.74
	model 2	2v1	1.87	5.89	0.32	0.753	-10.24	13.97
		3v1	5.19	6.66	0.78	0.443	-8.50	18.89
	model 3	2v1	1.96	6.11	0.32	0.752	-10.65	14.56
		3v1	6.03	7.08	0.85	0.403	-8.59	20.65
	model 4	2v1	1.664	6.201	.268	.791	-11.163	14.491
		3v1	4.460	7.571	.589	.562	-11.201	20.121
DBP	model 1	2v1	-4.03	4.70	-0.86	0.398	-13.60	5.54
		3v1	-5.52	5.15	-1.07	0.292	-16.01	4.97
	model 2	2v1	-3.12	4.53	-0.69	0.497	-12.44	6.20
		3v1	-3.84	5.13	-0.75	0.461	-14.38	6.70
	model 3	2v1	-2.10	4.67	-0.45	0.656	-11.74	7.53
		3v1	-1.95	5.41	-0.36	0.722	-13.12	9.22
	model 4	2v1	-1.917	4.750	-.404	.690	-11.744	7.910
		3v1	-.947	5.800	-.163	.872	-12.945	11.050
BG	model 1	2v1	0.02	0.30	0.06	0.953	-0.60	0.63
		3v1	0.11	0.33	0.34	0.736	-0.56	0.79
	model 2	2v1	0.12	0.33	0.37	0.716	-0.56	0.80
		3v1	0.27	0.37	0.73	0.473	-0.49	1.04
	model 3	2v1	0.00	0.31	0.01	0.994	-0.63	0.63
		3v1	0.01	0.35	0.04	0.967	-0.72	0.75
	model 4	2v1	-.021	.305	-.070	.945	-.653	.610
		3v1	-.113	.373	-.304	.764	-.884	.657
Crisk	model 1	2v1	-1.00	1.51	-0.66	0.513	-4.07	2.07
		3v1	-0.60	1.65	-0.36	0.719	-3.96	2.76
	model 2	2v1	-0.24	1.28	-0.19	0.851	-2.87	2.38
		3v1	-0.06	1.44	-0.04	0.968	-3.03	2.91
	model 3	2v1	-0.16	1.29	-0.12	0.905	-2.82	2.51
		3v1	-0.12	1.50	-0.08	0.937	-3.22	2.98
	model 4	2v1	-.065	1.299	-.050	.961	-2.752	2.623
		3v1	.366	1.586	.231	.820	-2.916	3.647

Test of between subject effects inflammation and cytokines

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Period2 * intervention	ADIPONECTIN	1.91	2.00	0.96	0.16	0.853
Model 1	HsCRP	1.96	2.00	0.98	0.24	0.784
	TNF	1.99	2.00	1.00	0.04	0.957
	ILsix	0.37	2.00	0.19	0.01	0.989
	BNP	13.90	2.00	6.95	0.19	0.831
Period2 * intervention	ADIPONECTIN	6.07	2.00	3.03	0.59	0.565
Model 2	HsCRP	4.46	2.00	2.23	0.55	0.583
	TNF	4.36	2.00	2.18	0.17	0.848
	ILsix	9.43	2.00	4.71	0.26	0.771
	BNP	75.06	2.00	37.53	1.34	0.283
Period2 * intervention	ADIPONECTIN	2.66	2.00	1.33	0.29	0.749
Model 3	HsCRP	4.03	2.00	2.01	0.77	0.478
	TNF	2.65	2.00	1.32	0.10	0.907
	ILsix	20.27	2.00	10.13	0.57	0.577
	BNP	58.18	2.00	29.09	1.08	0.357
Period2 * intervention	ADIPONECTIN	1.55	2.00	0.77	0.16	0.850
Model 4	HsCRP	1.18	2.00	0.59	0.25	0.782
	TNF	0.05	2.00	0.02	0.00	0.998
	ILsix	33.56	2.00	16.78	1.06	0.367
	BNP	66.85	2.00	33.42	1.21	0.321

Parameter estimates inflammation and cytokines

							95% Confidence Interval	
			B	Std. Error	t	Sig.	Lower Bound	Upper Bound
ADIPONECTIN	Model 1	2v1	0.42	1.89	0.22	0.826	-3.45	4.29
		3v1	-0.72	2.01	-0.36	0.722	-4.83	3.39
	Model 2	2v1	-0.80	1.88	-0.42	0.676	-4.71	3.11
		3v1	-2.22	2.08	-1.07	0.296	-6.53	2.08
	Model 3	2v1	0.19	1.84	0.10	0.921	-3.65	4.02
		3v1	-1.17	2.05	-0.57	0.576	-5.44	3.11
	Model 4	2v1	0.20	1.87	0.10	0.918	-3.73	4.12
		3v1	-0.90	2.17	-0.41	0.684	-5.43	3.64
HsCRP	Model 1	2v1	-0.86	1.55	-0.56	0.583	-4.02	2.31
		3v1	0.20	1.64	0.12	0.903	-3.16	3.57
	Model 2	2v1	-1.56	1.66	-0.94	0.358	-5.01	1.89
		3v1	-0.16	1.83	-0.09	0.930	-3.96	3.63
	Model 3	2v1	-0.16	1.40	-0.12	0.909	-3.08	2.76
		3v1	1.48	1.56	0.95	0.353	-1.77	4.74
	Model 4	2v1	-0.19	1.33	-0.14	0.890	-2.97	2.60
		3v1	0.77	1.54	0.50	0.622	-2.45	3.99
TNF	Model 1	2v1	-0.54	3.65	-0.15	0.883	-8.02	6.93
		3v1	0.62	3.88	0.16	0.873	-7.32	8.57
	Model 2	2v1	0.09	3.00	0.03	0.976	-6.14	6.32
		3v1	1.67	3.31	0.50	0.619	-5.19	8.53
	Model 3	2v1	0.06	3.16	0.02	0.985	-6.54	6.66
		3v1	1.33	3.53	0.38	0.711	-6.04	8.69
	Model 4	2v1	0.02	3.13	0.01	0.995	-6.53	6.57
		3v1	0.20	3.62	0.05	0.958	-7.39	7.78
ILsix	Model 1	2v1	-0.44	3.21	-0.14	0.891	-7.03	6.14
		3v1	-0.37	3.42	-0.11	0.914	-7.37	6.63
	Model 2	2v1	-2.48	3.51	-0.71	0.487	-9.75	4.79
		3v1	-1.98	3.86	-0.51	0.614	-9.99	6.03
	Model 3	2v1	-3.59	3.65	-0.98	0.337	-11.21	4.02
		3v1	-3.62	4.07	-0.89	0.385	-12.12	4.88
	Model 4	2v1	-3.67	3.43	-1.07	0.299	-10.85	3.52
		3v1	-5.61	3.97	-1.41	0.174	-13.92	2.71
BNP	Model 1	2v1	1.81	4.71	0.38	0.703	-7.83	11.45
		3v1	3.00	5.00	0.60	0.554	-7.25	13.25
	Model 2	2v1	4.70	4.39	1.07	0.296	-4.41	13.80
		3v1	7.81	4.84	1.61	0.121	-2.22	17.84
	Model 3	2v1	4.70	4.47	1.05	0.305	-4.62	14.02
		3v1	7.20	4.99	1.44	0.164	-3.20	17.60
	Model 4	2v1	4.73	4.54	1.04	0.310	-4.77	14.23
		3v1	8.06	5.25	1.54	0.141	-2.93	19.05

Between subject effects: interaction of time period*intervention group for glucose control and liver function markers

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig
Period * intervention	HbA1c	.062	2	.031	.896	.432
	insulin	1540.822	2	770.411	.341	.717
	HOMAIR	1.526	2	.763	.306	.741
Model 1	ALP	154.883	2	77.441	.934	.418
	ALT	122.673	2	61.336	1.572	.245
	GammaGT	.508	2	.254	.031	.970
	Albumin	17.031	2	8.516	.344	.715
	Bilirubin	13.165	2	6.582	.649	.539
Period * intervention	HbA1c	.048	2	.024	.518	.614
	insulin	4183.554	2	2091.777	2.120	.182
	HOMAIR	4.532	2	2.266	2.342	.158
Model 2	ALP	21.555	2	10.777	.144	.868
	ALT	104.807	2	52.403	1.281	.329
	GammaGT	1.833	2	.917	.152	.862
	Albumin	23.291	2	11.646	.539	.603
	Bilirubin	13.041	2	6.520	.892	.447
Period * intervention	HbA1c	.059	2	.029	.541	.608
	insulin	**8670.115**	**2**	**4335.058**	**24.275**	**.001**
	HOMAIR	**6.502**	**2**	**3.251**	**8.641**	**.017**
Model 3	ALP	23.048	2	11.524	.157	.858
	ALT	62.449	2	31.224	.975	.430
	GammaGT	1.937	2	.969	.175	.843
	Albumin	6.778	2	3.389	.127	.883
	Bilirubin	26.081	2	13.041	1.962	.221
Period * intervention	HbA1c	.036	2	.018	1.188	.378
	insulin	**8932.946**	**2**	**4466.473**	**31.317**	**.001**
	HOMAIR	**6.785**	**2**	**3.392**	**10.052**	**.018**
Model 4	ALP	26.978	2	13.489	.159	.857
	ALT	61.480	2	30.740	.858	.478
	GammaGT	2.024	2	1.012	.169	.849
	Albumin	5.173	2	2.587	.153	.862
	Bilirubin	29.412	2	14.706	3.715	.103

Parameter estimates glucose control and liver function

			B	Std. Error	Sig.	95% Confidence Interval	
		Time point comparison				Lower Bound	Upper Bound
HbA1c	Model 1	2v1	-0.13	0.18	0.459	-0.51	0.25
		3v1	-0.43	0.33	0.214	-1.15	0.28
	Model 2	2v1	-0.16	0.23	0.499	-0.70	0.37
		3v1	-0.48	0.50	0.368	-1.62	0.67
	Model 3	2v1	-0.15	0.25	0.577	-0.76	0.47
		3v1	-0.83	0.84	0.363	-2.89	1.23
	Model 4	2v1	-0.14	0.13	0.353	-0.48	0.21
		3v1	-0.62	0.45	0.224	-1.77	0.53
ALP	Model 1	2v1	-4.31	8.58	0.624	-22.85	14.24
		3v1	-22.16	16.22	0.195	-57.20	12.88
	Model 2	2v1	-0.35	9.26	0.971	-21.71	21.01
		3v1	-10.11	19.97	0.626	-56.16	35.93
	Model 3	2v1	0.91	9.23	0.925	-21.68	23.50
		3v1	-15.60	30.92	0.632	-91.26	60.07
	Model 4	2v1	0.81	9.93	0.938	-24.72	26.35
		3v1	-17.22	33.49	0.629	-103.30	68.86
ALT	Model 1	2v1	-9.35	5.89	0.136	-22.07	3.36
		3v1	1.33	11.12	0.906	-22.69	25.36
	Model 2	2v1	-9.15	6.86	0.219	-24.97	6.66
		3v1	4.08	14.78	0.790	-30.01	38.17
	Model 3	2v1	-7.94	6.10	0.241	-22.86	6.98
		3v1	2.75	20.43	0.897	-47.23	52.73
	Model 4	2v1	-8.02	6.45	0.269	-24.61	8.56
		3v1	1.26	21.75	0.956	-54.64	57.16
GammaGT	Model 1	2v1	0.23	2.70	0.934	-5.61	6.07
		3v1	-0.94	5.11	0.857	-11.97	10.10
	Model 2	2v1	-1.07	2.64	0.696	-7.15	5.01
		3v1	-2.86	5.68	0.628	-15.97	10.24
	Model 3	2v1	-1.44	2.53	0.590	-7.64	4.75
		3v1	0.02	8.48	0.998	-20.74	20.77
	Model 4	2v1	-1.40	2.64	0.618	-8.18	5.38
		3v1	0.76	8.89	0.935	-22.08	23.60
Albumin	Model 1	2v1	-3.89	4.69	0.422	-14.01	6.24
		3v1	-2.59	8.85	0.775	-21.72	16.54
	Model 2	2v1	-3.01	4.98	0.562	-14.50	8.48
		3v1	-10.91	10.74	0.340	-35.68	13.86
	Model 3	2v1	-2.66	5.57	0.650	-16.28	10.97
		3v1	-5.31	18.65	0.785	-50.94	40.31
	Model 4	2v1	-2.45	4.44	0.605	-13.85	8.96
		3v1	-1.73	14.96	0.912	-40.18	36.71
Bilirubin	Model 1	2v1	-2.89	3.00	0.353	-9.38	3.59
		3v1	-5.27	5.67	0.369	-17.52	6.98
	Model 2	2v1	-3.37	2.90	0.279	-10.05	3.32
		3v1	0.86	6.25	0.894	-13.55	15.27
	Model 3	2v1	-3.58	2.78	0.245	-10.37	3.22
		3v1	10.24	9.30	0.313	-12.53	33.00
	Model 4	2v1	-3.47	2.14	0.167	-8.98	2.04
		3v1	12.09	7.23	0.155	-6.49	30.67

Appendix 7

Unpresented data from chapter 5: Study 3

Mean (±SD) cardiometabolic risk factor values across quantiles of GI and GL for males

	GI				GL RES			
	1 (54.62)	2 (62.82)	F	P	1 (100.07)	2 (120.19)	F	P
HDL	1.08 (0.26)	1.20 (0.31)	0.22	.639	1.08 (0.26)	1.20 (0.31)	2.22	.145
			1.38	.248			1.69	.202
TG	0.73 (0.37)	0.91 (0.49)	0.03	.868	0.77 (0.39)	0.87 (0.48)	0.78	.383
			1.89	.177			0.94	.340
WC	76.93 (9.08)	81.88 (14.33)	3.05	.089	80.10 (15.16)	78.97 (9.07)	0.01	.956
BG	4.94 (0.55)	4.83 (0.43)	0.54	.468	4.94 (0.57)	4.83 (0.41)	0.24	.626
			0.38	.542			0.24	.631
SBP	118.11 (9.17)	117.85 (12.89)	1.86	.181	119.32 (10.68)	116.75 (11.64)	1.16	.289
			0.04	.850			0.69	.410
DBP	70.73 (8.97)	70.27 (7.76)	1.67	.202	70.73 (8.78)	70.27 (7.95)	0.01	.937
			0.12	.731			0.01	.960
Crisk	-0.80 (2.43)	0.19 (3.50)	0.24	.631	-0.34 (3.76)	-0.23 (2.86)	0.03	.865
			1.75	.194			0.15	.698

Mean (±SD) cardiometabolic risk factor values across quantiles of GI and GL for females

	GI				GL RES			
	1 (55.58)	2 (62.39)	F	P	1 (96.37)	2 (88.55)	F	P
HDL	1.08 (0.24)	1.21 (0.44)	0.07	.793	1.15 (0.47)	1.14 (0.24)	0.25	.624
			0.03	.858			0.23	.636
TG	1.36 (1.41)	0.82 (0.22)	1.04	.321	1.02 (0.49)	1.13 (1.30)	0.26	.619
			0.84	.372				
WC	-	-			-	-		
	83.39 (14.85)	85.97 (13.66)	0.27	.610	81.55 (13.36)	87.27 (14.48)	0.42	.526
BG	4.87 (0.67)	4.57 (0.35)	0.77	.393	4.63 (0.48)	4.79 (0.59)	0.93	.347
			0.71	.410			0.97	.336
SBP	111.54 (12.24)	109.50 (9.39)	0.77	.392	109.17 (9.37)	111.53 (11.86)	0.22	.644
			0.43	.522			0.20	.662
DBP	75.29 (8.43)	72.64 (7.10)	1.01	.329	73.53 (7.99)	74.23 (7.80)	0.11	.749
			0.67	.424			0.10	.751
Crisk	1.98 (4.05)	0.33 (2.55)	1.62	.219	0.95 (3.93)	1.26 (3.04)	0.02	.882
			0.54	.471			0.02	.891

Mean (±SD) cardiometabolic risk factor values across quantiles of GI and GL for normal weight participants.

	GI				GL RES			
	1 (54.20)	2 (61.18)	F	P	1 (68.58)	2 (94.38)	F	P
HDL	1.19 (0.24)	1.21 (0.42)	0.70	.409	1.09 (0.26)	1.30 (0.38)	2.49	.124
			1.14	.294			2.24	.114
TG	0.64 (0.17)	0.82 (0.33)	2.18	.149	0.69 (0.27)	0.78 (0.29)	0.93	.342
			2.69	.110			1.05	.312
WC	73.20 (7.42)	74.18 (6.14)			73.67 (5.75)	73.75 (7.63)		
			0.17	.684			0.83	.369
BG	4.96 (0.57)	4.66 (0.40)	1.47	.234	4.83 (0.58)	4.78 (0.44)	0.33	.572
			3.00	.092			0.14	.713
SBP	115.10 (9.34)	113.39 (12.07)	1.01	.322	115.50 (9.85)	113.02 (12.64)	0.00	.954
			0.25	.618			0.01	.946
DBP	70.20 (9.34)	68.72 (7.36)	0.38	.540	69.28 (7.73)	69.57 (8.96)	0.04	.835
			0.34	.567			0.05	.828
Crisk	-1.44 (1.84)	-1.25 (2.41)	0.04	.850	-1.40 (2.06)	-1.28 (2.24)	0.69	.412
			0.39	.538			0.88	.354

Mean (±SD) cardiometabolic risk factor values across quantiles of GI and GL for overweight participants.

	GI				GL RES			
	1 (56)	2 (62.90)	F	P	1 (91.62)	2 (93.40)	F	P
HDL	0.97 (0.20)	1.13 (0.27)	2.00	.173	1.00 (0.25)	1.10 (0.25)	0.12	.730
			2.21	.153			0.24	.630
TG	1.41 (1.41)	1.03 (0.52)	1.72	.205	1.08 (0.50)	1.30 (1.31)	0.03	.875
			1.83	.191			0.04	.846
WC	92.11 (8.02)	94.02 (14.06)	0.01	.927	93.02 (15.70)	93.28 (7.33)	0.02	.878
BG	4.95 (0.59)	4.76 (0.47)	0.87	.362	4.88 (0.49)	4.82 (0.57)	0.21	.655
			1.04	.321			0.03	.859
SBP	120.96 (7.88)	113.28 (8.06)	3.23	.088	118.85 (12.34)	115.00 (11.41)	0.51	.484
			3.43	.079			0.00	.978
DBP	75.35 (7.88)	75.25 (5.92)	0.06	.814	76.00 (8.19)	74.72 (5.50)	0.01	.945
			0.17	.686			0.38	.546
Crisk	3.15 (3.58)	2.21 (2.82)	1.57	.225	2.80 (3.83)	2.49 (2.61)	1.57	.225
			1.80	.195			1.80	.195